Supplementary Material (ESI) for Chemical Communications

The biological targets of acivicin inspired 3-chloro- and 3-bromodihydroisoxazole scaffolds

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Supplementary Information

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.

Column chromatography was performed on Merck silica gel (Acros Organics 0.035 – 0.070 mm, mesh 60 Å). For TLC analysis, Merck TLC Silica gel 60 F_{254} aluminum sheets were used.

^{1}H NMR spectra were recorded on a Varian NMR-System 600 (600 MHz) or a Burker AV-500 (500 MHz). Two-dimensional spectra were obtained with a a Burker AV-500 (500 MHz). and ^{13}C NMR spectra were measured with a Varian NMR-System 600 (600 MHz), a Varian NMR-System 300 (300 MHz) or a Burker AV-360 (360 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}).

HPLC analysis was accomplished with a Waters 2695 separations module, for analytical runs with an X-Bridge BEH130 C18 column (4.6 x 100 mm) or an X-Bridge BEH130 PREP C18 column (10 x 150 mm) for preparative applications, and a Waters 2996 PDA detector.

Syntheses

The acivicin inspired probe library was prepared according to a procedure described by Halling et al. from dihaloformaldoximes and unsaturated alcohols.[1]

Glyoxylic acid aldoxime (11)

48.7 mL of an aqueous solution of 50% (w/w) glyoxylic acid (32.4 g, 437 mmol) was added stepwise to 25.3 mL of a stirred aqueous solution of 50% (w/w) NH_2OH (13.6 g, 413 mmol). The reaction mixture was stirred for 16 h at room temperature. The reaction mixture was extracted with 3 x 50 mL diethyl ether and the combined organic layers were dried over $MgSO_4$, filtrated and the solvent evaporated. Glyoxilic acid aldoxime (26.1 g, 71%) was obtained as a colorless solid.

^{1}H NMR (400 MHz, acetone-d_6) δ 7.48 (s, 1 H, C<u>H</u>N).

^{13}C NMR (150 MHz, acetone-d_6) δ 164.5 (<u>C</u>OOH), 143.0 (<u>C</u>HN).

Dibromoformaldoxime (12)

Glyoxilic acid aldoxime **11** (26.1 g, 293 mmol) was dissolved in 520 mL water and cooled to 4°C in an ice bath. Then, Br_2 (20.8 mL, 406 mmol) was added under continuous stirring in small portions over 50 min to prevent an uncontrolled reaction. After addition of Br_2 the ice bath was removed and the reaction mixture was stirred for 3 h. After extraction with 3 x 150 mL diethyl ether, the combined organic layers were washed with 100 mL saturated sodium thiosulphate solution, dried over $MgSO_4$, filtrated and the solvent removed. Dibromoformaldoxime (45.0 g, 76%) was obtained as yellow solid. The product was directly consumed in the following reactions without further purification.

Dichloroformaldoxime (13)

Glyoxilic acid aldoxime **11** (5.80 g, 65.1 mmol) was dissolved in 63 mL 1,2-dimethoxyethane and heated to 55°C. Then, *N*-chlorosuccinimide (16.9 g, 127 mmol) was added stepwise and the reaction mixture was heated to 110°C for 10 min while stirring vigorously. Vehement gas evolution was observed during heating. The reaction mixture was cooled to room temperature. The solid parts of the suspension were filtered off and the yellow filtrate was immediately used in the following reactions without further concentration or purification assuming complete conversion.

General procedure for the synthesis of 3-bromo-2-isoxazolines.

Preparation of 3-bromo-5-(hydroxymethyl)-2-isoxazoline (14)

Dibromoformaldoxime **12** (1.0 g, 4.9 mmol) was added to a solution of allyl alcohol (1.25 mL, 18.4 mmol) in 46.4 mL ethyl acetate. After addition of anhydrous, freshly triturated K_2CO_3 (4.0 g, 29 mmol), the reaction mixture was stirred for 48 h at room temperature. Then, the reaction mixture was diluted with 130 mL H_2O and extracted with 3 x 130 mL diethyl ether. The combined organic layers were dried over $MgSO_4$, filtrated and the solvent evaporated. The product (652 mg, 73 %) was obtained by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.24.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.80 (dddd, *J* = 3.1, 4.1, 8.2, 10.9 Hz, 1 H, C<u>H</u>-CH_2O), 3.88 (dd, *J* = 3.1, 12.5 Hz, 1 H, C(<u>H</u>)HOH), 3.65 (dd, *J* = 4.1, 12.5 Hz, 1 H, C(H)<u>H</u>OH), 3.26 (dd, *J* = 10.8, 17.2 Hz, 1 H, CBr-C(<u>H</u>)H), 3.19 (dd, *J* = 8.2, 17.2 Hz, 1 H, CBr-C(H)<u>H</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 137.8 (N=<u>C</u>Br), 81.9 (<u>C</u>H-CH_2OH), 63.0 (<u>C</u>H_2OH), 42.6 (CBr-<u>C</u>H_2).

GC RT: 5.8 min, EI-MS (m/z): 180.9592 $[M_{isotop.1}]^+$ (calc.: 180.9561), 178.9574 $[M_{isotop.2}]^+$ (calc.: 178.9582), 149.9369 $[M_{isotop.1}\text{-}CH_2OH]^+$ (calc.: 149.9378), 147.9395 $[M_{isotop.2}\text{-}CH_2OH]^+$ (calc.: 147.9398).

***cis*-3-Bromo-4-(hydroxymethyl)-5-methyl-2-isoxazoline (16a) and *cis*-3-bromo-5-(hydroxymethyl)-4-methyl-2-isoxazoline (16b)**

The reaction was performed with dibromoformaldoxime **12** (1.0 g, 4.9 mmol), 2-buten-1-ol (crotyl alcohol *cis*/*trans* 1:19, 2.1 mL, 25 mmol) and K_2CO_3 (4.0 g, 29 mmol) in 46.4 mL ethyl acetate. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.26 yielded the product (325 mg, 34.0%).

Proton signal ratio **16a**/**16b**: 1.3/1.

cis-3-Bromo-4-(hydroxymethyl)-5-methyl-2-isoxazoline **16a**:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.75 (qd, *J* = 6.3, 8.3, 1 H, C<u>H</u>-CH_3), 3.87 (dd, *J* = 5.6, 11.2 Hz, 1 H, C(<u>H</u>)HOH), 3.82 (dd, *J* = 3.8, 11.2 Hz, 1 H, C(H)<u>H</u>OH), 3.08 (ddd, *J* = 3.8, 5.6, 8.3, 1 H, C<u>H</u>-CH_2OH), 1.46 (d, *J* = 6.3 Hz, 3 H, CH_3).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.9 (N=CBr), 80.9 (CH-CH_3), 61.1 (CH-CH_2OH), 60.5 (CH-CH_2OH), 20.2 (CH_3).

cis-3-Bromo-5-(hydroxymethyl)-4-methyl-2-isoxazoline **16b**:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.33 (ddd, *J* = 3.1, 4.1, 8.5 Hz, 1 H, CH-CH_2O), 3.90 (dd, *J* = 3.2, 12.6 Hz, 1 H, C(H)HOH), 3.68 (dd, *J* = 4.2, 12.6 Hz, 1 H, C(H)HOH), 3.36 (qd, *J* = 7.2, 8.5, 1 H, CH-CH_3), 1.30 (d, *J* = 7.2 Hz, 3 H, CH_3).

^{13}C NMR (151 MHz, $CDCl_3$) δ 144.6 (N=CBr), 88.4 (CH-CH_2OH), 62.1 (CH-CH_2OH), 48.1 (CH-CH_3), 16.1 (CH_3).

DEI-MS (m/z): 195.9794 $[M_{isotop.1}+H]^+$ (calc.: 195.9796), 193.9806 $[M_{isotop.2}+H]^+$ (calc.: 193.9817), 163.9544 $[M_{isotop.1}-CH_3O]^+$ (calc.: 163.9534), 161.9553 $[M_{isotop.2}-CH_3O]^+$ (calc.: 161.9555), 149.9374 $[M_{isotop.1}-C_2H_5O+H]^+$ (calc.: 149.9378), 147.9390 $[M_{isotop.2}-C_2H_5O+H]^+$ (calc.: 147.9398).

cis-3-Bromo-5-ethyl-4-(hydroxymethyl)-2-isoxazoline (18a) and _cis_-3-bromo-4-ethyl-5-(hydroxymethyl)-2-isoxazoline (18b)

The reaction was performed with dibromoformaldoxime **12** (1.0 g, 4.9 mmol), *cis*-2-penten-1-ol (0.95 mL, 9.4 mmol) and K_2CO_3 (4.0 g, 29 mmol) in 46.4 mL ethyl acetate. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.32 yielded the product (248 mg, 24 %).

Proton signal ratio **18a**/**18b**: 1.2/1.

3-Bromo-5-ethyl-4-(hydroxymethyl)-2-isoxazoline **18a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.59 (ddd, *J* = 5.0, 9.0, 9.6 Hz, 1 H, CH-CH_2-CH_3), 3.90 (dd, *J* = 5.6, 11.6 Hz, 1 H, C(H)HOH), 3.85 (dd, *J* = 3.6, 11.6 Hz, 1 H, C(H)HOH), 3.30 (ddd, *J* = 3.5, 5.6, 9.4 Hz, 1 H, CH-CH_2OH), 1.97-1.63 (m, 2 H, CH_2-CH_3), 1.09 (t, *J* = 7.4 Hz, 3 H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 140.1 (N=CBr), 85.6 (CH-CH_2-CH_3), 60.4 (CH_2OH), 56.2 (CH-CH_2OH), 21.8 (CH_2-CH_3), 10.9 (CH_3).

3-Bromo-4-ethyl-5-(hydroxymethyl)-2-isoxazoline **18b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.74 (ddd, *J* = 4.6, 5.4, 10.0 Hz, 1 H, CH-CH_2OH), 3.83 (dd, *J* = 5.3, 12.6 Hz, 1 H, C(H)HOH), 3.81 (dd, *J* = 4.7, 12.6 Hz, 1 H, C(H)HOH), 3.32 (ddd, *J* = 4.5, 9.4, 10.0 Hz, 1 H, CH-CH_2-CH_3), 1.97-1.63 (m, 2 H, CH_2-CH_3), 1.09 (t, *J* = 7.5 Hz, 3 H, CH_3)

^{13}C-NMR (151 MHz, $CDCl_3$) δ 144.6 (N=CBr), 83.5 (CH-CH_2OH), 58.1 (CH_2OH), 54.3 (CH-CH_2-CH_3), 19.1 (CH_2-CH_3), 12.8 (CH_3).

DEI-MS (m/z): 208.9862 $[M_{isotop.1}]^+$ (calc.: 208.9874), 206.9881 $[M_{isotop.2}]^+$ (calc.: 206.9895), 177.9718 $[M_{isotop.1}-CH_3O]^+$ (calc.: 177.9691), 175.9751 $[M_{isotop.2}-CH_3O]^+$ (calc.: 175.9711), 149.9340 $[M_{isotop.1}-C_3H_8O+H]^+$ (calc.: 149.9378), 147.9352 $[M_{isotop.2}-C_3H_8O+H]^+$ (calc.: 147.9398).

cis-3-Bromo-4-(hydroxymethyl)-5-phenyl-2-isoxazoline (20a) and _cis_-3-bromo-5-(hydroxymethyl)-4-phenyl-2-isoxazoline (20b)

The reaction was performed with dibromoformaldoxime **12** (1.0 g, 4.9 mmol), cinnamyl alcohol (1.26 g, 9.4 mmol) and K_2CO_3 (4.0 g, 29 mmol) in 46.4 mL ethyl acetate. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.48 yielded the product (190 mg, 15 %).

Proton signal ratio **20a**/**20b**: 3/1.

cis-3-Bromo-4-(hydroxymethyl)-5-phenyl-2-isoxazoline **20a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.42-7.22 (m, 5 H, H_{arom}), 5.63 (d, *J* = 8.6 Hz, 1 H, CH-Phe), 4.02 (dd, *J* = 5.0, 11.4 Hz, 1 H, C(H)HOH), 3.89 (dd, *J* = 3.6, 11.4 Hz, 1 H, C(H)HOH), 3.43 (ddd, *J* = 3.6, 5.0, 8.6 Hz, 1 H, CH-CH_2OH).

cis-3-Bromo-5-(hydroxymethyl)-4-phenyl-2-isoxazoline **20b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.42-7.22 (m, 5 H, H_{arom}), 4.67 (ddd, *J* = 3.1, 3.7, 8.0 Hz, 1 H, CH-CH_2OH), 4.45 (d, *J* = 8.0 Hz, 1 H, CH-Phe), 3.96 (dd, *J* = 3.1, 12.7 Hz, 1 H, C(H)HOH), 3.73 (dd, *J* = 3.8, 12.7 Hz, 1 H, C(H)HOH).

DEI-MS (m/z): 257.9919 $[M_{isotop.1}+H]^+$ (calc.: 257.9953), 255.9965 $[M_{isotop.2}+H]^+$ (calc.: 255.9973), 225.9677 $[M_{isotop.1}-CH_3O]^+$ (calc.: 225.9691), 223.9684 $[M_{isotop.2}-CH_3O]^+$ (calc.: 223.9711), 77.0378 $[C_6H_5]^+$ (calc.: 77.0391).

Preparation of (3-bromo-2-isoxazolin-5-yl)methyl hex-5-ynoate (1)

The isoxazoline alcohol **14** (40 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (60.8 µL, 0.44 mmol) and hex-5-ynoic acid chloride (57.4 mg, 0.44 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (43.2 mg, 71%) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.10.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.91 (dddd, *J* = 4.0, 5.2, 7.4, 11.0 Hz, 1 H, C<u>H</u>-CH_2O), 4.28 (dd, *J* = 4.0, 12.1 Hz, 1 H, C(<u>H</u>)HO), 4.19 (dd, *J* = 5.2 , 12.1 Hz, 1 H, C(H)<u>H</u>O), 3.34 (dd, *J* = 10.9, 17.3 Hz, 1 H, CBr-C(<u>H</u>)H), 3.05 (dd, *J* = 7.4, 17.3 Hz, 1 H, CBr-C(H)<u>H</u>), 2.51 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.28 (dt, *J* = 2.7, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.98 (t, *J* = 2.7 Hz, 1 H, C≡C<u>H</u>), 1.86 (ψ-quint., 6.9 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>).
^{13}C-NMR (151 MHz, $CDCl_3$) δ = 172.6 (<u>C</u>=O), 136.8 (N=<u>C</u>Br), 83.0 (<u>C</u>≡CH), 78.8 (<u>C</u>H-CH_2O), 69.3 (C≡<u>C</u>H), 64.2 (<u>C</u>H_2O), 43.5 (CBr-<u>C</u>H_2), 32.6 (C(O)-<u>C</u>H_2), 23.4 (C(O)-CH_2-<u>C</u>H_2), 17.8 (<u>C</u>H_2-C≡CH).
GC RT: 0.2 min, EI-MS (m/z): 276.0053 $[M_{isotop.1}+H]^+$ (calc.: 276.0058), 274.0071 $[M_{isotop.2}+H]^+$ (calc.: 274.0079).

cis-(3-Bromo-5-methyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (<u>2a</u>) and _cis_-(3-bromo-4-methyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (<u>2b</u>)

The isoxazoline alcohol **<u>16a/b</u>** (43 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (65.4 µL, 0.47 mmol) and hex-5-ynoic acid chloride (61.7 mg, 0.47 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (55.4 mg, 87%) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.16.
Proton signal ratio **<u>2a</u>**/**<u>2b</u>**: 1.3/1.
Regioisomers were separated by preparative HPLC: mobile phase (HPLC grade)
A = water + 0.1 % TFA, B = acetonitrile + 0.1 % TFA. Gradient (T_{min}): T_0, B = 30 %; T_{80}, B = 60 %; T_{85}, B = 100 %; T_{90}, B = 100 %; T_{100}, B = 0 %; T_{100}, B = 0 %. Retention time (**<u>2a</u>**) = 18.9 min; retention time (**<u>2b</u>**) = 20.4 min.
cis-(3-Bromo-5-methyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **<u>2a</u>**
^{1}H NMR (500 MHz, DMSO-d_6) *δ* 4.58 (qd, *J* = 6.2, 8.6 Hz, 1 H, C<u>H</u>-CH_3), 4.25 (dd, *J* = 3.8, 11.7 Hz, 1 H, C(<u>H</u>)HO), 4.19 (dd, *J* = 4.8, 11.7 Hz, 1 H, C(H)<u>H</u>O), 3.49 (ddd, *J* = 3.9, 4.6, 8.6 Hz, 1 H, C<u>H</u>-CH_2O), 2.81 (t, J = 2.6 Hz, 1 H, CH_2-C≡C<u>H</u>), 2.41 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.20 (dt, *J* = 2.6, 7.1 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.69 (ψ-quint., *J* = 7.2 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>), 1.35 (d, *J* = 6.25 Hz, 3 H, C<u>H_3</u>).

^{13}C NMR (360 MHz, DMSO-d_6) *δ* 172.0 (<u>C</u>(O)-CH_2), 139.4 (N=<u>C</u>Br), 83.5 (<u>C</u>≡CH), 80.2 (<u>C</u>H-CH_3), 71.7 (C≡<u>C</u>H), 60.8 (<u>C</u>H_2O), 57.0 (<u>C</u>H-CH_2O), 32.1 (C(O)-<u>C</u>H_2), 23.3 (C(O)-CH_2-<u>C</u>H_2), 19.4 (<u>C</u>H_3), 17.0 (<u>C</u>H_2-C≡CH).

cis-(3-Bromo-4-methyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **<u>2b</u>**

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.50 (ddd, *J* = 3.2, 5.8, 8.0 Hz, 1 H, C<u>H</u>-CH_2O), 4.28 (dd, *J* = 3.2, 12.2 Hz, 1 H, C(<u>H</u>)HO), 4.19 (dd, *J* = 5.6, 12.0 Hz, 1 H, C(H)<u>H</u>O), 3.39-3.31 (m, 1 H, C<u>H</u>-CH_3), 2.82 (t, *J* = 2.6 Hz, 1 H, CH_2-C≡C<u>H</u>), 2.43 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.21 (dt, *J* = 2.5, 7.0 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.70 (ψ-quint., *J* = 7.1 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>), 1.21 (d, *J* = 7.1 Hz, 3 H, C<u>H_3</u>).

^{13}C NMR (360 MHz, DMSO-d_6) δ 172.2 (<u>C</u>(O)-CH_2), 144.3 (N=<u>C</u>Br), 84.8 (<u>C</u>H-CH_2O), 83.6 (<u>C</u>≡CH), 71.8 (C≡<u>C</u>H), 63.6 (<u>C</u>H_2O), 48.4 (<u>C</u>H-CH_3), 32.2 (C(O)-<u>C</u>H_2), 23.4 (C(O)-CH_2-<u>C</u>H_2), 17.0 (<u>C</u>H_2-C≡CH), 15.6 (<u>C</u>H_3).

DEI-MS (m/z): 290.0159 $[M_{isotop.1}+H]^+$ (calc.: 290.0215), 288.0228 $[M_{isotop.2}+H]^+$ (calc.: 288.0235), 67.0527 $[C_5H_7]^+$ (calc.: 67.0548).

cis-(3-Bromo-5-ethyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (<u>3a</u>) and (3-Bromo-4-ethyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (<u>3b</u>)

The isoxazoline alcohol **<u>18a/b</u>** (46.3 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (70.4 µL, 0.51 mmol) and hex-5-ynoic acid chloride (66.4 mg, 0.51 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (36.3 mg, 55%) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.19.
Proton signal ratio **<u>3a</u>**/**<u>3b</u>**: 1.2/1.
Regioisomers were separated by preparative HPLC: mobile phase (HPLC grade)
A = water + 0.1 % TFA, B = acetonitrile + 0.1 % TFA. Gradient (T_{min}): T_0, B = 30 %; T_{80}, B = 60 %; T_{85}, B = 100 %; T_{90}, B = 100 %; T_{100}, B = 0 %; T_{100}, B = 0 %. Retention time (**<u>3a</u>**) = 24.4 min; retention time (**<u>3b</u>**) = 26.2 min.

cis-(3-Bromo-5-ethyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **<u>3a</u>**

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.56 (ddd, *J* = 4.8, 9.2, 9.8 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 4.23 (dd, *J* = 5.1, 12.2 Hz, 1 H, C(<u>H</u>)HO), 4.16 (dd, *J* = 3.6, 12.2 Hz, 1 H, C(H)<u>H</u>O), 3.72 (ddd, *J* = 3.7, 4.9, 9.9 Hz, 1 H, C<u>H</u>-CH_2O), 2.80 (t, *J* = 2.3 Hz, 1 H, CH_2-C≡C<u>H</u>), 2.39 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.19 (dt, *J* = 2.6, 7.1 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.75-1.64 (m, 4 H, C(O)-CH_2-C<u>H_2</u> and C<u>H_2</u>-CH_3), 0.97 (t, *J* = 7.4 Hz, 3 H, C<u>H_3</u>).

^{13}C NMR (360 MHz, DMSO-d_6) δ 171.8 (<u>C</u>(O)-CH_2), 140.1 (N=<u>C</u>Br), 84.4 (<u>C</u>H-CH_2-CH_3), 83.4 (<u>C</u>≡CH), 71.7 (C≡<u>C</u>H), 58.6 (<u>C</u>H_2O), 52.3 (<u>C</u>H-CH_2O), 32.2 (C(O)-<u>C</u>H_2), 23.2 (C(O)-CH_2-<u>C</u>H_2), 21.3 (<u>C</u>H_2-CH_3), 17.0 (<u>C</u>H_2-C≡CH), 10.7 (<u>C</u>H_3).

cis-(3-Bromo-4-ethyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **<u>3b</u>**

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.87 (ddd, J = 3.3, 6.6, 9.9 Hz, 1 H, C<u>H</u>-CH_2O), 4.27 (dd, J = 3.3, 12.3 Hz, 1 H, C(<u>H</u>)HO), 4.17 (dd, J = 6.7, 12.3 Hz, 1 H, C(H)<u>H</u>O), 3.53 (ddd, J = 4.9, 8.2, 9.8 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 2.81 (t, J = 2.7 Hz, 1 H, CH_2-C≡C<u>H</u>), 2.42 (t, J = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.20 (dt, J = 2.6, 7.1 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.69 (ψ-quint., J = 7.3 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>), 1.68-1.53 (m, 2 H, C<u>H_2</u>-CH_3), 0.99 (t, J = 7.4 Hz, 3 H, C<u>H_3</u>).

^{13}C NMR (360 MHz, DMSO-d_6) δ 171.9 (<u>C</u>(O)-CH_2), 144.0 (N=<u>C</u>Br), 83.5 (<u>C</u>≡CH), 80.4 (<u>C</u>H-CH_2O), 71.7 (C≡<u>C</u>H), 61.4 (<u>C</u>H_2O), 53.1 (<u>C</u>H-CH_2-CH_3), 32.2 (C(O)-<u>C</u>H_2), 23.3 (C(O)-CH_2-<u>C</u>H_2), 18.5 (<u>C</u>H_2-CH_3), 17.0 (<u>C</u>H_2-C≡CH), 11.9 (<u>C</u>H_3).

DEI-MS (m/z): 304.0341 $[M_{isotop.1}+H]^+$ (calc.: 304.0371), 302.0382 $[M_{isotop.2}+H]^+$ (calc.: 302.0392), 191.9890 $[M_{isotop.1}+C_6H_7O_2]^+$ (calc.: 191.9847), 189.9920 $[M_{isotop.1}+C_6H_7O_2]^+$ (calc.: 189.9868).

cis-(3-Bromo-5-phenyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (<u>4a</u>) and _cis_-(3-bromo-4-phenyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (<u>4b</u>)

The isoxazoline alcohol **<u>20a/b</u>** (57.0 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (86.6 µL, 0.62 mmol) and hex-5-ynoic acid chloride (81.8 mg, 0.63 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (16.8 mg, 22%) was obtained by column chromatography on silica gel with the solvent mixing ratio: _iso_-hexane / ethyl acetate 7:1, R_f = 0.27.
Proton signal ratio **<u>4a</u>**/**<u>4b</u>**: 3.1/1.

cis-(3-Bromo-5-phenyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **<u>4a</u>**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.43-7.34 (m, 5 H, H_{arom}), 5.47 (d, J = 8.8 Hz, 1 H, C<u>H</u>-Phe), 4.43 (dd, J = 3.7, 11.7 Hz, 1 H, C(<u>H</u>)HO), 4.35 (dd, J = 5.6, 11.7 Hz, 1 H, C(H)<u>H</u>O), 3.59 (ddd, J = 3.7, 5.6, 8.9 Hz, 1 H, C<u>H</u>-CH_2O), 2.51 (t, J = 7.2 Hz, 2 H, C(O)-C<u>H_2</u>), 2.27 (dt, J = 2.7, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.99 (t, J = 2.6 Hz, 1 H, C≡C<u>H</u>), 1.85 (ψ-quint., J = 7.2 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.6 (<u>C</u>=O), 138.4 (C_q), 137.8 (C_q), 129.0 (2x <u>C</u>H_{arom}), 129.0 (<u>C</u>H_{arom}), 125.9 (2x <u>C</u>H_{arom}), 85.8 (<u>C</u>H-Phe), 83.0 (<u>C</u>≡CH), 69.4 (C≡<u>C</u>H), 61.1 (<u>C</u>H_2O), 60.1 (<u>C</u>H-CH_2O), 32.5 (C(O)-<u>C</u>H_2), 23.3 (C(O)-CH_2-<u>C</u>H_2), 17.8 (<u>C</u>H_2-C≡CH).

cis-(3-Bromo-4-phenyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **<u>4b</u>**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.43-7.34 (m, 3 H, H_{arom}), 7.22-7.20 (m, 2 H, H_{arom}), 4.79 (ddd, J = 4.3, 5.0, 7.1 Hz, 1 H, C<u>H</u>-CH_2O), 4.38 (dd, J = 4.3, 12.1 Hz, 1 H,

C(H)HO), 4.30 (dd, *J* = 5.0, 12.1 Hz, 1 H, C(H)HO), 4.26 (d, *J* = 7.1 Hz, 1 H, CH-Phe), 2.52 (t, *J* = 7.4 Hz, 2 H, C(O)-CH_2), 2.27 (dt, *J* = 2.7, 6.9 Hz, 2 H, CH_2-C≡CH), 1.98 (t, *J* = 2.6 Hz, 1 H, C≡CH), 1.86 (ψ-quint., *J* = 7.2 Hz, 2 H, C(O)-CH_2-CH_2).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.6 (C=O), 141.1 (C_q), 135.7 (C_q), 129.5 (2x CH_{arom}), 128.7 (CH_{arom}), 127.8 (2x CH_{arom}), 86.9 (CH-CH_2O), 82.9 (C≡CH), 69.4 (C≡CH), 63.6 (CH_2O), 61.4 (CH-Phe), 32.6 (C(O)-CH_2), 23.4 (C(O)-CH_2-CH_2), 17.8 (CH_2-C≡CH).
DEI-MS (m/z): 350.0380 $[M_{isotop.2}+H]^+$ (calc.:350.0392), 239.9787 $[M_{isotop.1}-C_6H_7O_2]^+$ (calc.:239.9847), 237.9770 $[M_{isotop.2}-C_6H_7O_2]^+$ (calc.:237.9868), 67.0548 $[C_5H_7]^+$ (calc.: 67.0548), 77.0328 $[C_6H_5]^+$ (calc.: 77.0391).

General procedure for the synthesis of 3-chloro-2-isoxazolines.

Preparation of 3-chloro-5-(hydroxymethyl)-2-isoxazoline (15)

9 mL of a solution of dichloroformaldoxime in 1,2-dimethoxyethane **13** (calc. 1.25 mol/L, 11.3 mmol) was added to allyl alcohol (1.25 mL, 18.4 mmol). After addition of anhydrous, freshly triturated K_2CO_3 (4.0 g, 29 mmol), the reaction mixture was stirred for 48 h at room temperature. Then, the reaction mixture was diluted with 90 mL ice cold H_2O and extracted with 3 x 100 mL diethyl ether. The solvent of the combined organic layers was evaporated and the product (27 mg, 1.8%) was obtained by column chromatography on silica gel with a TLC optimized solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.21.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.86 (dddd, *J* = 3.1, 4.1, 8.4, 10.7 Hz, 1 H, CH-CH_2OH), 3.85 (dd, *J* = 3.1, 12.6 Hz, 1 H C(H)HOH), 3.64 (dd, *J* = 4.1, 12.6 Hz, C(H)HOH), 3.20 (dd, *J* = 10.7, 17.1 Hz, 1 H, CCl-C(H)H), 3.15 (dd, *J* = 8.4, 17.2 Hz, 1 H, CCl-C(H)H).
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 149.5 (N=CCl), 82.6 (CH-CH_2OH), 63.0 (CH_2OH), 39.6 (CCl-CH_2).

***cis*-3-Chloro-4-(hydroxymethyl)-5-methyl-2-isoxazoline (17a) and *cis*-3-chloro-5-(hydroxymethyl)-4-methyl-2-isoxazoline (17b)**

The reaction was performed with 2-buten-1-ol (crotyl alcohol *cis*/*trans* 1:19, 2.1 mL, 25 mmol) as described above. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.31 yielded the product (270 mg, 16%).

Proton signal ratio **17a**/**17b**: 1/1.

cis-3-Chloro-4-(hydroxymethyl)-5-methyl-2-isoxazoline **17a**:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.78 (qd, *J* = 6.3, 8.4 Hz, 1 H, C<u>H</u>-CH_3), 3.87 (dd, *J* = 5.2, 11.3 Hz, 1 H, C(<u>H</u>)HOH), 3.80 (dd, *J* = 3.9, 11.2 Hz, 1 H, C(H)<u>H</u>OH), 3.03 (ddd, *J* = 4.0, 5.5, 8.9 Hz, 1 H, C<u>H</u>-CH_2OH), 1.43 (d, *J* = 6.3 Hz, 3 H, C<u>H</u>$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 128.3 (N=<u>C</u>Cl), 81.7 (<u>C</u>H-CH_3), 60.1 (<u>C</u>H_2OH), 58.9 (<u>C</u>H-CH_2OH), 20.3 (<u>C</u>H_3).

cis-3-Chloro-5-(hydroxymethyl)-4-methyl-2-isoxazoline **17b**:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.39 (ddd, *J* = 3.2, 4.0, 8.7 Hz, 1 H, C<u>H</u>-CH_2OH), 3.87 (dd, *J* = 3.3, 12.2 Hz, 1 H, C(H)<u>H</u>OH), 3.65 (dd, *J* = 4.1, 12.6 Hz, 1 H, C(<u>H</u>)HOH), 3.32 (qd, *J* = 7.1, 8.5 Hz, 1 H, C<u>H</u>-CH_3), 1.30 (d, *J* = 7.1 Hz, 3 H, C<u>H</u>$_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ 130.1 (N=<u>C</u>Cl), 89.4 (<u>C</u>H-CH_2OH), 62.1 (<u>C</u>H_2OH), 45.6 (<u>C</u>H-CH_3), 15.5 (<u>C</u>H_3).

DEI-MS (m/z): 149.0240 $[M_{isotop.2}]^+$ (calc.: 149.0244), 120.0058 $[M_{isotop.1}\text{-}CH_3O]^+$ (calc.: 120.0030), 118.0094 $[M_{isotop.2}\text{-}CH_3O]^+$ (calc.: 118.0060), 105.9933 $[M_{isotop.1}\text{-}C_2H_6O+H]^+$ (calc.: 105.9874), 103.9959 $[M_{isotop.2}\text{-}C_2H_6O+H]^+$ (calc.: 103.9903).

cis-3-Chloro-5-ethyl-4-(hydroxymethyl)-2-isoxazoline (<u>19a</u>) and _cis_-3-chloro-4-ethyl-5-(hydroxymethyl)-2-isoxazoline (<u>19b</u>)

The reaction was performed with *cis*-2-penten-1-ol (0.95 mL, 9.4 mmol) as described above. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.38 yielded the product (49 mg, 3 %).

Proton signal ratio **<u>19a</u>**/**<u>19b</u>**: 1.2/1.

cis-3-Chloro-5-ethyl-4-(hydroxymethyl)-2-isoxazoline **<u>19a</u>**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.65 (ddd, *J* = 4.9, 9.0, 9.6 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 3.92 (dd, *J* = 5.6, 11.6 Hz, 1 H, C(<u>H</u>)HOH), 3.88 (dd, *J* = 3.7, 11.6 Hz, 1 H, C(H)<u>H</u>OH), 3.27 (ddd, *J* = 3.7, 5.4, 9.8 Hz, 1 H, C<u>H</u>-CH_2OH), 1.97-1.78 (m, 2 H, CH_2CH_3), 1.09 (t, *J* = 7.4 Hz, 3 H, C<u>H</u>$_3$).

cis-3-Chloro-4-ethyl-5-(hydroxymethyl)-2-isoxazoline **<u>19b</u>**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.81 (ddd, *J* = 4.3, 5.7, 10.0 Hz, 1 H, C<u>H</u>-CH_2OH), 3.83 (dd, *J* = 5.6, 12.5 Hz, 1 H, C(<u>H</u>)HOH), 3.81 (dd, *J* = 4.3, 12.4 Hz, 1 H, C(H)<u>H</u>OH), 3.30 (ddd, *J* = 5.3, 9.0, 10.2 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 1.97-1.78 (m, 2 H, CH_2CH_3), 1.09 (t, *J* = 7.4 Hz, 3 H, C<u>H</u>$_3$).

DEI-MS (m/z): 165.0367 $[M_{isotop.1}]^+$ (calc.: 165.0371), 163.0402 $[M_{isotop.2}]^+$ (calc.: 163.0400).

cis-3-Chloro-4-(hydroxymethyl)-5-phenyl-2-isoxazoline (21a) and _cis_-3-chloro-5-(hydroxymethyl)-4-phenyl-2-isoxazoline (21a)

The reaction was performed with cinnamyl alcohol (1.26 g, 9.4 mmol) as described above. Standard workup and purification by column chromatography on silica gel with the solvent mixing ratio: CH_2Cl_2 / MeOH 40:1, R_f = 0.46 yielded the product (128 mg, 6 %).

Proton signal ratio **21a**/**21b**: 2.7/1.

cis-3-Chloro-4-(hydroxymethyl)-5-phenyl-2-isoxazoline **21a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.42-7.25 (m, 5 H, H_{arom}), 5.70 (d, *J* = 8.7 Hz, 1 H, C<u>H</u>-Phe), 4.03 (dd, *J* = 5.0, 11.3 Hz, 1 H, C(<u>H</u>)HOH), 3.90 (dd, *J* = 3.6, 11.3 Hz, 1 H, C(H)<u>H</u>OH), 3.41 (ddd, *J* = 3.7, 4.9, 8.7 Hz, 1 H, C<u>H</u>-CH_2OH).

cis-3-Chloro-5-(hydroxymethyl)-4-phenyl-2-isoxazoline **21b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.42-7.25 (m, 5 H, H_{arom}), 4.73 (ddd, *J* = 3.2, 3.6, 8.1 Hz, 1 H, C<u>H</u>-CH_2OH), 4.44 (d, *J* = 8.1 Hz, 1 H, C<u>H</u>-Phe), 3.96 (dd, *J* = 3.1, 12.7 Hz, 1 H, C(<u>H</u>)HOH), 3.73 (dd, *J* = 3.7, 12.7 Hz, 1 H, C(H)<u>H</u>OH).

DEI-MS (m/z): 211.0400 $[M_{isotop.2}]^+$ (calc.: 211.0400), 182.0160 $[M_{isotop.1}\text{-}CH_3O]^+$ (calc.: 182.0187), 180.0178 $[M_{isotop.2}\text{-}CH_3O]^+$ (calc.: 180.0216).

(3-Chloro-2-isoxazolin-5-yl)methyl hex-5-ynoate (5)

The isoxazoline alcohol **15** (23.6 mg, 0.18 mmol) was dissolved in 0.7 mL $CHCl_3$ and 0.8 mL CH_2Cl_2. Subsequently, triethylamine (47.4 µL, 0.34 mmol) and hex-5-ynoic acid chloride (44.4 mg, 0.34 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (11.5 mg, 28 %) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.14.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.98 (dddd, *J* = 4.0, 5.2, 7.6, 11.0 Hz, 1 H, C<u>H</u>-CH_2O), 4.29 (dd, *J* = 4.0, 12.1 Hz, 1 H, C(<u>H</u>)HO), 4.20 (dd, *J* = 5.2, 12.1 Hz, 1 H, C(H)<u>H</u>O), 3.30 (dd, *J* = 11.0, 17.2 Hz, 1 H, CCl-C(<u>H</u>)H), 3.01 (dd, *J* = 7.6, 17.2 Hz, 1 H, CCl-C(H)<u>H</u>), 2.52 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.28 (dt, *J* = 2.7, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.98 (t, *J* = 2.7 Hz, 1 H, C≡C<u>H</u>), 1.86 (ψ-quint., *J* = 7.1 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 172.6 (C=O), 148.6 (N=CCl), 83.0 (C≡CH), 79.5 (CH-CH_2O), 69.3 (C≡CH), 64.2 (CH_2O), 40.5 (CCl-CH_2), 32.5 (C(O)-CH_2), 23.4 (C(O)-CH_2-CH_2), 17.8 (CH_2-C≡CH).

DEI-MS (m/z): 230.0529 $[M_{isotop.2}+H]^+$ (calc.: 230.0584), 229.0506 $[M_{isotop.2}]^+$ (calc.: 229.0498), 126.0632 $[C_7H_9O_2+H]^+$ (calc. 126.0681), 119.9991 $[M_{isotop.1}-C_6H_7O_2]^+$ (calc.: 120.0030), 117.9994 $[M_{isotop.2}-C_6H_7O_2]^+$ (calc.: 18.0060).

cis-(3-Chloro-5-methyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (6a) and _cis_-(3-chloro-4-methyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (6b)

The isoxazoline alcohol **17a/b** (33.2 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (65.4 µL, 0.47 mmol) and hex-5-ynoic acid chloride (61.7 mg, 0.47 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (16.9 mg, 31 %) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.15.

Proton signal ratio **6a**/**6b**: 1.1/1.

cis-(3-Chloro-5-methyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **6a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.68 (qd, *J* = 6.3, 8.5 Hz, 1 H, CH-CH_3), 4.34 (dd, *J* = 3.9, 11.6 Hz, 1 H, C(H)HO), 4.24 (dd, *J* = 6.1, 11.6 Hz, 1 H, C(H)HO), 3.20 (ddd, *J* = 3.9, 5.8, 8.3 Hz, 1 H, CH-CH_2O), 2.50 (t, *J* = 7.6 Hz, 2 H, C(O)-CH_2), 2.28 (dt, *J* = 2.7, 6.9 Hz, 2 H, CH_2-C≡CH), 1.98 (t, *J* = 2.6 Hz, 1 H, C≡CH), 1.85 (ψ-quint., *J* = 7.2 Hz, 2 H, C(O)-CH_2-CH_2), 1.47 (d, *J* = 6.3 Hz, 3 H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.6 (C=O), 148.9 (N=CCl), 82.9 (C≡CH), 81.9 (CH-CH_3), 69.4 (C≡CH), 60.9 (CH_2O), 56.0 (CH-CH_2O), 32.5 (C(O)-CH_2), 23.3 (C(O)-CH_2-CH_2), 20.1 (CH_3), 17.8 (CH_2-C≡CH).

cis-(3-Chloro-4-methyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **6b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.51 (ddd, *J* = 4.1, 5.3, 8.2 Hz, 1 H, CH-CH_2O), 4.32 (dd, *J* = 4.1, 12.1 Hz, 1 H, C(H)HO), 4.23 (dd, *J* = 5.5, 12.1 Hz, 1 H, C(H)HO), 3.18 (qd, *J* = 7.2, 8.1 Hz, 1 H, CH-CH_3), 2.52 (t, *J* = 7.4 Hz, 2 H, C(O)-CH_2), 2.28 (dt, *J* = 2.7, 6.9 Hz, 2 H, CH_2-C≡CH), 1.98 (t, *J* = 2.6 Hz, 1 H, C≡CH), 1.86 (ψ-quint., *J* = 7.2 Hz, 2 H, C(O)-CH_2-CH_2), 1.34 (d, *J* = 7.1 Hz, 3 H, CH_3).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.6 (C=O), 153.7 (N=CCl), 86.2 (CH-CH_2O), 83.0 (C≡CH), 69.3 (C≡CH), 63.5 (CH_2O), 47.0 (CH-CH_3), 32.5 (C(O)-CH_2), 23.4 (C(O)-CH_2-CH_2), 17.8 (CH_2-C≡CH), 15.7 (CH_3).

DEI-MS (m/z): 246.0739 $[M_{isotop.1}+H]^+$ (calc.: 246.0711), 244.0746 $[M_{isotop.2}+H]^+$ (calc.: 244.0741).

cis-(3-Chloro-5-ethyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (7a) and _cis_-(3-chloro-4-ethyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (7b)

The isoxazoline alcohol **19a/b** (36.4 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (70.4 µL, 0.51 mmol) and hex-5-ynoic acid chloride (66.4 mg, 0.51 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (14.6 mg, 26 %) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.18.

Proton signal ratio **7a**/**7b**: 1.1/1.

cis-(3-Chloro-5-ethyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **7a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.67 (ddd, *J* = 4.9, 9.0, 9.7 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 4.36 (dd, *J* = 6.1, 11.9 Hz, 1 H, C(<u>H</u>)HO), 4.27 (dd, *J* = 4.0, 11.8 Hz, 1 H, C(H)<u>H</u>O), 3.45 (ddd, *J* = 4.1, 6.1, 10.0 Hz, 1 H, C<u>H</u>-CH_2O), 2.52 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.30 (dt, *J* = 2.6, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 2.01 (t, *J* = 2.7 Hz, 1 H, C≡C<u>H</u>), 1.91-1.85 (m, 2 H, C(O)-CH_2-C<u>H_2</u>), 1.87-1.64 (m, 2 H, C<u>H_2</u>-CH_3), 1.12 (t, *J* = 7.5 Hz, 3 H, C<u>H_3</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.4 (<u>C</u>=O), 150.1 (N=<u>C</u>Cl), 85.9 (<u>C</u>H-CH_2-CH_3), 82.9 (<u>C</u>≡CH), 69.3 (C≡<u>C</u>H), 58.6 (<u>C</u>H_2O), 51.0 (<u>C</u>H-CH_2O), 32.6 (C(O)-<u>C</u>H_2), 23.4 (C(O)-CH_2-<u>C</u>H_2), 21.9 (<u>C</u>H_2-CH_3), 17.8 (<u>C</u>H_2-C≡CH), 10.7 (<u>C</u>H_3).

cis-(3-Chloro-4-ethyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **7b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 4.93 (ddd, *J* = 3.9, 7.0, 10.1 Hz, 1 H, C<u>H</u>-CH_2O), 4.45 (dd, *J* = 3.9, 12.1 Hz, 1 H, C(<u>H</u>)HO), 4.26 (dd, *J* = 7.1, 12.0 Hz, 1 H, C(H)<u>H</u>O), 3.34 (ddd, *J* = 5.1, 8.3, 9.9 Hz, 1 H, C<u>H</u>-CH_2-CH_3), 2.54 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.31 (dt, *J* = 2.6, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 2.01 (t, *J* = 2.7 Hz, 1 H, C≡C<u>H</u>), 1.91-1.85 (m, 2 H, C(O)-CH_2-C<u>H_2</u>), 1.80-1.54 (m, 2 H, C<u>H_2</u>-CH_3), 1.12 (t, *J* = 7.5 Hz, 3 H, C<u>H_3</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.7 (<u>C</u>=O), 153.6 (N=<u>C</u>Cl), 83.0 (<u>C</u>≡CH), 81.8 (<u>C</u>H-CH_2O), 69.3 (C≡<u>C</u>H), 61.6 (<u>C</u>H_2O), 52.2 (<u>C</u>H-CH_2-CH_3), 32.5 (C(O)-<u>C</u>H_2), 23.4 (C(O)-CH_2-<u>C</u>H_2), 18.8 (<u>C</u>H_2-CH_3), 17.8 (<u>C</u>H_2-C≡CH), 12.3 (<u>C</u>H_3).

DEI-MS (m/z): 258.0924 $[M_{isotop.2}+H]^+$ (calc.: 258.0897), 148.0357 $[M_{isotop.1}-C_6H_7O_2]^+$ (calc.: 148.0343), 146.0360 $[M_{isotop.2}-C_6H_7O_2]^+$ (calc.: 146.0373), 118.9915 $[M_{isotop.1}-C_8H_{12}O_2]^+$ (calc.: 118.9952), 116.9960 $[M_{isotop.2}-C_8H_{12}O_2]^+$ (calc.: 116.9981).

cis-(3-Chloro-5-phenyl-2-isoxazolin-4-yl)methyl hex-5-ynoate (8a) and _cis_-(3-chloro-4-phenyl-2-isoxazolin-5-yl)methyl hex-5-ynoate (8b)

The isoxazoline alcohol **21a/b** (47.0 mg, 0.22 mmol) was dissolved in 1.4 mL CH_2Cl_2. Subsequently, triethylamine (86.6 µL, 0.62 mmol) and hex-5-ynoic acid chloride (81.8 mg, 0.63 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. The product (13.6 mg, 20 %) was obtained by column chromatography on silica gel with the solvent mixing ratio: *iso*-hexane / ethyl acetate 7:1, R_f = 0.26.
Proton signal ratio **8a**/**8b**: 3.6/1.

cis-(3-Chloro-5-phenyl-2-isoxazolin-4-yl)methyl hex-5-ynoate **8a**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.43-7.35 (m, 5 H, H_{arom}), 5.53 (d, *J* = 8.9 Hz, 1 H, C<u>H</u>-Phe), 4.45 (dd, *J* = 3.7, 11.8 Hz, 1 H, C(<u>H</u>)HO), 4.37 (dd, *J* = 5.5, 11.7 Hz, 1 H, C(H)<u>H</u>O), 3.57 (ddd, *J* = 3.7, 5.5, 9.1 Hz, 1 H, C<u>H</u>-CH_2O), 2.51 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.27 (dt, *J* = 2.6, 6.9 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.99 (t, *J* = 2.7 Hz, 1 H, C≡C<u>H</u>), 1.85 (ψ-quint., *J* = 7.1 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.8 (<u>C</u>=O), 148.6 (C_q), 138.3 (C_q), 129.1 (<u>C</u>H $_{arom}$), 129.0 (2x <u>C</u>H_{arom}), 125.9 (2x <u>C</u>H_{arom}), 86.6 (<u>C</u>H-Phe), 83.0 (<u>C</u>≡CH), 69.4 (C≡<u>C</u>H), 60.7 (<u>C</u>H_2O), 57.8 (<u>C</u>H-CH_2O), 32.5 (C(O)-<u>C</u>H_2), 23.3 (C(O)-CH_2-<u>C</u>H_2), 17.7 (<u>C</u>H_2-C≡CH).

cis-(3-Chloro-4-phenyl-2-isoxazolin-5-yl)methyl hex-5-ynoate **8b**:

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.43-7.35 (m, 3 H, H_{arom}), 7.23-7.21 (m, 2 H, H_{arom}), 4.85 (ddd, *J* = 4.4, 4.9, 7.3 Hz, 1 H, C<u>H</u>-CH_2O), 4.38 (dd, *J* = 4.2, 12.3 Hz, 1 H, C(<u>H</u>)HO), 4.30 (dd, *J* = 5.0, 12.1 Hz, 1 H, C(H)<u>H</u>O), 4.24 (d, *J* = 7.3 Hz, 1 H, C<u>H</u>-Phe), 2.52 (t, *J* = 7.4 Hz, 2 H, C(O)-C<u>H_2</u>), 2.27 (dt, *J* = 2.5, 6.7 Hz, 2 H, C<u>H_2</u>-C≡CH), 1.98 (t, *J* = 2.5 Hz, 1 H, C≡C<u>H</u>), 1.86 (ψ-quint., *J* = 7.2 Hz, 2 H, C(O)-CH_2-C<u>H_2</u>).

^{13}C-NMR (151 MHz, $CDCl_3$) δ 172.8 (<u>C</u>=O), 151.5 (C_q), 135.4 (C_q), 129.5 (2x <u>C</u>H_{arom}), 128.7 (<u>C</u>H_{arom}), 127.8 (2x <u>C</u>H_{arom}), 87.7 (<u>C</u>H-CH_2O), 83.0 (<u>C</u>≡CH), 69.4 (C≡<u>C</u>H), 63.5 (<u>C</u>H_2O), 58.8 (<u>C</u>H-Ph), 32.5 (C(O)-<u>C</u>H_2), 23.3 (C(O)-CH_2-<u>C</u>H_2), 17.7 (<u>C</u>H_2-C≡CH).

DEI-MS (m/z): 308.0922 $[M_{isotop.1}+H]^+$ (calc.: 308.0868), 306.0891 $[M_{isotop.2}+H]^+$ (calc.: 306.0897), 194.0341 $[M_{isotop.2}-C_6H_7O_2]^+$ (calc.: 194.0373), 182.0235 $[M_{isotop.1}-C_7H_9O_2]^+$ (calc.: 182.0186), 180.0246 $[M_{isotop.2}-C_7H_9O_2]^+$ (calc.: 180.0216).

Bacterial strains and culture

Listeria welshimeri SLCC 5334 serovar 6b (DSMZ, Germany) and the *Staphylococcus aureus* strains NCTC 8325 (Institute Pasteur, France) and Mu50 (Institute Pasteur, France) were maintained in brain-heart broth (BHB) medium. *Bacillus licheniformis* ATCC 14580 (DSMZ, Germany) and *Pseudomonas putida* KT2440 (ATCC, USA) were maintained in Luria-Bertani (LB) medium. All strains were grown at 37 °C.

In situ experiments

For analytical and preparative in situ studies, bacteria were grown in BHB or LB medium and harvested 1 h after reaching stationary phase by centrifugation. Depending on the cell density at stationary phase 2 mL of *B*. *licheniformis*, *L*. *welshimeri* and *P*. *putida* strains and 1 mL of *S*. *aureus* strains were harvested for analytical and 10 mL and 5 mL for preparative studies, respectively. After washing with PBS, the cells were resuspended in 100 µL and 500 µL PBS for analytical and preparative experiments. Unless indicated otherwise bacteria were incubated for 2 h with varying concentrations of probe at RT for analytical studies and with concentrations of 100 µM and 200 µM probe for preparative studies. Subsequently, the cells were washed three times with 1 mL PBS and lysed by sonication in 100 µL and 500 µL PBS with a Bandelin Sonopuls with 3x 20 sec. pulsed at 70% max. power under ice cooling. Membrane and cytosolic fractions were separated by centrifugation at 13.000 rpm for 10 min. Membrane pellets were resuspended in 100 µL and 500 µL PBS.

In analytical experiments 43 µL proteome were used to append a fluorescent rhodamine reporter tag via click chemistry (CC), such that once CC reagent were added, the total reaction volume was 50 µL. The volumes of CC reagents are given below for analytical experiments and can be accordingly adjusted for preparative scale. For preparative purposes, the total volume of 500 µL was consumed per experiment. Reporter tagged-azide reagents (13 µM rhodamine-azide (1 µL) for analytical or 20 µM rhodamine-biotin-azide for preparative scale) were added followed by 1 mM TCEP (1 µL) and 100 µM (3 µL) ligand (TBTA, tris-(benzyltriazolylmethyl)amine). Samples were gently vortexed and the cycloaddition initiated by the addition of 1 mM $CuSO_4$ (1 µL). The reactions were incubated at room temperature for 1 h.[2, 3]

For analytical gel electrophoresis, 50 µL 2× SDS loading buffer were added and 50 µL applied on 10% polyacrylamide gels. After SDS-PAGE fluorescence was recorded in a Fujifilm Las-4000 Luminescent Image Analyzer with a Fujinon VRF43LMD3 Lens and a 575DF20 filter.

Preparative 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 at 0° C. 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 200 µL of pre-chilled methanol and resuspended by sonication. Subsequently, the pellet was dissolved in 1 mL PBS with 0.4% 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/0.4% 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]

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 X_{corr} vs. charge state filter was set to X_{corr} 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] Minimum P-values of each run as well as the total number of obtained peptides are reported in Table S3.

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, alternatively by colony PCR with cells directly from the corresponding cryostocks. *att*B1 forward primer and *att*B2 reverse primer were designed to yield *att*B-PCR Products needed for Gateway® Technology:

1-Pyrroline-5-carboxylate dehydrogenase (PCDH) from *B. licheniformis*
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GAC AAC ACC TTA CAA ACA C
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA AAA CAT TTC GCT GAT CGT TTT

Bifunctional aldehyde-CoA / alcohol dehydrogenase (AADH) from *L. welshimeri*
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GGC AAT TAA AGA AAA TGC GGC
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TCA AAC CCC TTT GTA AGC TTC AA

3-Oxoacyl-[acyl-carrier-protein] synthase III (FabH) from *L. welshimeri*
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GAA CGC AGG AAT TTT AG
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA TTT ACC CCA ACG AAT AAT

3-Oxoacyl-[acyl-carrier-protein] synthase III (FabH) from *S. aureus* Mu50
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTA CTT TGG TGC ATA TGC ACC AG
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TAT TGT CAT TGC GCC CCA A

Aldehyde dehydrogenase (ADH) from *P. putida*
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GAC CAC CCT GAC CC
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA CAG CTT GAT CCA GGT C

Aldehyde dehydrogenase (AldA) from *S. aureus* NCTC 8325
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GGC AGT AAA CGT TCG A
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG CTA GTA CAA ACC TTT TAA AGC

Aldehyde dehydrogenase (ADH) from *S. aureus* NCTC 8325
forward primer: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GAA TAT CAT TGA GCA AAA ATT TT
reverse primer: 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA ATT TTT AAA GAA AGC TTT GAT G

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 LB medium supplemented with kanamycin. 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 (100 µg mL^{-1}) and target gene expression was induced with 0.2 µg mL^{-1} anhydrotetracyclin for 2 h at 37°C.

Binding site of bifunctional aldehyde-CoA / alcohol dehydrogenase (AADH)

To discover the binding site of the 3-bromodihydroisoxazole probe **1** in its target enzyme AADH, purified enzyme was labeled by the probe and after tryptic digest the peptide fragments were analysed by MS. 50 µg of AADH (0.53 nmol) in 17 µL PBS were incubated for 60 min at room temperature with 1 µL of 1 mM probe **1** (1 nmol) in DMSO. 80 µL of 25 mM NH_4HCO_3 were added and the buffer was exchanged three times with 100 µL of 25 mM NH_4HCO_3 to wash away unreacted probe using a centrifugal filter device (30 kDa molecular weight cut-off) and adjusted to 25 mM NH_4HCO_3 giving a total volume of 100 µL. Then, 1 µL trypsin (0.5 µg, 9550 units, sequencing grade modified, Promega) were added to 50 µl of the AADH solution and incubated for 16 h at 37°C. The sample was analysed by LC-MS with MS-MS and high resolution mass spectra. The spectra of the modified peptides are shown in figure 5 and the corresponding peptides in table S2.

IC_{50}-Determination of AADH

The reaction mixture (95 µL total volume) contained 100 mM Tris-HCl, 0.10 M KCl, 10 mM β-mercaptoethanol and 0.63 µg AADH and different concentrations of the probes **1**, **2a/b**, **3a/b** and **4a/b**. All samples were pre-incubated with the probe or DMSO (control) for 15 min at room temperature. The reaction was started by adding 0.66 mM β-NAD^+ (3.3 µL) and 1.60 mM acetaldehyde (1.6 µL) and was performed at room temperature. The product formation was monitored by measuring the absorption increase at λ = 340 nm in the reaction and control mixture respectively. All measurements were carried out as triplicates. The results are shown in figure S2 and figure 8.

Antibacteria activity assay

Overnight-cultures of *Pseudomonas putida*, *Bacillus licheniformis*, *Listeria welshimeri* and *Staphylococcus aureus* NCTC 8325 were used to innoculate LB medium (100 µl/well) in a 96 well plate. The antibacterial activity was determined only for probes with strong labeling pattern in the corresponding proteomes. For

Pseudomonas putida probes **1** and **2a/b**, for *Bacillus licheniformis* probe **1** and **5**, for *Listeria welshimeri* probes **1** and **3a/b** and for *Staphylococcus aureus* NCTC 8325 probes **1**, **3a/b** and **6** were added at 500 µM, each. All samples and controls without probes were carried out in triplicates. The samples were incubated in a 96 well plate for 16 h at 37°C. The optical density of each sample was compared with its controls.

Labeling of γ-glutamyltranspeptidase (γ-GT)

To 500 ng of purified γ-GT in 50 µl PBS probe **1** (50 µM) was added and the mixture incubated for 1 h at room temperature. For comparison 500 ng of purified AADH and PCDH were treated likewise with 50 µM of probe **1**. CC reagents were added and the fluorescence recorded after SDS-PAGE (supporting figure S3).

Labeling of recombinant proteins

An equivalent of 100 µL OD_{600} = 2.0 of *E. coli* BL21 after expression of their corresponding recombinant proteins was harvested by centrifugation and resuspended in 50 µL PBS. A control contained the same amount of non-induced cells. Cells were lysed by sonication with a Bandelin Sonopuls with 3x 20 sec. pulsed at 70% max. power under ice cooling. Membrane and cytosolic fractions were separated by centrifugation at 13.000 rpm for 10 min. 43 µl of the cytosolic fraction were incubation with 1 µL probe at different concentrations (200 µM for probe **1** and 100 µM for probe **3a/b**) followed by CC and SDS-PAGE. For heat controls the proteome was denatured with 2 µL of 21.5% SDS (1% in total) at 95 °C for 7 min and cooled to room temperature before the probe was applied.

Table S1: List of labeled target enzymes.

Organism	**Identified enzyme**	**Enzyme class**	**Function**
B. licheniformis	1-pyrroline-5-carboxylate dehydrogenase (PCDH)	Oxido-reductase	glutamate/proline metabolism
	alcohol dehydrogenase (AlcDH)	Oxido-reductase	alcohol metabolism
L. welshimeri	bifunctional aldehyde-CoA / alcohol dehydrogenase (AADH)	Oxido-reductase	alcohol/aldehyde metabolism
	3-oxoacyl-[acyl-carrier-protein] synthase III (FabH)	Acyl-transferase	fatty acid biosynthesis
P. putida	aldehyde dehydrogenase (ADH)	Oxido-reductase	aldehyde metabolism
S. aureus NCTC 8325	aldehyde dehydrogenase (AldA)	Oxido-reductase	aldehyde metabolism
	aldehyde dehydrogenase (ADH)	Oxido-reductase	aldehyde metabolism
	3-oxoacyl-[acyl-carrier-protein] synthase III (FabH)	Acyl-transferase	fatty acid biosynthesis

Table S2: MS results of tryptically digested AADH after labeling with probe **1**. Modified cysteine residues (C#) are highlighted in red.

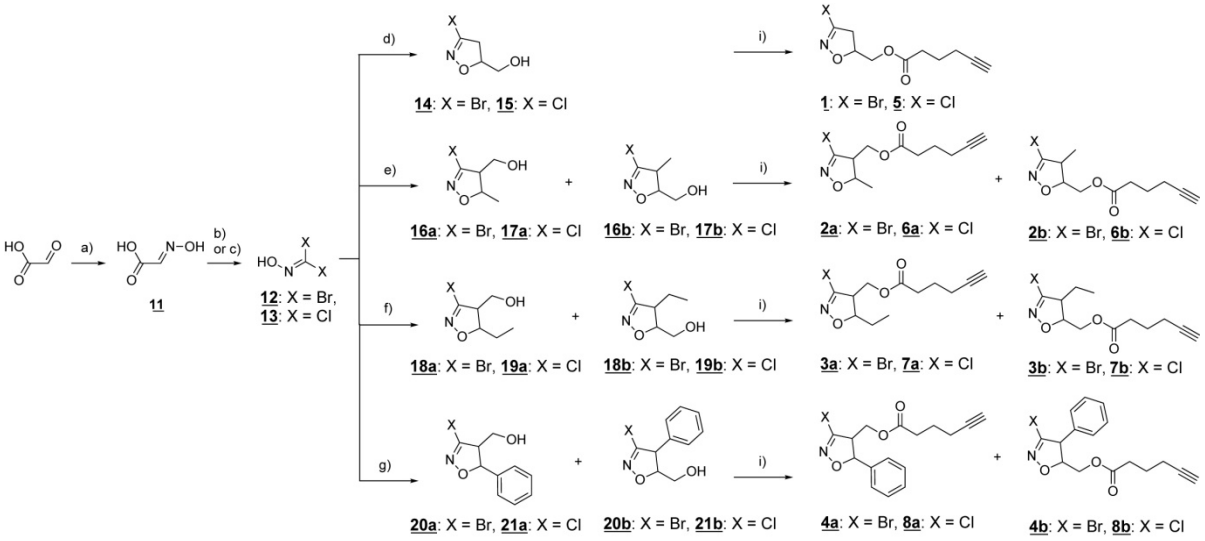
Found peptide ion	Assigned peptide Sequence	assigned AADH sequence position
b_4^{+1}	SFDQ	248 to 251
b_7^{+1}	SFDQGMI	248 to 254
b_9^{+1}	SFDQGMIC#A	248 to 256
b_{10}^{+1}	SFDQGMIC#AS	248 to 257
b_{11}^{+1}	SFDQGMIC#ASE	248 to 258
b_{12}^{+1}	SFDQGMIC#ASEQ	248 to 259
b_{13}^{+1}	SFDQGMIC#ASEQA	248 to 260
b_{14}^{+1}	SFDQGMIC#ASEQAV	248 to 261
y_9^{+1}	SEQAVIVDK	257 to 265
y_{11}^{+1}	C#ASEQAVIVDK	255 to 265
y_{12}^{+1}	IC#ASEQAVIVDK	254 to 265
y_{14}^{+1}	GMIC#ASEQAVIVDK	252 to 265

Table S3: Target proteins identified by mass spectrometry.

Species	Protein	Protein ID	MW	R	min. p Value	NP
B. licheniformis ATCC 14580	1-Pyrroline-5-carboxylate dehydrogenase (PCDH)	Q65NN2	56655	1	$2.2 \cdot 10^{-15}$	14
				2	$5.6 \cdot 10^{-10}$	19
				3	$7.0 \cdot 10^{-07}$	5
				4	$1.9 \cdot 10^{-08}$	6
	Alcohol dehydrogenase (hypothetical), (AlcDH)	Q65CW1	95008	1	$2.5 \cdot 10^{-13}$	19
				2	$3.3 \cdot 10^{-15}$	27
				3	$6.7 \cdot 10^{-14}$	24
L. welshimeri SLCC 5334 serovar 6b	Bifunctional aldehyde-CoA/alcohol dehydrogenase (AADH)	YP_849847	94594	1	$2.2 \cdot 10^{-12}$	25
				2	$4.2 \cdot 10^{-11}$	30
	3-Oxoacyl-[acyl-carrier-protein] synthase III (FabH)	A0AKV5	34029	1	$4.2 \cdot 10^{-13}$	9
				2	$2.0 \cdot 10^{-07}$	3
				3	$3.0 \cdot 10^{-12}$	7
				4	$4.9 \cdot 10^{-13}$	12
				5	$8.3 \cdot 10^{-15}$	14

P. putida KT2440	Aldehyde dehydrogenase (ADH)	Q88CA3	53054	1 2	$7.7 \cdot 10^{-07}$ $5.6 \cdot 10^{-15}$	4 15
S. aureus NCTC 8325	Aldehyde dehydrogenase (putative) (AldA)	Q2G1J0	53659	1	$3.1 \cdot 10^{-12}$	5
	Aldehyde dehydrogenase (putative) (ADH)	Q2FWX9	51741	1 2	$1.2 \cdot 10^{-08}$ $7.7 \cdot 10^{-09}$	3 5
	3-Oxoacyl-[acyl-carrier-protein] synthase III (FabH)	Q2FZS0	33879	1 2 3	$1.7 \cdot 10^{-08}$ $1.9 \cdot 10^{-06}$ $5.7 \cdot 10^{-11}$	2 3 7

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



Scheme S1: Synthesis of the probes **1** to **8**. a) NH_2OH, H_2O, 16h, rt, b) for dibromide: Br_2, H_2O, 3h, 0°C to rt, c) for dichloride: *N*-chloro succinimide, DME, 10 min, 110°C to rt, d) allyl alcohol, K_2CO_3, EtOAc, 24 h, rt, e) *cis*/*trans* crotyl alcohol, K_2CO_3, EtOAc, 24 h, rt, f) *cis*-2-penten-1-ol, K_2CO_3, EtOAc, 24 h, rt, g) *trans* cinnamyl alcohol, K_2CO_3, EtOAc, 24 h, rt, h) hexynol K_2CO_3, EtOAc, 24 h, rt, i) hexynoic acid chloride, DCM, TEA, 16h, rt.

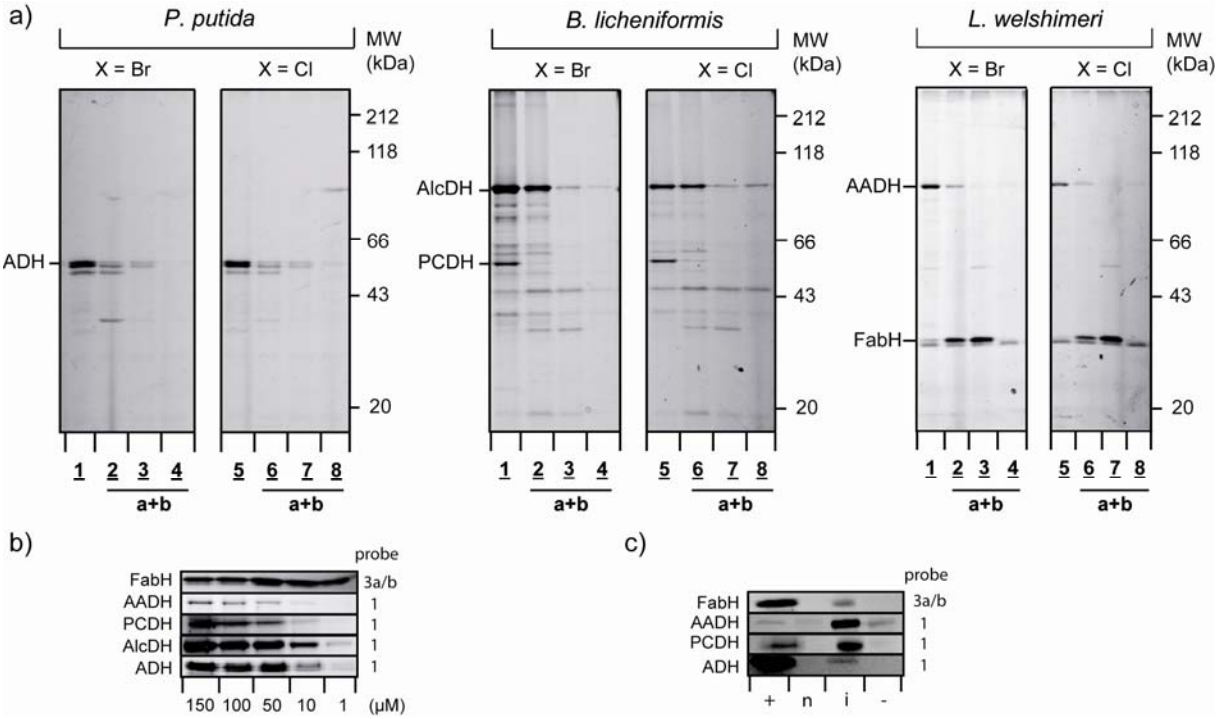


Figure S1: a) *In situ* labeling of *B. licheniformis*, *P. putida*, *L. welshimeri* using probes **1** to **8** (1h incubation). Aldehyde dehydrogenase (ADH, 53.0 kDa) was detected in *P. putida*, whereas alcohol dehydrogenase (AlcDH, 94.9 kDa) and 1-pyrroline-5-carboxylate dehydrogenase (PCDH, 56.7 kDa) were detected in *B. licheniformis*. Bifunctional aldehyde-CoA / alcohol dehydrogenase (AADH, 94.5 kDa) and 3-oxoacyl-[acyl-carrier-protein] synthase III (FabH, 34.0 kDa) were detected in *L. welshimeri*. b) Titration of probes **1** and **3** in *in situ* labeling experiments. The final concentration of the probe is given in µM. c) *In vitro* labeling of recombinant, overexpressed target enzymes (+: positive control using native proteome, n: not induced, i: induced, -: heat control using induced proteome).

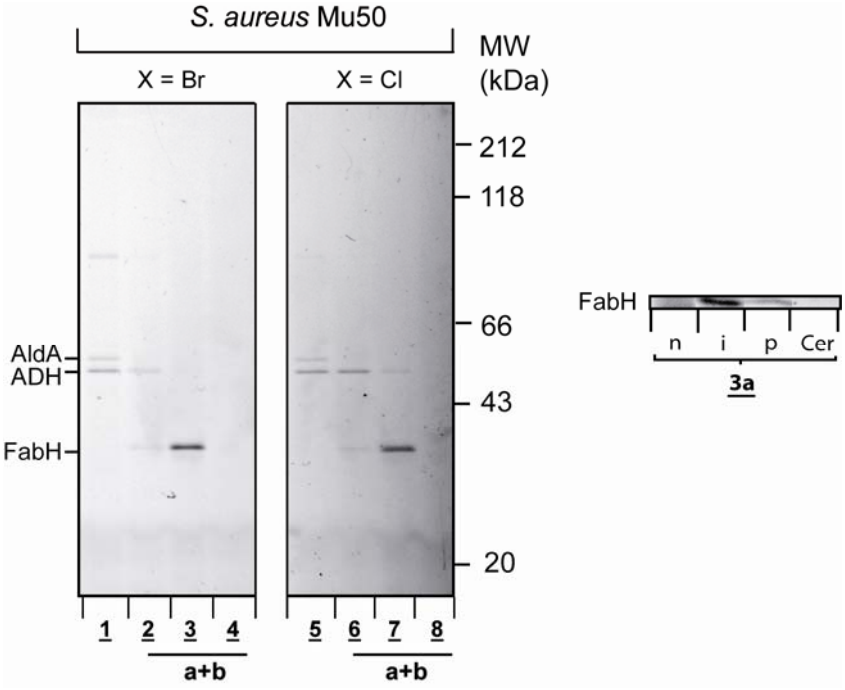
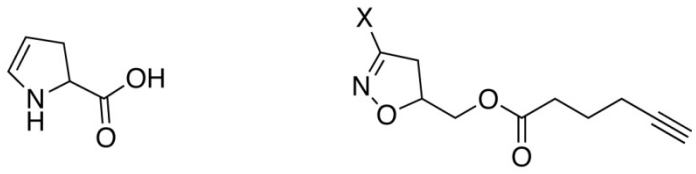


Figure S2: Labeling of the MRSA strain Mu50 by the probe library. FabH from *S. aureus* Mu50 was recombinantly overexpressed and labelled with probe **3a** before (n) and after induction (i). Active site labelling was confirmed by a pre-incubation of FabH with a 100 fold excess of cerulenin (Cer), a specific FabH inhibitor, and subsequent labelling with **3a**. While the purified enzyme without competitive inhibitor (p) was labelled, the cerulenin (Cer) treated sample did not show any labelling which confirms the probe specificity.



Pyrroline-5-carboxylate

1: X = Br; **5**: X = Cl

Figure S3: Structure of PCDH's substrate pyrroline-5-carboxylate and probes **1** and **5**.

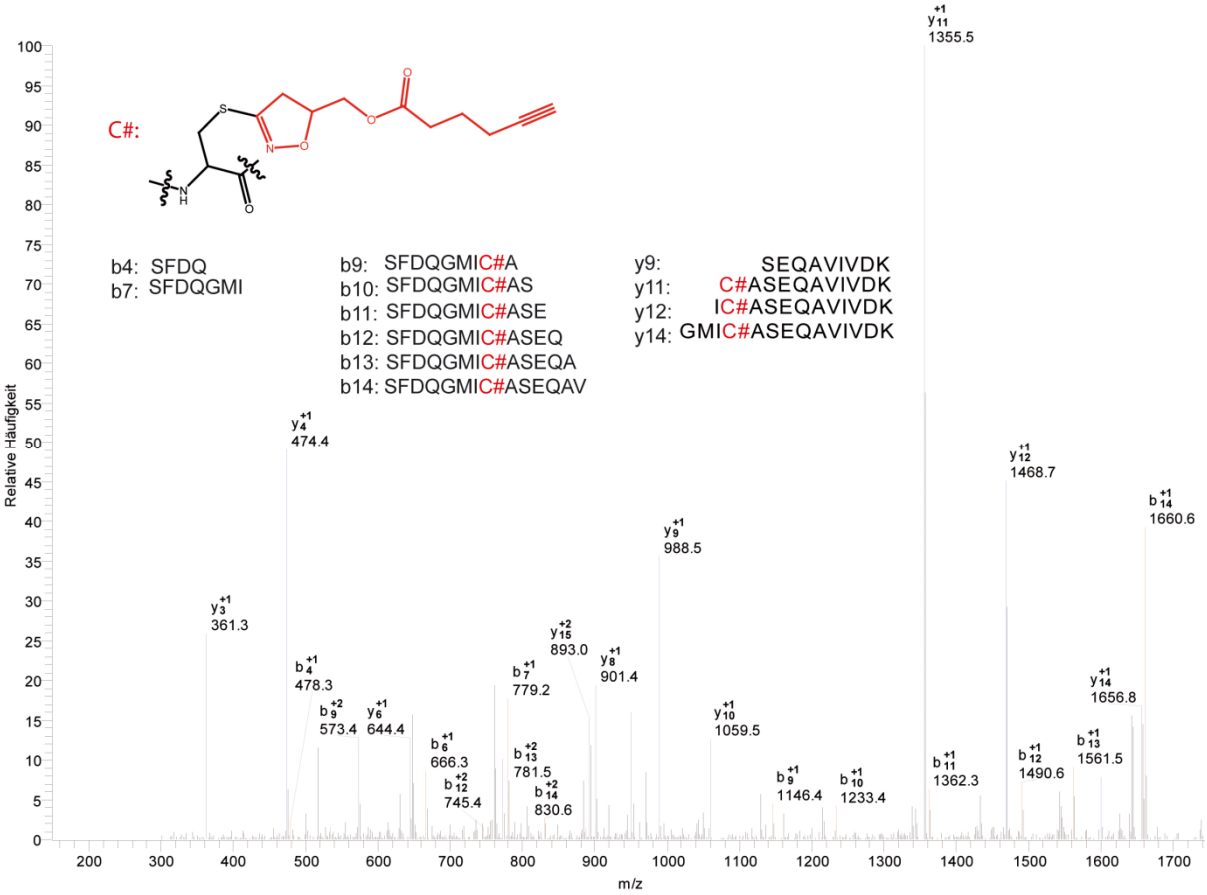


Figure S4: Detection of the modified C255 (C#) of AADH by MS.

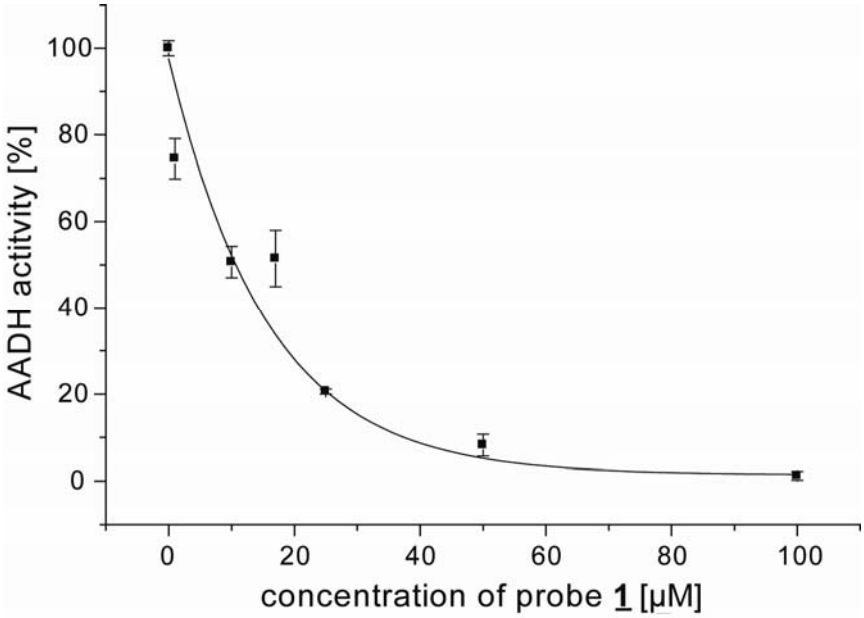


Figure S5: IC_{50} measurement of AADH using probe **1**.

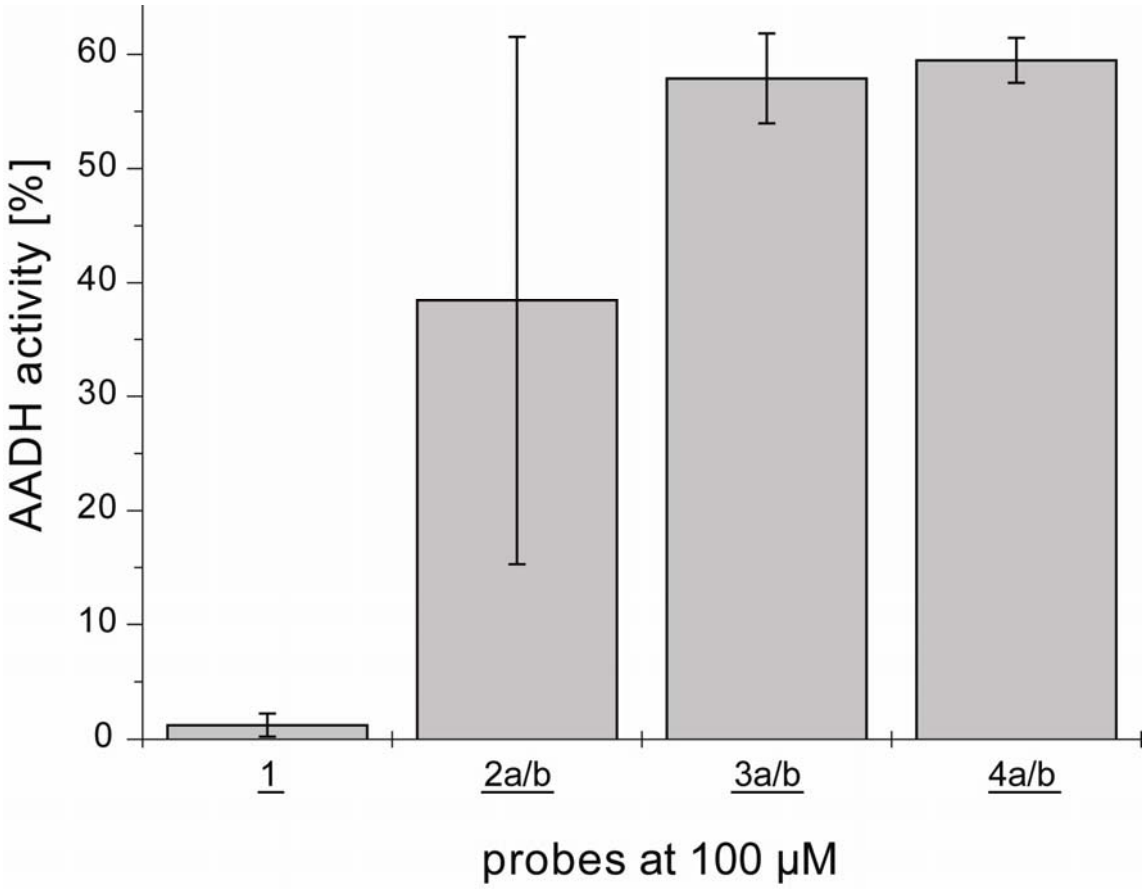


Figure S6: Activity of AADH at 100 µM probe concentration.

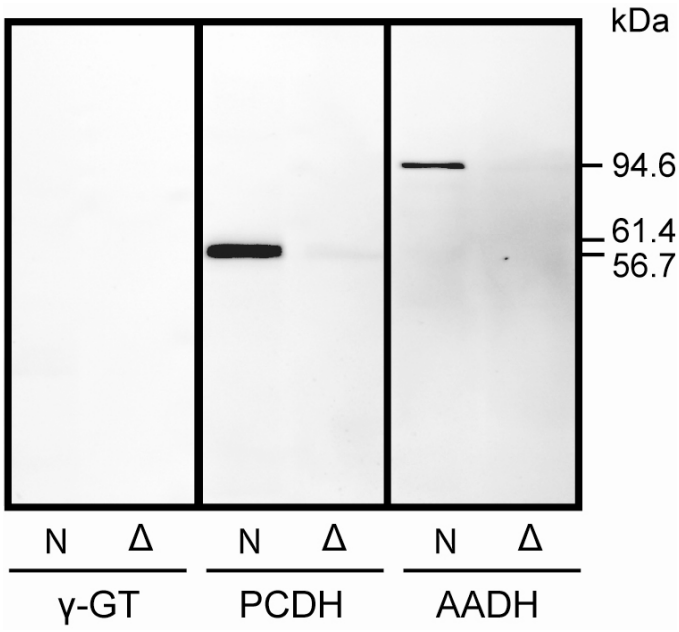
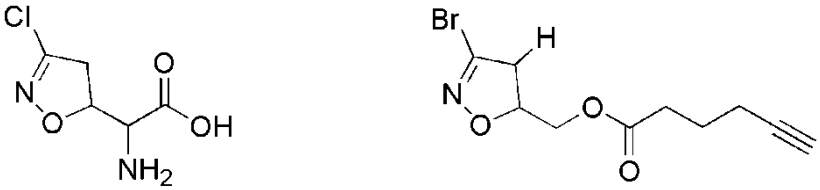


Figure S7: γ-GT is not labeled by 50 μM probe **1** while PCDH and AADH are strongly labeled (N: native protein, Δ: Heat control).



Acivicin **1**

Figure S8: Structure of acivicin and probe **1**.

References

1. Halling, K.; Thomsen, I.; Torssell, K. B. G., Synthesis of 3-Hydroxy-4-amino Acids and Butyrolactones via the Isoxazoline Route. *Liebigs Ann. Chem.* **1989**, 958-990.

2. Böttcher, T.; Sieber, S. A., Beta-lactones as privileged structures for the active-site labeling of versatile bacterial enzyme classes. *Angew Chem Int Ed Engl* **2008,** 47, (24), 4600-3.

3. Speers, A. E.; Adam, G. C.; Cravatt, B. F., Activity-based protein profiling in vivo using a copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition. *J. Amer. Chem. Soc.* **2003,** 125, 4686-4687.

4. Sieber, S. A.; Niessen, S.; Hoover, H. S.; Cravatt, B. F., Proteomic profiling of metalloprotease activities with cocktails of active-site probes. *Nat Chem Biol* **2006,** 2, (5), 274-81.

5. Mirza, S. P.; Halligan, B. D.; Greene, A. S.; Olivier, M., Improved method for the analysis of membrane proteins by mass spectrometry. *Physiol Genomics* **2007,** 30, (1), 89-94.