Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information:

Species selective diazirine positioning in tag-free photoactive quorum sensing probes

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Contents

General: All chemical reagents were purchased from Sigma Aldrich or Acros and used without further purification. Solvents were dried with an MBraun solvent purification system. Reactions were monitored by TLC using commercially available glass plates precoated with silica (0.25 mm, Merck 60 F254), which were developed using potassium permanganate. Flash chromatography was performed on Merck 40-63 µm silica gel. NMR analyses were performed using a Bruker Avance DPX400 or alternatively with a Bruker Avance DMX500. Spectra were calibrated on residual solvent signal. HRMS of probes **3** and **4** were obtained using an LTQ XL Orbitrap ETD with nano ESI (Thermo). Other intermediates were analyzed using an LCQ Fleet mass spectrometer (Thermo Scientific) with ESI source, connected to a Surveyor Plus HPLC System (Thermo Scientific). Spectra were collected in positive ion mode and analyzed by Xcalibur software (Thermo Scientific).

Overexpression of LasR ligand binding domain in *E. coli* BL21 (procedure for purification with Ni^{2+} affinity chromatography)

The expression of full length LasR was found to yield largely insoluble proteins, in the presence and in the absence of the native ligand C12.[42] Therefore, expression was performed using a plasmid with the pETM-11 vector encoding for a LasR-LBD (ligand binding domain) His-tagged construct, spanning Met-1 to Lys-173 (obtained from M. Bottomley *et. al.*). The LasR-LBD was overexpressed in *E. coli* BL-21 cells, incubated with either native C12, probe **2-4** or both (to stabilze the receptor) and purified by Ni^{2+} affinity chromatography as described elsewhere.[42] The purification process was monitored by SDS-PAGE and the molecular mass of the purified proteins was confirmed by mass spectrometry. **Procedure**: a 10 mL culture of *E. coli* in LB medium was grown to an optical density ($OD_{600\ nm}$) of 0.4; 10 µM C12 (or 5 µM probe **2-4**, or 5 µM probe **2-4** + **10** µM C12) was added and expression was induced at 21 ^{0}C using 0.2 mM of isopropyl 1-thio-β-D-galactopyranoside (IPTG). After reaching $OD_{600\ nm}$ of 1.4 (approx. 6 hours) cells were centrifuged at 4000 rpm for 10 min. at 4 ºC, washed and resuspended in PBS. The sample was irradiated with UV for 20 minutes on ice. Cells were centrifuged and resuspended in lysis buffer containing 50 mM Tris-HCl at pH 8, 300 mM NaCl, 0.2 % (v/v) Triton, 0.75 ug/mL DNAse-inhibitor, 0.05 mM $MgCl_2$, 0.01 mM $CaCl_2$, 5 mM imidazole and 0.01 % (v/v) protein inhibitor cocktail. Cells were incubated for 60 minutes at 37 ^{0}C and then pelleted at 4000 rpm for 10 min. The supernatants were purified using Ni-NTA spin columns (QIAGEN) following the procedures described in the provided manual . SDS PAGE was performed using a NuPAGE Surelock Xcell, on NuPAGE Novex Bis-Tris Pre-Cast gels purchased from Invirogen (NP0342). Expression was performed either at small scale, using Ni-NTA spin columns (31314, QIAGEN) or at large scale using Ni^{2+} prepacked cartridges

(Bio-Scale, Mini Profinity IMAC cartrige, 732-4612, BIO-RAD) fitted to a AKTAprime plus purification system (GE Heathcare).

Overexpression of LasR ligand binding domain in *E. coli* BL21 (procedure for fishing experiments)

Electrocompetent *E. coli* BL21 cells were transfected with pET 28 plasmid containing the LasR-LBD. After transfection, cells were grown overnight at 37 °C in LB medium containing 50 µg/mL kanamycin (LB/KAN). After overnight growth cells were plated onto LB/KAN agar plates at 37 °C. Cells transfected with empty pET 28 plasmid dealt as controls.

For overexpression of LasR-LBD a single colony of the transfected BL21 cells was transferred into liquid LB/KAN and grown overnight at 37 °C. The next morning the overnight culture was diluted 1:100 into fresh medium, aliquoted into smaller volumes, protected from light and 25 µM of probe or control solution was added. The cells were then incubated at 37 °C until they reached an OD_{600}~0.6. At this point 0.4 mM IPTG was added to each cell culture and the plasmid was induced at 21 °C over night. The following morning cells were pelleted for 25 minutes at 4000 rpm. The supernatant was discarded and the pellet washed once with PBS. Again the supernatant was discarded and the pellet was re-suspended in 3 mL fresh PBS. Cell cultures were then transferred into 5 mL petri dishes and UV irradiated at 365 nm for 10 minutes. Thereafter, ~1-2 mg lysozyme and protease inhibitor were added to each cell suspension and cells were lysed by sonication for a total of 2 minutes in cycles of 20” pulses, interspersed with 40” cooling. After sonication the crude lysate was centrifuged for 25 minutes at 4000 rpm at 4 °C. The supernatant was transferred into a fresh tube and kept at 4 °C or for longer storage at -20 °C.

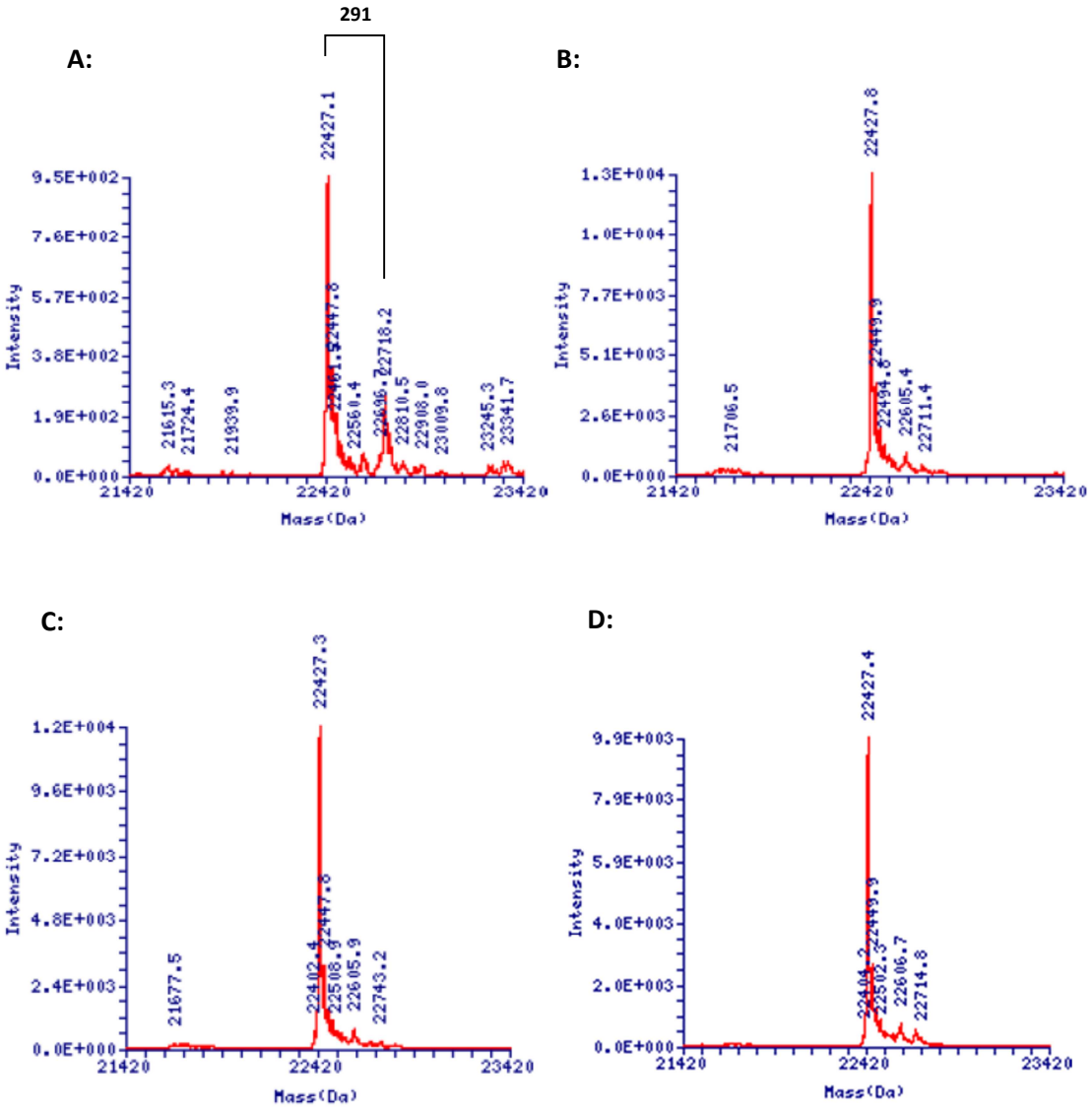


Figure S1: Deconvoluted ESI mass spectra for: (**A**) LasR-LBD, expressed in the presence of diazirine probe **3** (20 µM); (**B**) LasR-LBD, expressed in the presence of both probe **3** (20 µM) and C12 (20 µM). (**C**) LasR-LBD, expressed in the presence of C12 (20 µM); (**D**) LasR-LBD, expressed in the presence of both probe **4** (20 µM) and C12 (20 µM).

CLICK chemistry using rhodamine azide (RhN_3)

To get a relative estimate of protein concentration and allow a fair comparison between samples we took 10 µL of each sample to use in a BCA protein assay (Pierce®BCA Protein Assay Kit; THERMO Scientific, Rockford, IL). To label proteins that covalently bound probes **2-4**, we then set up a click reaction between the alkyne present on our probes and Rhodamine azide (RhN_3). According to the sample with the lowest protein concentration samples were diluted in PBS (no less than 1:10), to a total volume of 89 µL. To this volume of sample we then added 50 µM RhN_3 (from a 50 mM stock in DMSO), 1 mM tris(2-carboxyethyl)phosphine (TCEP; from a 50 mM solution prepared fresh every time in DDW), 100 µM tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, from a 1.7 mM stock in 1:5 DMSO:n-butanol). Samples were briefly mixed before adding

1 mM $CuSO_4$ (form a 50 mM stock in DDW). After a final brief mix, samples were incubated for a minimum of 90 minutes but never longer than over night at room temperature (RT) under constant agitation.

For visualization, samples were then prepared for SDS polyacrylamide gel electrophoretic separation. To 20 µL click reaction we added 5 µL gel loading buffer and 2 µL reducing agent. Samples were then heated to 70 °C for 10 minutes before loading them onto a NuPAGE® Bis-Tris 12 % Precast Gel (Invitrogen Corp., Grand Island, NY). For molecular weight determination a Precision Plus Protein Prestained Dual Color Standard (Bio-Rad Laboratories Inc.) was run in parallel to our samples. After electrophoresis, performed in dark conditions, RhN_3 labeled bands were revealed with a Fujifilm LAS-3000 Imager.

CLICK chemistry using biotin azide (Biotin-N_3) and affinity purification using streptavidin agarose.

To label proteins that covalently bind the diazirine alkyne C12 probes, and affinity purify them using streptavidin agarose beads (Invitrogen Corp., Grand Island, NY) we set up a click reaction between the alkyne present on our probes and azide-PEG3-Biotin Conjugate (Jena Bioscience GmbH, Jena, Germany). According to the sample with the lowest protein concentration samples were diluted in PBS (used as undiluted as possible), to a total volume of 870 µL. To this volume of sample we then added 150 µM biotin-N_3 (from a 5 mM stock in DDW), 1 mM TCEP, 100 µM TBTA. Then the samples were briefly mixed before adding 1 mM $CuSO_4$. After a final brief vortex, samples were incubated over night at RT under constant agitation.

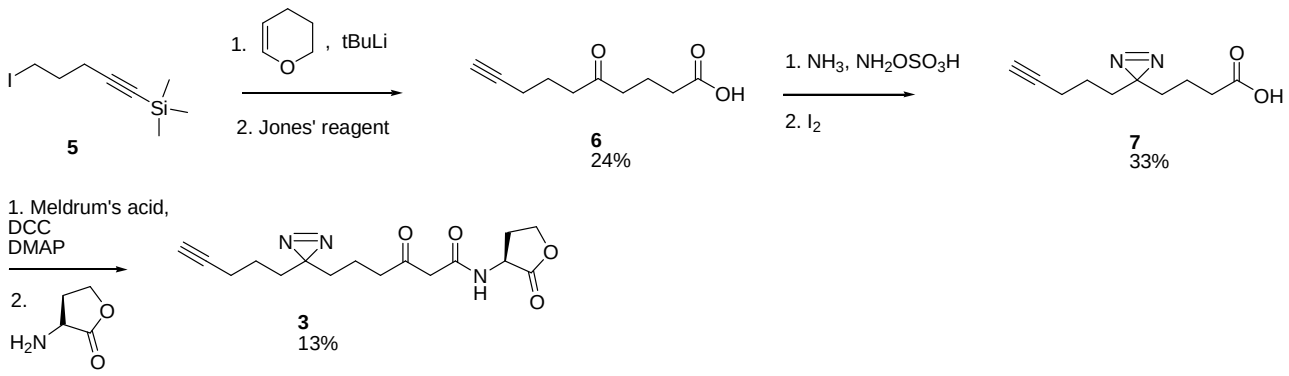
For affinity purification for each sample 200 µL of streptavidin agarose resin were transferred into 0.8 mL Pierce®Centrifuge Columns (Pierce Biotechnology, Rockford, IL), placed into 1.5 mL eppendorf tubes. The resin was washed 5 times with PBS before it was transferred into the click solution. Click solution and beads were incubated for 90-120 minutes at RT under constant gentle end over end rotation. After incubation samples were transferred back into the centrifuge columns and gently spun down. The run through was collected and the agarose beads washed 4 times with 350 µL PBS each – collecting each wash in a separate tube. Then the beads were incubated with 350 µL 0.1 % SDS in PBS for 10 minutes at RT under constant gentle rotation. Thereafter the beads were washed three times with 350 µL PBS each, followed by three washes with 350 µL DDW each. For Western Blot analysis samples were eluted adding 300 µL reducing SDS-PAGE sample running buffer and heating samples for 10 minutes at 60 °C, then washing them once with 300 µL PBS, before adding another 300 µL loading buffer and heating them to 90 °C for 10 minutes.

All eluted samples were transferred into separate 500 µL Amicon Ultra Centrifugal Filters (10 kDa cut-off; Milipore Corp., Billerica, MA). Samples were concentrated ~50 times (to about 30-40 µL), collected in low protein binding centrifuge tubes and stored at -20 °C until further processing.

Western blotting

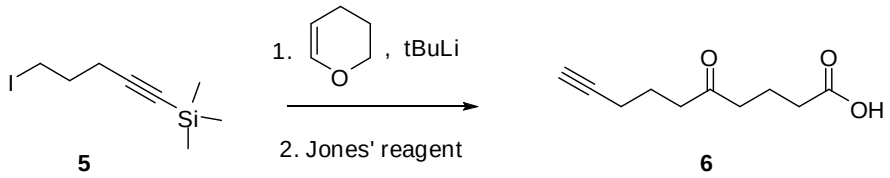
After elution with sample loading buffer and concentration, 10 µL sample were diluted 1:1 in PBS. After adding 2 µL reducing agent, samples were heated to 90 °C for 10 minutes and then loaded onto a NuPAGE® Bis-Tris 12 % Precast Gel (Invitrogen Corp., Grand Island, NY). After electrophoresis the gel was briefly pre-incubated in transfer buffer (Invitrogen Laboratories) containing 10 % methanol (when two gels were blotted at once the buffer contained 20 % methanol). PVDF membrane (Millipore Corporation, Billerica, MA), cut to size, was briefly soaked in 100 % methanol, rinsed with DDW and pre-incubated in transfer buffer. Western blotting was done at 40 V for 60 minutes using an XCell *SureLock*® Mini-Cell (Invitrogen Corp, San Diego, CA). After blotting, the membrane was washed three times in PBS and then blocked in 4 % skim milk/PBS (Sigma) for 2-4 hours at RT. After blocking the membrane was transferred into a solution of 1% skim milk in PBS/ 0.05 % Tween 20 containing Streptavidin-HRP (1:2000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for a minimum of 90 min at RT, or overnight at 4 °C. Specific binding of affinity purified sample was detected with SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific Inc., Rockford IL) using a Fujifilm LAS-3000 Imager.

Synthesis of N-((*S*)-tetrahydro-2-oxofuran-3-yl)-3-oxo-6-(3-(pent-4-ynyl)-*3H*-diazirin-3-yl)hexanamide (probe 3):



Scheme S1: Synthesis of diazirine alkynyl **probe 3**.

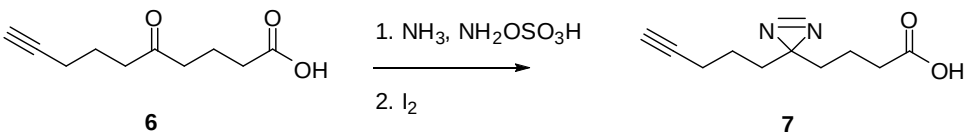
Synthetic procedures:



5 **6**

5-oxodec-9-ynoic acid (6):

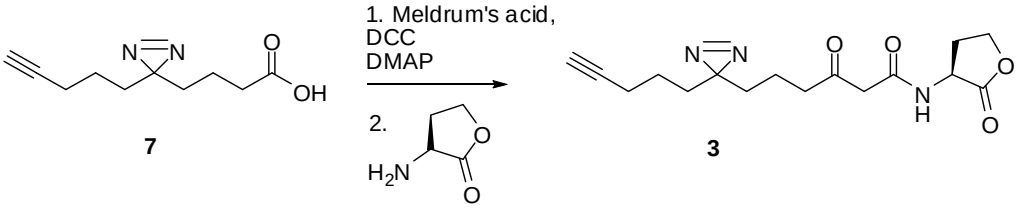
Compound **6** was prepared following a procedure described by Hodgson *et al.*[1] A stirred solution of 3,4-dihydro-2H-pyran (1.05 gr, 12.5 mmol) in THF (5 mL), was cooled to -78 °C followed by dropwise addition of t-BuLi (7.3 mL of a 1.7 M solution in pentane, 12.5 mmol) via syringe. The solution was warmed to 5 °C over 1 h before being recooled to -78 °C. Commercially available 5-iodo-1-pentynyl)trimethylsilylsilane (**5**) (1.66 gr, 6.23 mmol) was added dropwise via syringe, and the resulting solution was warmed to 25 °C and then stirred for 16 h. The reaction mixture was recooled to 0 °C and quenched by careful addition of saturated aq. NH_4Cl solution (1 mL). The aqueous phase was extracted with Et_2O (3*10 mL), and the combined organic layers were dried ($MgSO_4$) before concentration under reduced pressure. The resultant crude alkylated dihydrofuran was dissolved in THF (20 mL) and Jones reagent (6.5 mL of a 2.7 M aq. solution) was added dropwise under vigorous stirring. After 18 h the reaction mixture was diluted with Et_2O (20 mL) and H_2O (20 mL) and stirred vigorously for 30 min. The aqueous phase was separated and extracted with Et_2O (4*20 mL), and the combined organic components were washed with H_2O (3*75 mL) and extracted with 10 % aq NaOH solution (4*20 mL). The combined basic portions were cooled to 0 °C and acidified to pH 1 with concentrated HCl. The cloudy aqueous component was extracted with CH_2Cl_2 (4*20 mL). Combined organic components were dried ($MgSO_4$), concentrated under reduced pressure and purified by silica gel chromatography (1:2 EtOAc : hexanes) to yield **6** as a yellowish solid (0.273 g, 1.5 mmol, 24 % over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54-2.47 (m, 4H), 2.35 (t, J =7.2 Hz, 2H), 2.19 (dt, J_1=6.86, J_2=2.64, 2H), 1.93 (t, J=2.64, 1H), 1.86 (quintet, J=7.2, 2H), 1.75 (quintet, J=7.0 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 209.5, 179.0, 83.4, 69.1, 41.4, 41.0, 32.9, 22.0, 18.4, 17.6.



6 **7**

6-diazirine-9-decynoic acid (7):

Anhydrous ammonia (10 mL) was condensed into a roundbottomed flask containing **6** (0.273 g, 1.5 mmol) at dry ice temperature. The mixture was stirred at 35-40 °C for 5 h. The solution was cooled with dry ice, and a solution of hydroxylamine-*O*-sulfonic acid (0.20 g, 1.84 mmol) in anhydrous methanol (1.4 mL) was added over a period of 30 minutes. The dry ice bath was removed, and the mixture was refluxed with stirring at -35 °C for 1 h. The ammonia was then allowed to evaporate overnight. The resulting slurry was filtered and the filter cake washed with several portions of methanol. The combined solution was rotary evaporated. The residue of the diaziridine derivative was dissolved in dichloromethane (1 mL) and treated with triethylamine (0.25 mL). A solution of iodine (0.11 g, 1 mmol) in dichloromethane (1.7 mL) was slowly added under stirring until the appearance of a persistent orange-brown coloration. The mixture was chromatographed on a column of silica gel (0 %-15 % EtOAc in DCM) to yield **7** as a yellow oil (0.095 g, 0.49 mmol, 33 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (t, *J*=7.00, 2H), 2.16 (dt, J_1=6.90, J_2=2.64, 2H), 1.95 (t, *J*=2.64, 1H), 1.54-1.43 (m, 6H), 1.36-1.29 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 178.4, 83.2, 68.9, 33.0, 32.1, 31.5, 27.9, 22.6, 18.9, 17.8.

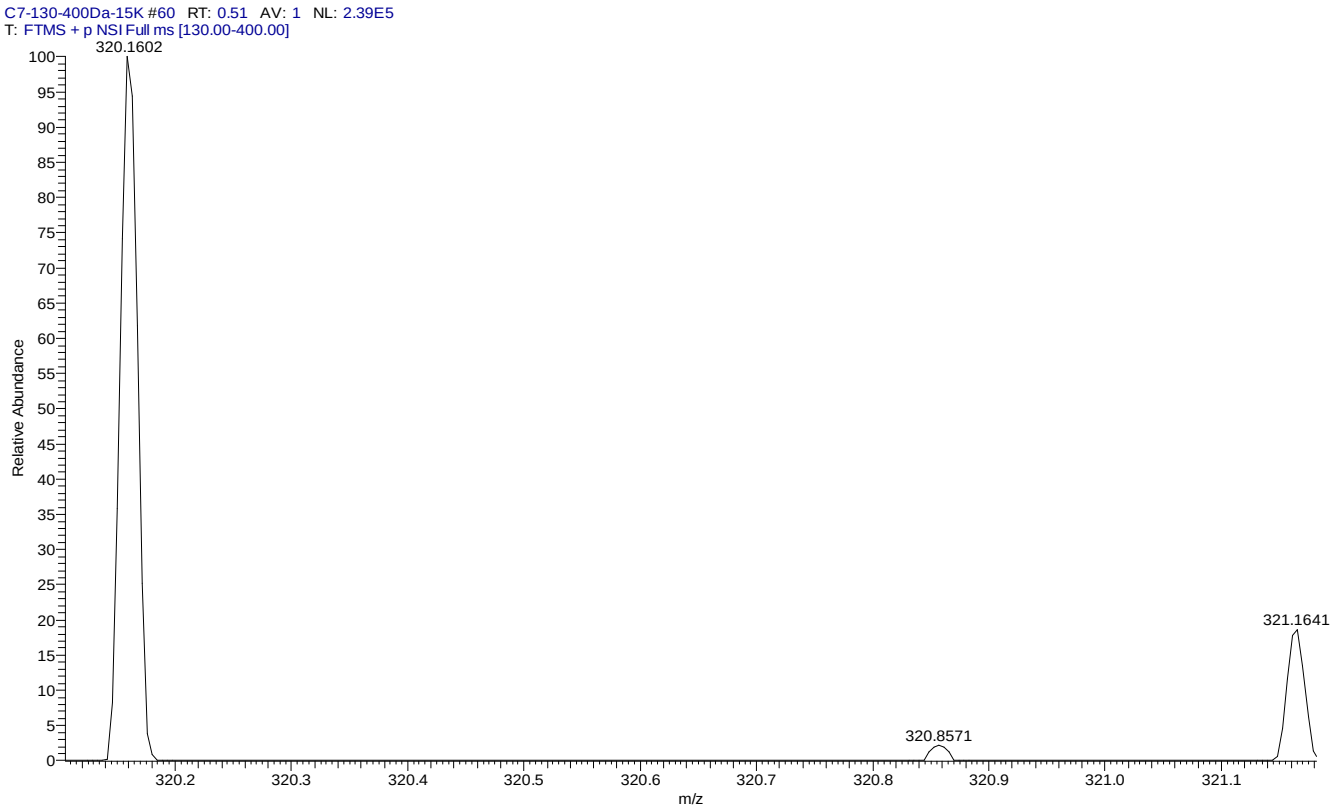


N-((*S*)-tetrahydro-2-oxofuran-3-yl)-3-oxo-6-(3-(pent-4-ynyl)-*3H*-diazirin-3-yl)hexanamide (probe 3):

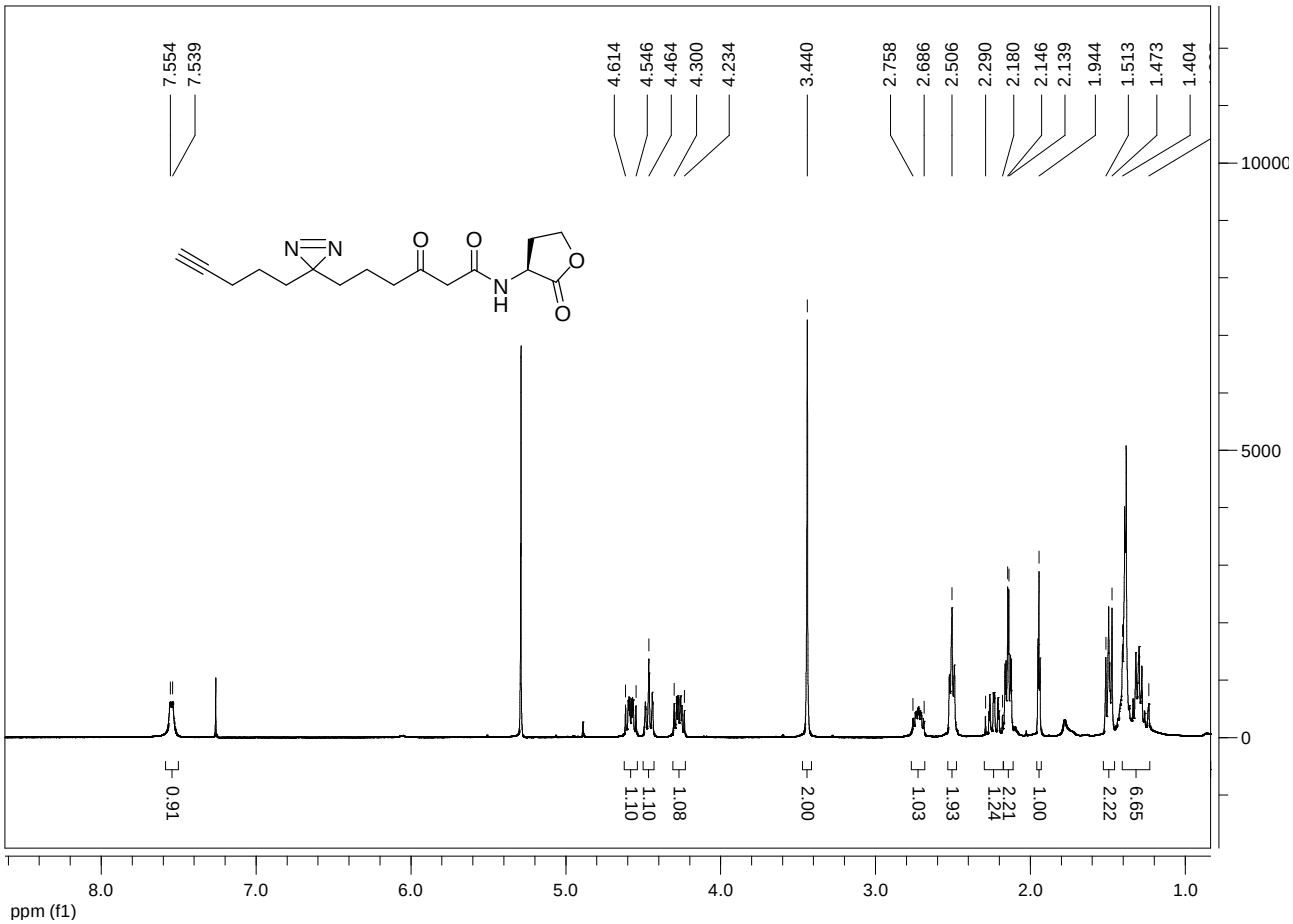
Compound **3** was prepared following procedure described by Amara et al.[120] N-(dimethylamino)pyridine (DMAP) (0.063 gr, 0.54 mmol), N,N-dichlorohexylcarbodiimide (DCC) (0.114 gr, 0.56 mmol), compound **7** (0.095 g, 0.49 mmol) and Meldrum's acid (0.072 gr, 0.50 mmol) where disulved in 5 mL of dichloromethane. The resulting solution was stirred overnight and then filtered to remove N,N-dicyclohexyl urea formed in the reaction. The filtrate was concentrated in vacuo. The resulting residue was dissolved in acetonitrile 4 mL and α-amino-γ-butyrolactone hydrobromide (0.091 gr, 0.50 mmol) was added. The mixture was stirred at RT for 1 hour and at 60 ^{0}C for 4 additional hours. The resulting solution was diluted with ethyl acetate 10 mL, and washed with saturated sodium bicarbonate solution, 1 M sodium hydrogen sulfate solution and brine. The organic phase was dried over magnesium sulfate filtered and concentrated in vacuo. The mixture was chromatographed on a column of silica gel (50 %-70 % EtOAc in hexanes) to yield the probe 3

as a yellow solid (0.021 g, 0.066 mmol, 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55(d, *J*=6.1, 1H), 4.61-4.55 (m, 1H), 4.46 (dt, J_1=9.1, J_2=1.3, 1H), 4.30-4.23 (m, 1H), 3.44 (s, 2H), 2.76-2.69 (m, 1H), 2.51 (t, *J*=6.8, 2H), 2.29-2.18 (m, 1H), 2.15 (dt, J_1=6.9, J_2=2.6, 2H), 1.94 (t, *J*=2.6, 1H), 1.51-1.47 (m, 2H), 1.40-1.24 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 205.1, 174.9, 166.2, 83.3, 69.9, 65.9, 49.0, 48.5, 42.6, 31.9, 31.4, 29.6, 28.0, 22.6, 17.8, 17.5. HRMS: $C_{16}H_{21}N_3O_4$ calc. (MH+): 320.1605; found: 320.1602 (MH+).

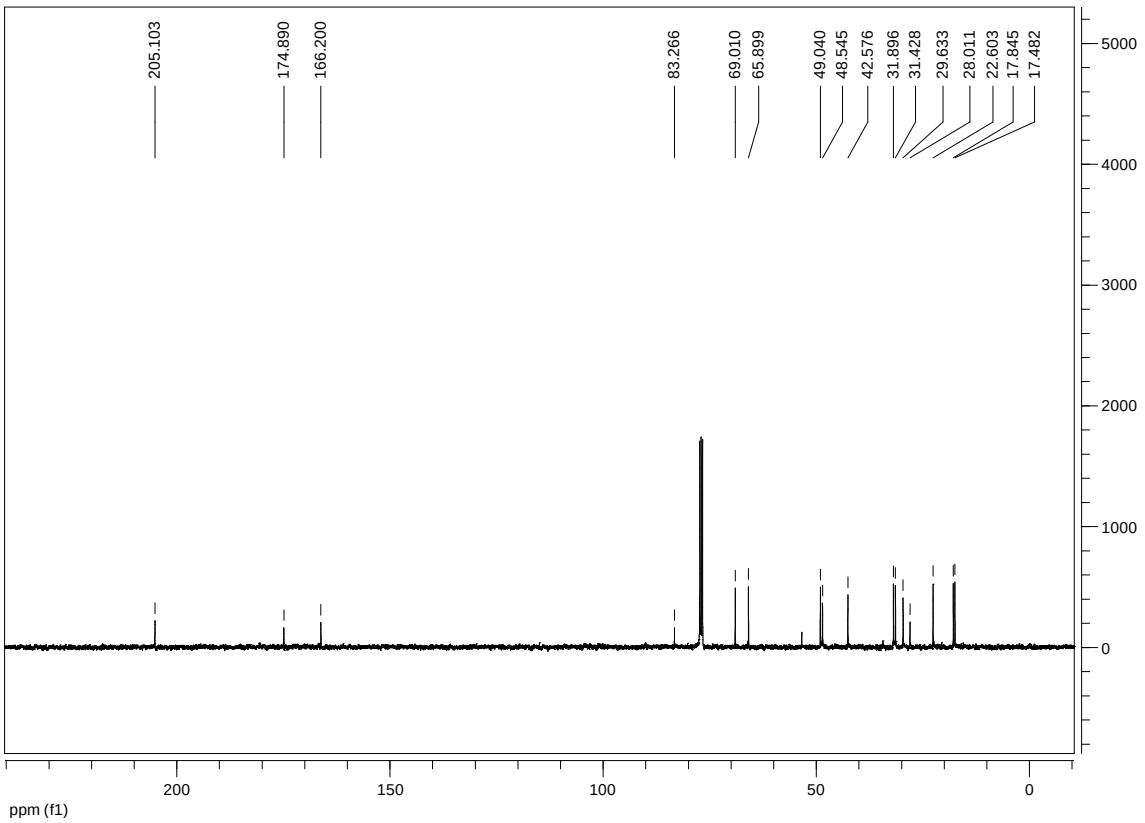
HRMS results:



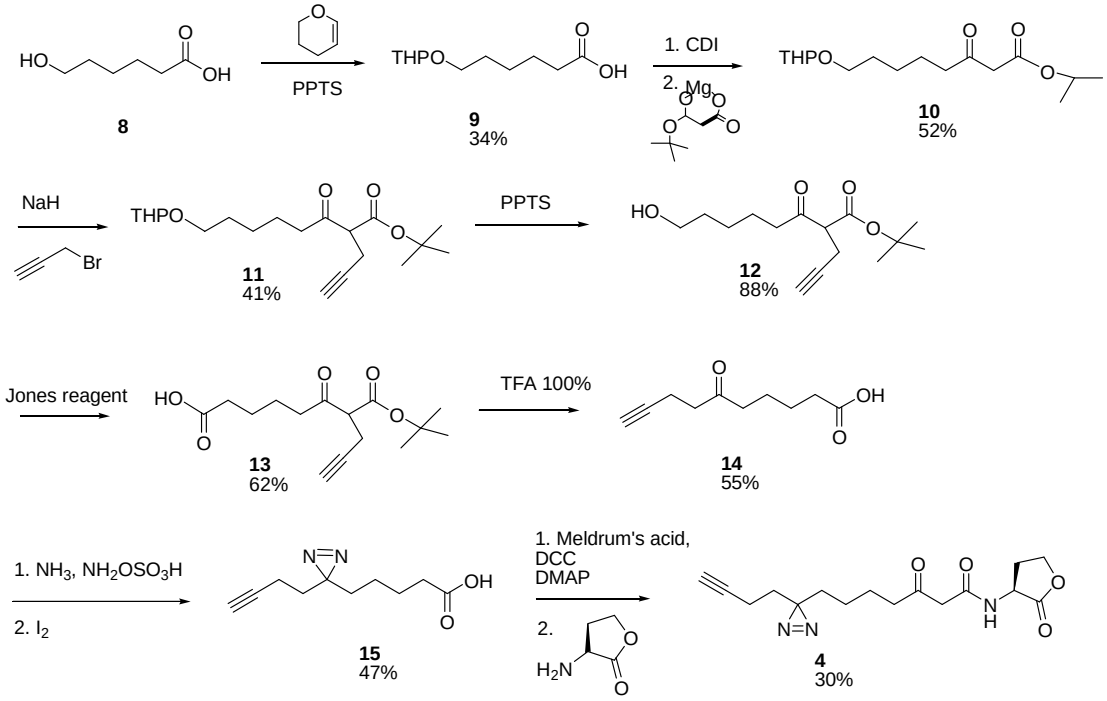
H^{1}NMR:



C^{13}NMR

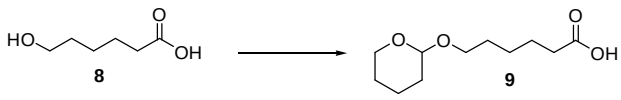


Synthesis of 7-(3-(but-3-ynyl)-*3H*-diazirin-3-yl)-N-(S)-(2-oxo-tetrahydrofuran-3-yl)-3-oxoheptanamide (probe 4):



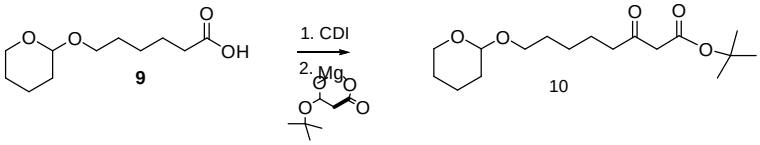
Scheme 3S: Synthesis of diazirine alkynyl **probe 4**.

Synthetic procedures:



6-(tetrahydro-*2H*-pyran-2-yloxy)hexanoic acid (9):

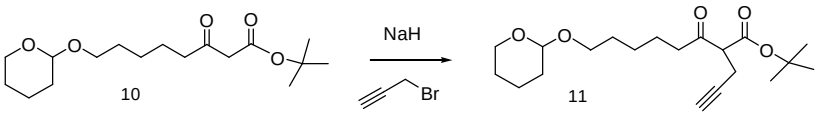
A solution of 1.8 gr (13.6 mmol) of hydroxycaproic acid **8** in dry dichloromethane (15 mL) was stirred at RT under nitrogen and 32.5 mg of pyridinium *p*-toluenesulfonate was added, followed by drop wise addition of dihydropyran (1.3 mL, 17.1 mmol). The mixture was stirred at ambient temperature o/n. After solvent evaporation, the reaction mixture was diluted with 30 mL ethyl acetate. The mixture was extracted with aqueous sodium hydroxide (1 M; 2*20 mL). The combined sodium hydroxide extracts were acidified with concentrated hydrochloric acid, and extracted with ethyl acetate. The combined organic extracts were dried under $MgSO_4$, concentrated under reduced pressure and purified by silica gel chromatography (0 % - 15 % EtOAc in hexanes) to yield **9** as a yellowed oil (1.0 g, 4.6 mmol, 34 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53-4.55 (m, 1H), 3.77-3.84 (m, 1H), 3.66-3.72 (m, 1H), 3.43-3.49 (m, 1H), 3.31-3.37 (m, 1H), 2.31 (t, *J* =7.5 Hz, 2H), 1.80-1.34 (m, 12H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 179.0, 98.7, 67.1, 62.2, 33.7, 30.6, 29.2, 25.6, 25.3, 24.3, 19.5. ESI: $C_{11}H_{20}O_4$ calc. (M+): 216.1; found: 239.2 (MNa+).



***tert*-butyl 3-oxo-8-(tetrahydro-*2H*-pyran-2-yloxy)octanoate (10):**

A: To a solution of 6-(Tetrahydro-2*H*-pyran-2-yloxy)hexanoic acid **9**, (2.0 g , 9.2 mmol) in dry dichloromethane (21 mL) under argon, CDI (1.7 gr, 10 mmol) was added at RT. The mixture was stirred at RT for 4 hours, then the solvent was evaporated and the mixture was diluted with dry THF (21 mL).

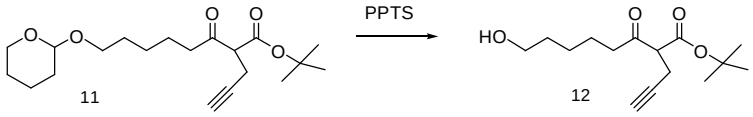
B: To a solution of mono-tert-butyl malonate (1.7 mL) in dry THF (21 mL) at 0 ºC under argon, isopropyl magnesium chloride (2M in THF, 10.4 mL, 21 mmol) was added dropwise. After 30 minutes at 0 ºC the solution was heated at 50 ºC for 30 minutes, then was cooled again at 0 ºC and solution A was added via cannula. The mixture was left at RT and was stirred o/n, then was quenched with dilute HCl (25 mL) and aqueous phase was extracted with ethyl acetate (2*25 mL). The combined organic fractions were extracted with NaOH (1 M, 33 mL). The organics were dried over $MgSO_4$, concentrated under reduced pressure and purified by silica gel chromatography (0 % - 15 % EtOAc in hexanes) to yield **10** as a yellowed oil (1.52 g, 4.85 mmol, 52 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.54-4.55 (m, 1H), 3.82-3.86 (m, 1H), 3.96-3.74 (m, 1H), 3.46-3.50 (m, 1H), 3.34-3.39 (m, 1H), 3.32 (s, 2H), 2.52 (t, *J*=7.4 Hz, 2H), 1.49-1.83 (m, 10H), 1.45 (s, 9H), 1.32-1.41 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.2, 166.4, 98.8, 81.8, 67.2, 62.3, 50.6, 42.7, 30.7, 29.4, 27.9, 25.7, 25.4, 23.2, 19.6. ESI: $C_{17}H_{30}O_5$ calc. (M+): 314.2; found: 337.2 (MNa+).



***tert*-butyl 3-oxo-2-(prop-2-ynyl)-8-(tetrahydro-*2H*-pyran-2-yloxy)octanoate (11):**

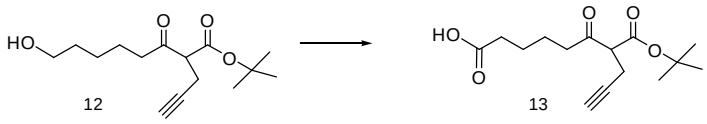
Compound **10** (1.52 g, 4.85 mmol) was dissolved in 10 mL dry THF and stirred. Under argon atmosphere NaH (111 mg, 4.6 mmol) was added carefully and stirred for 3 hours. Propargyl bromide (0.550 mL, 80 wt. % solution in toluene, 3.7 mmol) was diluted with 0.55 mL THF and added to a stirred solution during 5 hours. After completion of the reaction, unreacted NaH was neutralized with water, reaction mixture was acidified with 2-3 drops of concentrated HCl, extracted with ethyl acetate (2*20 mL), dried over $MgSO_4$, concentrated under reduced pressure

and purified by silica gel chromatography (0% - 15% EtOAc in hexanes) to yield **11** as a yellowed oil (0.70 g, 2.0 mmol, 41 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.53-4.54 (m, 1H), 3.80-3.86 (m, 1H), 3.68-3.80 (m, 1H), 3.58 (t, *J*=7.5 Hz, 1H), 3.44-3.50 (m, 1H), 3.32-3.38 (m, 1H), 2.49-2.67 (m, 4H), 1.95 (t, *J*=2.6, 1H), 1.47-1.82 (m, 10H), 1.43 (s, 9H), 1.31-1.39 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.8, 167.2, 98.9, 82.6, 80.8, 70.0, 67.3, 62.4, 58.5, 42.5, 30.7, 29.6, 27.9, 25.8, 25.5, 23.3, 19.7, 17.4. ESI: $C_{20}H_{32}O_5$ calc. (M+): 352.2; found: 375.2 (MNa+).



***tert*-butyl 8-hydroxy-3-oxo-2-(prop-2-ynyl)octanoate (12):**

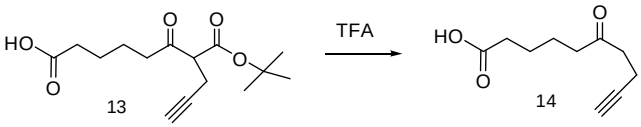
A solution of THP ether **11** (0.58 g, 1.7 mmol) and pyridinium *p*-toluenesulfonate (6 mg, 0.02 mmol) in ethanol (2 mL) was stirred at 55 ºC for 3 hours. The solvent was evaporated in vacuo, and the residue was chromatographed on a silica gel colounm (EtOAc : hexanes, 1:1 ratio) to yield **12** as a yellowed oil (0.39 g, 1.5 mmol, 88 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.66-3.58 (m, 3H), 2.72-2.47 (m, 4H), 1.97 (t, *J*=2.7, 1H), 1.68-1.52 (m, 4H), 1.45 (s, 9H), 1.41-1.33 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.7, 167.1, 82.5, 80.7, 69.9, 62.5, 58.3, 42.4, 32.3, 27.8, 25.1, 22.9, 17.3. ESI: $C_{15}H_{24}O_4$ calc. (M+): 268.2; found: 291.1 (MNa+).



7-(*tert*-butoxycarbonyl)-6-oxodec-9-ynoic acid (13):

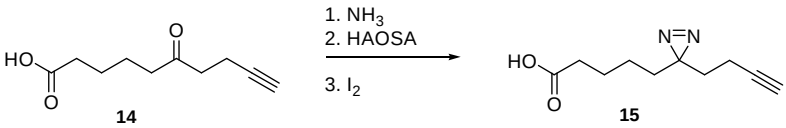
Jones reagent was added to a stirred solution of compound **12** (0.39 g, 1.5 mmol) in acetone (1.5 mL), with external cooling until the orange colour of the oxidant persisted. After the addition was complete, the reaction was stirred at RT for 30 min, and then diluted with water (5 mL). The aqueous mixture was extracted with ether and the combined ethereal extracts back extracted with aqueous sodium hydroxide (1 M ; 2 x 35 mL). The combined sodium hydroxide extracts were acidified with concentrated hydrochloric acid, and extracted with ether. The combined etheral extracts were dried under $MgSO_4$, concentrated under reduced pressure and purified by silica gel chromatography (EtOAc : DCM, 2:8) to yield **13** as a white solid (0.26 g, 0.9 mmol, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.60 (t, *J*=7.53, 1H), 2.72-2.48 (m, 4H), 2.36 (t, *J*=6.9, 2H), 1.97 (t,

J=2.7, 1H), 1.68-1.61 (m, 4H), 1.45 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.3, 179.0, 167.1, 82.6, 80.6, 70.0, 58.3, 42.0, 33.6, 27.8, 23.9, 22.6, 17.3. ESI: $C_{15}H_{22}O_5$ calc. (M+): 282.2; found: 305.1 (MNa+).



6-oxodec-9-ynoic acid (14):

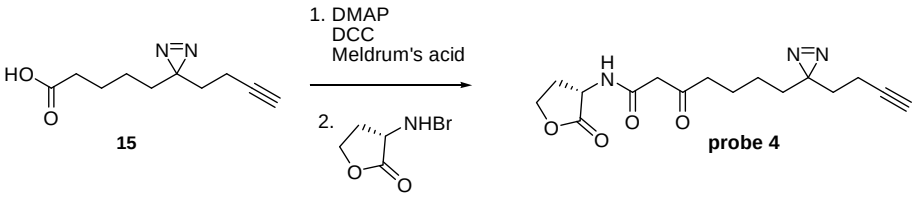
Compound **13** (0.26 g, 0.9 mmol) was disolved in trifluoroacetic acid (1.5 mL) and stirred for 20 minutes. The trifluooracetic acid was evaporated and the crude was purified by silica gel chromatography (10 % - 50 % EtOAc in hexanes) to yield **14** as a white solid (0.09 g, 0.5 mmol, 55 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.66 (t, *J*=7.25, 2H), 2.49-2.42 (m, 4H), 2.38 (t, *J*=7.03, 2H), 1.95 (t, *J*=2.7, 1H), 1.68-1.61 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 208.2, 178.7, 82.9, 68.7, 42.2, 41.2, 33.5, 24.0, 22.8, 12.9. ESI: $C_{10}H_{14}O_3$ calc. (M+): 182.2; found: 205.2 (MNa+).



6-diazirine-9-decynoic acid (15):

Anhydrous ammonia (10 mL) was condensed into a roundbottomed flask containing **14** (0.12 g, 0.66 mmol) at dry ice temperature. The mixture was stirred at 35-40 ºC for 5 h. The solution was cooled with dry ice, and a solution of hydroxylamine-*O*-sulfonic acid (0.088 g, 0.81 mmol) in anhydrous methanol (0.6 mL) was added over a period of 30 min. The dry ice bath was removed, and the mixture was refluxed with stirring at -35 °C for 1 h. The ammonia was then allowed to evaporate overnight. The resulting slurry was filtered and the filter cake washed with several portions of methanol. The combined solution was rotary evaporated. The residue of the diaziridine derivative was dissolved in dichloromethane (0.35 mL) and treated with triethylamine (0.11 mL). A solution of iodine (0.11 g, 1 mmol) in dichloromethane (0.75 mL) was slowly added with stirring until the appearance of a persistent orange-brown coloration. The mixture was chromatographed on a column of silica gel (10 %-30 % EtOAc in hexanes) to yield **15** as a yellow oil (0.062 g, 0.32 mmol, 47 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (t, *J*=7.39, 2H), 2.02-1.97 (m, 3H), 1.65-1.55 (m, 4H), 1.48-1.44 (m, 2H), 1.19-1.11 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 178.9, 82.7, 69.0, 33.5,

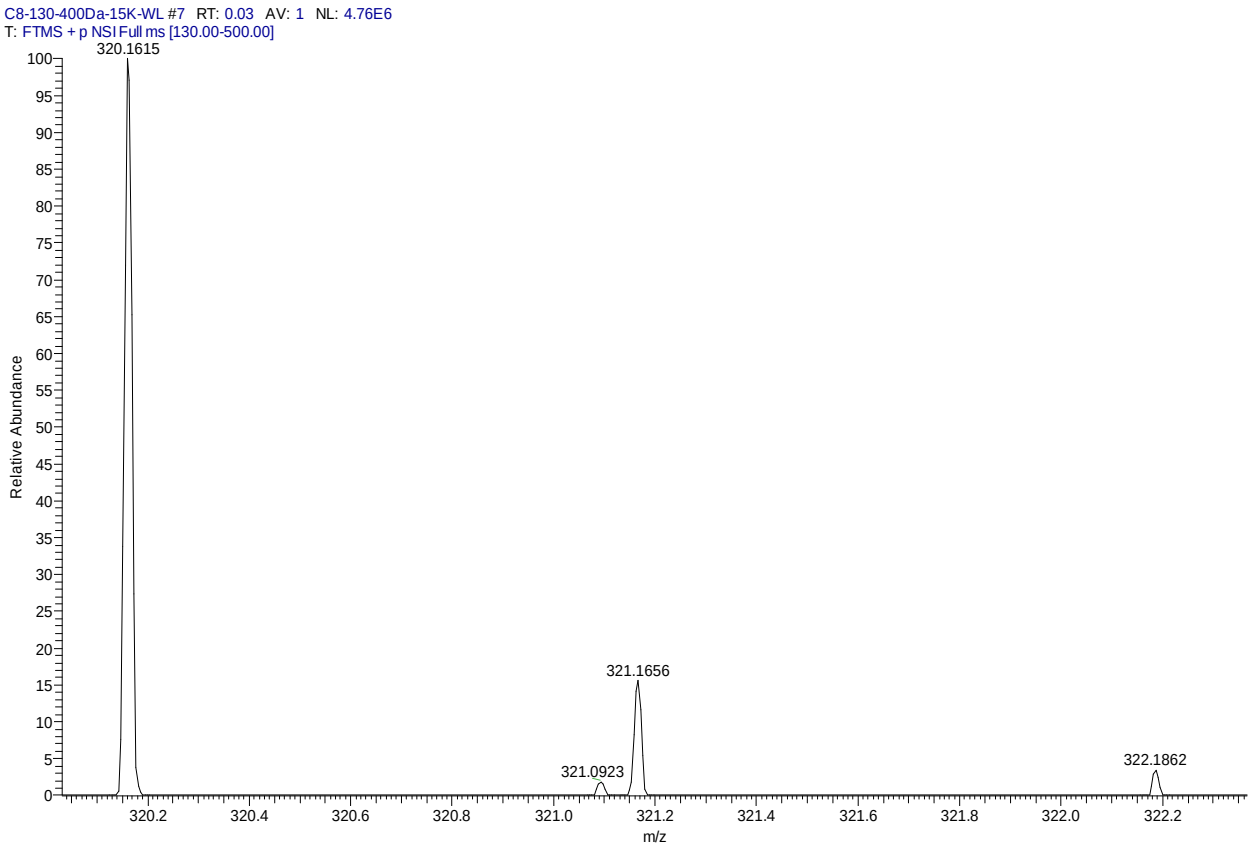
32.3, 32.2, 27.9, 24.0, 23.2, 13.2. ESI: $C_{10}H_{14}N_2O_2$ calc. (M+): 194.1; found: 167 (MH+ - N_2, without diazirine).



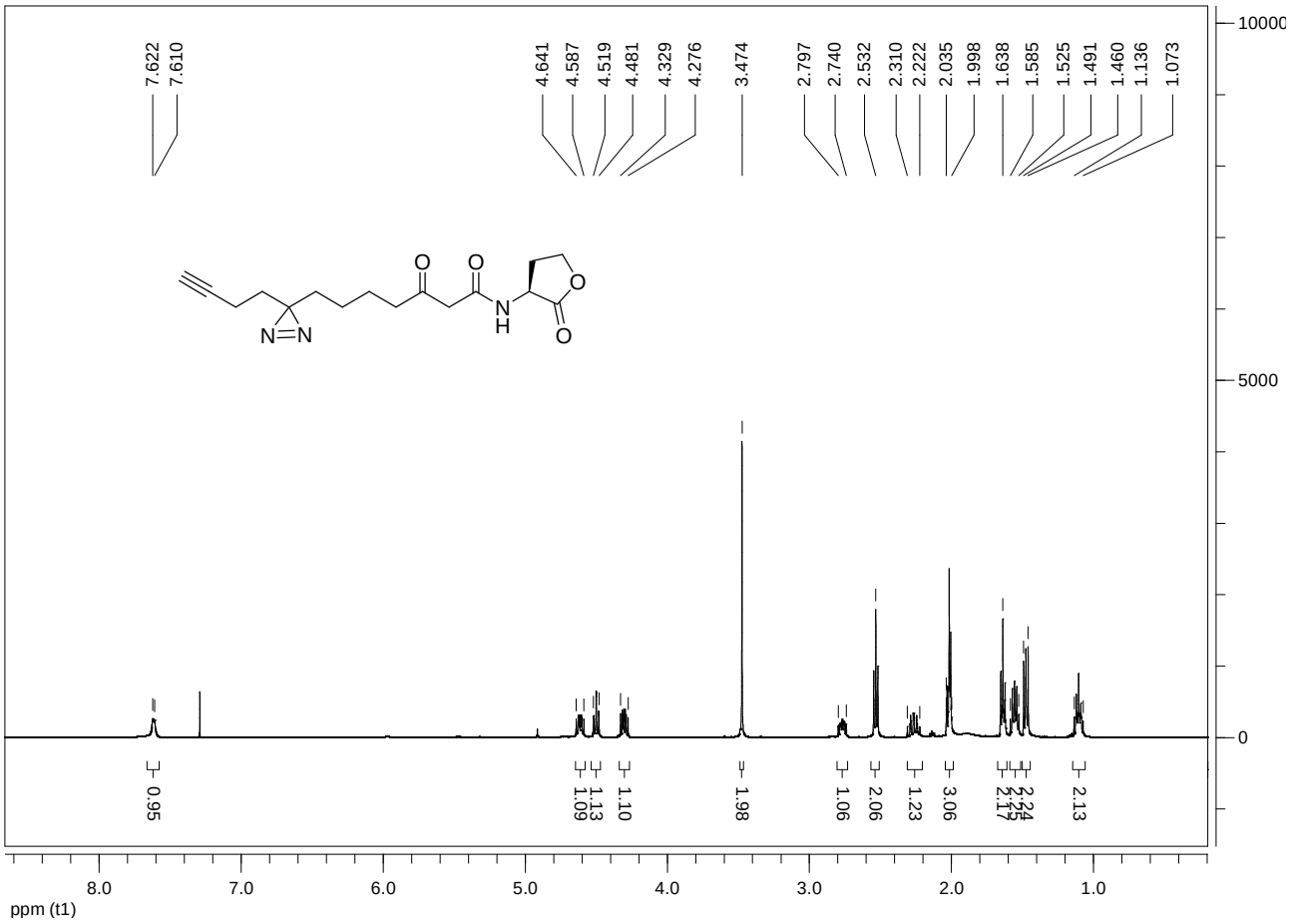
7-(3-(but-3-ynyl)-*3H*-diazirin-3-yl)-N-(S)-(2-oxo-tetrahydrofuran-3-yl)-3-oxoheptanamide (probe 4):

Probe **4** was prepared following procedure described by Amara *et al.*[2] N-(dimethylamino)pyridine (DMAP) (0.043 gr, 0.35 mmol), N,N-dichlorohexylcarbodiimide (DCC) (0.076 gr, 0.37 mmol), compound **15** (0.32 mmol) and Meldrum's acid (0.048 gr, 0.33 mmol) where disulved in 3 mL of dichloromethane. The resulting solution was stirred overnight and then filtered to remove N,N-dicyclohexyl urea formed in the reaction. The filtrate was concentrated in vacuo. The resulting residue was dissolved in acetonitrile 3 mL and α-amino-γ-butyrolactone hydrobromide (0.061 gr, 0.33 mmol) was added. The mixture was stirred at RT for 1 hour and at 60 ^{0}C for 4 additional hours. The resulting solution was diluted with ethyl acetate 10 mL, and washed with saturated sodium bicarbonate solution, 1 M sodium hydrogen sulfate solution and brine. The organic phase was dried over magnesium sulfate filtered and concentrated in vacuo. The mixture was chromatographed on a column of silica gel (50 %-70 % EtOAc in hexanes) to yield the probe **4** as a yellow solid (0.021 g, 0.066 mmol, 19 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J*=6.2, 1H), 4.64-4.59 (m, 1H), 4.5 (dt, J_1=9.1, J_2=1.3, 1H), 4.33-4.28 (m, 1H), 3.47 (s, 2H), 2.80-2.74 (m, 1H), 2.53 (t, *J*=7.2, 2H), 2.31-2.22 (m, 1H), 2.04-2.00 (m, 3H), 1.66-1.62 (m, 2H), 1.59-1.53 (m, 2H), 1.49-1.46 (m, 2H), 1.14-1.07 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 205.7, 174.9, 166.3, 82.8, 69.2, 66.0, 49.1, 48.4, 43.4, 32.4, 32.3, 29.8, 28.0, 23.2, 22.7, 13.3. ESI: $C_{16}H_{21}N_3O_4$ calc. (MH+): 320.1605; found: 320.1615 (MH+).

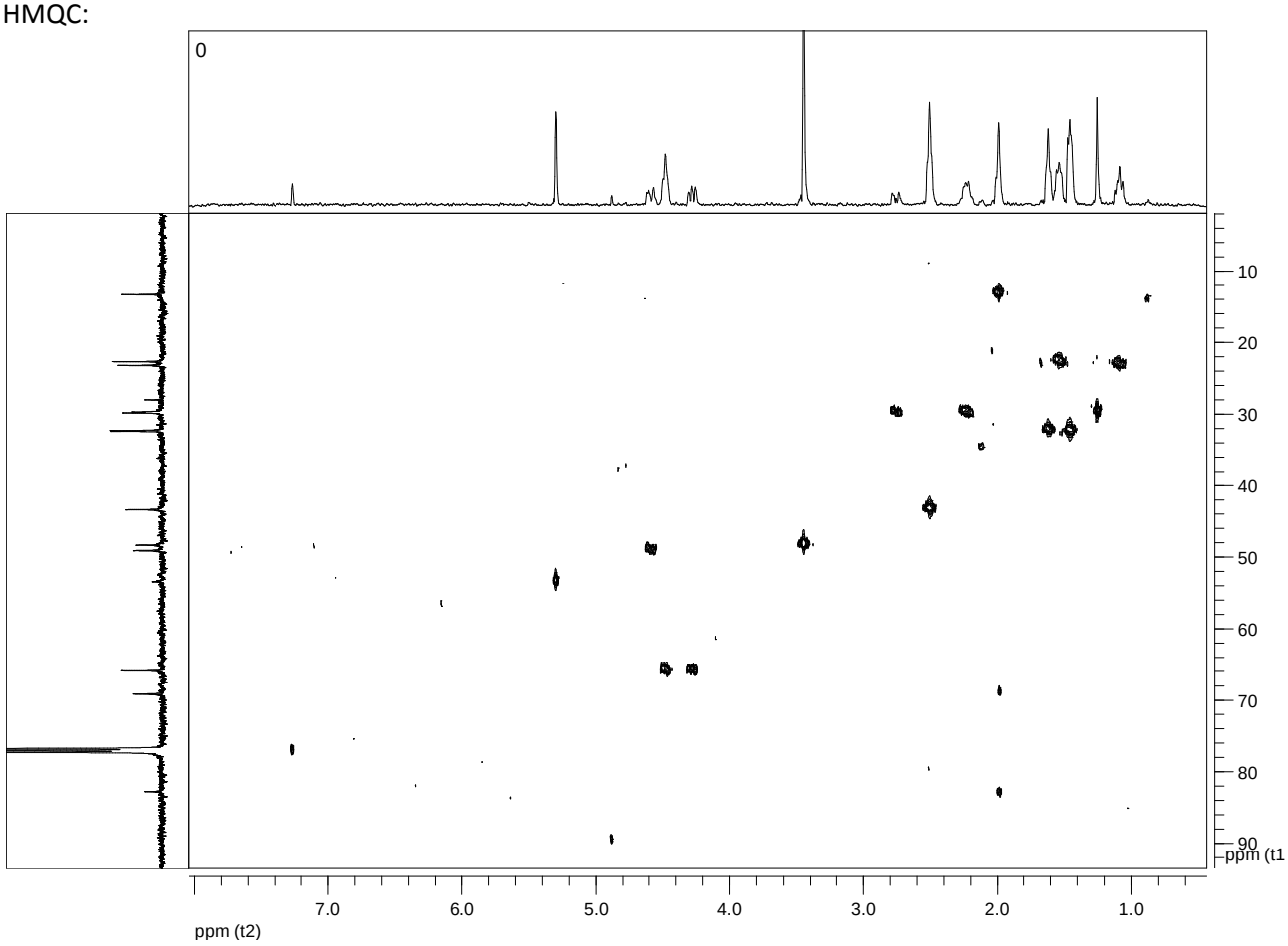
HRMS results:


C8-130-400Da-15K-WL #7 RT: 0.03 AV: 1 NL: 4.76E6
T: FTMS + p NSI Full ms [130.00-500.00]
320.1615
321.1656
321.0923
322.1862
Relative Abundance
m/z

H^1NMR:



HMQC:



Mass spectra of the **probes 3-4** were obtained using an LTQ XL Orbitrap ETD with nano ESI (Thermo). Other intermediates (**5-15**) were analyzed using an LCQ Fleet mass spectrometer (Thermo Scientific) with ESI source, connected to a Surveyor Plus HPLC System (Thermo Scientific). Spectra were collected in positive ion mode and analyzed by Xcalibur software (Thermo Scientific).

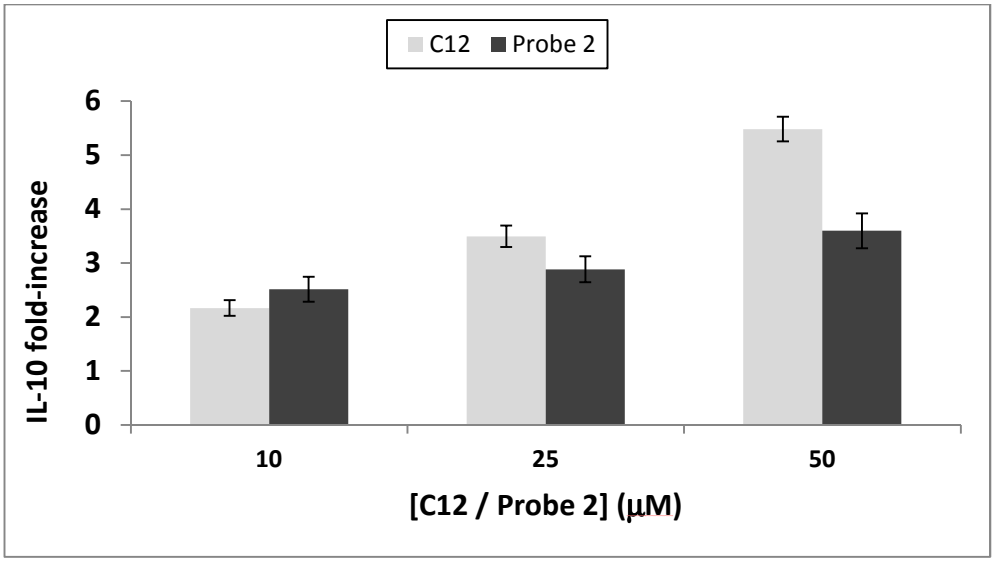


Fig. S2. Probe 2 and C12 increase LPS-stimulated IL-10 expression in RAW264.7 macrophages. Mouse macrophage RAW264.7 cells were incubated for 5 hours at 37°C with LPS (10 ng/ml), together with either probe 2 or C12 (0, 10, 25 or 50 μM). IL-10 secretion was measured by ELISA. Each data point represents the mean ± S.D. (n=6), relative to treatment with LPS alone. IL-10 production in resting cells or by C12 or probe 2 alone was undetectable (< 40 pg/ml).

Mouse RAW264.7 macrophage cells, obtained from American Type Culture Collection (ATCC, Rockville, MD), were grown to 80-90% confluence in DMEM medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 1250 U/ml nystatin (hereafter culture medium), and with 10% FBS, at 37°C in a humidified incubator with 5% CO_2. The cells were maintained for 48 hours prior to the experiment in 96-well plates, at $1.5 \cdot 10^5$ cells per well, in culture medium supplemented with 5% FBS, up to a confluence of 90%. The culture medium was replaced 2 hours before treatment in order to avoid an artifact on signaling caused by medium replacement. Following a 5 hours treatment with Lipopolysaccharide (LPS; *Escherichia coli* serotype 055:B5) and either C12 or probe 2, IL-10 secretion to the medium was measured by ELISA using reagents sets, according to the manufacturer's instructions, and a microplate reader (Bio-Tek, Winooski, Vermont).

References:

1. D. M. Hodgson, A. H. Labande, F. Y. Pierard, and M. A. Exposito Castro, The scope of catalytic enantioselective tandem carbonyl ylide formation-intramolecular [3 + 2] cycloadditions., *J. Org. Chem.* 68, 6153 (**2003**).
2. N. Amara, R. Mashiach, D. Amar, P. Krief, S. A. Spieser, M. J. Bottomley, A. Aharoni and M. M. Meijler, Covalent Inhibition of Bacterial Quorum Sensing, *J. Am. Chem. Soc.*, 131(30), 10610 (**2009**).