Supporting Information

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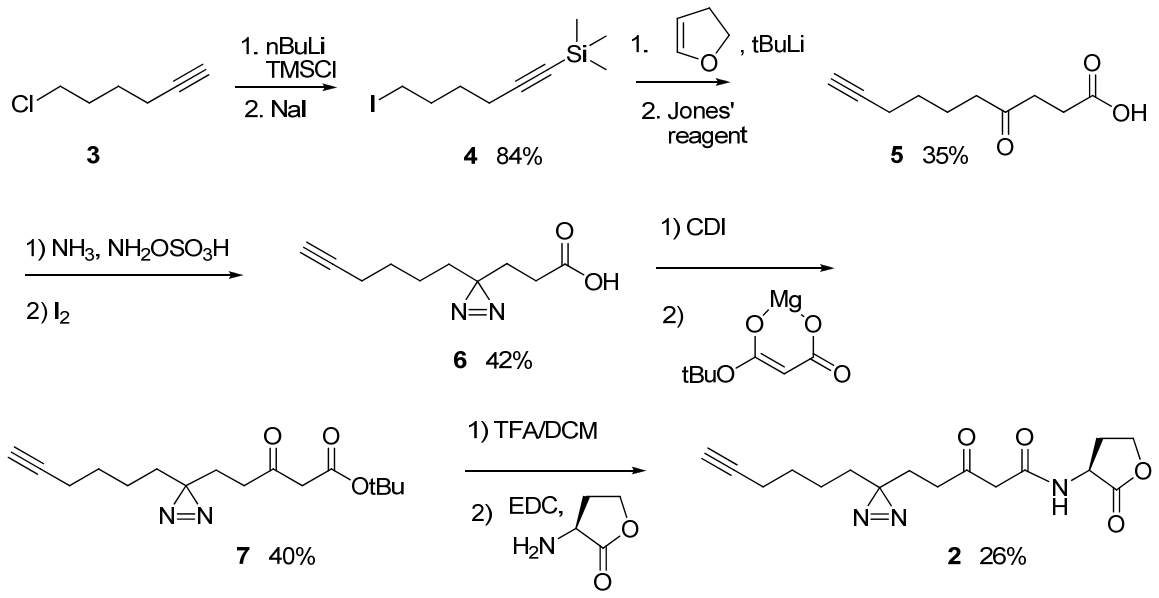
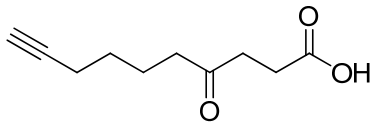
General methods

All chemical reagents were purchased from Sigma-Aldrich or Acros and used without further purification. THF was distilled from sodium/benzophenone. Reactions were monitored by TLC using commercially available glass backed plates precoated with silica (0.25 mm, Merck 60 F254), that were commonly stained using potassium permanganate. Flash chromatography was performed using Merck 40-63 μm silica gel. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance DPX200 or alternatively on a Bruker Avance DMX500 spectrometer. ^{1}H and ^{13}C chemical shifts are reported with respect to TMS and spectra were calibrated on residual solvent signal.

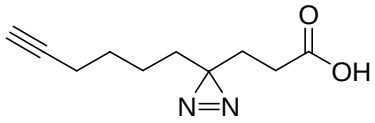
Synthesis of 5-(3-(hex-5-ynyl)-*3H*-diazirin-3-yl)-*N*-*((S)*-2-oxo-tetrahydrofuran-3-yl)-3- oxopentanamide (2)



Synthetic procedures

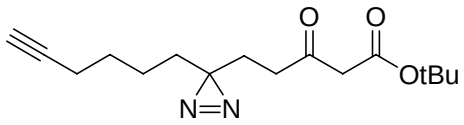


4-oxodec-9-ynoic acid (**5)** was prepared by a known procedure[1] via lithiation of 2,3-dihydrofuran and alkylation with TMS-protected alkyne iodide **4**, followed by direct oxidation of the crude hydroxynonynone using Jones' reagent[1].



4-diazirine-9-decynoic acid (6) was prepared following procedures described by Husain *et al.*[2] Anhydrous ammonia (3.5 mL) was condensed into a round bottomed flask. 4-

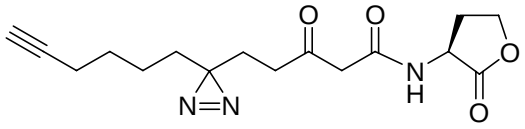
oxodec-9-ynoic acid **5** (0.54 g, 2.9 mmol) was added dissolved in small amount of anhydrous methanol. The mixture was stirred at -35-40 ºC for 5 h. The solution was cooled with dry ice, and a solution of hydroxylamine-*O*-sulfonic acid (0.38 g, 3.3 mmol) in anhydrous methanol (2 mL) was added over a period of 30 min. The dry ice bath was removed, and the mixture was refluxed with stirring at -35 ºC for 1 h. The mixture was allowed to warm slowly to room temperature and stirred overnight. The ammonia was then allowed to evaporate. The resulting slurry was filtered and the filter cake washed with several portions of methanol. The combined solution was concentrated *in vacuo*. The crude aziridine residue was dissolved in dichloromethane (1.7 mL) and treated with triethylamine (0.5 mL). A solution of iodine (0.5 g, 3.9 mmol) in dichloromethane (3 mL) was slowly added with stirring until the appearance of a persistent orange-brown color. The mixture was purified by flash chromatography on a column of silica gel using as eluent dichloromethane/ethyl acetate 4/1. Yield: 42%. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.20 – 2.12 (m, 4H); 1.95 (t, 1H, *J* = 2.62 Hz); 1.75 (t, 2H, *J* = 7.76 Hz); 1.55 – 1.39 (m, 4H); 1.31 – 1.14 (m, 2H). ^{13}C NMR (200 MHz, $CDCl_3$): δ = 178.14; 83.83; 68.63; 32.18; 29.64; 28.22; 27.82; 27.75; 22.85; 18.12.



1-*tert*-butyl-3-oxo-6-diazirine-11-dodecynoate (7) was prepared following a modified procedure based on reactions described by Kaufmann *et al.*[3]

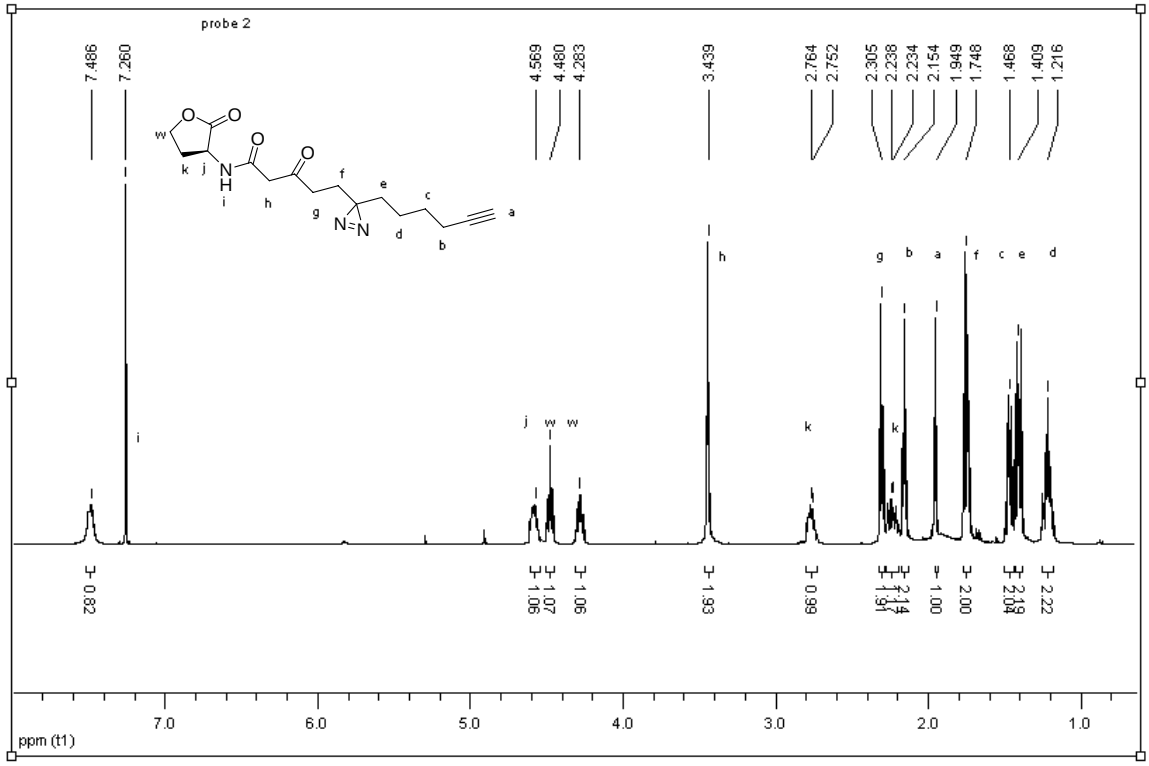
A: To a solution of 4-diazirine-9-decynoic acid **6** (0.22 g, 1.1 mmol) in dry THF (2.5 mL) uder argon, CDI (0.21 g, 1.3 mmol) was added at room temperature. The mixture was stirred at room temperature for 4 hours. B: To a solution of mono *tert*-butyl malonate (0.2 g, 1.3 mmol) in dry THF (2.5 mL) at 0 ºC under argon, isopropyl magnesium chloride (2 M in THF, 1.2 mL, 2.4 mmol) was added dropwise. After 30 minutes at 0 ºC the solution was heated at 50 ºC for 30 minutes, then was cooled again to 0 ºC and solution A was added via cannula. The mixture was left warming to room temperature and was stirred for 16 hours, then quenched with HCl 1 M (6 mL). The aqueous phase was extracted with ethyl acetate (3x12 mL). The organic layers were combined and washed with NaOH 1M,

dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash chromatography using as eluent hexane/ethyl acetate (0-10%). Yield: 40 %. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.32 (s, 2H); 2.30 (t, 2H, *J* = 7.45 Hz); 2.13 (dt, 2H, *J* = 7.02 Hz, *J* = 2.64 Hz); 1.94 (t, 1H, *J* = 2.64 Hz); 1.74 (t, 2H, *J* = 7.45 Hz); 1.54 (m, 2H); 1.47 (s, 9H); 1.41 (t, 2H, J = 8.00 Hz); 1.25 – 1.19 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ =201.43; 166.12; 83.87; 82.17; 68.58; 50.54; 36.58; 32.48; 29.67; 28.26; 27.77; 26.34; 22.91; 18.14.



5-(3-(hex-5-ynyl)-*3H*-diazirin-3-yl)-*N*-*((S)*-2-oxo-tetrahydrofuran-3-yl)-3-oxopentanamide (2) was prepared following a modified procedure based on reactions described by Kaufmann *et al*.[3] The *tert*-butyl protecting group was removed by stirring 1-*tert*-butyl-3-oxo-6-diazirine-11-dodecynoate in TFA/DCM 1:1 for 20 min. After solvent evaporation, the resulting 3-oxo-6-diazirine-11-dodecynoic acid (0.091 g, 0.385 mmol) was dissolved in 1,4-dioxane and EDCI (0.090 g, 0.467 mmol), HOBT (0.0453 g, 0.333 mmol) and homoserine lactone hydrobromide (0.061g, 0.333 mmol) were added alongside a few drops of water (3.33mL) at room temperature. Triethylamine (0.093mL, 0.667 mmol) was added and the solution was stired at room temperature for 3 hours. The reaction mixture was diluted with ethyl acetate (30 mL), washed with water, dried over $MgSO_4$, the solvent was evaporated and the crude was purified by flash chromatography using as eluent ethyl acetate/hexane 4/1. Yield: 26%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.49 (s, 1H); 4.57 (m, 1H); 4.48 (t, 1H, *J* = 8.95 Hz); 4.28 (m, 1H); 3.44 (s, 2H); 2.76 (m, 1H); 2.30 (t, 2H, *J* = 7.21 Hz); 2.24 (m, 1H); 2.15 (dt, 2H, *J* = 7.01 Hz, *J* = 2.60 Hz); 1.95 (t, 1H, *J* = 2.60 Hz); 1.75 (t, 2H, *J* = 7.21 Hz); 1.47 (m, 2H); 1.41 (t, 2H, *J* = 7.90 Hz); 1.22 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ = 204.22; 174.80; 165.99; 83.91; 68.70; 65.96; 49.17; 48.42; 37.50; 32.50; 29.84; 27.995; 27.79; 26.15; 22.94; 18.19. ESI: $C_{16}H_{21}N_3O_4$ calc. (MH^+): 320.2; found: 320.1, 342.1 (MNa^+).

^{1}H-NMR


probe 2
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7.260
4.589
4.480
4.283
3.439
2.764
2.752
2.305
2.238
2.234
2.154
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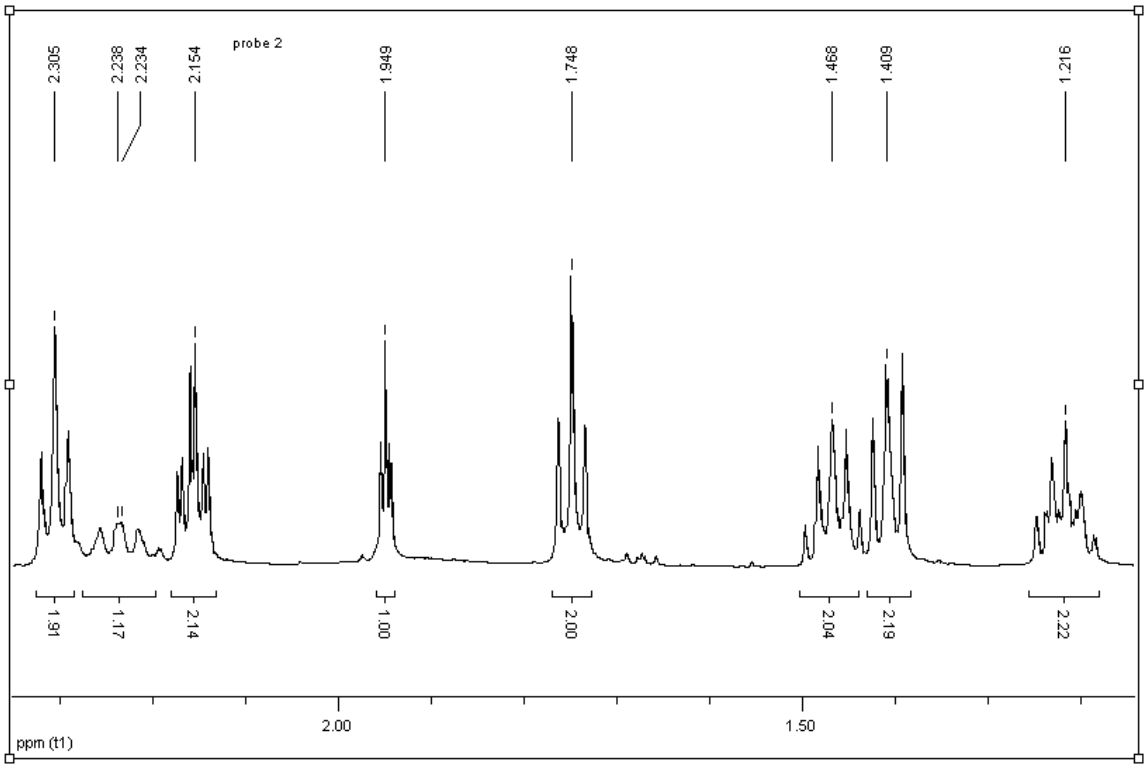
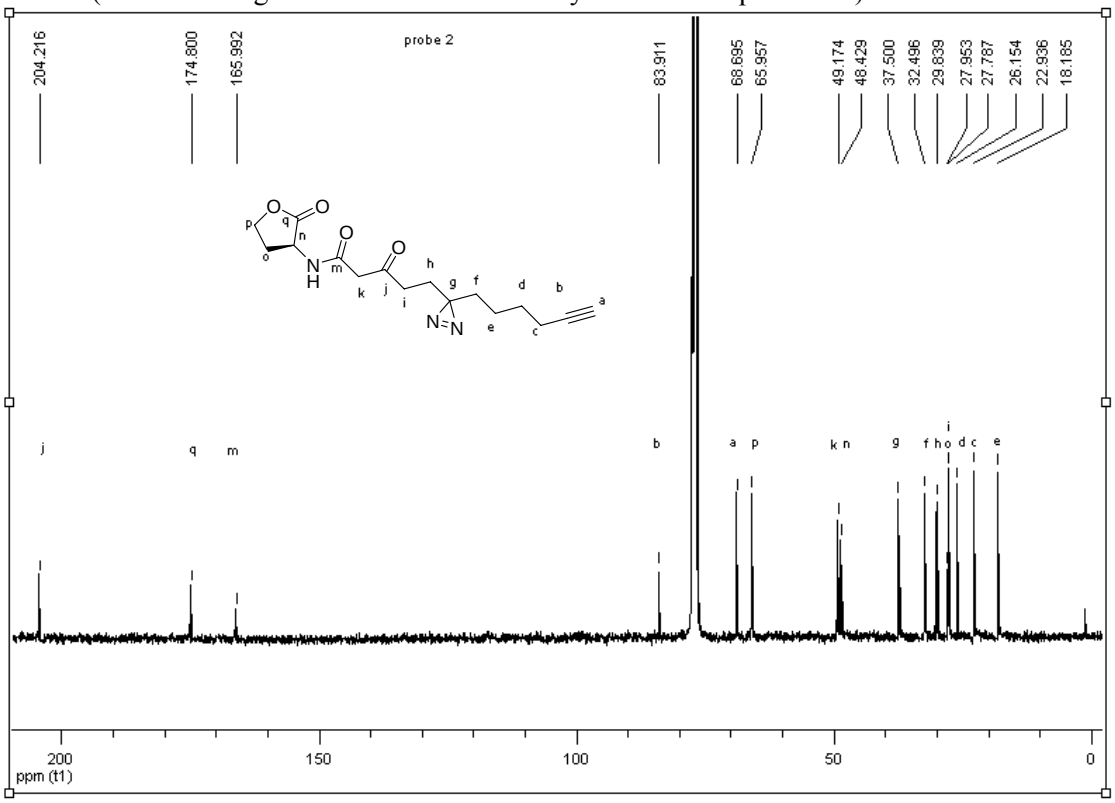
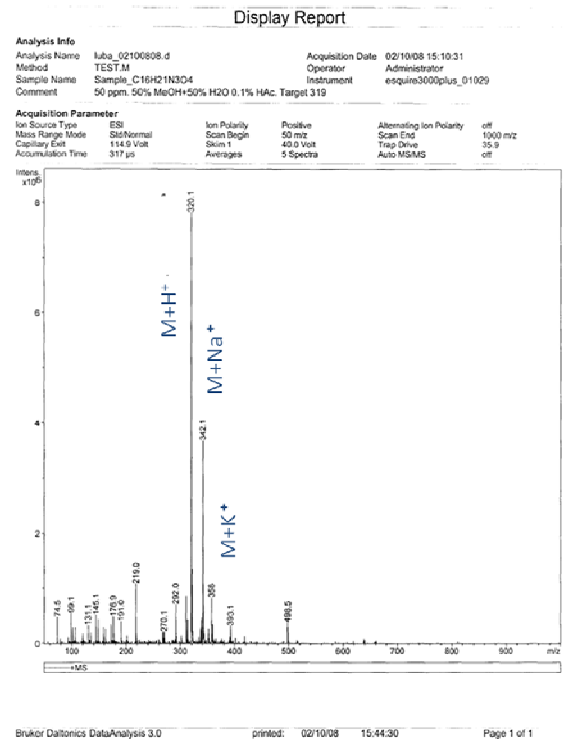
1.748
1.468
1.409
1.216
ppm (t1)


probe 2
2.305
2.238
2.234
2.154
1.949
1.748
1.468
1.409
1.216
1.91
1.17
2.14
1.00
2.00
2.04
2.19
2.22
ppm (t1)

^{13}C NMR (tentative assignments – not confirmed by 2D-NMR experiments)



MS analysis: ESI



Biological evaluations

Activity test in biosensor *E. coli* pSB1075. The biosensor strain carrying a bioluminescence reporter plasmid (lux) was grown overnight in 5 mL LB medium with 50 μg/ml ampicillin at 37°C. The bacterial culture was subsequently diluted 100 times in LB medium. 200 μL of the bacterial suspension was added to wells of a 96-well microtiter plate. Conditions were tested as follow: 5 μM of probe **2** was added, 5 μM of 3-oxo-C_{12}-AHL (C12), LB medium or LB medium with 0.5% DMSO. The plate was incubated at 37°C, while shaking at 60 rpm for 3 hours, followed by measurement of luminescence.

Effects of 3-oxo-C_{12}-AHL (C12) and probe 2 on germ tube formation in *C. albicans*. *C. albicans* SC 5314 were grown overnight in 5 mL yeast nitrogen broth (YNB) medium at 30°C shaking (120 rpm). Cells were spun down at 14000 rpm for 5 min, resuspended in saline phosphate buffer (PBS), pH 7.4, followed by OD_{600} measurement. The cell suspension was diluted in YNB to receive OD_{600} = 0.01 and added to 12 wells plate. 200, 100, 50, 25, 12.5, 6.25, 3.12 and 0 μM of probe **2** and C12 were added to the plates following by incubation at 37°C, shaking at 80 rpm for 3 hours. Every sample was examined using an inverted light microscope – numbers of yeasts and hyphal cells were counted.

Elastase activity was determined using the elastin Congo red (ECR) assay, as previously described[4, 5] with minor modifications. The supernatant of a *P. aeruginosa ΔlasI* strain (kindly provided by D. A. Hogan) grown in tryptic soy broth (TSB) for 5 hours with various concentrations of C12 or probe **2** was collected by centrifugation. 20 mg of ECR (Sigma) was added to each tube containing 900 μl of ECR buffer (100mM Tris, 1mM $CaCl_2$, pH 7.5) and 100 μl of culture supernatant. The mixtures were incubated at 37^{o}C for 18 hours while shaking at 150 rpm after which the suspention was centrifuged at 10,000 x *g* for 15 min. The absorbance at 490 nm of the supernatants was measured using a spectrophotometer. Background absorbance was subtracted and uninoculated TSB was used as a negative control.

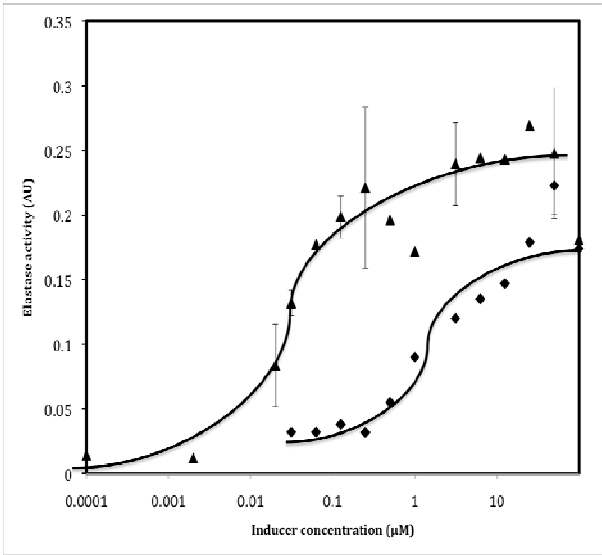


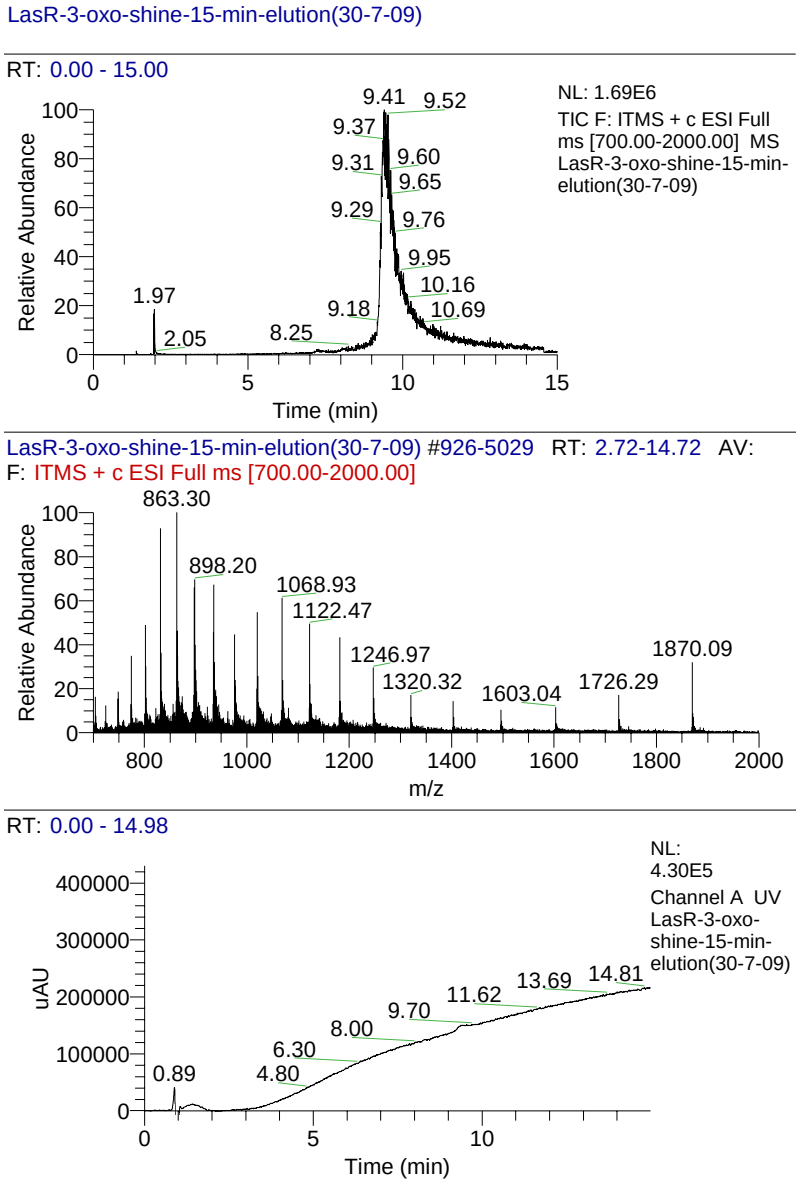
Figure S1. Elastase activity induced by C12 (diamonds) and probe **2** (triangles) in *P. aeruginosa* LasI. Lines are drawn to guide the eye. IC_{50} values calculated for C12 = 1.0 μM and for probe **2** = 0.03 μM. The stronger induction of elastase activity by **2** compared with C12 is not understood by us, and is the subject of further investigations.

Expression of LasR-LBD. The expression of full length LasR was found to yield largely insoluble protein in the presence or absence of the native ligand C12.[6] Therefore expression was performed using a plasmid with the pETM-11 vector encoding for a shortened, His_6- tagged LasR construct, LasR-LBD (ligand binding domain), spanning Met-1 to Lys-173. The plasmid was transferred *E.coli* BL-21, and cells were plated on LB agar plates containing kanamycin (50 μg/mL). Proteins were expressed in the presence of either native C12, probe **2** or both and purified by Ni^{2+} affinity chromatography as described elsewhere.[4] The purification process was monitored by SDS-PAGE electrophoresis and the molecular mass of the purified proteins was confirmed by LC/MS (Thermo-Finnigan LTQ). Following the procedure reported by Amara *et al.*[7], one milliliter of an overnight grown cell culture was used to inoculate 10 mL of rich LB medium containing kanamycin (50 μg/mL) and 10 μM of C12 (or 20 μM probe **2**, or 20 μM probe **2** + 20 μM C12). Cells were grown to an optical density at 600 nm (OD_{600}) of 0.4, after which expression was induced at 21°C by addition of 0.2 mM

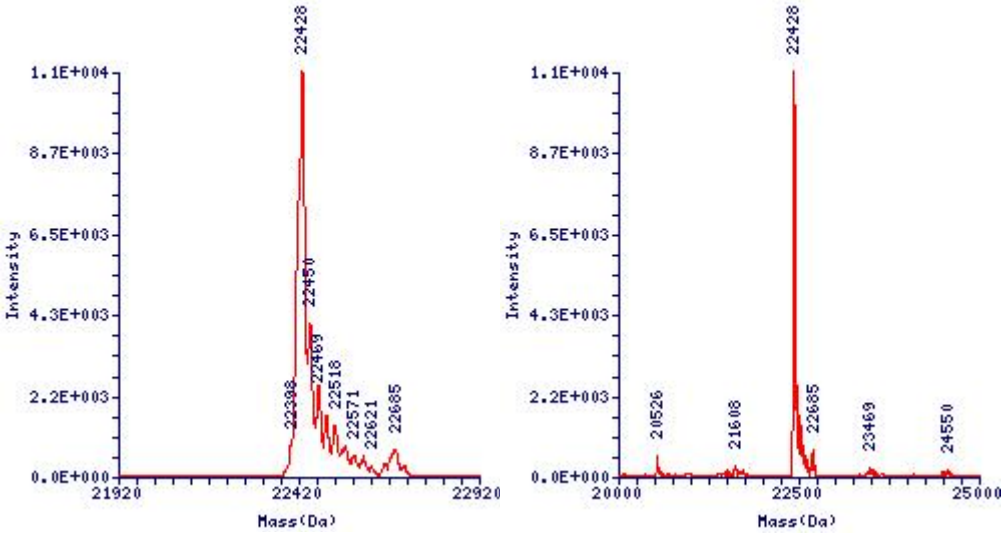
isopropyl 1-thio-β-D-galactopyranoside (IPTG). After reaching an OD_{600} of 1.4 (approx. 6 hours) cells were centrifuged at 4000 rpm at 4°C, washed and resuspended in PBS. Samples were was irradiated with a hand-held UV lamp (360 nm) for 20 min on ice. Cells were centrifuged and resuspended in lysis buffer containing 50 mM tris-HCl at pH 8, 300 mM NaCl, 0.2 % (v/v) triton, 0.75 μg/mL DNAse-I, 0.05 mM $MgCl_2$, 0.01 mM $CaCl_2$, 5 mM imidazole and 0.01 % (v/v) protein inhibitor cocktail. Cells were incubated 60 minutes at 37 °C and centrifuged at 4000 rpm. The supernatants were purified using Ni-NTA spin colums following kit procedures by QIAGEN. SDS page was performed using a NuPAGE Surelock Xcell, on NuPAGE Novex Bis-Tris Pre-Cast gels purchased from Invitrogen (NP0342).

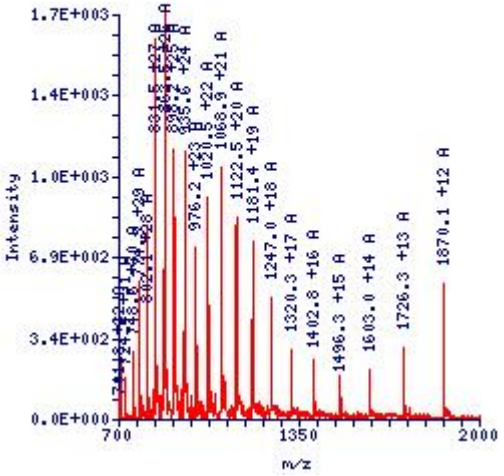
Mass spectra were obtained using an LCQ Fleet mass spectrometer (Thermo Scientific) with ESI source, connected to a Surveyor Plus HPLC System (Thermo Scientific). Spectra were collected in positive ion mode and analyzed by Xcalibur software (Thermo Scientific).

MS data, experiment **A**
probe: C12 (file: LasR-3-oxo-shine-15-min-elution(30-7-09))

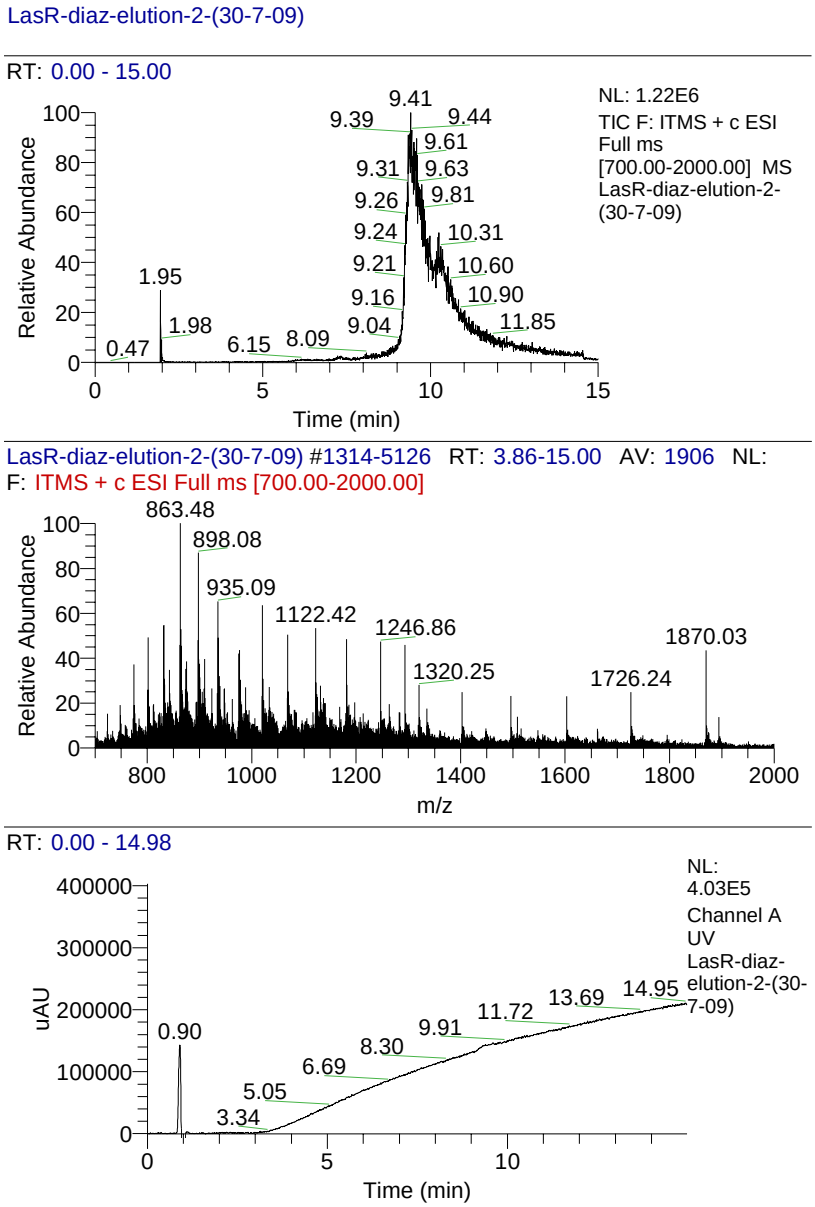


Deconvolution of MS data obtained in experiment **A** (using ProMass for Xcalibur)

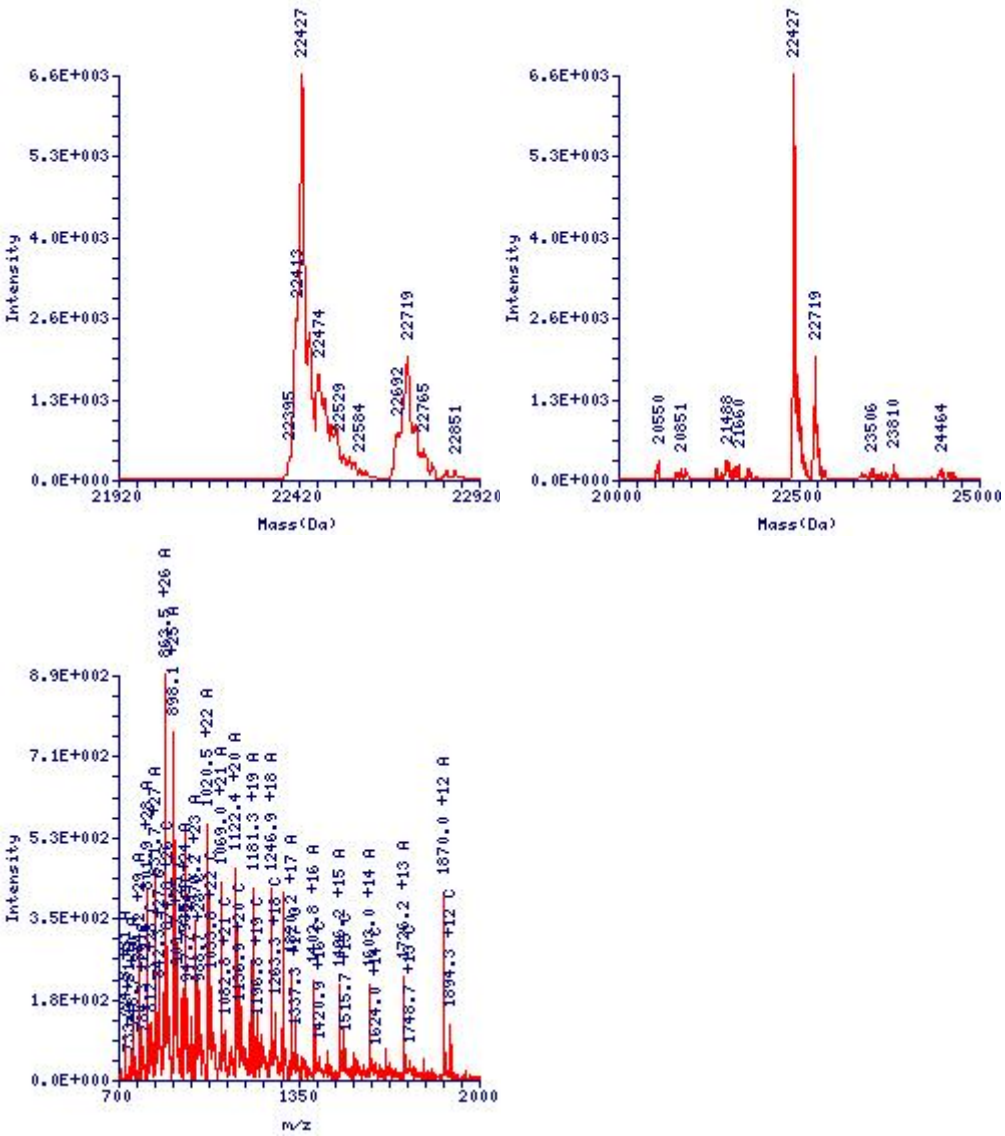




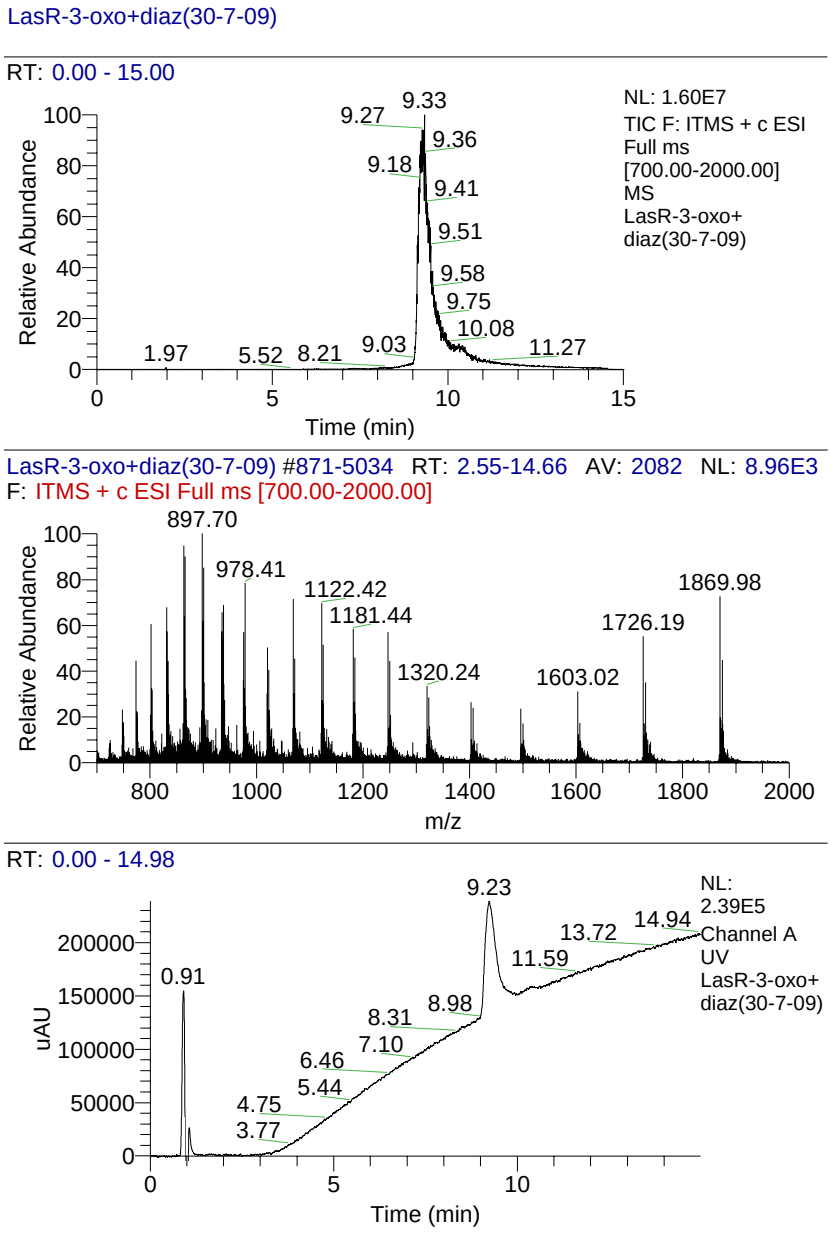
MS data, experiment **B**
probe: diazirine (file: LasR-diaz-elution-2(30-7-09))



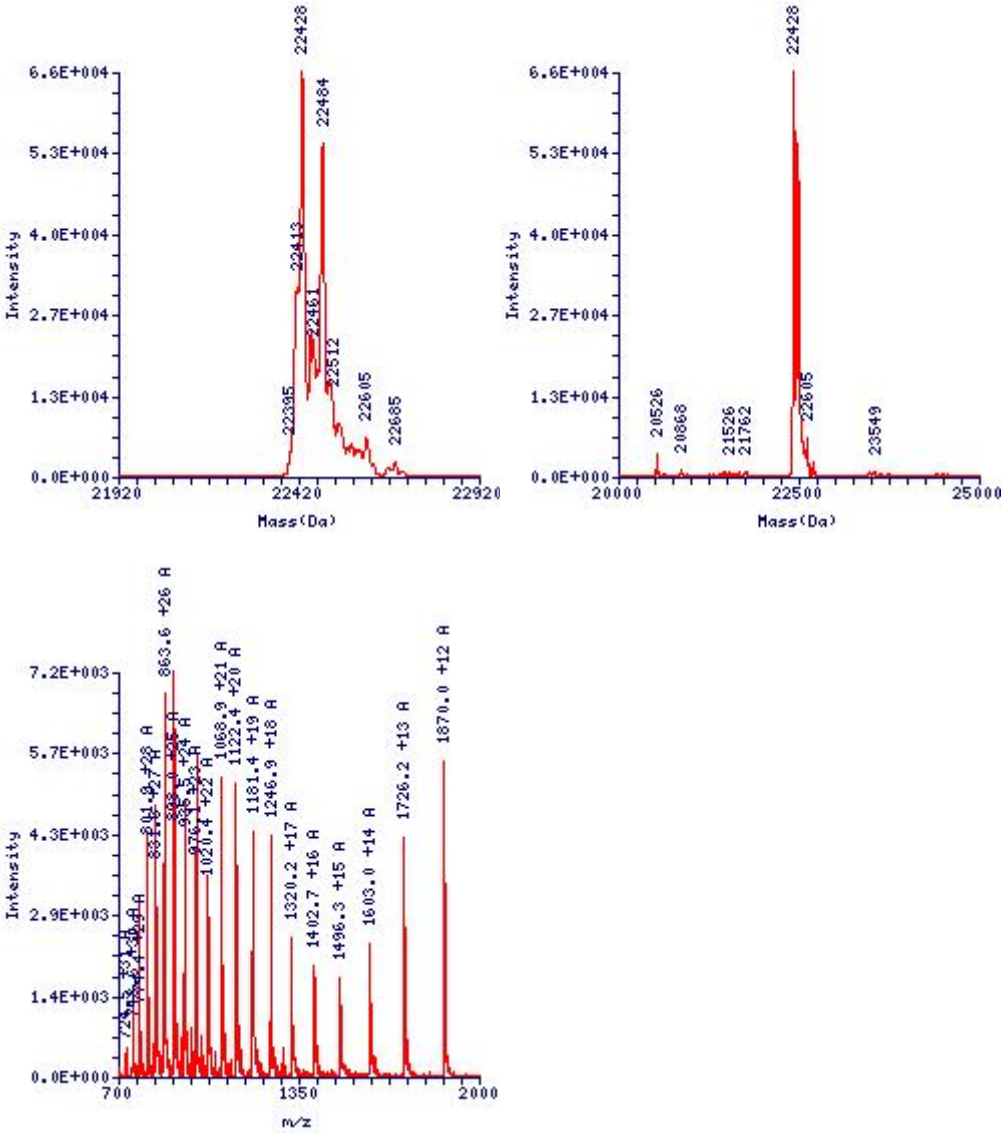
Deconvolution of MS data obtained in experiment **B** (using ProMass for Xcalibur)



MS data, experiment **C**
probe: C12 + diazirine (file: LasR-3-oxo+diaz(30-7-09))



Deconvolution of MS data obtained in experiment **C** (using ProMass for Xcalibur)



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4) S. R. Petermann, C. Doetkott, and L. Rust. *Clin. Diagn. Lab Immunol.* 2001, **8**, 632-636.

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