Supporting Information

Optical Control of Lysophosphatidic Acid Signaling

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Reagents and Instrumentation

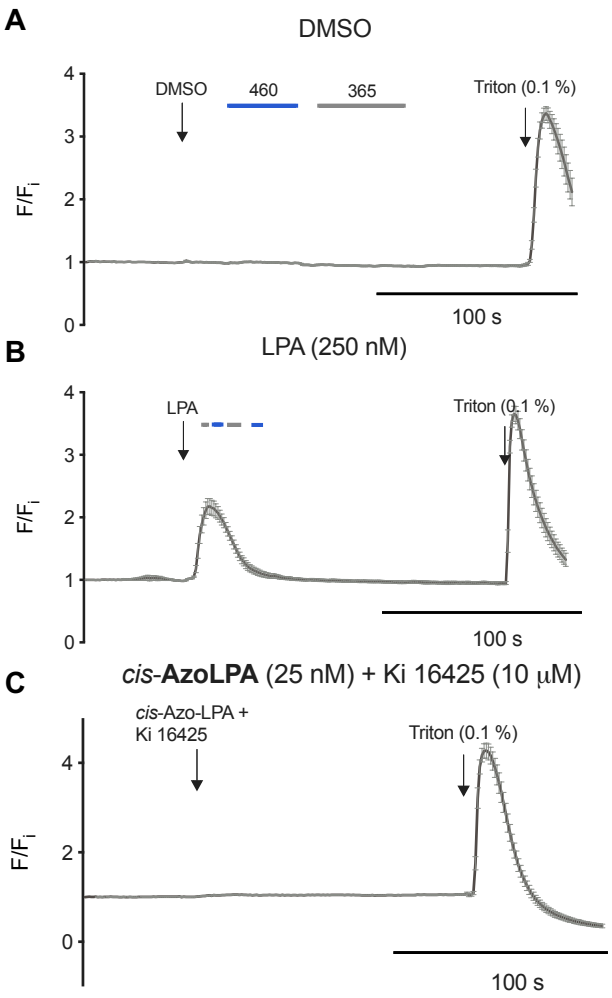
All reagents and solvents were purchased from commercial sources (Sigma-Aldrich, TCI Europe N.V., Strem Chemicals, etc.) and were used without further purification. Solvents were obtained from Fisher Scientific. Reactions were monitored by thin layer chromatography (TLC) on pre-coated, Merck Silica gel 60 F_{254} glass backed plates and the chromatograms were first visualized by UV irradiation at λ = 254 nm. Flash silica gel chromatography was performed using silica gel (SiO_2, particle size 40-63 μm) purchased from SiliCycle. NMR spectra were measured on a BRUKER Avance III HD 400 (equipped with a CryoProbe^{TM}). Multiplicities in the following experimental procedures are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. ^{1}H chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the residual protium in the NMR solvent (THF-d_8: δ = 1.72). ^{13}C chemical shifts are expressed in ppm (δ scale) and are referenced to the carbon resonance of the NMR solvent (THF-d_8: δ = 25.31). NOTE: Due to the *trans*/*cis* isomerization of some compounds containing an azobenzene functionality, more signals were observed in the ^{1}H and ^{13}C spectra than would be expected for the pure *trans*-isomer. Only signals for the major *trans*-isomer are reported.

Photophysical Characterization

UV-Vis spectra were recorded using a Varian Cary 50 Bio UV-Visible Spectrophotometer. Photoswitching was achieved using 365 nm or 460 nm LED light sources. The LEDs were pointed directly onto the top of the sample cuvette with **AzoLPA** (50 μM in DMSO). An initial spectrum was recorded (dark-adapted state, black) and then again following illumination at 365 nm for 30 s (*cis*-adapted state, gray). A third spectrum was recorded after irradiation at 470 nm for 30 s (*trans*-adapted state, blue). Absorption at 340 nm was recorded over several switching cycles whilst alternating illumination at 365 nm and 460 nm with. The light source was directly pointed onto the top of the sample cuvette.

Ca^{2+} Imaging

Ca^{2+} imaging was conducted using non-transfected HEK293T cells, which express LPA_{1-3} endogenously.[1] **AzoLPA** and other compounds were added from a 2x solution and a final concentration of 1% DMSO in Ringer's solution (Sigma K4002). X-Rhod5F (Invitrogen X23984) was used as Ca^{2+} dye. The dye was loaded with 1% DMSO in Ringer's solution for 30 minutes. Cells were washed two times and equilibrated for 10 minutes before the imaging experiment was started.



Supplementary Figure 1 | Optical control of endogenous LPA receptors in HEK293T cells. Ca^{2+} responses after treatment with DMSO (C), LPA (18:1) (D), *cis*-**AzoLPA** and Ki16425, irradiation with light, and after treatment with Triton X100. Data from at least twenty cells and a minimum of two independent experiments.

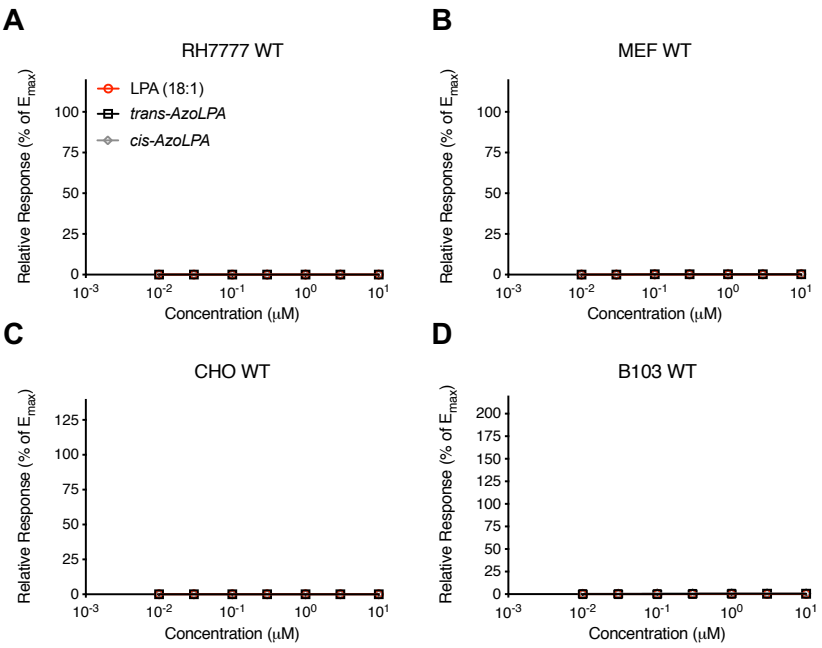
Ca^{2+} Mobilization

Ca^{2+} mobilization assays were carried out as previously described.[2] Briefly, cells stably expressing LPA_{1-5} receptors were plated in poly-L-lysine coated 96 well microplates (25,000 cells/well) and cultured overnight. The culture medium was replaced with Krebs buffer for 2 - 3 h before assays. The transfected cells were loaded with Fura-2/AM in Krebs buffer containing 0.01% pluronic acid for 30 min, and rinsed with Krebs buffer before measuring Ca^{2+} mobilization. The Ca^{2+} responses were measured using a Flex Station III fluorescent plate reader (Molecular Devices, Sunnyvale, CA). The ratio of peak emissions at 510 nm after 2 min of ligand addition was determined for excitation wavelengths of 340 nm/380 nm. All samples were run in at least in triplicate, and assays were performed at least two times for each receptor. The Ca^{2+} response of *trans*-**AzoLPA** was recorded in the dark-adapted state without prior illumination. The Ca^{2+} response of *cis*-**AzoLPA** was recorded in the same plate from the same stock solution. Between measurements for *trans*-**AzoLPA** and *cis*-**AzoLPA**, the compound addition well plate was removed from the plate reader shortly and illuminated for 90 s at 365 nm light. The responses were measured and reported in terms of maximal activation (E_{max}) and potency (EC_{50}):

LPA receptor activation by LPA (18:1), *trans*-AzoLPA and *cis*-AzoLPA

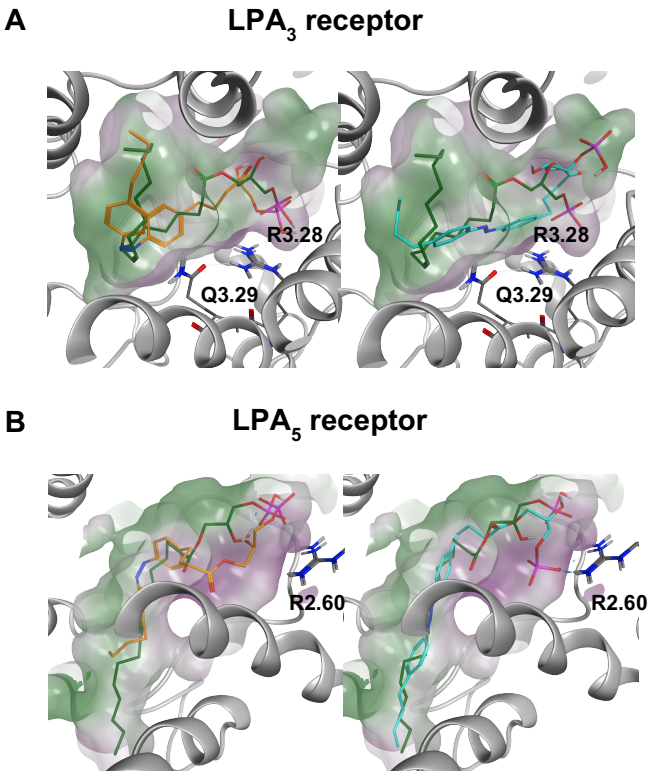
	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5
Compound	E_{max}, EC_{50}	E_{max}, EC_{50}	E_{max}, EC_{50}	E_{max}, EC_{50}	E_{max}, EC_{50}
LPA (18:1)	100%, 39.1	100%, 4.5	100%, 150	100%, 61.5	100%, 345
trans-**AzoLPA**	9%, 157	64%, 542	49%, 3076	55%, -	113%, 21.8
cis-**AzoLPA**	77%, 238	88%, 66.3	37%, 1166	87%, 35.2	143%, 58.4

Intracellular Ca^{2+} mobilization in response to LPA, *trans*-**AzoLPA** and *cis*-**AzoLPA** was measured in cells expressing LPA_{1-5}. The responses are reported in terms E_{max} (% of LPA maximal response) and EC_{50} (nM).



Supplementary Figure 2 | Ca^{2+} Mobilization Control Experiment with non-transfected cell lines. Fura2-AM Ca^{2+} imaging in cells. Dose response of LPA (18:1), *trans*-**AzoLPA** and *cis*-**AzoLPA** in RH7777 (A), MEF (B), CHO (C), and B103 (D) cells. The experiments were run in triplicates. Error bars represent mean +/- S.D.

Molecular Docking of AzoLPA



Supplementary Figure 3 | Molecular Docking of AzoLPA. Computationally predicted poses for **LPA(18:1)** (green), *trans*-**AzoLPA** (cyan) and *cis*-**AzoLPA** (orange) docked into LPA_3 receptor (A) and LPA_5 receptor (B). Pocket surfaces are highlighted in green (hydrophobic) and violet (hydrophilic). The numerals represent key residues involved in target engagement according to the Ballesteros-Weinstein system.

Molecular Docking

Homology models were constructed using MOE 2018.01. Conformations of extracellular loop 2 (EL2) in the LPA_4 and LPA_5 receptor models were sampled and loop models selected using Rosetta, as described by Wink et al.,[3] due to uncharacterized atomic coordinates of six amino acids in EL2 of the template crystal structure. Docking of LPA (18:1), *cis*-**AzoLPA** and *trans*-**AzoLPA** in both the singly- and doubly- charged states was done using MOE 2018.01 with 400 of 1000 sampled poses refined with to allow induced fit with the surrounding residues.[3]

Neurite Retraction Experiments

Cell culture

NG108-15 cells were maintained in DMEM supplemented by 10% fetal bovine serum, 0.1mM hypoxanthine, 400nM aminopterin, 0.016mM thymidine, 1.5g/L sodium bicarbonate and 100µg/mL of penicillin, 100µg/mL of streptomycin, at 37°C, in an atmosphere of 5% CO_2.

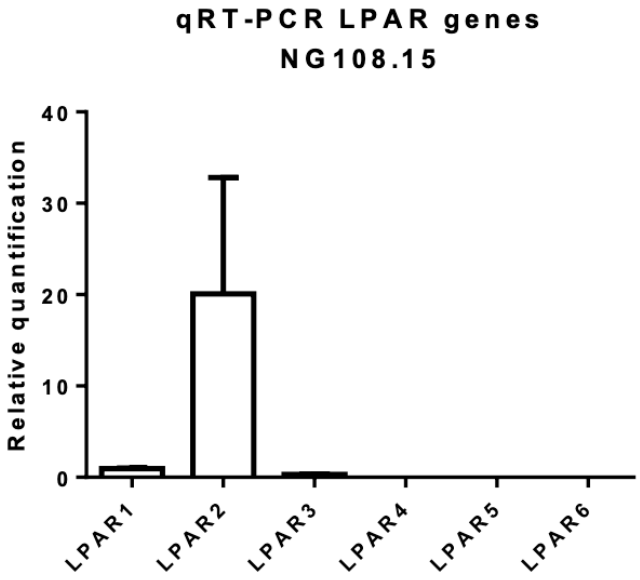
Morphological assays

Cells were serum-deprived for 24 to 48h prior the morphological assay at a density of 1-2 x 104/cm2 in plastic 45mm dishes or 6 well plate. *Trans*- or *cis*-AzoLPA (1µM, 500nM, 100nM or 30nM) were added to the medium while the cells were observed for 30min. Cell rounding was determined with ImageJ by measuring each cell area and comparing the total surface with t=0.

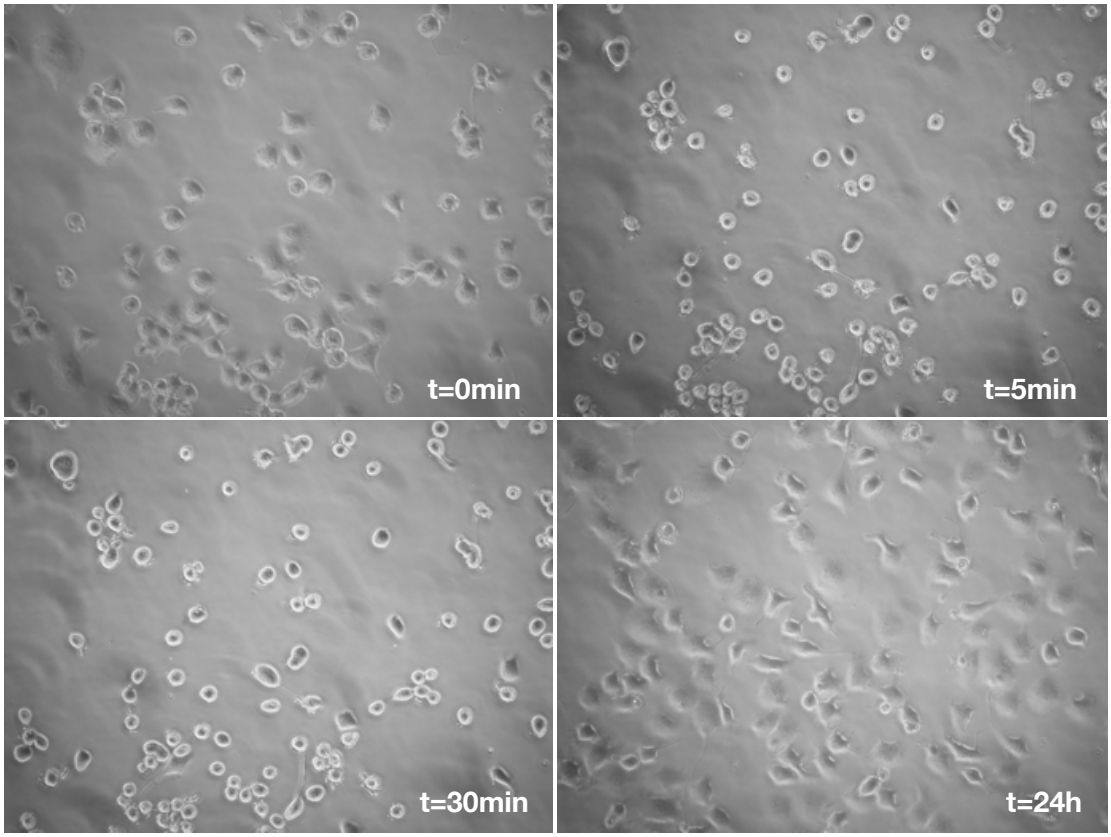
qRT-PCR

Total RNA was isolated from NG108-15 cell line using the RNeasy® MicroKit (Qiagen), treated with RNase-free DNase Set 1500 kunitz units (Qiagen) and quantified by NanoDropOne (Thermo scientific). 1µg of total RNA was reverse-transcripted using RevertAid RT kit (Thermo scientific), according the manufacturer's protocol. RT-PCR was performed with a QuantStudio 6 Flex instrument (applied biosystems by life technologies) using PowerUp™ SYBR™ Green Master Mix (applied biosystems by Thermo Fisher Scientific). LPARs primers used are listed in the table below. For normalization, the four house-keeping genes GAPDH, αSMA, PGK1 and β-tubulin were used. Relative levels of expression of each LPA receptors were determined with the method of $2^{-\Delta\Delta Ct}$.

Gene	Forward sequence 5'→ 3'	Reverse sequence 5'→ 3'
$LPAR_1$	GCTGGACCTAGCAGGCTTAC	TGCCAGGCACAAAAAGCAAT
$LPAR_2$	TGTTCAGCCGCTCCTACTTG	AATGCCCCCAGAATGATGACA
$LPAR_3$	GTCTTAGGCGCCTTCGTGG	TTGCACGTTACACTGCTTGC
$LPAR_4$	GCGAGTTGCCAGTTTACACG	TTGAGTGCCCAAGAAAGAGTGT
$LPAR_5$	CGCTCGCTCCCTTATCTCAG	TGGAGACCTTCTTGGGACCT
$LPAR_6$	CATCTGTGCCCTCAAAGTGA	AAAAGATCCGAAATGGCAAA
GAPDH	CTGCACCACCAACTGCTTAG	GGGCCATCCACAGTCTTCT
αSMA	CCTGGCTTCGCTGTCTACCT	TTGCGGTGGACGATGGA
PGK1	ATGTCGCTTTCCAACAAGCTG	GCTCCATTGTCCAAGCAGAAT
β-tubulin	AAACCGTAGCCATGAGGGAAA	TCCCAGAACTTAGCACCGAT

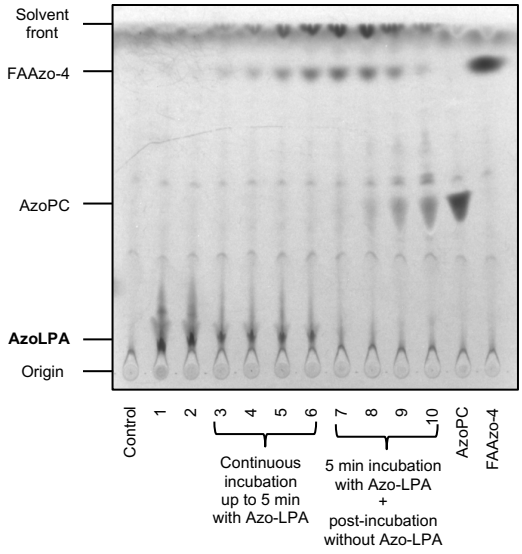


Supplementary Figure 4 | qRT-PCR of LPAR genes in NG108.15 cells employed for neurite retraction experiments.



Supplementary Figure 5 | Viability assessment of NG108.15 cells with AzoLPA. Brightfield images of NG108.15 cells after t = 0 min, 5 min, 30 min, and 24 h after addition of *cis*-**AzoLPA** (1 μM).

Metabolic Fate of AzoLPA in HeLa



Control → Non-labeled cell extract
1 - Control + Azo-LPA (6 nmol)
2 - Azo-LPA (6 nmol) spiked into non-labeled cells during lipid extraction
3 - 15 s incubation with Azo-LPA on live cells
4 - 45 s incubation with Azo-LPA on live cells
5 - 2 min incubation with Azo-LPA on live cells
6 - 5 min incubation with Azo-LPA on live cells
7 - 5 min incubation with Azo-LPA + wash + 5 min
8 - 5 min incubation with Azo-LPA + wash + 20 min
9 - 5 min incubation with Azo-LPA + wash + 60 min
10 - 5 min incubation with Azo-LPA + wash + 150 min
11 - Control + Azo-PC (6 nmol)
12 - Control + Faazo-4 (6 nmol)

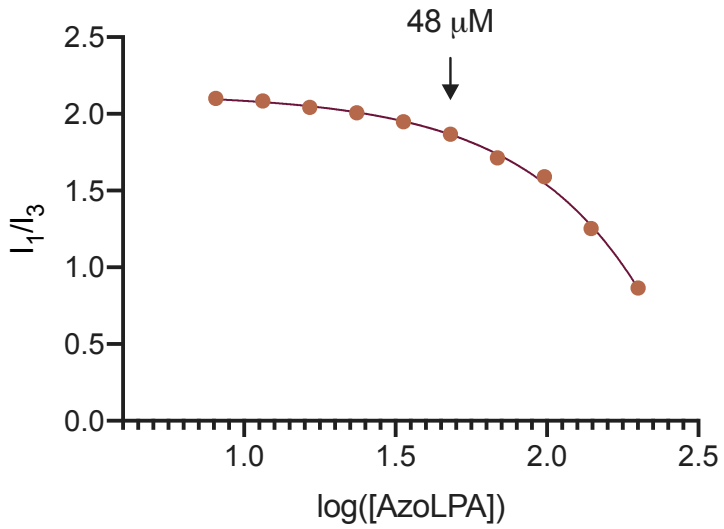
Supplementary Figure 6 | Metabolic Assessment of AzoLPA stability at different time points in live cells (HeLa).

TLC analysis of AzoLPA metabolism in HeLa

HeLa Kyoto cells were seeded at 45% confluency on 35 mm dishes and cultured for 22 hours in DMEM low glucose supplemented with 10% (v/v) FBS. Prior to compound addition, cells were washed twice in HEPES-buffered loading medium (20 mM HEPES pH 7.4, 140 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ supplemented with 0.2% (w/v) D-glucose and 2 mM L-glutamine). Azo-LPA from a stock of 5 mM in PBS was added to a final concentration of 50 uM in HEPES-buffered loading medium. Cells were either incubated continuously with **AzoLPA** for up to 5 min, or washed and then incubated further without compound for up to 150 min following an initial 5 min incubation with **AzoLPA**. Prior to harvest, cells were quickly washed in ice-cold D-PBS (w/o Ca^{2+} and Mg^{2+}) and then quenched by addition of ice-cold 10% (w/v) trichloroacetic acid in water. Dishes were scraped, then the cellular material was transferred into 2 mL polypropylene tubes (Eppendorf DNA-Lobind) and centrifuged at 20.000 x g for 3 min at 4 ℃. The pellet was washed twice in ice-cold 5% (w/w) trichloroacetic acid + 10 mM EDTA in water by vortex mixing and consecutive centrifugations at 20.000 x g for 3 min at 4 ℃. Lipid extraction was started by addition of 726 uL of chloroform:methanol:12.1 M HCl 40/80/1. Samples were vortexed for 15 min at 4 ℃. Then, 720 uL of chloroform was added, followed by 5 min vortex at 4 ℃. 354 uL of 1 M HCL was added to induce phase separation. Samples were vortexed for 2 min at 4 ℃ and centrifuged at 1000 x g for 5 min at 4 ℃. The lower phase was

transferred to a fresh tube, to which 702 uL of theoretical upper phase (chloroform:methanol:1.185 M HCL 3/48/47) was added. Samples were vortexed for 15 s at 4 ℃ and centrifuged at 1000 x g for 3 min at 4 ℃. Finally, the lower phase was transferred again into a fresh tube and dried in a refrigerated CentriVap (Labconco) at -4 ℃ for several hours. Lipids were re-dissolved in chloroform and spotted onto fluorescently coated (F254) HPTLC silica gel 60 glass plates from Merck. Lipid standards were spotted after mixing with non-labeled cell extract to determine their migration in presence of cellular lipids. In one sample, **AzoLPA** was spiked into non-labeled cells at the beginning of lipid extraction to check whether there is breakdown during lipid extraction. The plate was developed in chloroform:methanol:water 65/25/4. Imaging was performed with a Bio-Rad Chemidoc Touch Imaging System using the ethidium bromide channel.

Critical Micellar Concentration

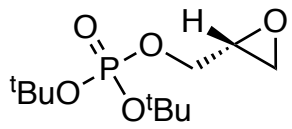


Supplementary Figure 7 | Determination of the CMC of *trans*-AzoLPA using pyrene fluorescence.

The CMC (critical micellar concentration) of **AzoLPA** was measured fluorometrically by monitoring the incorporation of pyrene in PBS (pH = 7.4) with 1% DMSO. A solution with 1 μM pyrene and varying concentrations of *trans*-**AzoLPA** was irradiated at 332 nm using a HORIBA fluorometer and the emission was recorded. The ratio of intensities at 372 nm (I_1) and 380 nm (I_3) were plotted against the lipid concentration.

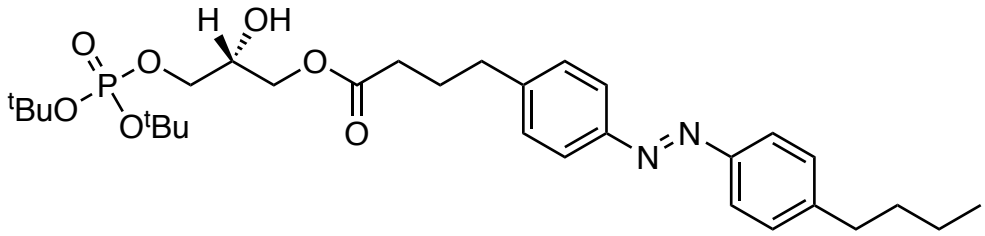
Synthesis of AzoLPA

(*R*)-di-*tert*-butyl (oxiran-2-ylmethyl) phosphate (1)[4]



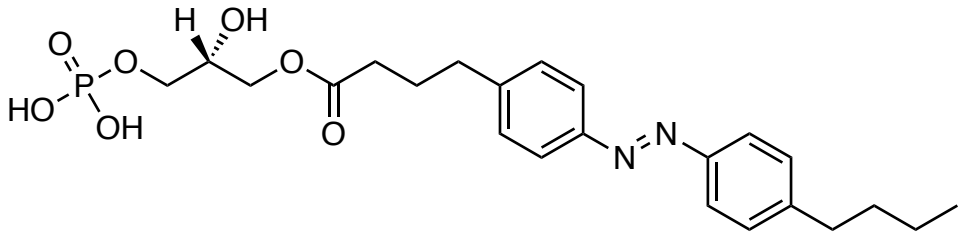
(*S*)-Glycidol (159 μL, 2.40 mmol, 1.0 equiv.) was dissolved in dry DCM (60.0 mL) under argon atmosphere. Di-*tert*-butyl diisopropylphosphoramidite (1.00 g, 3.60 mmol, 1.5 equiv.) and 1*H*-tetrazole (0.45 M solution in MeCN, 16 mL) were added and the solution was stirred for 50 minutes at room temperature. The mixture was cooled to 0 °C and mCPBA (1.20 g, 7.20 mmol, 3.0 equiv.) was added. The mixture was stirred for 30 min at 0 °C and 30 min at room temperature, washed with aqueous $Na_2S_2O_3$ solution (10%), $NaHCO_3$ solution (saturated), dried, filtered, and the solvent was evaporated under reduced pressure. The crude mixture was purified by flash column chromatography (3:1:0.1% hexanes:ethyl acetate:triethylamine) to yield **(*R*)-di-*tert*-butyl (oxiran-2-ylmethyl) phosphate** as colorless oil (306 mg, 1.15 mmol, 48%). The NMR spectra matched those reported in the literature.[4]

(*R*)-3-((di-*tert*-butoxyphosphoryl)oxy)-2-hydroxypropyl (*E*)-4-(4-((4-butyl-phenyl)diazenyl)phenyl)butanoate (2)



FAAzo-4[5] (32.4 mg, 0.100 mmol, 1.0 equiv) and FAAzo-4Cs (136 mg, 298 mmol, 3.0 equiv; cesium salt obtained through addition of equimolar amounts of CsOH to **FAAzo-4**) were dissolved in dry N'N-dimethylformamide (2 mL). **(*R*)-di-*tert*-butyl (oxiran-2-ylmethyl) phosphate (1)** (27.0 mg, 0.101 mmol, 1.0 equiv) was added and the reaction mixture was stirred at 80 ºC for 2 h. H_2O was added and the product was extracted with EtOAc, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude mixture was purified by flash column chromatography (ethyl acetate + 1% triethylamine) to yield **(*R*)-3-((di-*tert*-butoxyphosphoryl)oxy)-2-hydroxypropyl (*E*)-4-(4-((4-butylphenyl)diazenyl)phenyl)butanoate** (**2**) as orange oil (47.0 mg, 79.6 mmol, 80%). ^{1}H NMR (400 MHz, THF-d_8) δ 7.82 (dd, *J* = 8.3, 3.9 Hz, 4H), 7.34 (dd, *J* = 13.2, 8.3 Hz, 4H), 4.59 (d, *J* = 4.8 Hz, 1H), 4.12 – 4.03 (m, 2H), 3.91 (q, *J* = 6.2 Hz, 3H), 2.79 – 2.62 (m, 4H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.97 (p, *J* = 7.5 Hz, 2H), 1.64 (q, *J* = 7.8 Hz, 2H), 1.45 (s, 18H), 1.42 – 1.35 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, THF-d_8) δ 172.99, 152.11, 151.95, 147.09, 146.12, 130.00, 129.81, 123.60, 123.55, 82.20 (d, *J* = 6.6 Hz), 69.05 (d, *J* = 7.2 Hz), 68.58 (d, *J* = 6.2 Hz), 65.67, 36.29, 35.67, 34.46, 33.82, 30.05 (d, *J* = 4.3 Hz), 27.38, 23.22, 14.27. ^{31}P NMR (162 MHz, $CDCl_3$) δ -7.95. HRMS: m/z calcd. for $C_{31}H_{48}N_2O_7P^+$ ($[M+H]^+$): 591.3194, found: 591.3203.

(*R*)-2-hydroxy-3-(phosphonooxy)propyl (*E*)-4-(4-((4-butylphenyl)diazenyl)-phenyl)butanoate (AzoLPA)



(*R*)-3-((di-*tert*-butoxyphosphoryl)oxy)-2-hydroxypropyl (*E*)-4-(4-((4-butylphenyl)-diazenyl)phenyl)butanoate (**2**) (15.0 mg, 25.4 μmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (3 mL). TFA (0.75 mL) was added and the solution the mixture was stirred for 10 min at room temperature. MeOH (40 μL) was added and the mixture was stirred for 10 min at room temperature. Toluene (10 mL) was added and the solvents were removed under reduced pressure. The residue was dissolved in MeCN and washed with hexanes. MeCN was removed under reduced pressure and **(*R*)-2-hydroxy-3-(phosphonooxy)propyl (*E*)-4-(4-((4-butylphenyl)diazenyl)-phenyl)butanoate** (**AzoLPA**) was obtained as orange solid (10.1 mg, 21.1 mmol, 83%). ^{1}H NMR (400 MHz, THF-d_8) δ 7.81 (dd, *J* = 8.2, 2.7 Hz, 4H), 7.34 (dd, *J* = 13.0, 8.2 Hz, 4H), 4.10 (m, 2H), 3.95 (m, 3H), 2.70 (dt, *J* = 15.7, 7.6 Hz, 4H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.96 (p, *J* = 7.4 Hz, 2H), 1.64 (p, *J* = 7.7 Hz, 2H), 1.38 (h, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, THF-d_8) δ 172.16, 151.12, 150.98, 146.11, 145.22, 129.04, 128.84, 122.64, 122.59, 68.14 (d, *J* = 7.0 Hz), 67.33 (d, *J* = 5.2 Hz), 64.67, 35.32, 34.71, 33.50, 32.86, 26.40, 22.25, 13.30. ^{31}P NMR (162 MHz, THF) δ 2.00. HRMS: m/z calcd. for $C_{23}H_{32}N_2O_7P^+$ ($[M+H]^+$): 479.1942, found: 479.1945.

References

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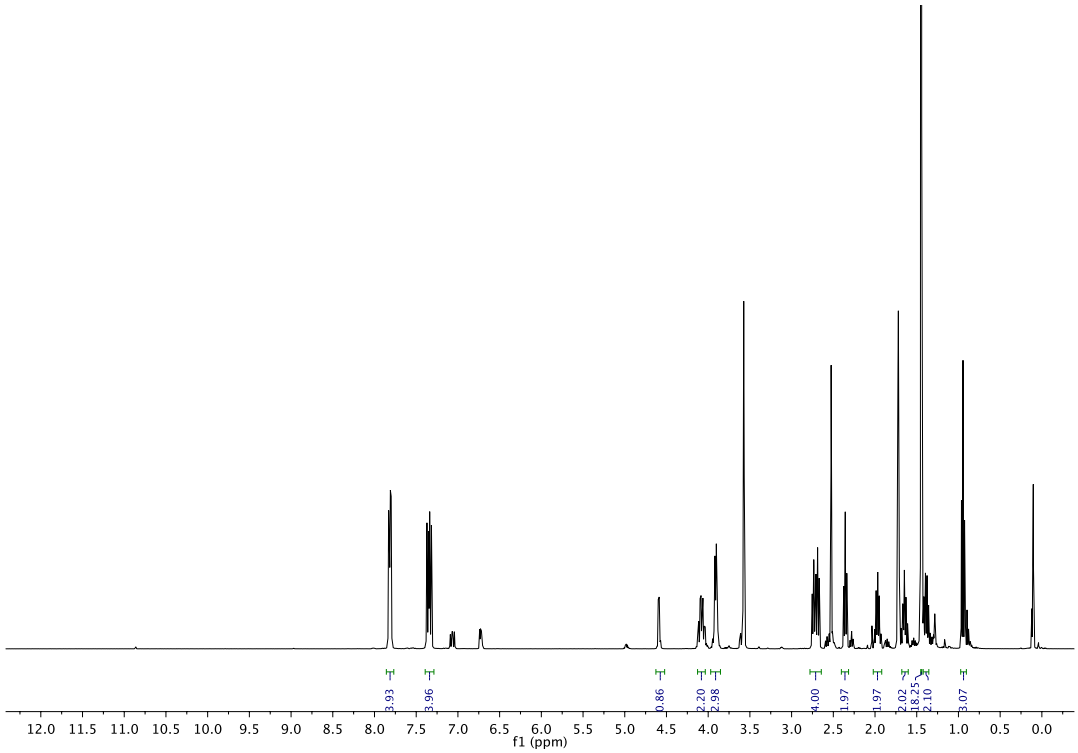
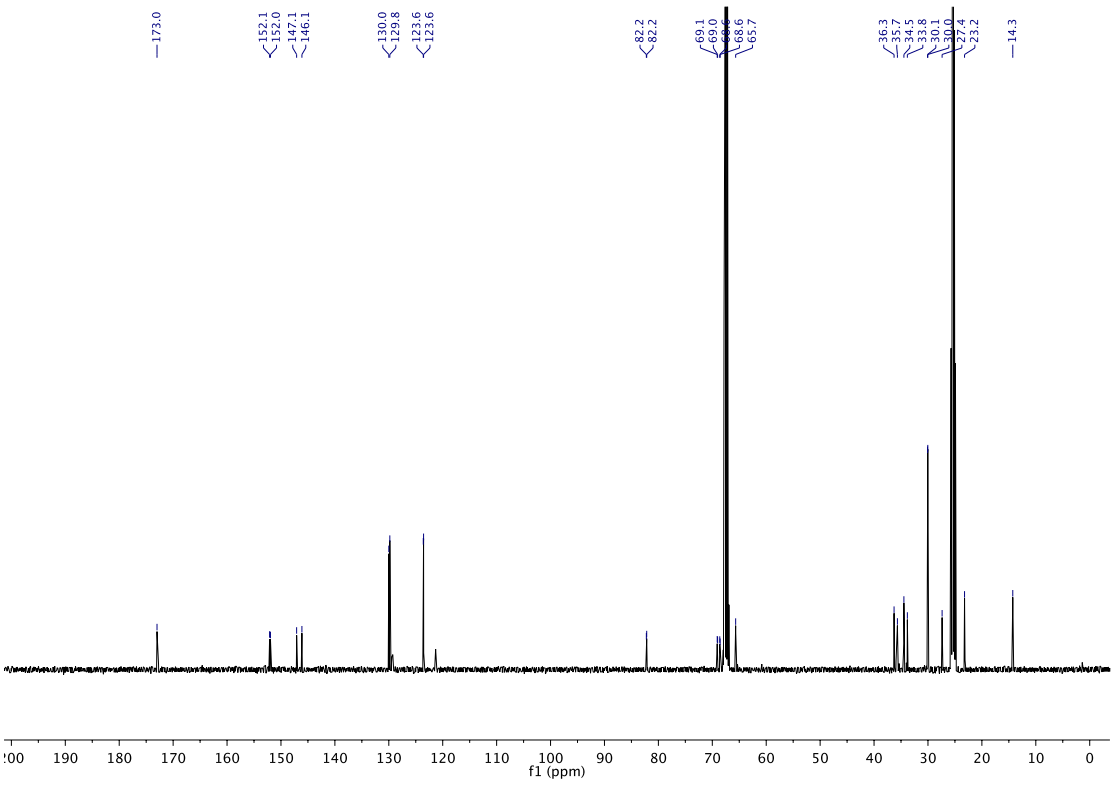
^{1}H,^{13}C, and ^{31}P NMR Spectra

(*R*)-3-((di-*tert*-butoxyphosphoryl)oxy)-2-hydroxypropyl (*E*)-4-(4-((4-butylphenyl)-diazenyl)phenyl)butanoate (2)

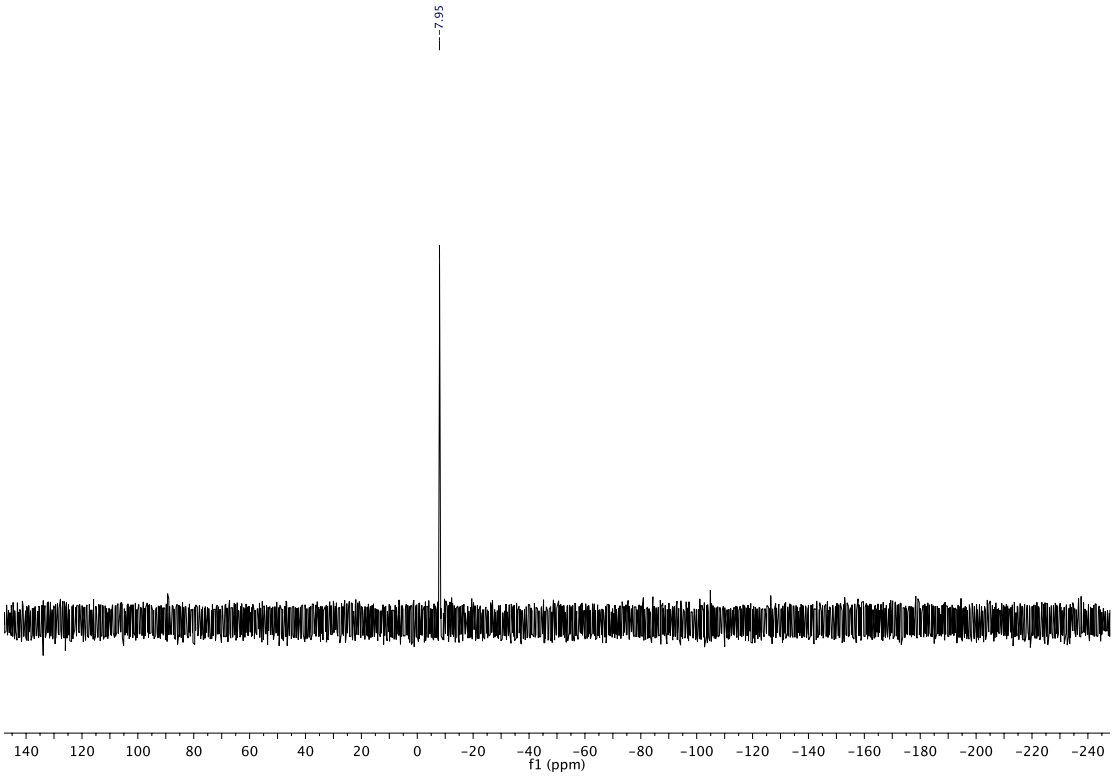
^{1}H NMR



^{13}C NMR

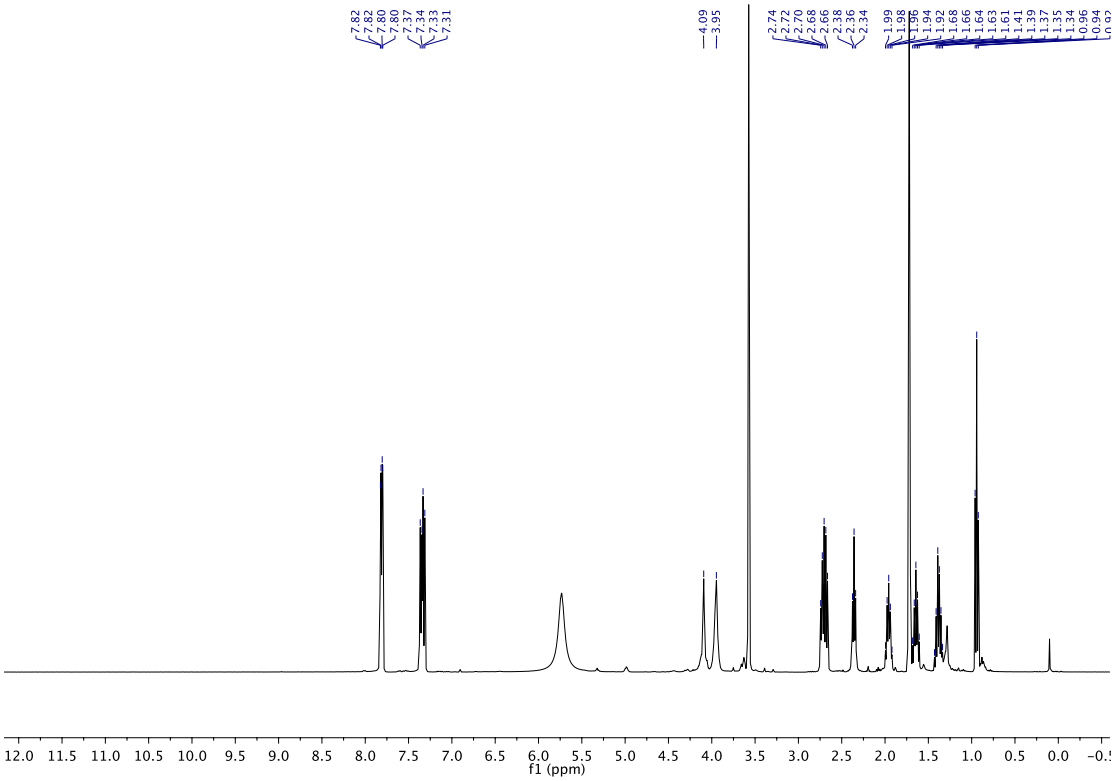


^{31}P NMR

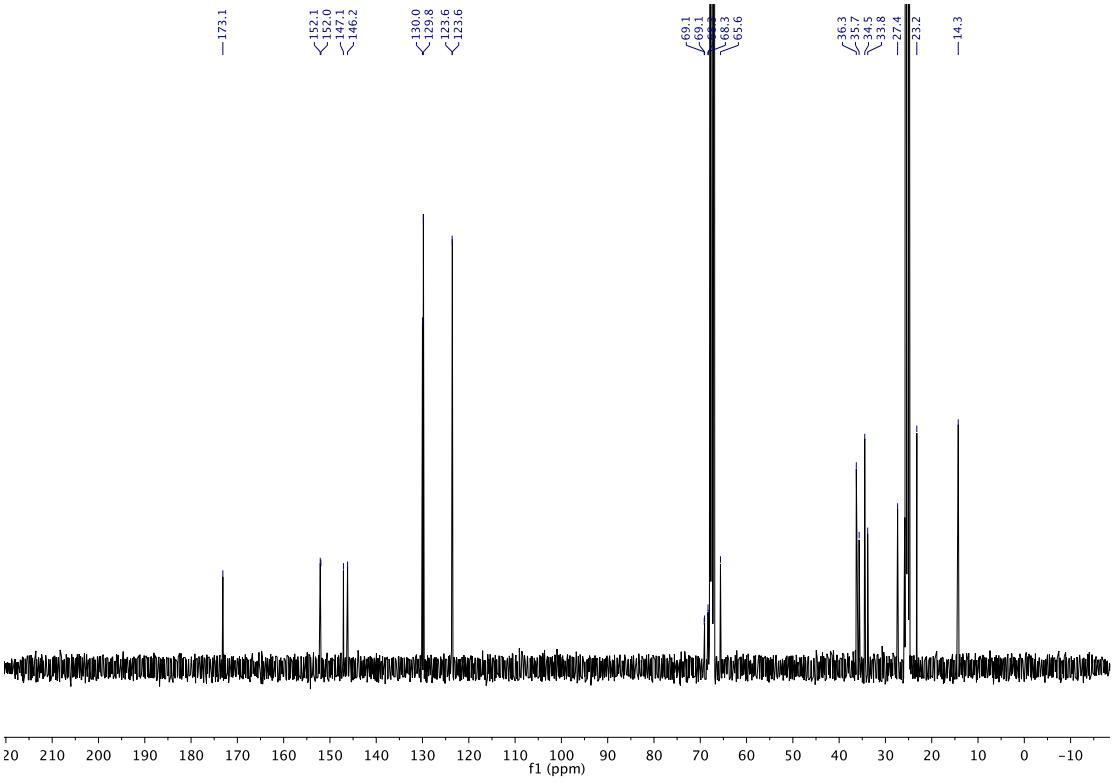

7.95
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
f1 (ppm)

(*R*)-2-hydroxy-3-(phosphonooxy)propyl (*E*)-4-(4-((4-butylphenyl)diazenyl)-phenyl)butanoate (AzoLPA)

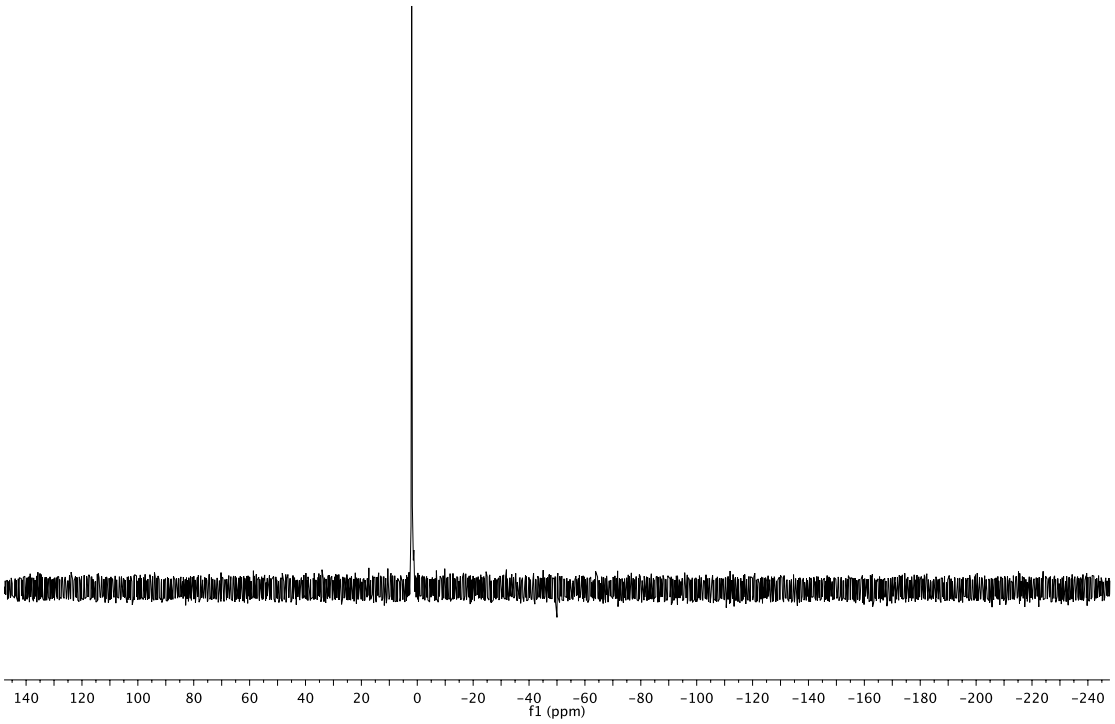
^{1}H NMR



^{13}C NMR



^{31}P NMR


140
120
100
80
60
40
20
0
−20
−40
−60
−80
−100
−120
−140
−160
−180
−200
−220
−240
f1 (ppm)