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Electronic Supplementary Information

Upon the photostability of 8-nitro-cGMP and its caging as a 7-dimethylaminocoumarinyl ester

Ayan Samanta, Martin Thunemann, Robert Feil and Thorsten Stafforst*

Interfaculty Institute of Biochemistry, University of Tübingen, Auf der Morgenstelle 15, D-72076 Tübingen (Germany). Tel. (+) 49 7071 29 75376; webpage: http:// www.ifib.uni-tuebingen.de/research/stafforst.html

E-mail: thorsten.stafforst @uni-tuebingen.de

List of abbreviations:

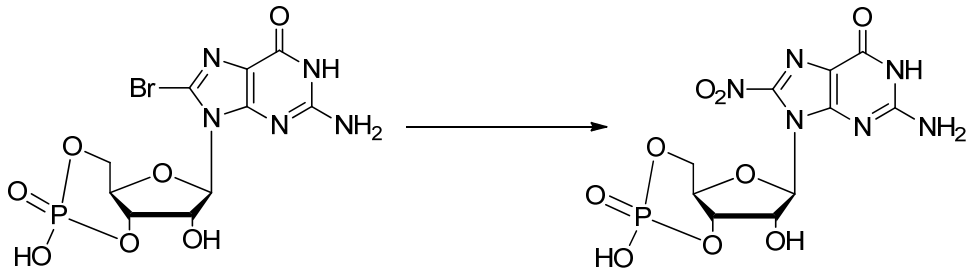
HPLC-ESI-HRMS = High performance liquid chromatography electron spray ionisation high-resolution mass spectrometry.

General methods:

All substrates and reagents required for synthesis and biochemical studies were purchased from commercial providers and used without any further purification. Thin layer chromatography (TLC) or HPLC was used for monitoring reactions. TLCs were performed on silica gel sheets (60 F_{254}, 0.2 mm, 5 x 10 cm, Merck) and visualised under UV light. Column chromatographic purifications of organic compounds were carried out on self-packed column of silica gel (0.04-0.063 mm/230-240 mesh). HPLC assays were accomplished on a Shimadzu Class-VP system (SCL-10A VP) equipped with a fixed wavelength UV detector (SPD-20AV). LC-ESI-HRMS analyses were carried out on a Bruker maXis4G ESI mass spectrometer combined with a DIONEX UltiMate 3000 HPLC system equipped with a multi-wavelength detector. High-resolution mass spectra were obtained by internal calibration (enhanced quadratic and HPLC) using sodium formate as a calibrant. For LC, samples were resolved on a Nucleosil 100 C_{18} column (3 µm, 2 × 100 mm, Dr. Maisch GmbH, Germany) with a binary gradient of 0 – 100% solvent B in 20 min at a flow rate of 0.3 ml/min [solvent A: water with 0.06% formic acid, solvent B: acetonitrile with 0.1% formic acid, all solvents and additives were of LC-MS grade]. LC-HRMS data were analysed using DataAnalysis (Version 4.1) software (Bruker Daltonics). Coumarinyl alcohol (**9**) was synthesised following a protocol by Eckardt *et al*.[1] UV spectra were recorded in a Cary 300 UV-visible spectrophotometer (Varian). Microwave reactions were performed in CEM Discover Benchmate operated by Synergy v1.46 software. *In vitro* photochemical study and fluorescence measurements were accomplished on a QuantaMaster 7 (Photon Technology International) fluorescence spectrophotometer, equipped with automatic shutters and monochromators for excitation and emission as well as a peltier thermocontroller. All photochemical experiments were performed at 20^0C in degassed buffer. ^{1}H-NMR and ^{31}P-NMR spectra were recorded on AMX-600 (Bruker) and AVII+500 (Bruker) NMR spectrometers, respectively. Residual undeuterated solvent signal was used as internal calibration of NMR peaks. ^{1}H-NMR peaks were assigned from the respective 2D NMR data.

Syntheses:

Synthesis of 8-nitroguanosine 5´,3´-cyclic monophosphate (8-nitro-cGMP) (1):



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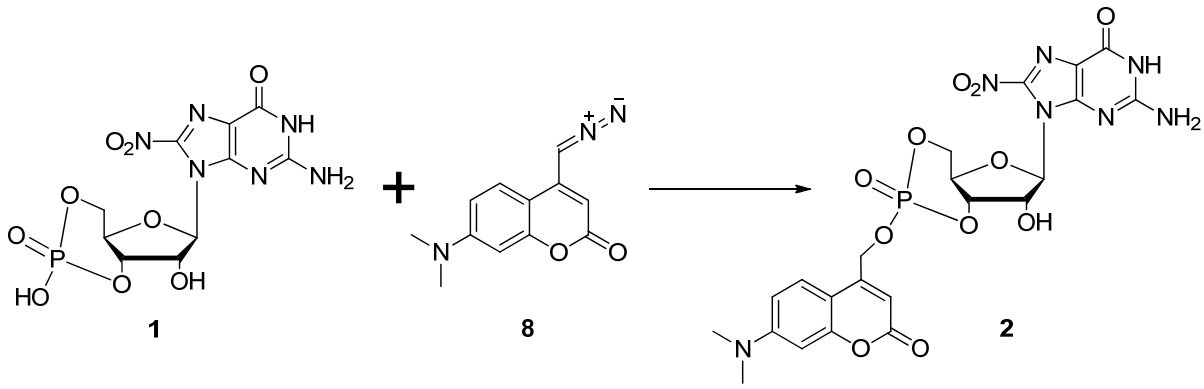
8-nitro-cGMP was synthesised by minor modification of a published procedure.[2] Briefly, a mixture of 8-bromo-cGMP (50 mg, 111 µmol, Biolog Germany) and sodium nitrite (35.3 mg, 500 µmol) in 1.4 ml of anhydrous DMSO containing 23 mM HCl was subjected to microwave heating at 100^0C. After 10 h of heating an additional quantity of sodium nitrite (43 mg, 600 µmol) was added and the heating was continued up to 30 h. The progress of the reaction can be monitored by HPLC for which samples were resolved on a Nucleodur 100 C_{18} column (5 µm, 125 mm x 4 mm, Macherey-Nagel) using a binary gradient of 5 to 20% of solvent B in 20 minutes [solvent B: 90% acetonitrile containing 0.1% TFA and solvent A: water containing 0.1% TFA, all solvents were of HPLC grade] at a flow-rate of 0.75 ml/min; t_R for starting material and product was 6.4 min and 7.2 min, respectively. The reaction mixture was purified directly by HPLC using similar eluent conditions. The yield of 8-nitro-cGMP was 27%. HPLC-ESI-HRMS: calc. $[M-H]^-$ $C_{10}H_{10}N_6O_9P$ = 389.0252, found 389.0249. UV: λ_{max} 395 nm (solvent: phosphate buffer pH 7.0)[2] (Fig. S2). ^{1}H-NMR (600 MHz, DMSO-d_6) δ 11.29 (s, 1H, NH), 7.18 – 6.97 (m, 2H, NH_2), 6.32 (s, 1H, H-1´), 5.94 (s, 1H, 2´-OH), 5.01 (dd, *J* = 9.8, 5.7 Hz, 1H, H-3´), 4.84 (d, *J* = 5.5 Hz, 1H, H-2´), 4.43 – 4.35 (m, 1H, H-5´), 4.25 (t, *J* = 9.4 Hz, 1H, H-5´´), 4.04 (td, *J* = 10.5, 5.2 Hz, 1H, H-4´) ppm.

Synthesis of DMACM-caged 8-nitro cGMP (2):



The coumarin-diazo compound (**8**) was synthesised according to a previously published procedure.[3]

A mixture of 8-nitro-cGMP (**1**) (28.4 µmol) and **8** (28.4 µmol, 6.5 mg) in anhydrous DMSO (227 µl) and acetonitrile (900 µl) was heated at 60^0C for 6 hour after which another equivalent of **8** (28.4 µmol, 6.5 mg) was added and the heating was continued for another 9 hours. 100 µl of acetonitrile was added to the reaction mixture and the heating was further continued for another 9 hours. The reaction can be monitored by HPLC. For this samples were resolved on a Nucleosil 100 C_{18} column (7 µm, 100 mm x 2 mm, Dr. Maisch GmbH, Germany) using a binary gradient of 0 to 50 % of solvent B in 20 minutes [solvent B: 90 % acetonitrile containing 0.1 % formic acid and solvent A: water containing 0.1 % formic acid, all solvents were of HPLC grade] at a flow-rate of 0.75 ml/min. t_R for DMACM-caged 8-nitro-cGMP axial and equatorial isomers were 13.2 min and 16 min, respectively and that for coumarinyl alcohol and 8-nitro-cGMP were 11.2 - 12.6 min (broad peak) and 5.8 min, respectively. The reaction mixture was purified directly by HPLC using similar eluent conditions. Due to the very broad and overlapping peak of the coumarinyl alcohol with the axial isomer, this isomer was extremely difficult to isolate in a large quantity with a very high purity as required for extensive NMR characterisation. The axial and the equatorial isomers could be assigned from their UV-absorption spectra as the axial isomers of DMACM-caged cyclic nucleotides were reported to have a slightly bathochromic λ_{max} in comparison to the corresponding equatorial isomers.[3] The UV-assignment of the isomers were further confirmed by the relative chemical shifts of the phosphorus atoms in the axial versus equatorial isomers, measured in ^{31}P-NMR as reported earlier.[4-6] The overall yield of **2** (both isomers, in nearly 50/50 ratio) was 22%. HPLC-ESI-HRMS: calc. $[M-H]^-$ $C_{22}H_{21}N_7O_{11}P$ = 590.1042, found 590.1048. UV: λ_{max} 386 and 395 nm (solvent: phosphate buffer)[3] for equatorial and axial isomer, respectively (Fig. S2). ^{31}P-NMR (162 MHz, DMSO-d_6, heteronuclear decoupled) δ -6.08 (axial isomer) ppm; δ -5.43 (equatorial isomer) ppm. ^{1}H-NMR (600 MHz, DMSO-d_6) (equatorial isomer, two rotamers in nearly 25% to 75% ratio) δ 11.32 (major rotamer) and 10.09 (minor

rotamer) (s, 1H, NH), 7.53 (major rotamer) and 8.23 (minor rotamer) (d, *J* = 9.0 Hz, 1H, coumarin H-5), 6.76 (major rotamer) and 6.80 (minor rotamer) (dd, *J* = 9.1, 2.5 Hz, 1H, coumarin H-6), 6.61 (major rotamer) and 6.64 (minor rotamer) (d, *J* = 2.5 Hz, 1H, coumarin H-8), 6.45 (major rotamer) and 6.68 (minor rotamer) (s, 1H, coumarin H-3), 6.17 (both rotamers) (s, 1H, H-1´), 6.13 (both rotamers) (br s, 1H, 2´-OH), 5.40 (both rotamers) (dd, *J* = 9.5, 5.3 Hz, 1H, H-3´), 5.34 (both rotamers) (dd, *J* = 7.8, 3.3 Hz, 2H, coumarin CH_2), 4.85 (both rotamers) (d, *J* = 5.4 Hz, 1H, H-2´), 4.77 – 4.72 (both rotamers) (m, 1H, H-5´), 4.49 (both rotamers) (dd, *J* = 8.4, 4.3 Hz, 2H, H-4´and H-5´´), 3.04 (major rotamer) and 3.05 (minor rotamer) (s, 6H, $N(CH_3)_2$,) ppm.

Irradiation experiment with 8-nitro cGMP (1):

A 2 ml, 27 µM solution (O.D. ~0.3 at 400 nm) of 8-nitro-cGMP in sodium phosphate buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.0) was irradiated in a fluorescence cuvette at 400±16 nm. After definite irradiation times (as indicated in Fig. 1) the UV spectra were recorded (220 – 600 nm) (Fig. S1) and aliquots of 50 µl were injected through a 20 µl analytical sample loop of the HPLC. A Nucleodur 100 C_{18} column (5 µm, 125 mm x 4 mm, Macherey-Nagel) was used along with a two-step binary gradient of 5 to 12 % of solvent B in 10 minutes followed by 12 to 80 % of solvent B in 10 minutes [solvent B: 90 % acetonitrile containing 0.1 % TFA and solvent A: water containing 0.1 % TFA, all solvents were of HPLC grade] at a flow-rate of 0.75 ml/min to resolve the decomposition products. The amounts of remaining 8-nitro-cGMP in each aliquot were determined by calculating the absolute HPLC peak area values for the UV-trace at 370 nm using Shimadzu Class-VP^{TM} data analysis software (Version 5.03) (Fig. 1).[7] t_R for 8-nitro-cGMP was 7.8 min.

The photo-decomposition of 8-nitro cGMP at 430 nm was monitored under exact same conditions as described above except for the irradiation wavelength which was 430±16 nm (Fig. S7).

Uncaging of DMACM-caged 8-nitro cGMP (2):

A 10 µM solution (O.D. ~0.3 at 400 nm) of DMACM-caged 8-nitro cGMP (**2**) in buffer was irradiated at 400±16 nm as described earlier. After definite irradiation times (as indicated in individual experiments) the UV spectra were recorded and aliquots were subjected to HPLC analyses to determine the amounts of liberated 8-nitro cGMP (**1**) (Fig. 1 and Fig. S3). For HPLC analyses samples were resolved on a Nucleosil 100 C_{18} column (7 µm, 100 mm x 2 mm, Dr. Maisch GmbH, Germany) using a binary gradient of 0 to 50 % of solvent B in 20 minutes [solvent B: 90 % acetonitrile containing 0.1 % formic acid and solvent A: water containing 0.1 % formic acid, all solvents were of HPLC grade] at a flow-rate of 0.75 ml/min. All other

conditions were maintained identical as earlier. t_R for DMACM-caged 8-nitro-cGMP axial and equatorial isomer was 13.2 min and 16 min, respectively and t_R for 8-nitro-cGMP was 5.8 min.

Similarly, the uncaging reaction at 430 nm could be monitored under exact same conditions as described above except for the irradiation wavelength which was 430±16 nm. Since the decomposition rate was expected to be slightly slower at higher wavelength, occasionally a few aliquots with longer irradiation times (as indicated in individual experiments) were collected to ensure reaching the plateau of the reaction kinetics (Fig. S5 and S6).

Solvolytic stability of the DMACM caging group:

A 10 µM (O.D. ~0.3 at 400 nm) solution of the DMACM-caged 8-nitro-cGMP (**2**) in sodium phosphate buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.0) was incubated overnight at room temperature in the dark and then subjected to HPLC analysis using eluent conditions as described for the uncaging reaction earlier. The experiment was performed for both isomers. No decomposition or hydrolysis of the starting material was detectable for either isomer (Fig. S8).

Determination of the quantum yield (ϕ) of DMACM-caged 8-nitro-cGMP (2) and 8-nitro-cGMP (1):

The photochemical quantum yield (ϕ) was determined by comparing the uncaging of DMACM-caged 8-bromo-cGMP axial isomer at 400 nm using relative methods.[7]

A 20 µM solution (O.D. ~0.3 at 400 nm) of the DMACM-caged 8-bromo-cGMP (Biolog Germany) axial isomer in sodium phosphate buffer (10 mM sodium phosphate, 100 mM NaCl, pH 7.0) was irradiated in a fluorescence cuvette at 400±16 nm as described earlier. After definite irradiation times (as indicated in Fig. S4) aliquots were subjected to HPLC analyses to determine the amounts of liberated 8-bromo-cGMP using eluent conditions as described for uncaging reaction earlier. The amounts of liberated 8-bromo-cGMP in each aliquot were determined by calculating the absolute HPLC peak area values for the UV-trace at 260 nm using Shimadzu Class-VP^{TM} data analysis software (Version 5.03) (Fig. S4). t_R for starting material was 13.4 min and the liberated 8-bromo-cGMP was 5.6 min.

The first-order half-life for the formation of 8-bromo-cGMP was calculated to be 13.7±0.9 seconds. Taking the known photoactivation efficiency $\varepsilon\times\phi$ = 4.96 $mM^{-1}cm^{-1}$ for the DMACM-caged 8-bromo-cGMP axial isomer[3] into account we estimated $\varepsilon\times\phi$ for the uncaging of DMACM-caged 8-nitro cGMP to be ≈ 1.88 $mM^{-1}cm^{-1}$ for the axial and ≈ 1.95 $mM^{-1}cm^{-1}$ for the equatorial isomer of DMACM-caged 8-nitro-cGMP at 400 nm from the respective half-lives of 36 seconds and 35 seconds measured for the axial and equatorial isomers, respectively. The respective photochemical quantum yields (ϕ) were

calculated to be 7.5±0.4% for the axial and 7.8±0.7% for the equatorial isomer of DMACM-caged 8-nitro-cGMP estimating an absorption coefficient (ε) ≈ 25 $mM^{-1}cm^{-1}$ for both isomers.[3,8] On the contrary, the photochemical quantum yield (ϕ) was calculated to be 0.18% for the photodecomposition (half-life = 67.4 min) of 8-nitro-cGMP taking an absorption coefficient of ε = 9.1 $mM^{-1}cm^{-1}$ into account.[8]

Preparation of cell extracts:

Immortalised mouse embryonic fibroblast cell lines derived from cGKI knockout[9] (genotype: $cGKI^{L-/L-}$) or cGKIα rescue[10] (genotype: $SM22^{+/I\alpha}$; $cGKI^{L-/L-}$) embryos were used in passages 30 to 40.[11] Cells were grown at 37°C in DMEM supplemented with 10% FCS and penicillin/streptomycin to 90-100% confluence and then serum-starved for 6 hours, followed by two washes with ice-cold PBS (135 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4). Then, 1 mL TN buffer (20 mM Tris/Cl pH 8.0, 100 mM NaCl) supplemented with 0.2 mM phenylmethylsulfonyl fluoride and a phosphatase inhibitor cocktail (PhosStop, Roche, 1 tablet per 10 mL buffer) was added per 75 cm^2 growth area. Cells were scraped off and the resulting cell suspension was sonicated (Bioruptor UCD-200, Diagenode, 5 cycles for 30 s at maximum power, interrupted by 30 s rest on ice). The cell extracts were centrifuged for 10 min at 4°C and 14000 rpm to remove cell debris. The supernatant was subjected to Bradford assay with BSA as calibration standard to determine the total protein concentration and was directly used in the *in vitro* VASP phosphorylation assay.

***In vitro* VASP phosphorylation assay:**

In vitro VASP phosphorylation assays were performed in a total volume of 100 µl containing 65 µl of the cell extract. Each reaction mixture was supplemented with MES buffer (50 mM 2-(N-morpholino)ethanesulfonic acid, 0.4 mM ethylene glycol tetraacetic acid, 1 mM Mg acetate, 10 mM NaCl, 0.1% BSA, pH 6.9), as well as dithiothreitol and ATP to final concentrations of 10 and 0.1 mM, respectively, followed by the addition of the test compound (4 mM stock solutions in DMSO/ACN 1:1) to a final concentration of 0.1 mM. For the reconstitution experiments, recombinant cGKIα expressed in Sf9 insect cells and purified as described[12] was prediluted in TN buffer and added to extracts of cGKI-deficient cells (15 ng/reaction). Samples were kept in the dark or were irradiated for 2 min at 400±16 nm as described above followed by incubation for 20 min at 30°C in the dark. Reactions were stopped by the addition of 25 µl protein loading buffer (320 mM Tris/Cl pH 6.8, 15% (w/v) SDS, 25% (v/v) 2-mercaptoethanol, 0.01% (w/v) bromophenol blue, 40% (v/v) glycerol). Samples were heated for 5 min to 95°C and subjected to Western blot analysis.

Western blot analysis:

SDS-PAGE and Western blotting was performed according to standard procedures. After semi-dry blotting, polyvinylidene fluoride membranes were blocked in 5% milk powder in TBS-T (5 mM Tris/Cl pH 8.2, 75 mM NaCl, 0.1% Tween-20) and incubated overnight at 4°C in primary antibody (rabbit anti-VASP, 1:1000; Cell Signaling, 9A2, order-no. 3132). Antibody binding was detected using horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit IgG, 1:2000; Cell Signaling, order-no. 7074) using a chemiluminescent substrate (Advansta WesternBright ECL, Biozym). Signals were recorded using a cooled CCD camera (Alpha-Imager, Bio-Rad Laboratories). Intensities of non-saturated bands were analysed using ImageJ (v 1.48, NIH) software.

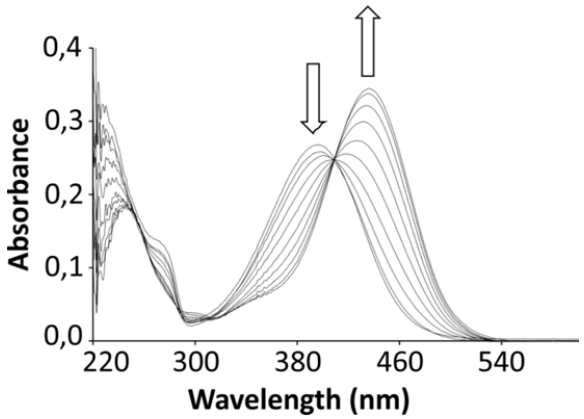


Fig. S1 Irradiation experiment with 8-nitro-cGMP (**1**) at 400 nm. 8-nitro-cGMP (27 µM) in degassed buffer was irradiated at 400 nm for 230 min. UV spectra of the irradiated reaction mixture at various time points.

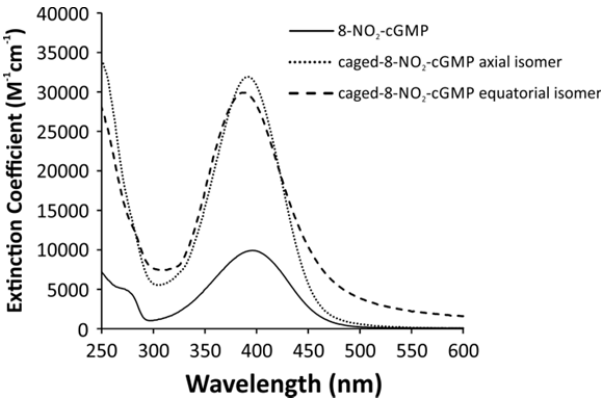


Fig. S2 UV spectra of 8-nitro-cGMP (**1**) (solid line), DMACM-caged 8-nitro-cGMP (**2**) axial isomer (dotted line) and DMACM-caged 8-nitro-cGMP (**2**) equatorial isomer (dashed line) in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl at concentration of 27 µM, 10 µM and 10 µM, respectively.

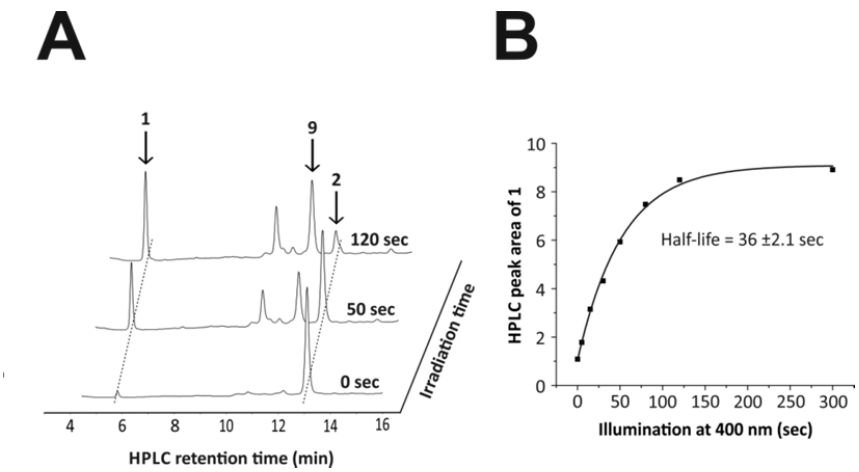


Fig. S3 Uncaging of DMACM-caged 8-nitro cGMP (**2**) at 400 nm. Axial isomer of the caged compound (10 µM) in buffer was irradiated at 400 nm for up to 300 sec. Aliquots at various time points were subjected to HPLC analyses to monitor the formation of the 8-nitro cGMP (**1**). A) Selected HPLC-traces (260 nm) of the irradiated reaction mixture; B) plot of the HPLC peak area values (traces at 370 nm) of the liberated 8-nitro cGMP (**1**) versus irradiation time revealed a first-order half-life of ~36 sec.

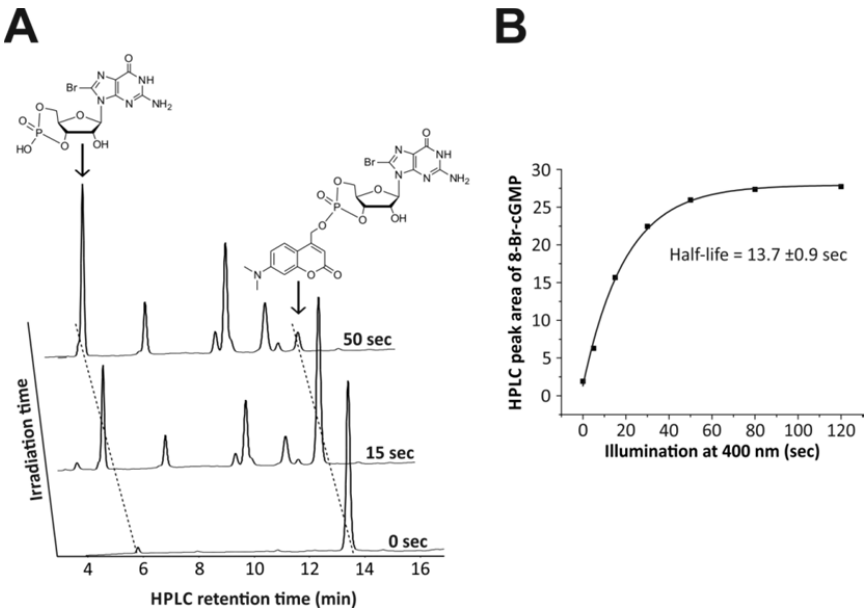


Fig. S4 Uncaging of DMACM-caged 8-bromo-cGMP at 400 nm for determination of quantum yield. Axial isomer of the caged compound (20 µM) in buffer was irradiated at 400 nm for up to 120 sec. Aliquots at various time points were subjected to HPLC analyses to monitor the formation of the 8-bromo cGMP. A) Selected HPLC-traces (260 nm) of the irradiated reaction mixture; B) plot of the HPLC peak area values (traces at 260 nm) of the liberated 8-bromo-cGMP versus irradiation time revealed a first-order half-life of ~14 sec.

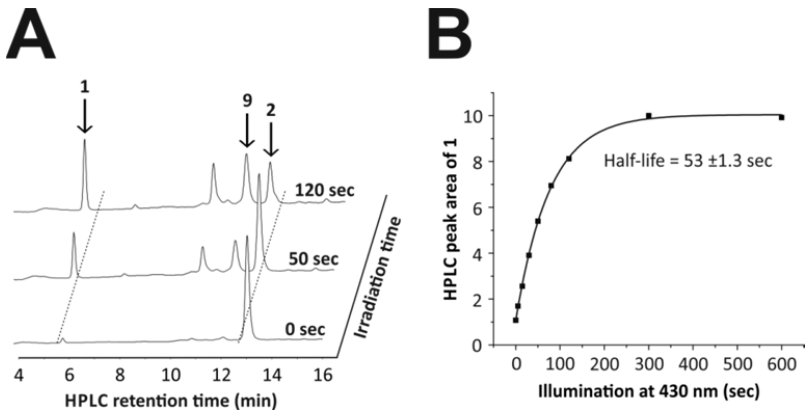


Fig. S5 Uncaging of DMACM-caged 8-nitro cGMP (**2**) at 430 nm. Axial isomer of the caged compound (10 µM) in buffer was irradiated at 430 nm for up to 600 sec. Aliquots at various time points were subjected to HPLC analyses to monitor the formation of the 8-nitro cGMP (**1**). A) Selected HPLC-traces (260 nm) of the irradiated reaction mixture; B) plot of the HPLC peak area values (traces at 370 nm) of the liberated 8-nitro cGMP (**1**) versus irradiation time revealed a first-order half-life of ~53 sec.

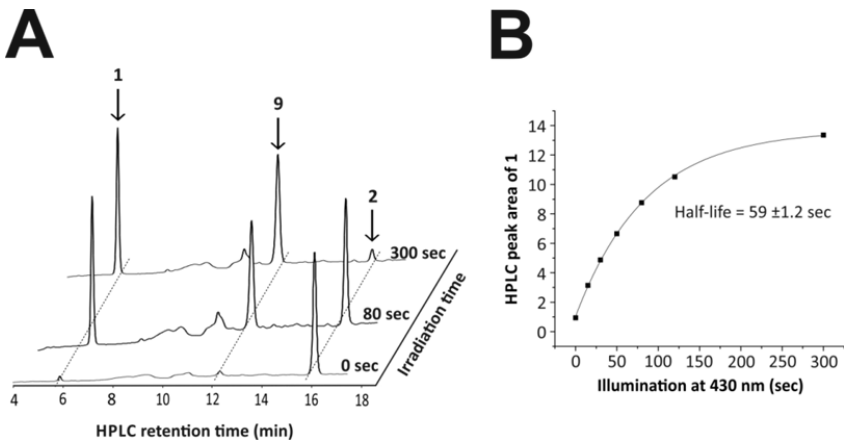


Fig. S6 Uncaging of DMACM-caged 8-nitro cGMP (**2**) at 430 nm. Equatorial isomer of the caged compound (10 µM) in buffer was irradiated at 430 nm for up to 300 sec. Aliquots at various time points were subjected to HPLC analyses to monitor the formation of the 8-nitro cGMP (**1**). A) Selected HPLC-traces (260 nm) of the irradiated reaction mixture; B) plot of the HPLC peak area values (traces at 370 nm) of the liberated 8-nitro cGMP (**1**) versus irradiation time revealed a first-order half-life of ~59 sec.

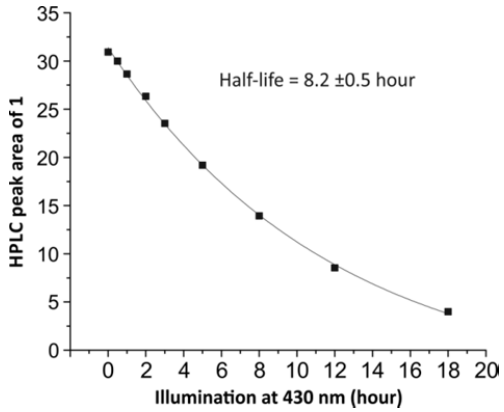


Fig. S7 Irradiation experiment with 8-nitro-cGMP (**1**) at 430 nm. 8-nitro-cGMP (27 µM) in degassed buffer was irradiated at 430 nm for 18 hour. Aliquots at various time points were subjected to HPLC analyses to monitor the decay of the starting material (**1**). Plot of the HPLC peak area values (traces at 370 nm) of the starting material (**1**) versus irradiation time revealed a first-order half-life of ~8 hour.

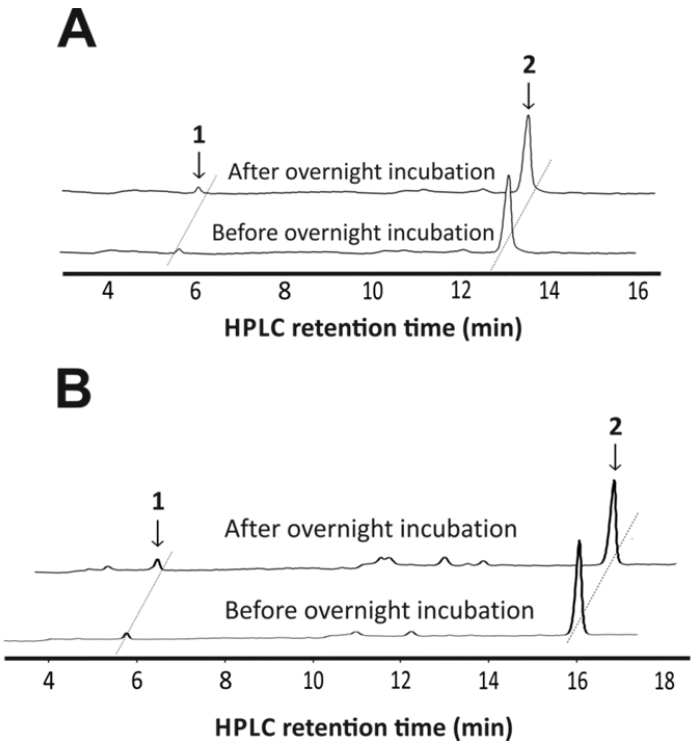


Fig. S8 Solvolytic stability of the caging group; (A) axial isomer and (B) equatorial isomer. A 10 µM solution of the DMACM-caged 8-nitro cGMP (**2**) was incubated overnight in the dark at room temperature in buffer and subjected to HPLC analysis to determine the amount of liberated 8-nitro cGMP (**1**) due to hydrolysis.

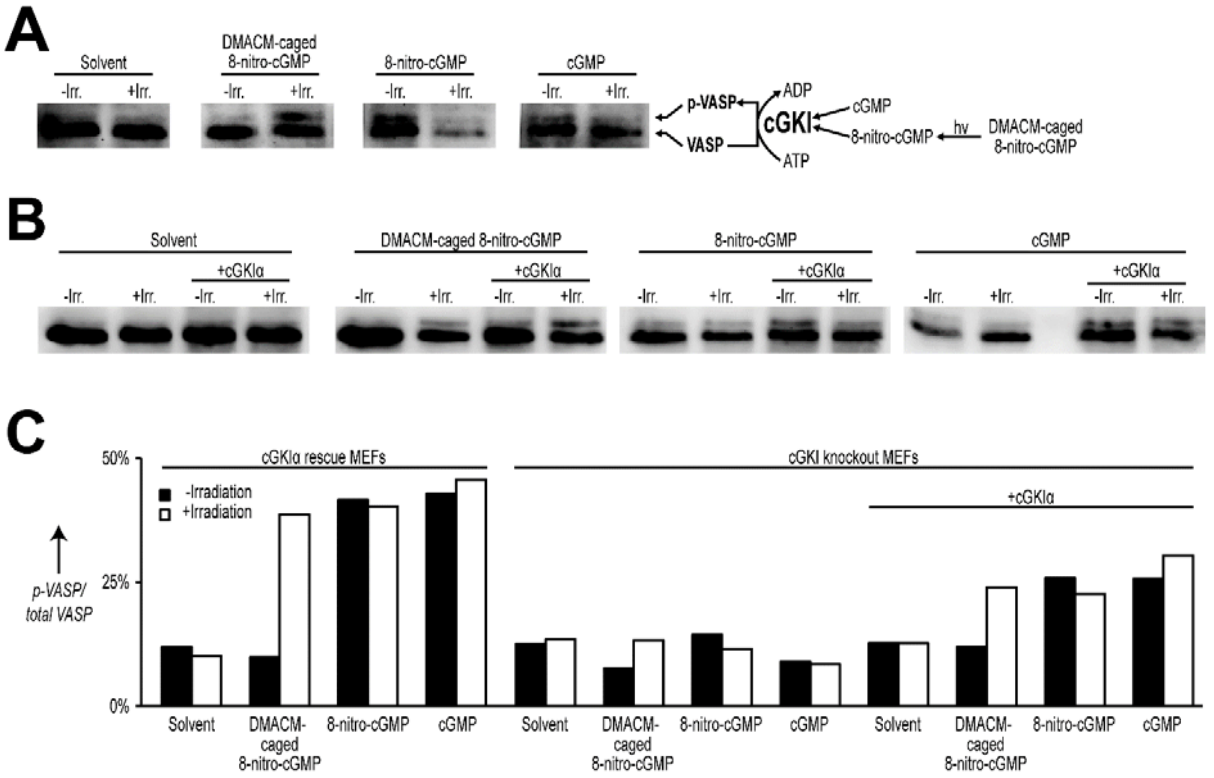


Fig. S9 Monitoring cGKI activation through detection of VASP phosphorylation in cell extracts upon incubation with cGMP, 8-nitro-cGMP (**1**), and DMACM-caged 8-nitro-cGMP (**2**). Cell extracts (**A**) from cGKIα-expressing mouse embryonic fibroblasts (MEFs) (cGKIα rescue MEFs, genotype: $SM22^{+/I\alpha}$; $cGKI^{L-/L-}$), or (**B**) from cGKI-deficient MEFs (cGKI knockout MEFs, genotype: $cGKI^{L-/L-}$), without or with addition of purified cGKIα (15 ng per reaction) were incubated in the dark with the indicated compounds diluted in acetonitrile/DMSO (1:1, solvent) for 20 min at 30°C. Before the incubation period, reaction mixtures were either illuminated for 2 min at 400±16 nm (+Irr.) or kept in the dark (-Irr.). Western blot analysis was performed to detect phosphorylated VASP (p-VASP), which migrates at a higher molecular weight than non-phosphorylated VASP. 8 µg (A) and 14 µg (B) protein was loaded per lane. Antibody binding was detected using horseradish peroxidase (HRP)-coupled secondary antibodies and chemiluminescence. **(C)** Densiometric analysis of the images shown in A and B to estimate the relative proportion of p-VASP given as the ratio between band intensities of p-VASP and p-VASP + non-phosphorylated VASP that were determined with ImageJ.

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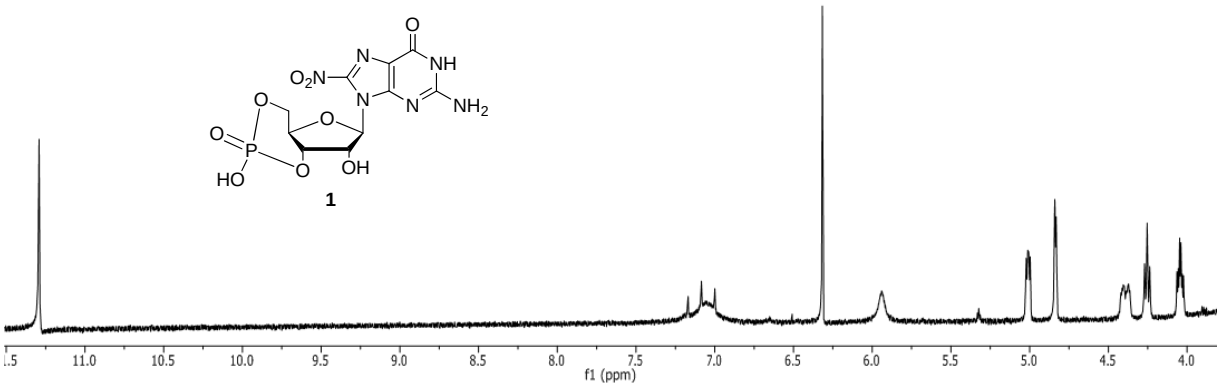
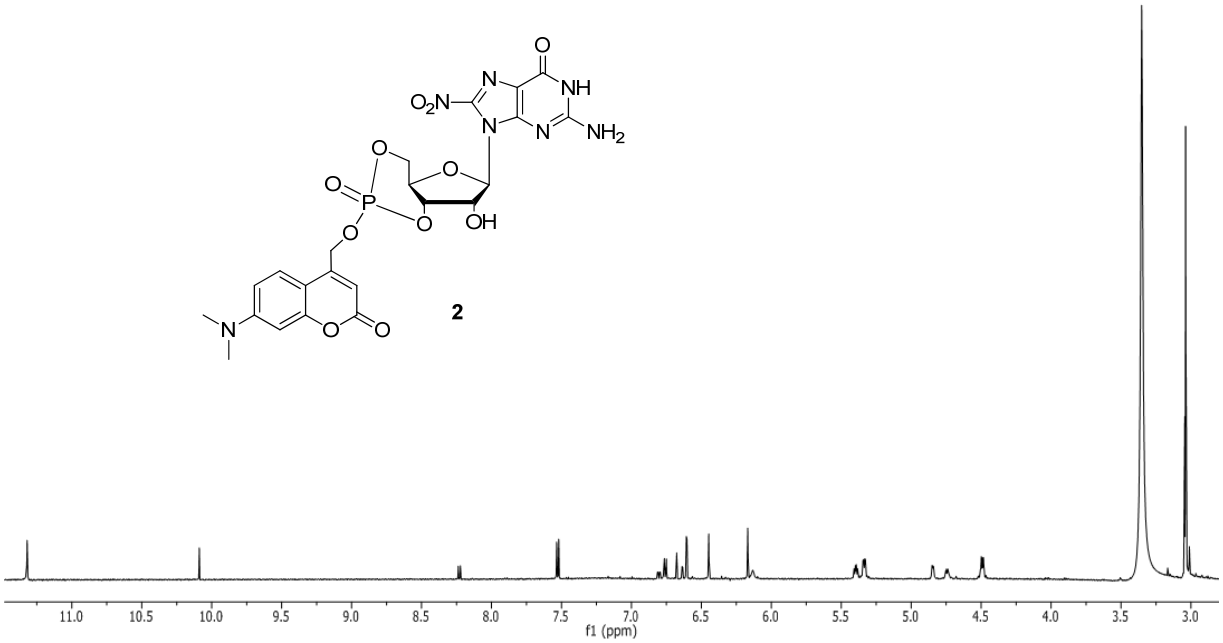
12. R. Feil, J. Kellermann and F. Hofmann, *Biochemistry*, 1995, **34**, 13152-13158.

Supplementary spectra:

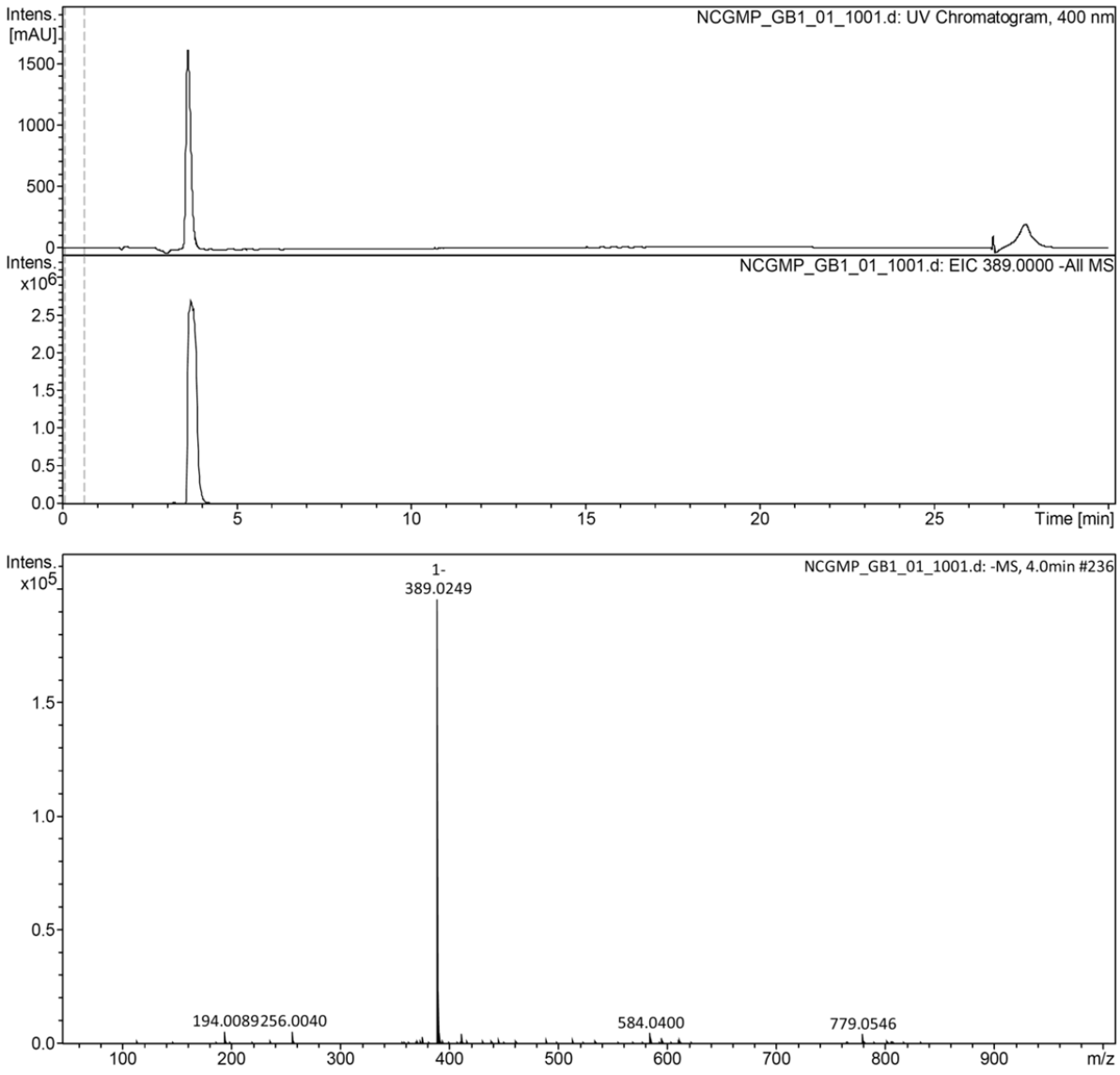
^{1}H NMR (600 MHz, DMSO-d_6) of 8-nitro-cGMP (**1**)



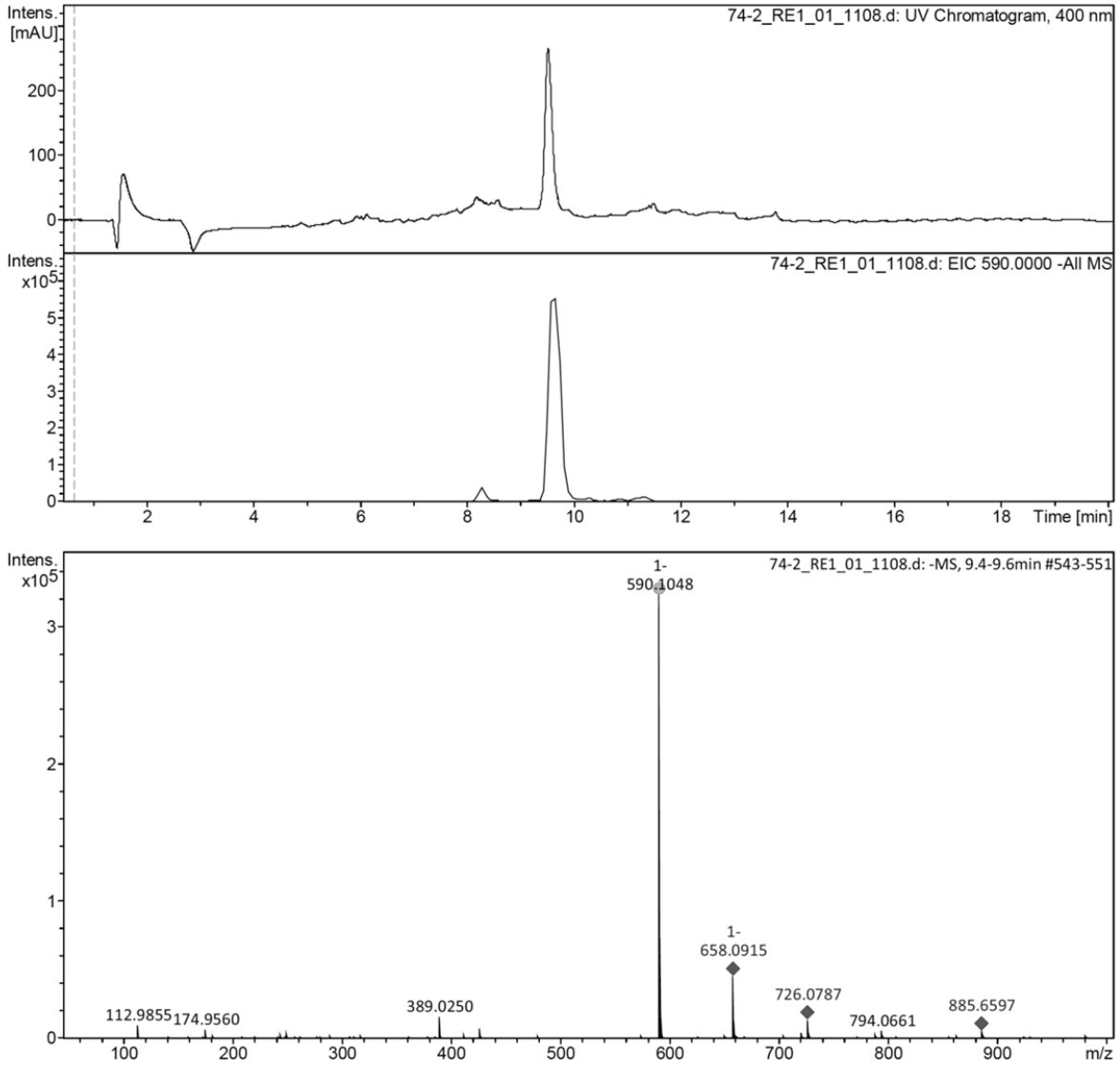
^{1}H NMR (600 MHz, DMSO-d_6) of DMACM-caged 8-nitro-cGMP (**2**) (equatorial isomer)



HPLC-ESI(negative mode)-HRMS analysis of 8-nitro-cGMP (**1**)

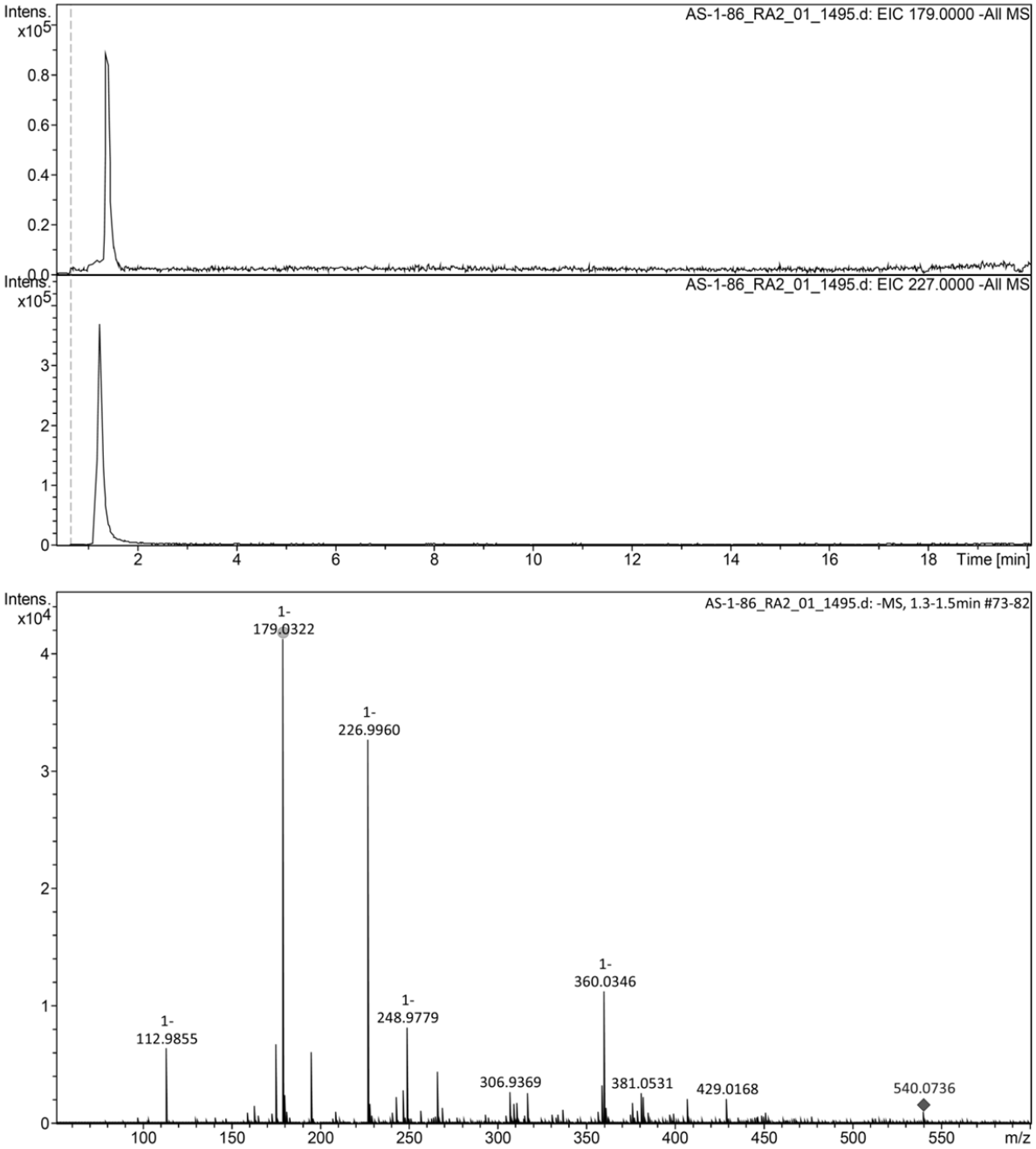


HPLC-ESI(negative mode)-HRMS analysis of DMACM-caged 8-nitro-cGMP (**2**)



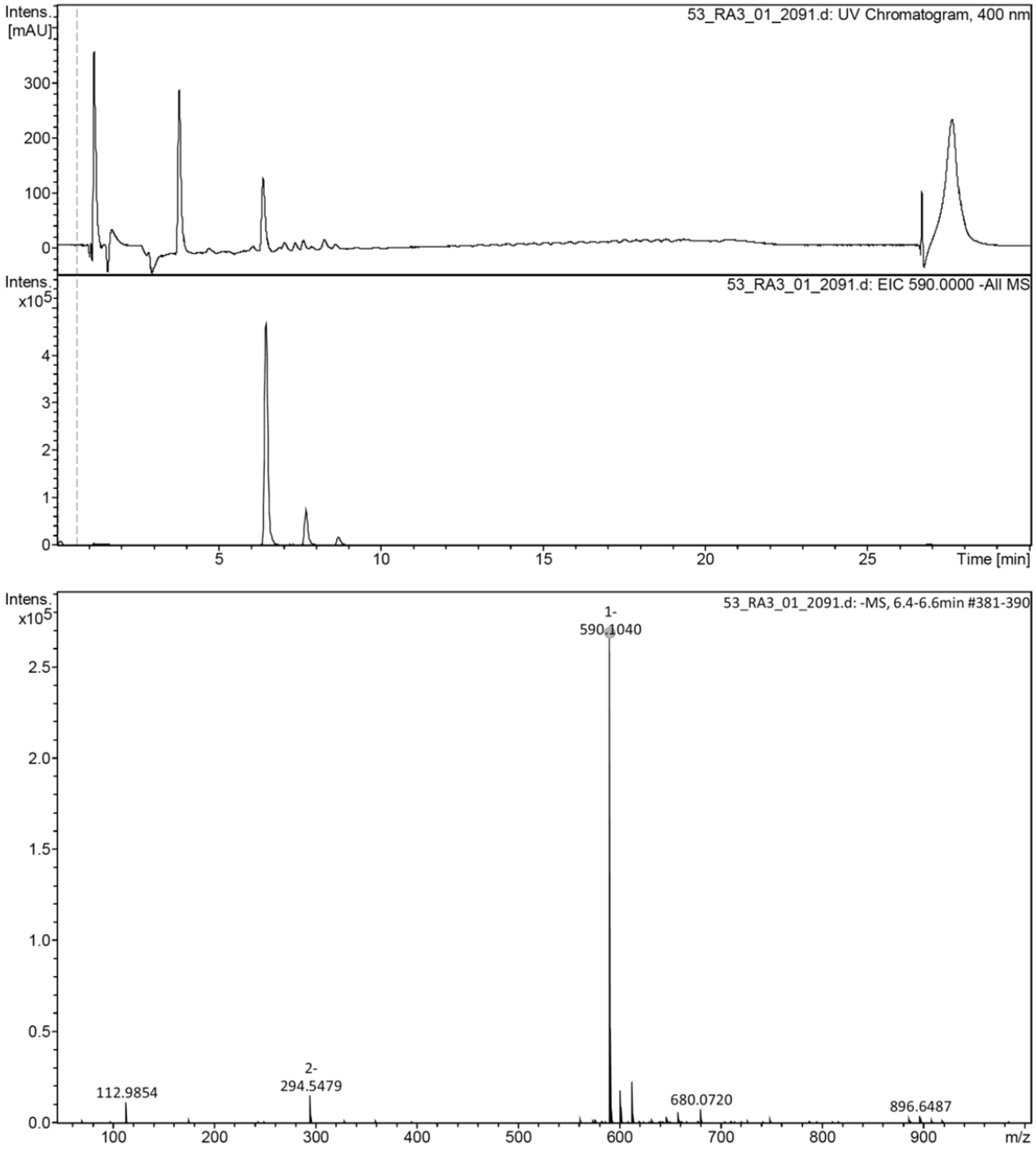
HPLC-ESI(negative mode)-HRMS analysis of photo-decomposed 8-nitro-cGMP (**1**)

Compound **3:** calc. $[M-H]^-$ $C_5H_3N_6O_2$ = 179.0323, found 179.0322 (error 0.7 ppm). Compound **4**: calc. $[M-H]^-$ $C_5H_8O_8P$ = 226.9962, found 226.9960 (error 1.0 ppm).



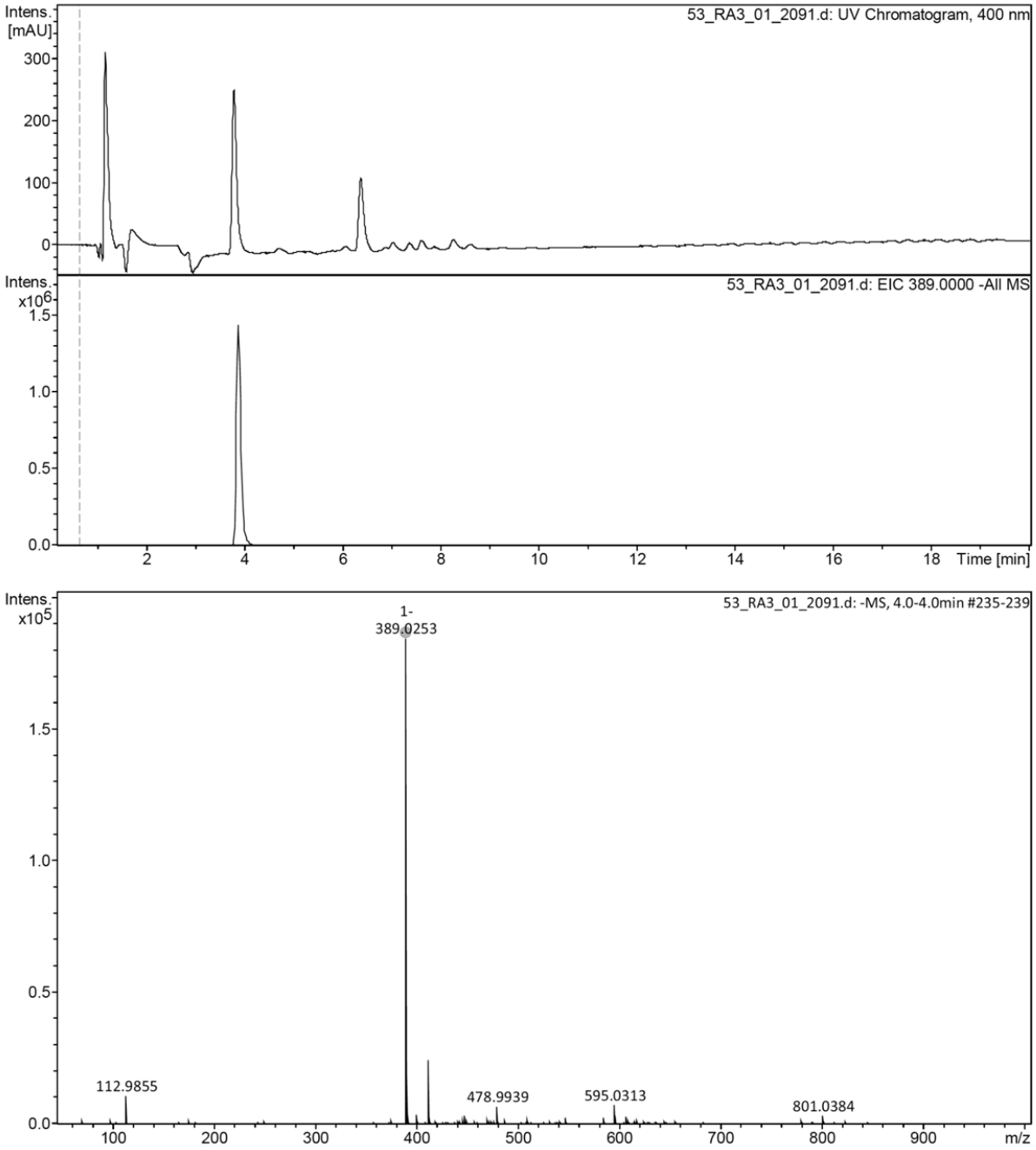
HPLC-ESI(negative mode)-HRMS analysis of uncaging reaction

Compound **2:** calc. $[M-H]^-$ $C_{22}H_{21}N_7O_{11}P$ = 590.1042, found 590.1040 (error 0.4 ppm).



HPLC-ESI(negative mode)-HRMS analysis of uncaging reaction

Compound **1:** calc. $[M-H]^-$ $C_{10}H_{10}N_6O_9P$ = 389.0252, found 389.0253 (error 0.1 ppm).



^{31}P NMR (162 MHz, DMSO-d_6) of DMACM-caged 8-nitro-cGMP (**2**) (equatorial isomer)