Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

β-Lactones as privileged structures for the active site labeling of versatile bacterial enzyme classes

Thomas Böttcher and Stephan A. Sieber

Materials

All chemicals were of reagent grade or better and used without further purification. Chemicals and solvents were purchased from Sigma Aldrich or Acros Organics. For all reactions, only commercially available solvents of purissimum grade, dried over molecular sieve and stored under Argon atmosphere were used. Solvents for chromatography and workup purposes were generally of reagent grade and purified before use by distillation. In all reactions, temperatures were measured externally. All experiments were carried out under nitrogen.

Column chromatography was performed on Merck silica gel (Acros Organics 0.035 – 0.070 mm, mesh 60 Å).

^{1}H NMR spectra were recorded on a Varian Mercury 200 (200 MHz), a Varian NMR-System 600 (600 MHz) or a Varian NMR-System 300 (300 MHz) and ^{13}C NMR spectra were measured with a Varian NMR-System 600 (600 MHz) and a Varian NMR-System 300 (300 MHz) and referenced to the residual proton and carbon signal of the deuterated solvent, respectively.

Mass spectra were obtained by GC-MS with a Varian 3400 gas chromatograph via a 25 m CS Supreme-5 capillary column (∅ 0.25 mm, layer 0.25 µm) with a gradient of 50 °C (1 min isotherm) to 300 °C (4 min isotherm), 25 °C min^{-1} coupled with a Finnigan MAT 95 mass spectrometer in EI mode (70 eV, 250 °C source). For DEI measurements, samples were directly desorbed from platinum wire (20 – 1600 °C, 120 °C min^{-1}). ESI spectra were recorded with a Thermo Finnigan LTQ FT. HPLC analysis was accomplished with a Waters 2695 separations module, a X-Bridge™ BEH130 C18 column (4.6×100 mm) and a Waters 2996 PDA detector. Mobile phase (HPLC grade): A = water, 0.1 % (v/v) TFA, B = acetonitril, 0.1 % (v/v) TFA. Gradient: T_0: A = 100%; T_{25}: A = 5%; T_{29}: A = 5%; T_{37}: A = 100%; T_{40}: A = 100%.

1. Synthesis of the β-lactone probe library

The C-3 unsubstitued β-lactone **A1** was prepared by a $Al(SbF_6)_3$ catalyzed [2 + 2] cycloaddition of a ketene generated in situ from acetyl chloride with an aldehyde according to Nelson et al., 1999.[1] However, this reaction failed in producing more complex C-3 substituted 2-oxetanones. Thus all other β-lactones of the library were synthesized according to a method developed by Danheiser and Nowick[2] (Figure 1A).

5-Hexynal (1).

A 25-mL, one-necked, round-bottomed flask was charged with a solution of Dess-Martin periodinane, DMP (848 mg, 2.0 mmol) in 10 mL of dichloromethane and then cooled in an ice bath while 5-hexyn-1-ol (217 µL, 2.0 mmol) was added dropwise over 1 min. The reaction was stirred for 5 min at 0 °C and after stirring additionally 3 h at room temperature the reaction was completed as monitored by TLC (n-pentane/diethyl ether 10:1; R_f = 0.39) Thereafter the suspension was transferred with 10 mL diethyl ether to a 50-mL falcon tube and centrifuged (4000 rpm; 10 min). The supernatant was collected by decantation and the residual pellet was washed with 20 mL diethyl ether. The combined organic solutions were concentrated under reduced pressure on a rotary evaporator (40 °C, >600 mbar). The mixture was purified by flash column chromatography on silica gel (n-pentane/diethyl ether 10:1). Evaporation of the solvent afforded 105 mg (55%) aldehyde **1** as colourless liquid.

^{1}H NMR (200 MHz, $CDCl_3$) δ 9.80 (t, *J* = 1.3 Hz, 1 H, -C<u>H</u>O), 2.60 (dt, *J* = 7.2, 1.3 Hz, 2 H, C<u>H</u>$_2$-CHO), 2.26 (dt, J = 6.8, 2.7 Hz, 2 H, HC≡C-C<u>H</u>$_2$), 1.97 (t, J = 2.6 Hz, 1 H, <u>H</u>C≡C), 1.84 (quint., J = 6.8 Hz, 2 H, C<u>H</u>$_2$-CH$_2$-CHO).

GC RT = 2.2 min, TIC-MS (m/z): 95.0485 $[M-H]^+$, Calc.: 95.0497.

General procedure for the preparation of S-Phenyl thioates from Acyl chlorides. Preparation of S-Phenyl 10-Undecenethioate (2).

A 50-mL, one-necked, round-bottomed flask was charged with 10 mL toluene, triethylamine (0.70 mL, 5.0 mmol) and thiophenol (0.51 mL, 5.0 mmol). A solution of 10-undecenoyl chloride (1.10 mL, 5.1 mmol) in 15 mL toluene was added by a pressure-equalizing dropping funnel over 30 min while stirring the solution with a magnetic stirrer.

Precipitating triethylammonium chloride made the reaction mixture turn to a turbid suspension. After 10 min of additional stirring the reaction was completed as monitored by TLC (iso-hexane/ethyl acetate 25:1, R_f = 0.43).

The organic mixture was washed twice with 25 mL saturated sodium hydrogen carbonate solution and once with 25 mL brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent evaporated in vacuo to yield 1.27 g (92%) S-phenyl 10-undecenethioate **2** as pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (app. S, 5 H, $C_6\underline{H}_5$), 5.83 (tdd, *J* = 16.9, 10.2, 6.7 Hz, 1 H, C$\underline{H}$=CH_2), 5.05-4.93 (m, 2H, CH=C$\underline{H}_2$), 2.67 (t, *J* = 7.5 Hz, 2 H, C(O)-C$\underline{H}_2$), 2.09-1.99 (m, 2 H, C$\underline{H}_2$-CH=CH_2), 1.73 (quint., *J* = 7.5 Hz, 2 H, C$\underline{H}_2$-CH_2-C(O)), 1.44-1.29 (m, 10 H, (C$\underline{H}_2$)$_5$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 197.8, 139.4, 134.7, 129.5, 129.4, 128.2, 114.4, 43.9, 34.0, 29.5, 29.4, 29.3, 29.2, 29.1, 25.8.

S-Phenyl 2-(4-Methoxyphenyl)ethanethioate (3).

2-(4-Methoxyphenyl)acetyl chloride (3.06 mL, 20.0 mmol) in 60 mL toluene was added to a solution of triethylamine (2.80 mL, 20.1 mmol) and thiophenol (2.04 mL, 20.0 mmol) in 40 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 25:1, R_f = 0.29. Workup yielded 4.99 g (97%) S-phenyl 2-(4-methoxyphenyl)ethanethioate **3** as crystalline yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (app. s, 5 H, $C_6\underline{H}_5$), 7.27 (d, *J* = 7.3 Hz, 2 H, phenylene C-$\underline{H}$), 6.91 (d, *J* = 8.7 Hz, 2 H, phenylene C-$\underline{H}$), 3.87 (s, 2 H, C(O)-C$\underline{H}_2$), 3.83 (s, 3 H, O-C$\underline{H}_3$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 196.1, 159.3, 134.7, 131.0, 129.6, 129.3, 128.1, 125.5, 114.4, 55.5, 49.5.

S-Phenyl Hexanethioate (4).

Hexanoyl chloride (2.76 mL, 19.7 mmol) in 60 mL toluene was added to a solution of triethylamine (2.80 mL, 20.1 mmol) and thiophenol (2.04 mL, 20.0 mmol) in 40 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 100:1, R_f = 0.20. Workup yielded 3.89 g (95%) S-phenyl hexanethioate **4** as pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (app. s, 5 H, $C_6\underline{H}_5$), 2.66 (t, *J* = 7.5 Hz, 2 H, C(O)-$C\underline{H}_2$), 1.72 (quint., *J* = 7.4 Hz, 2 H, C(O)-CH_2-$C\underline{H}_2$), 1.39-1.31 (m, 4 H, $(C\underline{H}_2)_2$-CH_3) 0.91 (t, *J* = 7.1, 2 H, $C\underline{H}_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 197.8, 134.7, 129.5, 129.4, 128.2, 43.9, 31.4, 25.5, 22.6, 14.1.

S-Phenyl 3,3-Dimethylbutanethioate (5).

3,3-Dimethylbutanoyl chloride (695 µL, 5.0 mmol) in 15 mL toluene was added to a solution of triethylamine (0.70 mL, 5.0 mmol) and thiophenol (0.51 mL, 5.0 mmol) in 10 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 25:1, R_f = 0.63. Workup yielded 941 mg (90%) S-phenyl 3,3-dimethylbutanethioate **5** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40 (app. s, 5 H, $C_6\underline{H}_5$), 2.55 (s, 2 H, C(O)-$C\underline{H}_2$), 1.08 (s, 9 H, $C(C\underline{H}_3)_3$) .

^{13}C NMR (75 MHz, $CDCl_3$) δ 196.1, 134.6, 129.5, 129.4, 128.7, 56.5, 32.1, 29.9.

S-Phenyl Isobutanethioate (6).

Isobutanoyl chloride (528 µL, 5.0 mmol) in 15 mL toluene was added to a solution of triethylamine (0.70 mL, 5.0 mmol) and thiophenol (0.51 mL, 5.0 mmol) in 10 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 25:1; R_f = 0.47. Workup yielded 653 mg (72%) S-phenyl isobutanethioate **6** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40 (app. s, 5 H, $C_6\underline{H}_5$), 2.87 (sept., *J* = 6.9 Hz, 1 H, $C\underline{H}(CH_3)_2$), 1.27 (d, *J* = 6.9 Hz, 6 H, $CH(C\underline{H}_3)_2$).

^{13}C NMR (600 MHz, $CDCl_3$) δ 202.1, 134.8, 129.4, 129.3, 128.1, 43.2, 19.6.

S-Phenyl Decanedithioate (7).

Sebacoyl chloride (267 µL, 1.25 mmol) in 7.5 mL toluene was added to a solution of triethylamine (350 µL, 2.5 mmol) and thiophenol (255 µL, 2.5 mmol) in 5 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 25:1, R_f = 0.19. Workup yielded 492 mg (98%) S-phenyl decanedithioate **7** as colourless solid.

^{1}H NMR (75 MHz, $CDCl_3$) δ 7.41 (app. s, 10 H, $(C_6\underline{H}_5)_2$), 2.65 (t, *J* = 7.4 Hz, 4 H, 2 x C(O)-$C\underline{H}_2$), 1.71 (quint., *J* = 7.2 Hz, 4 H, 2 x C(O)-CH_2-$C\underline{H}_2$), 1.45-1.26 (m, 8 H, $(C\underline{H}_2)_4$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 197.7, 134.7, 129.5, 129.4, 128.2, 43.9, 29.2, 29.1, 25.8.

S-Phenyl Cyclohexanecarbothioate (8).

Cyclohexanecarbonyl chloride (669 µL, 5.0 mmol) in 15 mL toluene was added to a solution of triethylamine (0.70 mL, 5.0 mmol) and thiophenol (0.51 mL, 5.0 mmol) in 10 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl acetate 25:1, R_f = 0.45. Workup yielded 1016 mg (92%) S-phenyl cyclohexanecarbothioate **8** as crystalline colourless solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (app. s, 5 H, $C_6\underline{H}_5$), 2.63 (tt, *J* = 11.4, 3.6 Hz, 1 H, $C\underline{H}$-C(O)), 2.05-1.19 (m, 10 H, $(C\underline{H}_2)_5$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 201.0, 134.8, 129.4, 129.3, 128.2, 52.7, 29.8, 25.8, 25.7.

S-Phenyl 2-(2-Naphthyl)ethanethioate (9).

2-(2-Naphthyl)acetyl chloride (308 mg, 1.5 mmol) in 4.5 mL toluene was added to a solution of triethylamine (0.21 mL, 1.5 mmol) and thiophenol (153 µL, 1.5 mmol) in 3 mL toluene. Reaction monitoring was accomplished by TLC: iso-hexane/ethyl

acetate 25:1, R_f = 0.33. Workup yielded 376 mg (90%) S-phenyl 2-(2-naphthyl)ethanethioate **9** as yellow solid.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.88 – 7.80 (m, 4 H, naphthyl C-H), 7.54-7.44 (m, 3 H, naphthyl C-H), 7.39 (app. s, 5 H, C_6H_5), 4.09 (s, 2 H, C(O)-CH_2)

^{13}C NMR (75 MHz, $CDCl_3$) δ 195.6, 134.7, 133.7, 132.9, 131.0, 129.7, 129.4, 128.9, 128.7, 128.0, 127.9, 127.8, 127.7, 126.5, 126.3, 50.6.

S-Phenyl 2-(3,4,5-Trimethoxyphenyl)ethanethioate (10).

A 10-mL, one-necked, round-bottomed flask was charged with 2.5 mL dichloromethane, (3,4,5-trimethoxyphenyl)acetic acid (566 mg, 2.5 mmol), DMAP (31 mg, 2.5 mmol) and thiophenol (1.0 mL, 9.9 mmol). The mixture was cooled in an ice bath while DCC (619 mg, 3.0 mmol) was added and stirred for 5 min. The reaction was allowed to warm up to room temperature and additionally stirred for 3 h.

After stirring, the resulting suspension was filtrated and the organic solution diluted with dichloromethane to a total volume of 30 mL. The organic mixture was washed twice with 30 mL 0.5 M HCl and once with 30 mL saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent evaporated in vacuo. The crude product was purified by column chromatography on silica gel (iso-hexane/ethyl acetate 50:11, R_f = 0.48). Evaporation of the solvent yielded 483.5 mg (61%) S-phenyl 2-(3,4,5-trimethoxyphenyl)ethanethioate **10** as white solid.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40 (app. s, 5 H, C_6H_5), 6.54 (s, 2 H, phenylene C-H), 3.87 (s, 6 H, 2 x CH_3), 3.5 (m, 5 H, CH_3, CH_2).

^{13}C NMR (151 MHz, $CDCl_3$) δ 195.5, 153.6, 137.7, 134.6, 129.7, 129.4, 129.0, 127.9, 106.9, 61.1, 56.4, 50.5.

4-(4-Pentyn-1-yl)oxetan-2-one (probe A1).

A 10-mL, one-necked, round-bottomed flask was charged with 600 µL dichloromethane, anhydrous $AlCl_3$ (10.6 mg, 0.08 mmol) and cooled to -30 °C in a water-isopropanol-dry ice bath. Then, diisopropylethylamine (41.8 µL, 0.24 mmol) and a pre-cooled solution of $AgSbF_6$ (82.4 mg, 0.24 mmol) in 600 µL

dichloromethane were added followed by diisopropylethylamine (69.8 µL, 0.40 mmol), acetyl chloride (42.6 µL, 0.60 mmol) and 5-hexynal **1** (38.4 mg, 0.40 mmol).

The reaction was complete after stirring for 2.5 h as monitored by TLC (iso-hexane/ethyl acetate 5:1; R_f = 0.23). The mixture was filtered, concentrated in vacuo and the product purified by flash column chromatography on silica gel (iso-hexane/ethyl acetate 5:1) to yield 15.6 mg (28%) 4-(4-pentyn-1-yl)oxetan-2-one **A1** as colourless oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.60-4.49 (m, 1 H, H-4), 3.54 (dd, *J* = 16.3, 5.8 Hz, 1 H, H-3), 3.10 (dd, *J* = 16.3, 4.3 Hz, 1 H, H-3), 2.28 (dt, *J* = 6.8, 2.7 Hz, 2 H, HC≡C-C$\underline{H}_2$), 1.99 (t, *J* = 2.6 Hz, 1 H, $\underline{H}$C≡C), 1.97-1.57 (m, 4 H, C$\underline{H}_2$-C$\underline{H}_2$-CH).

GC RT = 5.1 min, EI-MS (m/z): 137.0612 $[M-H]^+$, Calc.: 137.0603.

General procedure for the preparation of 2-Oxetanones from S-Phenyl thioates. Preparation of *trans*-3-(8-Nonen-1-yl)-4-(4-pentyn-1-yl)oxetan-2-one (probe D3).

A 10-mL, three-necked, round-bottomed flask equipped with a rubber septum, an nitrogen inlet adapter and a glass stopper was charged with 2 mL THF and diisopropylamine (36.5 µL, 0.26 mmol) and then cooled in an ice bath while n-butyllithium (96.0 µL of a 2.5 M solution in hexanes, 0.24 mmol) was injected via a syringe into the reaction mixture over 2 min. The mixture was stirred for 20 min by a magnetic stirrer at 0 °C.

Thereafter the ice bath was replaced with an acetone-dry ice bath and the reaction mixture was cooled to –78 °C. A solution of S-phenyl 10-undecenethioate **2** (55.3 mg, 0.20 mmol) in 250 µL THF was added dropwise via a syringe over 5 min. After stirring the mixture for 2 h at -78 °C, a solution of 5-hexynal **1** (19.2 mg, 0.20 mmol) in 250 µL THF was added dropwise over 25 min via a syringe which was cooled externally by a rubber tube filled with dry ice. The reaction mixture was stirred for 30 min and then gradually warmed up to 0 °C within 75 min. Then 1 mL half-saturated NH_4Cl solution was added.

The mixture was diluted with 30 mL diethyl ether and washed twice with 30 mL 10 % K_2CO_3 solution and once with 30 mL brine. The aqueous layers were re-extracted with 30 mL diethyl ether and the combined organic layers dried over $MgSO_4$, filtered and concentrated in vacuo. The product was purified by column chromatography on

silica gel according to TLC optimized solvent mixing ratios: iso-hexane/ethyl acetate 50:3, R_f = 0.31. 3.9 mg (7%) *trans*-3-(8-nonen-1-yl)-4-(4-pentyn-1-yl)oxetan-2-one **D3** was obtained as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 5.80 (tdd, *J* = 16.9, 10.1, 6.7 Hz, 1 H, C<u>H</u>=CH_2), 5.04-4.89 (m, 2H, CH=C<u>H_2</u>), 4.24 (dt, *J* = 6.5, 3.9 Hz, 1 H, H-4), 3.20 (ddd, *J* = 8.7, 6.8, 4.2 Hz, 1 H, H-3), 2.28 (dt, *J* = 6.9, 2.6 Hz, 2 H, HC≡C-C<u>H_2</u>), 1.99 (t, *J* = 2.6 Hz, 1 H, <u>H</u>C≡C), 2.05-1.31 (m, 18 H, (C<u>H_2</u>)$_2$ and (C<u>H_2</u>)$_7$).

GC RT = 9.0 min, DEI-MS (m/z): 262.1929 $[M]^+$, Calc.: 262.1933.

***trans*-3-(4-Methoxyphenyl)-4-(4-pentyn-1-yl)oxetan-2-one (probe E2).**

The reaction was performed with S-phenyl 2-(4-methoxyphenyl)ethanethioate **3** (51.7 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 5:1, R_f = 0.35) yielded 6.3 mg (13%) *trans*-3-(4-methoxyphenyl)-4-(4-pentyn-1-yl)oxetan-2-one **E2** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.5 Hz, 2 H, phenylene C-<u>H</u>), 6.91 (d, *J* = 8.7 Hz, 2 H, phenylene C-<u>H</u>), 4.51 (dt, *J* = 6.6, 4.3 Hz, 1 H, H-4), 4.38 (d, *J* = 4.3 Hz, 1 H, H-3), 3.81 (s, 3 H, O-C<u>H_3</u>), 2.29 (dt, *J* = 6.8, 2.7 Hz, 2 H, HC≡C-C<u>H_2</u>), 2.17-2.04 (m, 2 H, C<u>H_2</u>), 2.00 (t, J = 2.6 Hz, 1 H, <u>H</u>C≡C), 1.87-1.55 (m, 2 H, C<u>H_2</u>).

GC RT = 8.0 min, DEI-MS (m/z): 244.1090 $[M]^+$, Calc.: 244.1099.

***trans*-3-Butyl-4-(4-pentyn-1-yl)oxetan-2-one (probe G2).**

The reaction was performed with S-phenyl hexanethioate **4** (41.7 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 25:1, R_f = 0.19) yielded 4.8 mg (12%) *trans*-3-butyl-4-(4-pentyn-1-yl)oxetan-2-one **G2** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.25 (dt, *J* = 6.5, 4.0 Hz, 1 H, H-4), 3.19 (ddd, J = 8.6, 6.6, 4.0 Hz, 1 H, H-3), 2.28 (dt, *J* = 6.9, 2.6 Hz, 2 H, HC≡C-C<u>H_2</u>), 1.99 (t, *J* = 2.7 Hz, 1 H, <u>H</u>C≡C), 1.82-1.16 (m, 10H, (C<u>H_2</u>)$_3$ and (C<u>H_2</u>)$_2$) , 0.92 (t, *J* = 6.7 Hz, 3 H, C<u>H_3</u>).

GC RT = 6.7 min, TIC-MS (m/z): 193.1235 $[M-H]^+$, Calc.: 193.1229.

***cis* and *trans*-3-Dimethylethyl-4-(4-pentyn-1-yl)oxetan-2-one (probe L1 and LT1).**

The reaction was performed with S-phenyl 3,3-dimethylbutanethioate **5** (41.7 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 25:1, R_f^{cis} = 0.22, R_f^{trans} = 0.28) yielded 6.9 mg (17%) *cis*-3-dimethylethyl-4-(4-pentyn-1-yl)oxetan-2-one **L1** and 2.1 mg (5%) *trans*-3-dimethylethyl-4-(4-pentyn-1-yl)oxetan-2-one **LT1** as pale yellow oil.

For the *cis* isomer **L1**:

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.55 (dd, *J* = 13.6, 6.8 Hz, 1 H, H-4), 3.56 (d, J = 6.8 Hz, 1 H, H-3), 2.34-2.25 (m, 2 H, HC≡C-C$\underline{H}_2$), 2.13-2.02 (m, 2H, C$\underline{H}_2$), 1.98 (t, J = 2.7 Hz, 1 H, $\underline{H}$C≡C), 1.93-1.52 (m, 2H, C$\underline{H}_2$), 1.14 (s, 9 H, C(C$\underline{H}_3$)$_3$).

GC RT = 6.4 min, TIC-MS (m/z): 193.1224 $[M-H]^+$, Calc.: 193.1229.

For the *trans* isomer **LT1**:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.34 (dt, *J* = 7.8, 4.8 Hz, 1 H, H-4), 3.04 (d, *J* = 4.1 Hz, 1 H, H-3), 2.29 (m, 2 H, HC≡C-C$\underline{H}_2$), 1.99 (t, *J* = 2.6 Hz, 1 H, $\underline{H}$C≡C), 1.96-1.85 (m, 2H, C$\underline{H}_2$), 1.75-1.59 (m, 2H, C$\underline{H}_2$), 1.06 (s, 9 H, C(C$\underline{H}_3$)$_3$).

GC RT = 7.0 min, TIC-MS (m/z): 193.1205 $[M-H]^+$, Calc.: 193.1229.

^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 83.5, 74.1, 69.5, 67.6, 34.0, 30.9, 27.4, 24.4, 18.3.

***trans*-3,3-Dimethyl-4-(4-pentyn-1-yl)oxetan-2-one (probe M1).**

The reaction was performed with S-phenyl isobutanethioate **6** (36 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 50:3, R_f = 0.21) yielded 11.3 mg (34%) *trans*-3,3-dimethyl-4-(4-pentyn-1-yl)oxetan-2-one **M1** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.24 (t, *J* = 6.7 Hz, 1 H, H-4), 2.33-2.24 (m, 2 H, HC≡C-C$\underline{H}_2$), 1.98 (t, *J* = 2.7, 1 H, $\underline{H}$C≡C), 1.90-1.49 (m, 4 H, (C$\underline{H}_2$)$_2$), 1.42 (s, 3 H, C$\underline{H}_3$), 1.27 (s, 3 H, C$\underline{H}_3$).

GC RT = 5.4 min, TIC-MS (m/z): 166.0977 $[M-H]^+$, Calc.: 166.0994.

***trans*-S-Phenyl 8-(2-Oxo-4-(4-pentyn-1-yl)oxetan-3-yl)octanethioate (probe N1).**

The reaction was performed with S-phenyl decanedithioate **7** (38.7 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 25:3, R_f = 0.30) yielded 4.0 mg (5%) *trans*-S-phenyl 8-(2-Oxo-4-(4-pentyn-1-yl)oxetan-3-yl)octanethioate **N1** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.41 (app. s, 5 H, $C_6\underline{H}_5$), 4.25 (dt, *J* = 6.5, 4.0 Hz, 1 H, H-4), 3.20 (ddd, *J* = 8.5, 6.8, 3.9 Hz, 1 H, H-3), 2.65 (t, *J* = 7.4 Hz, 2 H, C(O)-C$\underline{H}_2$), 2.28 (dt, *J* = 6.9, 2.6 Hz, 2 H, HC≡C-C$\underline{H}_2$), 1.99 (t, *J* = 2.7 Hz, 1 H, $\underline{H}$C≡C), 1.95-1.28 (m, 16 H, (C$\underline{H}_2$)$_2$ and (C$\underline{H}_2$)$_6$).

HPLC RT (254 nm) = 27.05 min, ESI-MS (m/z): 373.1826 $[M+H]^+$, Calc.: 373.1837.

4-(4-Pentyn-1-yl)oxetan-2-one-3-spirocyclohexane (probe O1).

The reaction was performed with S-phenyl cyclohexanecarbothioate **8** (44.1 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 20:1, R_f = 0.28) yielded 10.1 mg (24%) 4-(4-pentyn-1-yl)oxetan-2-one-3-spirocyclohexane **O1** as pale yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.16 (dd, *J* = 9.8, 3.7 Hz, 1 H, H-4), 2.34-2.23 (m, 2H, HC≡C-C$\underline{H}_2$), 1.98 (t, J = 2.6 Hz, 1 H, $\underline{H}$C≡C), 1.94-1.33 (m, 11 H, C$\underline{H}$ and (C$\underline{H}_2$)$_5$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 174.9, 83.6, 82.9, 69.4, 58.3, 33.3, 29.1, 26.7, 25.5, 24.7, 23.0, 22.8, 18.3.

GC RT = 7.5 min, TIC-MS (m/z): 205.1222 $[M-H]^+$, Calc.: 205.1229.

***trans*-3-(2-Naphthyl)-4-(4-pentyn-1-yl)oxetan-2-one (probe P1).**

The reaction was performed with S-phenyl 2-(2-naphthyl)ethanethioate **9** (111.4 mg, 0.40 mmol). All reagents and solvents were scaled up accordingly. Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 100:11, R_f = 0.34) yielded 6.8 mg (6%) *trans*-3-(2-naphthyl)-4-(4-pentyn-1-yl)oxetan-2-one **P1** as pale yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.5 Hz, 1 H, naphthyl C-$\underline{H}$), 7.85-7.82 (m, 2 H, naphthyl C-$\underline{H}$), 7.76 (s, 1 H, naphthyl C-$\underline{H}$), 7.53-7.49 (m, 2 H, naphthyl C-$\underline{H}$) 7.36 (dd, *J* = 8.4, 1.8 Hz, 1 H, naphthyl C-$\underline{H}$), 4.66 (dt, *J* = 6.6, 4.3, 1 H, H-4), 4.61 (d, *J* =

4.3 Hz, 1 H, H-3), 2.32 (dt, *J* = 7.0, 2.6 Hz, 2 H, HC≡C-C$\underline{H}_2$), 2.19-2.15 (m, 2 H, C$\underline{H}_2$), 2.01 (t, *J* = 2.6 Hz, 1 H, $\underline{H}$C≡C), 1.84-1.69 (m, 2 H, C$\underline{H}_2$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 169.2, 133.6, 133.1, 130.0, 129.5, 128.1, 128.0, 127.0, 126.8, 126.7, 124.8, 83.3, 79.5, 69.8, 61.8, 33.7, 24.2, 18.3.

HPLC RT (254 nm) = 25.5 min, DEI-MS (m/z): 264.1160 $[M]^+$, Calc.: 264.1150.

***trans*-4-(4-Pentyn-1-yl)-3-(3,4,5-trimethoxyphenyl)oxetan-2-one (probe Q1).**

The reaction was performed with S-phenyl 2-(3,4,5-trimethoxyphenyl)ethanethioate **10** (63.7 mg, 0.20 mmol). Standard workup and purification by flash column chromatography (iso-hexane/ethyl acetate 5:2, R_f = 0.34) yielded 5.2 mg (9%) *trans*-4-(4-pentyn-1-yl)-3-(3,4,5-trimethoxyphenyl)oxetan-2-one **Q1** as pale yellow oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 6.46 (s, 2 H, phenylene C-$\underline{H}$), 4.55 (dt, *J* = 6.6, 4.3, 1 H, H-4), 4.36 (d, *J* = 4.3 Hz, 1 H, H-3), 3.86 (s, 6 H, 2 x C$\underline{H}_3$), 3.83 (s, 3 H, C$\underline{H}_3$), 2.31 (dt, *J* = 6.8, 2.7 Hz, 2 H, HC≡C-C$\underline{H}_2$), 2.17-2.06 (m, 2 H, C$\underline{H}_2$), 2.01 (t, *J* = 2.6 Hz, 1 H, $\underline{H}$C≡C), 1.86-1.61 (m, 2 H, C$\underline{H}_2$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 169.2, 154.1, 138.3, 128.2, 104.6, 83.3, 79.4, 69.8, 61.8, 61.1, 56.5, 33.6, 24.2, 18.3.

GC RT = 10.2 min, DEI-MS (m/z): 304.1295 $[M]^+$, Calc.: 304.1311.

HPLC RT (254 nm) = 20.2 min.

2) Preparation of proteomes

Proteomes of the bacterial strains *Escherichia coli* K12, *Bacillus licheniformis* ATCC 14580, *Bacillus subtilis* 168, *Listeria welshimeri* SLCC 5334 serovar 6b and *Pseudomonas putida* KT2440 were prepared from 1 L liquid cultures harvested 1 h after transition in the stationary phase by centrifugation at 13.000 rpm. All strains were grown in LB (Luria-Bertani broth) medium except *Listeria welshimeri*, which was maintained in BHB (brain-heart broth) medium. The bacterial cell pellets were washed with PBS, resuspended in 20 mL PBS and lysed by French press. As eukaryotic reference, the liver of a C3H mouse was homogenized in 2 mL PBS followed by sonication with a Bandelin Sonopuls with 4 x 15 sec. pulsed at 70% max. power.

3) Labeling of bacterial proteomes

Proteome samples were adjusted to a final concentration of 1 mg protein/mL by dilution in PBS prior to probe labeling. Experiments for visualization by 1D SDS-PAGE were carried out in 43 µL total volume and those for affinity enrichment in 1892 µL total volume, such that once CC reagents were added, the total reaction volume was 50 µL and 2 mL, respectively. Reactions were initiated by addition of the probe and allowed to incubate for 60 min at room temperature. For heat controls the proteome was denatured with 1 µL of 43% SDS at 95 °C for 6 min and cooled to room temperature before the probe was applied. Following incubation, reporter tagged-azide reagents (13 µM rhodamine-azide for analytical or 20 µM rhodamine-biotin-azide for preparative scale) were added followed by 1 mM TCEP and 100 µM ligand. Samples were gently vortexed and the cycloaddition initiated by the addition of 1 mM $CuSO_4$. The reactions were incubated at room temperature for 1 h.[3]

For analytical gel electrophoresis, 50 µL 2× SDS loading buffer were added and 50 µL applied on the gel. Fluorescence was recorded in a Fujifilm Las-3000 Fluoreszenz Darkbox with a Fujinon VRF 43LMD Lens, 605DF40 filter and 520 nm EPI excitation wavelength.

Reactions for enrichment were carried out together with a control lacking the probe to compare the results of the biotin-avidin enriched samples with the background of unspecific protein binding on avidin-agarose beads. After CC proteins were precipitated using an equal volume of pre-chilled acetone. Samples were stored on ice for 20 min and centrifuged at 13,000 rpm for 10 min. The supernatant was discarded and the pellet washed two times with 400 µL of pre-chilled methanol and resuspended by sonication. Subsequently, the pellet was dissolved in 1 mL PBS with 0.2% SDS by sonication and incubated under gentle mixing with 50 µL of avidin-agarose beads (Sigma-Aldrich) for 1 h at room temperature. The beads were washed three times with 1 mL of PBS/0.2% SDS, twice with 1 mL of 6 M urea and three times with 1 mL PBS. 50 µL of 2× SDS loading buffer were added and the proteins released for preparative SDS-PAGE by 6 min incubation at 95 °C. Gel bands were isolated, washed and tryptically digested as described previously.[4]

For analytical and preparative *in vivo* studies, bacteria were grown to stationary phase, pelleted by centrifugation (2 ml for analytical and 10 ml for preparative studies), resuspended with PBS and incubated for 2 h with varying concentrations of

probe at RT. Subsequently, the cells were lysed by sonication and separated into cytosolic and membrane fractions, followed by CC as described above.

4) Competitive labeling with PMSF and cerulenin

In competitive assays, a 100fold excess of PMSF or cerulenin was added to the proteome 15 min prior to lactone addition.

5) IC_{50} Determination

SFGH mediated proteolysis of the p-nitrophenylacetate leads to the release of p-nitrophenol which can be monitored at 400 nm. Various concentrations of lactone **G2** were added to a solution containing the substrate (2.4 mM) and enzyme (30 nM). Reactions were started by addition of the enzyme and subsequently monitored for 6 min. The mean average slopes of absorption (out of three independent experiments) versus lactone concentration were plotted and the concentration of 50% inhibition (IC_{50}) was estimated.

6) Mass spectrometry and bioinformatics

Tryptic peptides were loaded onto a Dionex C18 Nano Trap Column (100 µm) and subsequently eluted and separated by a Dionex C18 PepMap 100 (3 µm) column for analysis by tandem MS followed by high resolution MS using a coupled Dionex Ultimate 3000 LC-ThermoFinnegan LTQ-FT MS system.

The mass spectrometry data were searched using the SEQUEST algorithm against the corresponding databases via the software "bioworks". The search was limited to only tryptic peptides, two missed cleavage sites, monoisotopic precursor ions and a peptide tolerance of <10 ppm. Filters were set to further refine the search results. The Xcorr vs. charge state filter was set to Xcorr values of 1.5, 2.0 and 2.5 for charge states +1, +2 and +3, respectively. The number of different peptides has to be ≥ 2 and the peptide probability filter was set to < 0.001. These filter values are similar to others previously reported for SEQUEST analysis.[5] Maximum P-values and Xcorr values of each run as well as the total number of obtained peptides are reported in Table S1.

Table S1. Proteins identified by mass spectrometry

Species	Protein	Protein ID	MW	R	max. p Value	max. Xcorr	NP
Bacillus subtilis cytosol	Surfactin synthetase subunit 3 (SrfAC)	NP_388233	143727.1	1	$2.22 \cdot 10^{-15}$	5.31	20
				2	$1.00 \cdot 10^{-30}$	5.83	32
	Putative Esterase YjcH (SFGH)	CAB13043	27899.2	1	$3.33 \cdot 10^{-15}$	5.14	10
	Hypothetical protein yocD (Pep66)	CAB13809	36387.3	1	$7.54 \cdot 10^{-13}$	4.19	3
	Beta-ketoacyl-(acyl-carrier-protein) synthase II (KAS II)	NP_389016	43977.0	1	$1.00 \cdot 10^{-30}$	6.06	13
				2	$1.87 \cdot 10^{-11}$	5.27	9
	CTP Synthase (CTPS)	CAB15743	59680.7	1	$2.00 \cdot 10^{-15}$	4.43	16
				2	$1.00 \cdot 10^{-30}$	5.16	27
				3	$5.60 \cdot 10^{-14}$	4.04	15
	Paranitrobenzyl esterase (PNBE)	NP_391319	53952.3	1	$8.55 \cdot 10^{-15}$	5.19	5

				2	$2.30 \cdot 10^{-12}$	4.87	3
	Penicillin-binding protein 4* (PBP4*)	NP_391324	51436.8	1	$1.11 \cdot 10^{-14}$	6.16	12
				2	$7.91 \cdot 10^{-9}$	2.89	3
Bacillus licheni-formis cytosol	Beta-ketoacyl-(acyl-carrier-protein) synthase III (KAS III)	YP_078421	33855.1	1	$1.00 \cdot 10^{-30}$	5.52	4
				2	$3.33 \cdot 10^{-16}$	5.36	19
	Beta-ketoacyl-(acyl-carrier-protein) synthase II (KAS II)	YP_078422	43729.0	1	$6.66 \cdot 10^{-16}$	5.57	5
				2	$1.33 \cdot 10^{-15}$	5.36	15
	CTP Synthase (CTPS)	YP_093470	59770.8	1	$1.00 \cdot 10^{-30}$	5.14	11
				2	$2.78 \cdot 10^{-15}$	4.94	21
	Putative formate C-acetyltransferase (FCA)	YP_079294	83378.1	1	$2.32 \cdot 10^{-05}$	3.05	2
				2	$1.77 \cdot 10^{-13}$	6.63	12
	Putative esterase YitV (PutE)	YP_078404	29759.6	1	$4.00 \cdot 10^{-14}$	4.95	6
Escherichia coli cytosol	Aldehyde dehydrogenase B (ADB)	AAC76612	56270.5	1	$1.00 \cdot 10^{-30}$	5.99	18
				2	$3.33 \cdot 10^{-16}$	5.44	16
	Beta-ketoacyl-(acyl-carrier-protein) synthase II (KAS II)	NP_415613	43045.7	1	$1.00 \cdot 10^{-30}$	6.67	14
	Beta-ketoacyl-(acyl-carrier-protein) synthase I (KAS I)	NP_416826	42586.1	1	$1.11 \cdot 10^{-15}$	6.70	15
	Predicted esterase (SFGH)	AAC73458	31404.3	1	$3.28 \cdot 10^{-13}$	4.63	5
				2	$6.66 \cdot 10^{-14}$	4.85	2
	CTP Synthase (CTPS)	AAC75822	60374.1	1	$1.47 \cdot 10^{-10}$	4.72	4
				2	$5.55 \cdot 10^{-15}$	5.19	10

Listeria welshimeri cytosol	CTP Synthase, PyrG (CTPS)	YP_850706	59561.9	1	$4.61 \cdot 10^{-10}$	4.62	4
				2	$3.75 \cdot 10^{-5}$	2.65	2
	Beta-ketoacyl-(acyl-carrier-protein) synthase II (KAS II)	YP_850415	44189.0	1	$5.74 \cdot 10^{-13}$	6.34	8
				2	$6.84 \cdot 10^{-6}$	3.37	2
				3	$4.29 \cdot 10^{-8}$	4.05	6
	Lipase (Lip)	YP_850307	38500.3	1	$4.99 \cdot 10^{-10}$	4.42	3
	Thymidylate synthase, ThyA (ThyS)	YP_850091	36104.1	1	$6.03 \cdot 10^{-7}$	2.24	2
				2	$1.63 \cdot 10^{-9}$	3.48	2
	Tributyrin esterase, EstA (SFGH)	YP_850577	28894.1	1	$2.76 \cdot 10^{-6}$	2.48	2
Listeria welshimeri membrane	Beta-ketoacyl-(acyl-carrier-protein) synthase II (KAS II)	YP_850415	44189.0	1	$8.72 \cdot 10^{-10}$	3.63	2
	Lipase (Lip)	YP_850307	38500.3	1	$5.33 \cdot 10^{-15}$	5.12	12
				2	$4.44 \cdot 10^{-16}$	4.92	3
	Lipase/acylhydrolase putative (LipAc)	YP_850078	29611.4	1	$1.48 \cdot 10^{-11}$	4.37	10
				2	$1.19 \cdot 10^{-9}$	3.63	3
	ATP-dependent Clp protease proteolytic subunit (ClpP)	YP_850614	21591.0	1	$1.28 \cdot 10^{-8}$	3.91	4
				2	$2.28 \cdot 10^{-6}$	3.08	2

Mus musculus cytosol	Abhydrolase domain containing protein 14B (ABH)	NP_083907	22436.6	1 2 3	$1.38 \cdot 10^{-10}$ $1.11 \cdot 10^{-16}$ $1.11 \cdot 10^{-15}$	4.77 5.03 5.01	2 6 5
	Carboxylesterase 6 (CEI)	NP_598721	61900.3	1 2	$2.65 \cdot 10^{-13}$ $6.08 \cdot 10^{-13}$	5.02 4.16	7 3
	Carboxylesterase 5 (CEI)	NP_766347	62277.4	1 2	$2.21 \cdot 10^{-10}$ $5.19 \cdot 10^{-08}$	5.37 4.04	10 2
	Carboxylesterase 31 isoforms (CEI)	-	-	1 2	$6.82 \cdot 10^{-11}$ $5.79 \cdot 10^{-10}$	5.04 3.44	8 3
	Lysophospholipase-like protein 1 (LPL)	AAH52848	24671.4	1 2 3	$5.82 \cdot 10^{-7}$ $6.85 \cdot 10^{-8}$ $5.06 \cdot 10^{-5}$	3.23 3.53 3.05	2 2 2
	Esterase D/formylglutathione hydrolase (SFGH)	NP_058599	31299.3	1 2 3	$1.31 \cdot 10^{-12}$ $4.57 \cdot 10^{-11}$ $7.97 \cdot 10^{-13}$	4.86 5.76 5.07	5 5 4

P. putida cytosol	ATP-dependent Clp protease proteolytic subunit (ClpP)	NP_744449	23499.9	1	$5.55 \cdot 10^{-15}$	5.57	8
				2	$2.80 \cdot 10^{-13}$	5.39	5
				3	$1.13 \cdot 10^{-11}$	4.15	1
	Proline iminopeptidase (PIP)	NP_747129	36566.5	1	$7.44 \cdot 10^{-14}$	5.68	11
				2	$7.87 \cdot 10^{-10}$	3.75	3
	Acetyl-CoA hydrolase (ACoAH)	NP_742324	53923.8	1	$1.00 \cdot 10^{-30}$	4.80	7
				2	$6.51 \cdot 10^{-13}$	3.96	4
				3	$9.29 \cdot 10^{-12}$	3.99	6
	ATP-dependent protease, putative (PADP)	NP_742841	89621.9	1	$2.89 \cdot 10^{-14}$	5.00	24
	Beta-ketothiolase (BKT)	NP_745884	40962.4	1	$5.33 \cdot 10^{-07}$	2.80	2
				2	$4.79 \cdot 10^{-10}$	3.43	7
	Beta-ketoacyl-(acyl-carrier-protein) synthase I (KAS I)	NP_746292	43236.7	1	$1.00 \cdot 10^{-30}$	6.03	10
				2	$1.67 \cdot 10^{-14}$	5.33	6
				3	$3.33 \cdot 10^{-15}$	5.94	5
	Dienelactone hydrolase (DLH)	NP_746295	26252.3	1	$6.24 \cdot 10^{-12}$	2.71	2
				2	$3.31 \cdot 10^{-10}$	4.30	6
				3	$2.55 \cdot 10^{-9}$	4.07	2

This list of proteins shows Protein ID, molecular weight (MW) of the protein, the replicates (R) in which the proteins have been identified, the maximum p values, maximum Xcorr and the number of peptides (NP) found in each replicate.

7) Recombinant expression

The major hits of MS analysis were recombinantly expressed in *E. coli* as an internal control of the MS results by using the Invitrogen™ Gateway® Technology. Target genes were amplified from the corresponding genomes by PCR with an AccuPrime™

Pfx DNA Polymerase kit with 65 ng of genomic DNA, prepared by standard protocols. *att*B1 forward primer and *att*B2 reverse primer were designed to yield *att*B-PCR Products needed for Gateway® Technology:

Para-nitrobenzyl esterase *B. subtilis* 168

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GAC TCA TCA AAT AGT AAC G

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA TTC TCC TTT TGA AGG GAA

Penicillin-binding protein 4* *B. subtilis* 168

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GAA GCA GAA TAA AAG AAA GCA T

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG CTA CTT CGT ACG GAC CG

Aldehyde dehydrogenase B *E. coli* K12

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GAC CAA TAA TCC CCC TTC AGC

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCA GAA CAG CCC CAA CGG TTT AT

ATP-dependent Clp protease, proteolytic subunit, ClpP *P. putida* KT2440

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GTC CCG CAA TTC TTA TAT TCA GC

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCA GGA GGC CAG TTG CCG

CTP Synthase, *B. subtilis* 168

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GAC GAA ATA TAT TTT TGT AAC

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA CTT CTG ATT TGC AGC T

FabF beta ketoacyl carrier synthase II, *L. welshimeri*, serovar 6b - SLCC5334

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GGA TAA AAA AAG AGT AGT TG

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA GTC TTC TAT TCT TTT AAA TAC

Lipase *L. welshimeri* serovar 6b - SLCC5334

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GAA AAA TAC AAT AAA ATG G

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA CTT TTC TTC TAA GAA C

Proline iminopeptidase, *P. putida* KT2440

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GCA GAC CCT CTA CCC G

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCA TGC TTC TTC CAG AGG CAA

S-formylglutathione hydrolase, *E. coli* K12

forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CAT GGA ACT CAT TGA AAA ACA T

reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCA ACG CAT ATT CAG TTT ATT

PCR products were identified on agarose gels and gel bands were isolated and extracted with an E.Z.N.A.™ MicroElute™ Gel Extraction Kit. Concentrations of DNA were measured by a NanoDrop Spectrophotometer ND-1000. 100 fmol of purified *att*B-PCR product and 50 fmol of *att*P-containing donor vector pDONR™201 in TE buffer were used for *in vitro* BP recombination reaction with BP Clonase™ II enzyme mix to yield the appropriate *att*L-containing entry clone. After transformation in chemically competent One Shot® TOP10 *E. coli* (Invitrogen), cells were plated on LB agar plates containing 25 µg mL^{-1} kanamycin. Clones of transformed cells were selected and grown in kanamycin LB medium. Cells were harvested and plasmids were isolated using an E.Z.N.A.™ Plasmid Mini Kit. The corresponding *att*B-containing expression clone was generated by *in vitro* LR recombination reaction of approx. 50 fmol of the *att*L-containing entry clone and 50 fmol of the *att*R-containing destination vector pDest using LR Clonase™ II enzyme mix in TE buffer. The expression clone was transformed in chemically competent BL21 *E. coli* cells (Novagen) and selected on LB agar plates containing 100 µg mL^{-1} carbenicillin. Validity of the clones was confirmed by plasmid sequence analysis. Recombinant clones were grown in carbenicillin LB medium and target gene expression was induced with anhydrotetracyclin.

Labeled proteomes

Cytosol and membrane fractions of the proteome were labeled separately. However, except for *L. welshimeri* membrane fractions showed less intensive and specific labeling pattern compared to the cytosol.

Examples for heat denatured proteome controls are given in Figure S4. The majority of the labeled part of the proteome shows to be heat sensitive and thus can be regarded as results of specific activity based binding events. Labeling profiles for *cis* and *trans*-β-lactones and fluorescent gels of recombinantly expressed target proteins are shown in Figure S2 and S4, respectively.

The 1D SDS-PAGE gels for all proteoms and identified proteins assigned to the corresponding gel bands of *E. coli* (A), *P. putida* (B), *L. welshimeri* (C), *B. licheniformis* (D), *B. subtilis* (E) and mouse (F) are shown in Figure S3.

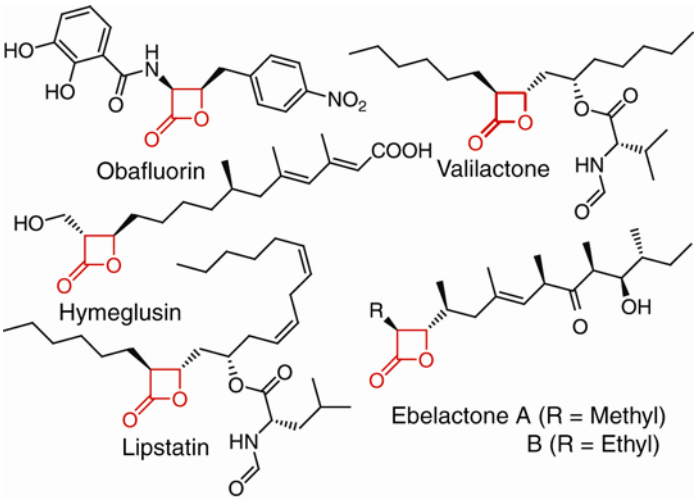


Figure S1: Structures of biologically active, naturally occurring β-lactones.

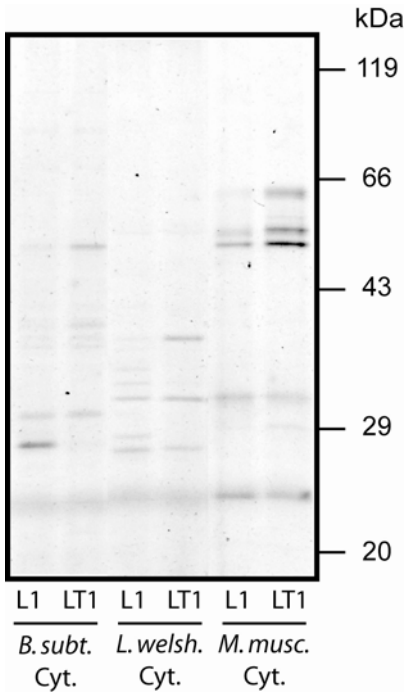
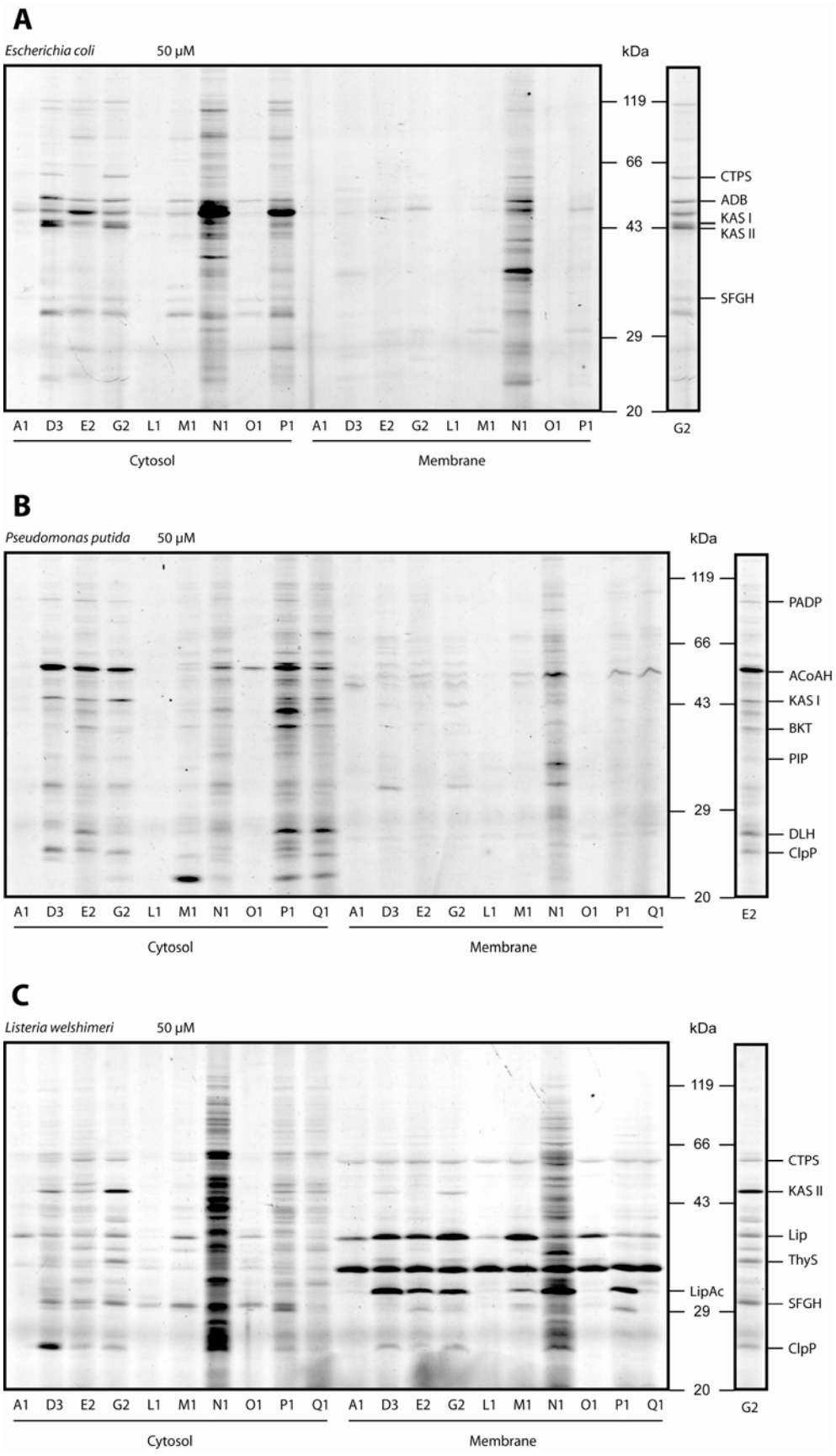


Figure S2: Comparison of the *cis* and *trans*-β-lactones L1 and LT1, respectively, reveals similar labeling profiles.


A
Escherichia coli
50 µM
kDa
119
66
43
29
20
CTPS
ADB
KAS I
KAS II
SFGH
A1 D3 E2 G2 L1 M1 N1 O1 P1 A1 D3 E2 G2 L1 M1 N1 O1 P1
G2
Cytosol
Membrane
B
Pseudomonas putida
50 µM
kDa
119
66
43
29
20
PADP
ACoAH
KAS I
BKT
PIP
DLH
ClpP
A1 D3 E2 G2 L1 M1 N1 O1 P1 Q1 A1 D3 E2 G2 L1 M1 N1 O1 P1 Q1
E2
Cytosol
Membrane
C
Listeria welshimeri
50 µM
kDa
119
66
43
LipAc
29
20
CTPS
KAS II
Lip
ThyS
SFGH
ClpP
A1 D3 E2 G2 L1 M1 N1 O1 P1 Q1 A1 D3 E2 G2 L1 M1 N1 O1 P1 Q1
G2
Cytosol
Membrane

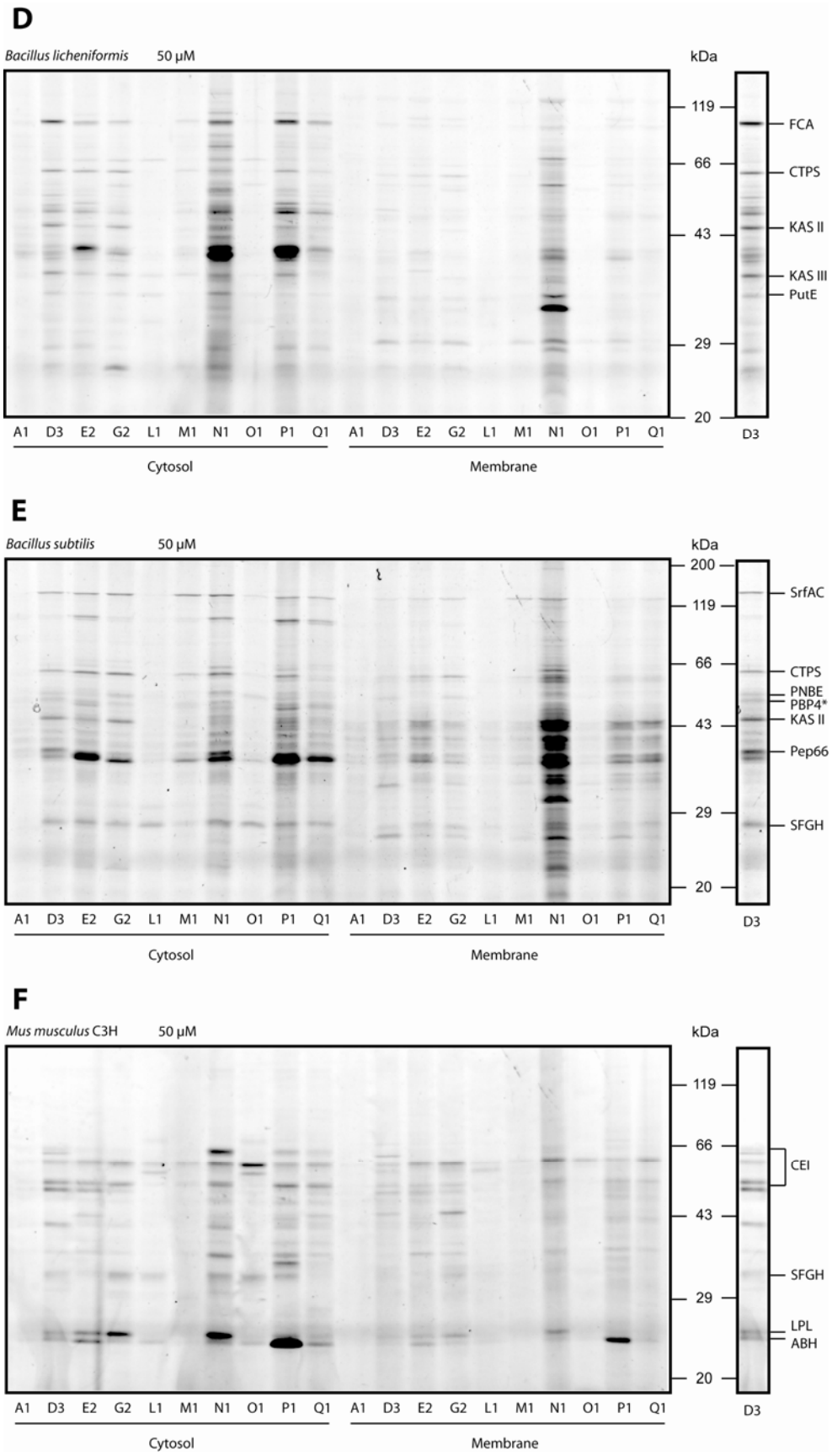


Figure S3: Labeling profiles of the proteomes in cytosol and membrane with the β-lactone library.

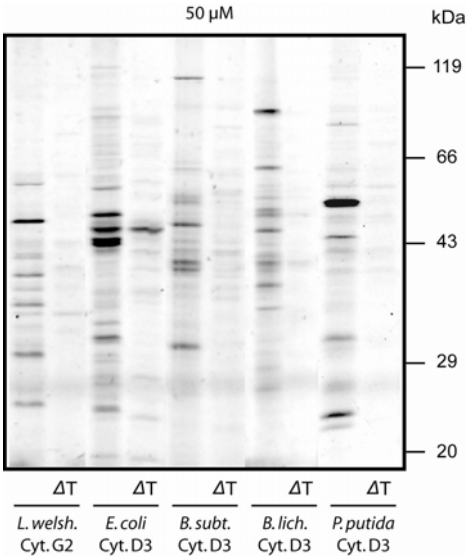


Figure S4: Representative examples of native proteomes with their corresponding heat denatured controls. The majority of the labeled part of the proteome shows to be heat sensitive and thus can be regarded as result of affinity binding events.

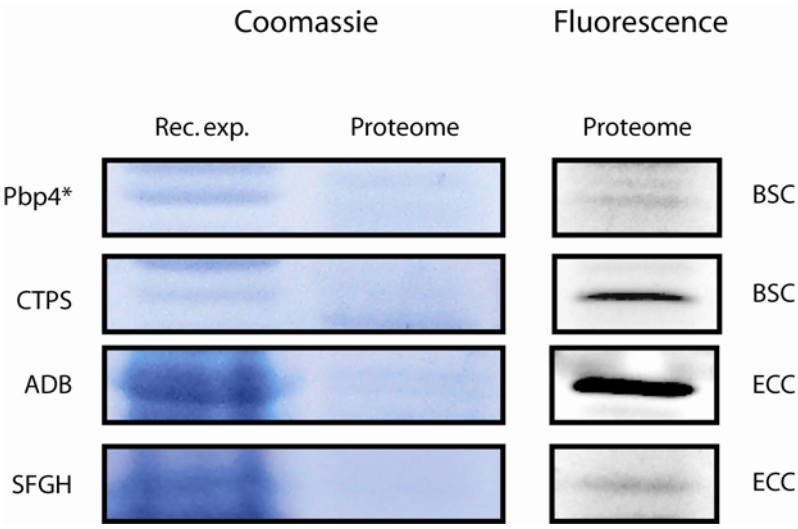


Figure S5: Comparison between relative protein intensities. Left side: visualized with coomassie staining. Right side: enzyme labeling visualized by fluorescence scanning. Most labeled proteins stained by coomassie are of very low abundance in the proteome, so that corresponding overexpressed enzymes were run next to them in order to identify the correct region of the gel.

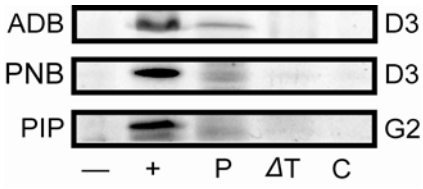


Figure S6: Recombinantly expressed enzymes in addition to Figure 2 (-: before induction, +: after induction, P: native proteome, ΔT: after induction/heat control, C: after induction/no probe).

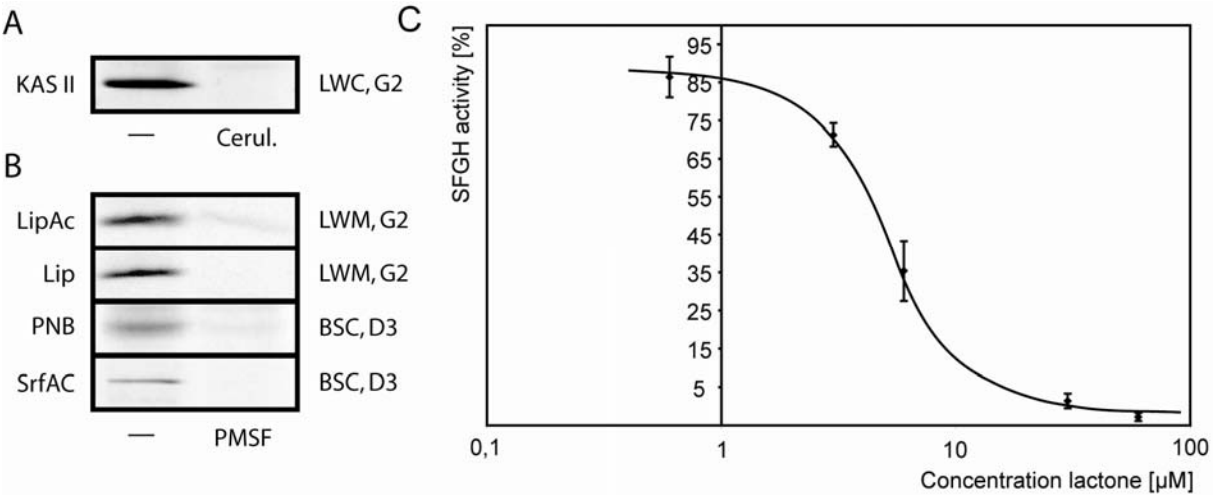


Figure S7: Active site competition assays and IC_{50}. A) Competition experiment with KAS II with and without 100 fold excess of cerulenin in presence of the lactone probe. B) Competition experiments with several serine proteases with and without 100 fold excess of PMSF in presence of the corresponding lactone probes (LWC: *L. welshimeri* cytosol, LWM: *L. welshimeri* membrane, BSC: *b. subtilis* cytosol). C) IC_{50} of 5 μM for SFGH.

Supplementary References

[1] z. W. S. G. Nelson, T. J. Peleen, K. L. Spencer, *Tetrahedron Letters* **1999**, 6535.
[2] R. L. Danheiser, Nowick, J. S., *J. Org. Chem.* **1991**, *56*, 1176.
[3] A. E. Speers, G. C. Adam, B. F. Cravatt, *J. Amer. Chem. Soc.* **2003**, *125*, 4686.
[4] S. A. Sieber, S. Niessen, H. S. Hoover, B. F. Cravatt, *Nat. Chem. Biol.* **2006**, *2*, 274.
[5] S. P. Mirza, B. D. Halligan, A. S. Greene, M. Olivier, *Physiol. Genomics* **2007**, *30*, 89.