Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 7120

www.rsc.org/obc

PAPER

Fluorescent analogs of the marine natural product psammaplin A: synthesis and biological activity†

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Received 11th May 2012, Accepted 6th July 2012
DOI: 10.1039/c2ob25909e

The symmetrical disulfide psammaplin A from the marine sponge *Pseudoceratina* sp. was synthesized and structurally altered by replacement of one of the α-(hydroxyimino)acyl units by a fluorescent 4-coumarinacetyl moiety. Thus, the first fluorescent analogs of psammaplin A were obtained. Structural variation also covered the substitution pattern of the phenyl ring. Cytotoxicity of psammaplin A against the mouse fibroblast cell line L-929 (IC_{50} 0.42 μg mL^{-1}) was about two-fold higher than that of the fluorescent hybrid compounds, whereas the disulfide containing two 4-coumarinacetyl moieties was inactive. Fluorescence microscopy revealed uptake of the 4-coumarinacetyl-α-(hydroxyimino)acyl hybrids into the cytoplasm leading to fluorescence in close proximity of the nuclear envelope, most likely in the Golgi apparatus. We did not observe strong fluorescence inside the nucleus, where most of the target histone deacetylases are located. We conclude that reduction of the disulfide probably takes place outside the nucleus. The psammaplin-derived thiol exhibited potent activity against histone deacetylase in the low nanomolar range, but diminished cytotoxicity. Antimicrobial activity of the new derivatives was also determined.

Introduction

The natural product psammaplin A (**1**) is a symmetrical disulfide with a cystamine linker functionalized on both sides by tyrosine-derived α-(hydroxyimino)acyl moieties. The compound was isolated in 1987 from the marine sponge *Psammaplysilla* (revised to *Pseudoceratina*) sp. by the Crews[1] and Scheuer[2] groups who reported cytostatic properties and activity against Gram-positive bacteria, respectively. Jung and co-workers determined ED_{50} and IC_{50} values between 100 nM and 1 μM against several cancer cell lines,[3] decreasing the percentage of human endometrial cancer cells in the S phase in favor of those in the G0/G1 and G2/M phases.[4] Psammaplin A (**1**) and its derivatives have become interesting for epigenetic therapy, since Bair, Crews and co-workers have identified **1** as a potent inhibitor of histone deacetylase (HDAC, IC_{50} 4.2 ± 2.4 nM), being more active than the benchmark natural product and hydroxamic acid trichostatin A.[5] Ho Jeong Kwon and co-workers[6] found that glutathione-depleted cells were insensitive against psammaplin A and concluded that **1** functions as a prodrug being reduced to the thiol inside the cell. This is supported by recent structure–activity studies by the Fuchter[7] and de Lera[8] groups. HDAC1 inhibition by the thiol proved to be stronger than by the disulfide and S-methylation of the thiol abolished the activity completely. Docking studies of psammaplin A-derived thiol **2** with the protein part of a trichostatin A–HDAC8 complex[8] and with HDAC1[7b] indicate that thiolate may serve as a ligand of Zn^{2+} (Fig. 1).

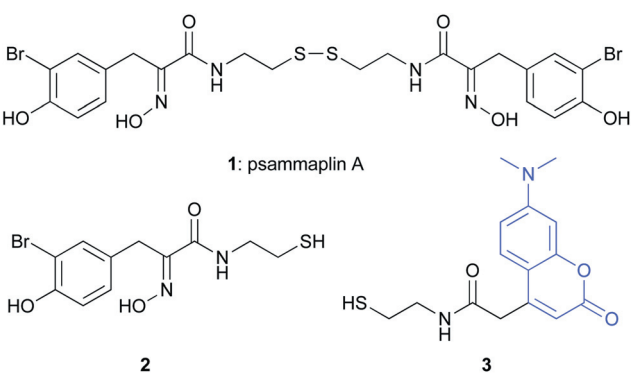

Fig. 1 Psammaplin A (**1**), reduced form **2**, and coumarin derivative **3**.

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†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of compounds **1**, **4**, **7–14**, **16**, **17**, **19**, **22–27**, **30**, **31**, **33** and **34**. See DOI: 10.1039/c2ob25909e

Results and discussion

Class I HDACs are located in the nucleus[9] and it has remained unclear whether reduction of the disulfide prodrug to the thiol takes place before or after the disulfide penetrates the nucleus. To address that question, we decided to synthesize fluorescent analogs of psammaplin A and to analyze their distribution in the cell by fluorescence microscopy. The architecture of the new fluorescent psammaplin analogs should allow reduction to a biologically active (**2**) and a fluorescent unit (**3**). If no fluorescence would be detected in the nucleus, reduction could either take place already outside the nucleus, or the coumarin unit would leave the nucleus rapidly after reduction. If, however, a fluorescent disulfide would bind inside the nucleus without reduction, this should be clearly visible under the microscope. The coumarin tag itself lacks strong biological activity and supports water solubility.[10] It was, of course, also to be tested whether our hybrid compounds were cytotoxic at all.

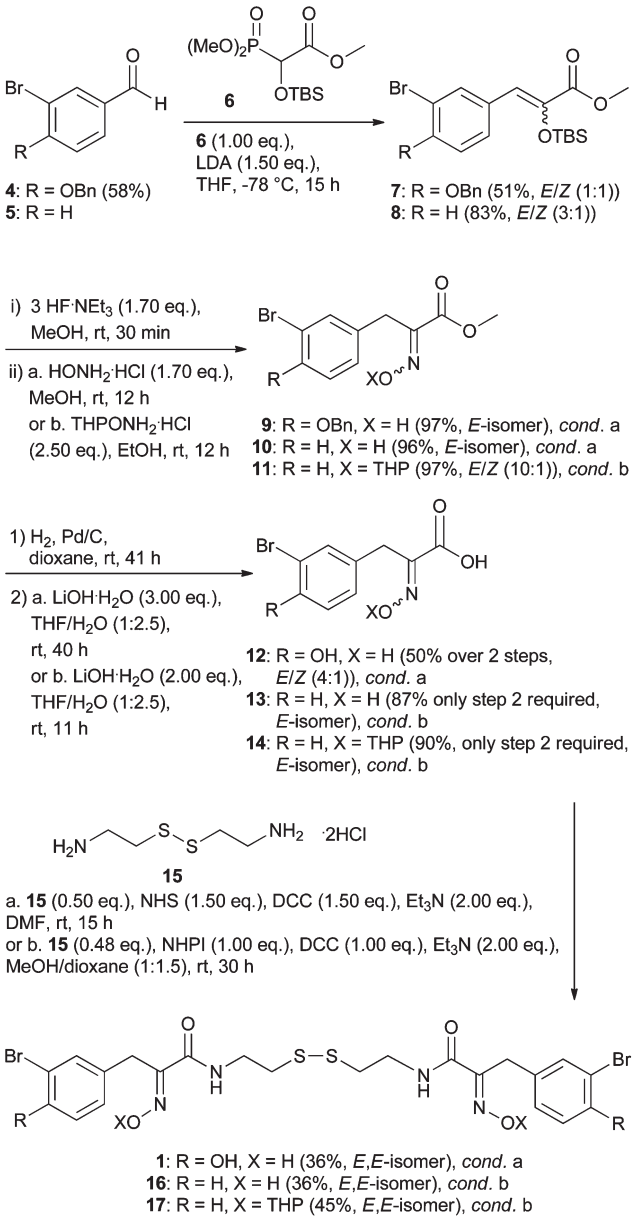
Synthesis

The synthesis of tyrosine-derived α-(hydroxyimino)amides from marine sponges has been reviewed comprehensively in 2010[11] and continues to be the subject of research.[7,8,12] An efficient route elongates substituted benzaldehydes by the Horner–Wadsworth–Emmons reaction employing Nakamura's α-OTBS-functionalized dimethylphosphonate **6**.[13] The resulting silylenolethers react smoothly to the corresponding oximes upon treatment with hydroxylamine derivatives.[14]

We synthesized psammaplin A (**1**) *via* the Horner–Wadsworth–Emmons (HWE) route originally established by Spilling.[14a] The HWE route has the advantage of making available derivatives of psammaplin A by varying the benzaldehyde building block. A similar approach was reported recently by Fuchter and co-workers.[7]

Bromination of *p*-hydroxybenzaldehyde[15] afforded an 8 : 1 mixture of 3-bromo and 3,5-dibromo-4-hydroxybenzaldehydes, which was benzylated without separation (Scheme 1). Column chromatography yielded aldehyde **4** (58%) together with its 3,5-dibrominated analog (7%). HWE reaction of **4** with phosphonate **6**[14a] gave ester **7** as *E*/*Z*-isomers (1 : 1, 51%). After desilylation of **7** with 3HF·NEt_3 oxime **9** was formed *in situ* by treatment with hydroxylamine hydrochloride (97%). Debenzylation (H_2/Pd–C) of **9** afforded the *E*/*Z*-hydroxyimino isomers in a ratio of 4 : 1 (51%). This monobrominated product was obtained recently by de Lera[8] after bromination with NBS of the corresponding debromo precursor. Saponification of the methyl ester with lithium hydroxide gave acid **12** (97%, *E*/*Z* 4 : 1), two equivalents of which were coupled in the final step with cystamine dihydrochloride (**15**) in the presence of NEt_3, DCC, and *N*-hydroxysuccinimide (NHS) in DMF[16] to afford the natural product psammaplin A (**1**, 36%, *E*,*E*-isomer, δ(benzylic CH_2) 28.4 in acetone-d_6). Psammaplin derivative **16** lacks the phenolic hydroxyl groups and was synthesized analogously to **1** starting from *m*-bromobenzaldehyde (**5**), with different amide coupling conditions employing *N*-hydroxyphthalimide (NHPI) instead of NHS and MeOH–dioxane (2 : 3) instead of DMF as solvent.[17]

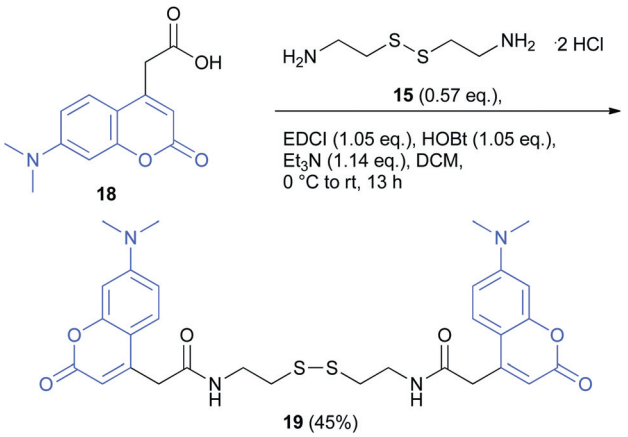
We also synthesized the doubly THP-protected didehydroxypsammaplin **17** using $THPONH_2$·HCl in the oxime forming step



Scheme 1 Synthesis of psammaplin A (**1**), didehydroxypsammaplin **16**, and THP-protected derivative **17**.

(Scheme 1). In all cases, the *E*-configuration of the oxime partial structures was major, as determined on the basis of NOESY experiments showing correlations between the oxime proton and the benzylic protons at 5-H and/or 6-H. *E*- and *Z*-isomers of THP-protected methyl ester **11** were separated by column chromatography and exhibit characteristic NMR shifts of the methylene group (acetone-d_6, *E*: δ_H 3.89, 4.01, δ_C 31.4 and *Z*: δ_H 3.72, 3.76, δ_C 37.2).

The coumarin unit of our fluorescent psammaplin moieties was synthesized following the Pechmann protocol by condensation of 1,3-acetonedicarboxylate and *m*-dimethylaminophenol, followed by saponification (LiOH–H_2O) of the ester affording 4-coumarinacetic acid **18** (21% over 2 steps).[18] Acid **18** was coupled with cystamine dihydrochloride (**15**) providing the symmetrical bis(coumarinyl) analog **19** of psammaplin A (45%, Scheme 2).



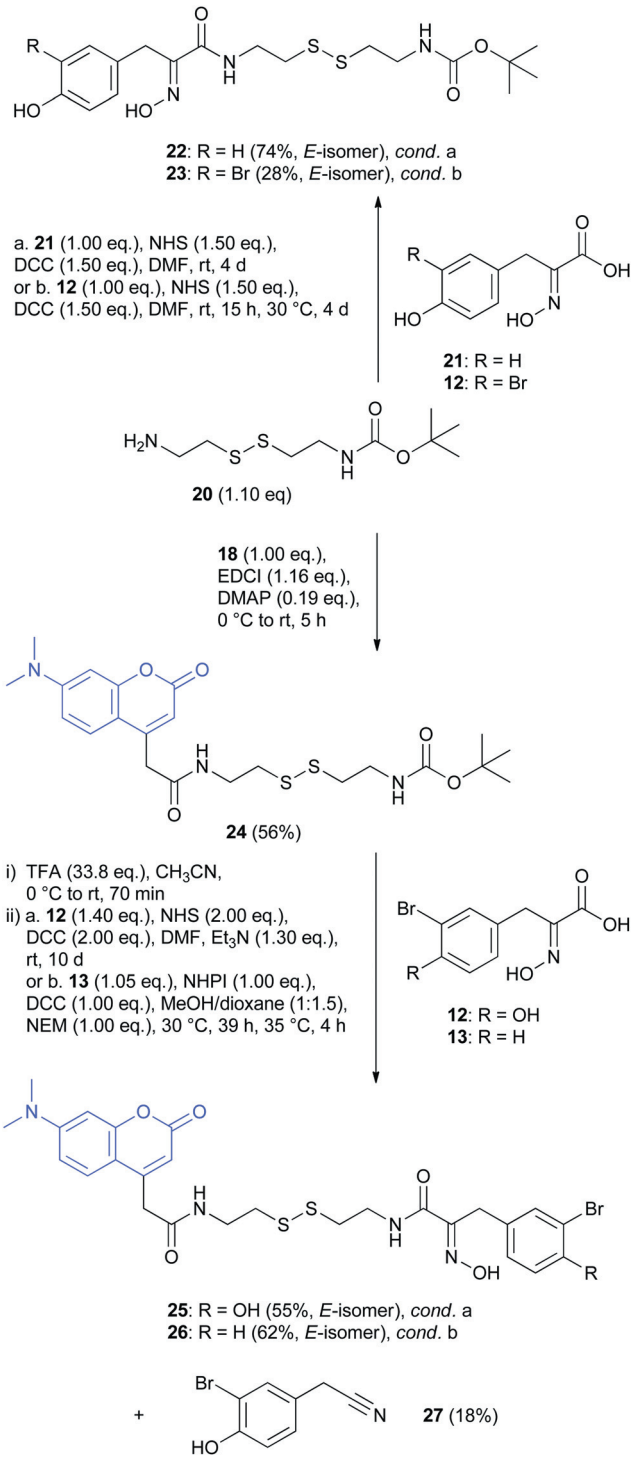
Scheme 2 Synthesis of the bis(coumarinyl) analog **19** of psammaplin A.

For the synthesis of non-symmetrical psammaplin derivatives, desymmetrization of cystamine was given preference over disulfide metathesis,[19] because the latter gives only a statistical 1 : 2 : 1 ratio of starting materials and hybrid products. Cystamine dihydrochloride (**15**) was mono-Boc protected to afford amine **20** (50%)[20] and then coupled with the 4-coumarinacetic acid **18** to disulfide **24** (56%, Scheme 3).[21] After Boc removal **24** was coupled *in situ* with α-(hydroxyimino) acids **12** and **13**, respectively, to afford the fluorescent hybrid psammaplin A derivatives **25** (55%) and **26** (62%), respectively. Mono-Boc-protected cystamine **20** was also coupled with α-(hydroxyimino) acids **21** and **12** to obtain amides **22** (74%) and **23** (28%). The *p*-hydroxy acid **21** (98%) was synthesized from *p*-hydroxyphenylpyruvic acid.[16]

As a side reaction of the amide couplings, decarboxylation and dehydration of the α-(hydroxyimino) acid to the benzylnitriles was observed, such as formation of **27**, which was isolated in 18% yield. This confirms an earlier finding by Spilling *et al.* converting α-(hydroxyimino) esters to the benzylnitrile in high yield by treatment of the free acid with TFA.[14a] In the presence of DCC–NEt_3, the nitrile is also known to be formed.

Interestingly, by changing the base from NEt_3 to pyrrolidine (**28**) in a coupling reaction with cystamine dihydrochloride we obtained the pyrrolidine-derived tertiary amide **30** (42%) as the only product. This reaction was then transferred to afford the corresponding derivative **31** by coupling the acid **21** with thiazolidine (**29**)[22] (40%, Scheme 4).

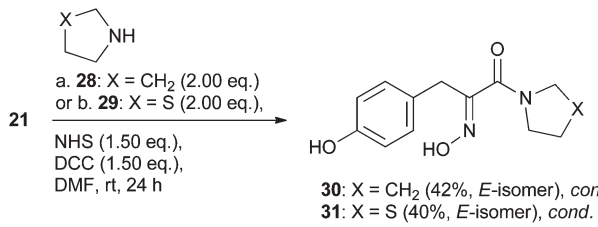
Thiol **33** was synthesized by treatment of disulfide **22** with dithiothreitol (DTT, **32**, 40%, RP chromatography)[12,14a] and was stable in methanol-d_4. In acetone-d_6 we observed formation of the symmetrical disulfide **34** (Scheme 5), indicated by intensity decrease of the signals of the methylene protons of the thiol (**33**, δ = 3.42 for NCH_2 and δ = 2.63 for SCH_2) in favour of those of the disulfide (**34**, δ = 3.56 for NCH_2 and δ = 2.87 for SCH_2).



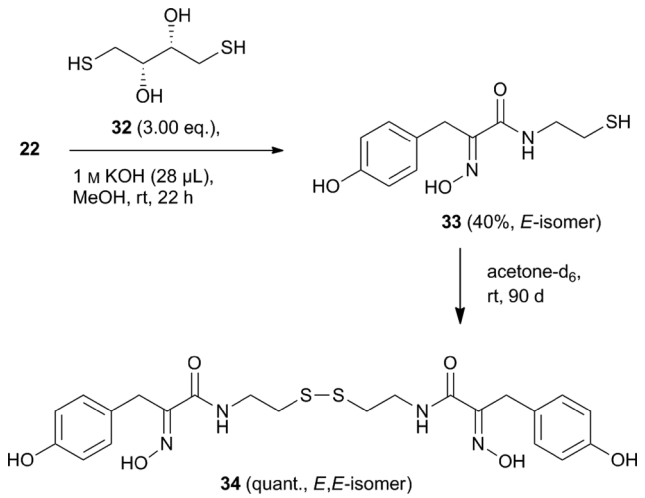
Scheme 3 Synthesis of fluorescent psammaplin hybrids **25** and **26** and of the psammaplin derivatives **22** and **23**.

Biological activity

Antibacterial activity. In the agar diffusion test, we observed moderate activity of the natural product psammaplin A (**1**) against Gram-positive bacteria. Against *Micrococcus luteus* the inhibition zone diameter was 11 mm (20 μg per disk, diameter 6 mm), corresponding to an IC_{50} of 6.7 μg mL^{-1} in a serial dilution assay. Similar activities were determined against *Mycobacterium phlei* (12 mm) and *Staphylococcus aureus* (12 mm), whereas Gram-negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were not sensitive. Only the *E. coli* TolC mutant



Scheme 4 Synthesis of pyrrolidine and thiazolidine derivatives **30** and **31**.



Scheme 5 Synthesis of thiol **33** and oxidation to disulfide **34**.

was slightly sensitive (8 mm). None of the other psammaplin A derivatives did show any inhibition at all.

Antibacterial activity of psammaplin A (**1**) against *S. aureus* compares well with earlier work. It was known that antibacterial activity of psammaplin A (**1**) is rather selective against Gram-positive *Staphylococcus aureus* and, to a lesser extent, *Streptococcus pyogenes*, but does not inhibit, for instance, Gram-negative *Pseudomonas aeruginosa*, as reported already by Scheuer and co-workers.[2] Activity against methicillin-resistant *S. aureus* (MRSA) was quantified by Sung-Il Yang and co-workers with average minimum inhibitory concentrations (MICs) ranging from 0.78 to 6.25 μg mL^{-1}. Gram-negative *Escherichia coli*, *Klebsiella oxytoca*, and *Enterobacter cloaca* were not sensitive against psammaplin A (**1**).[23] Nicolaou and co-workers found MICs of 5.47 μg mL^{-1} and 3.90 μg mL^{-1} against four strains of *S. aureus* and five strains of MRSA, respectively.[19] In our study, a *Micrococcus* species proved to be sensitive against psammaplin A (**1**) for the first time. It is somewhat surprising that none of the other synthesized psammaplin A analogs sharing the α-(hydroxyimino)acyl unit was active against Gram-positive bacteria, since Nicolaou and co-workers had identified several psammaplin A analogs with activity against MRSA, in which one of the α-(hydroxyimino)acyl units had been replaced by oxime-free moieties by disulfide metathesis.[19] The disulfide bridge is necessary for antibacterial activity. Differing from the activity pattern of HDAC inhibition (*vide infra*), Nicolaou identified a disulfide–thiol pair among which only the disulfide was antibacterial, but not the thiol.[19b] The newly discovered activity of psammaplin A (**1**) against *Mycobacterium phlei* may be related to results by Bewley and co-workers reporting an IC_{50} of 2.8 ± 0.5 μg mL^{-1} of psammaplin A (**1**) against the mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis*.[24]

Table 1 Cytotoxicity and HDAC inhibitory activity of the psammaplin derivatives

Compound	IC_{50} [a] (μg mL^{-1})	IC_{50} [a] (μM)	IC_{50} [b] (μM)
1	0.42	0.63	0.028
16	0.31	0.49	0.25
19	>40	Not active	>1.25
25	0.93	1.46	0.011
26	1.10	1.76	0.50
22	1.80	4.19	1.25
23	1.40	2.75	0.10
24	>40	Not active	>1.25
13	>40	Not active	>1.25
27	>40	Not active	>1.25
30	>40	Not active	>1.25
31	>40	Not active	>1.25
33	3.00	12.48	0.011
34	1.40	2.76	0.34
17	>40	Not active	>1.25

[a] Against the L-929 mouse fibroblast cell line. [b] Against HDAC.

Cytotoxicity. A few more of our compounds exhibit cytotoxic activity. Psammaplin A (**1**) was cytotoxic against the L-929 mouse fibroblast cell line (IC_{50} 0.42 μg mL^{-1}, Table 1). Didehydroxypsammaplin A (**16**) showed a similar cytotoxicity (IC_{50} 0.31 μg mL^{-1}). The fluorescent 4-coumarinacetyl-α-(hydroxyimino)acyl hybrids **25** and **26** retained cytotoxicity in the range 1 μg mL^{-1} (IC_{50}). Boc derivatives with only one α-(hydroxyimino)acyl moiety were also cytotoxic, among which phenol **22** (IC_{50} 1.80 μg mL^{-1}) was less active than psammaplin derivative **23** (IC_{50} 1.40 μg mL^{-1}). Bis- and mono(coumarinyl) analogs **19** and **24** lacking α-(hydroxyimino)acyl moieties were not active. Compounds without the disulfide bridge, such as acid **13**, nitrile **27**, *p*-hydroxyphenylacetonitrile, pyrrolidine derivative **30** and also thiazolidine derivative **31**, were not cytotoxic. Equally, derivative **17** containing THP-protected oximes was not active. Thiol **33** showed decreased cytotoxicity (IC_{50} 3 μg mL^{-1}) when compared to the corresponding disulfide **34**. Thus, cytotoxicity against L-929 was exhibited only by those psammaplin A derivatives, which contained a free oxime moiety and either a disulfide or a thiol. This agrees with previously reported studies. We also can confirm that for cytotoxicity only one α-(hydroxyimino)acyl moiety is required, because Boc derivatives **22** and **23** were cytotoxic. The phenolic hydroxy group of psammaplin A (**1**) was not necessary for cytotoxicity, since didehydroxypsammaplin A (**16**) was as active as the natural product. Absence of the bromine substituent (**34**) led to only a minor loss of cytotoxicity.

HDAC inhibition. Table 1 also lists the HDAC inhibitory activities of our compounds, which were obtained using a fluorometric HDAC assay using HeLa cell lysates as an HDAC source. Psammaplin A (**1**, IC_{50} 0.028 μM) and derivative **16** (IC_{50} 0.25 μM) showed HDAC inhibitory activity in the range of the potent natural product trichostatin A (IC_{50} 0.030 μM). The most

potent HDAC inhibitors among our compounds are the coumarin–psammaplin hybrid **25** (IC_{50} 0.011 μM) and thiol **33** (IC_{50} 0.011 μM), which was found to be 30-fold more potent than its disulfide derivative **34** (IC_{50} 0.34 μM). None of the non-cytotoxic compounds showed HDAC inhibition.

The observed cytotoxicities against L-929 correlate well with HDAC inhibition. As an exception, thiol **33** showed decreased cytotoxicity and increased HDAC inhibitory activity compared to the corresponding disulfide **34**, confirming Fuchter's finding[7b] that the thiols are more potent than the corresponding disulfides. However, thiol **33** was the less active compound in the cytotoxicity study.

While this work was in progress, a comprehensive study was reported by Altucci, de Lera, and co-workers who conclude that the apoptotic effect of **1** on the human leukemia cell line U937 is due to inhibition of Zn^{2+}-dependent HDACs, mainly of HDAC1, but also of HDAC4, not of HDAC6.[8] Fuchter and co-workers observed selectivity by a factor of more than 20 in favor of HDAC1 over HDAC6 and reported recently an IC_{50} of 45 nM against HDAC1 for **1** and an IC_{50} of 0.9 nM for the thiol.[7]

Fluorescence microscopy. Fluorescent hybrid compounds **25** and **26** are cytotoxic (IC_{50} 1 μg mL^{-1}), interestingly at approximately 50% of the cytotoxicity of psammaplin A (**1**). Since the coumarin part alone is not cytotoxic, as shown by bis- and mono (coumarinyl) analogs **19** and **24**, the cytotoxicity of **25** and **26** must be caused by the α-(hydroxyimino)acyl section. Fig. 2 shows the live cell images obtained of L-929 cells incubated with **25** and **26**, which were both taken up into the cytoplasm and led to fluorescence in close proximity of the nuclear envelope, probably in the Golgi apparatus. We observed only minor fluorescence inside the nucleus, where HDAC1 is located.[9] Non-cytotoxic bis- and mono(coumarinyl) analogs **19** and **24** lacking α-(hydroxyimino)acyl sections also caused fluorescence near the nuclear envelope (see the ESI†). Thus, the coumarin units of the hybrid and non-hybrid compounds are probably metabolized in a similar manner, indicating early cleavage of the disulfide bonds after penetrating the cells. The cytotoxic *N*-α-(hydroxyimino)acyl cysteamine unit, possibly glutathione-bound, would penetrate the nuclear envelope and inhibit HDAC1 in the nucleus.

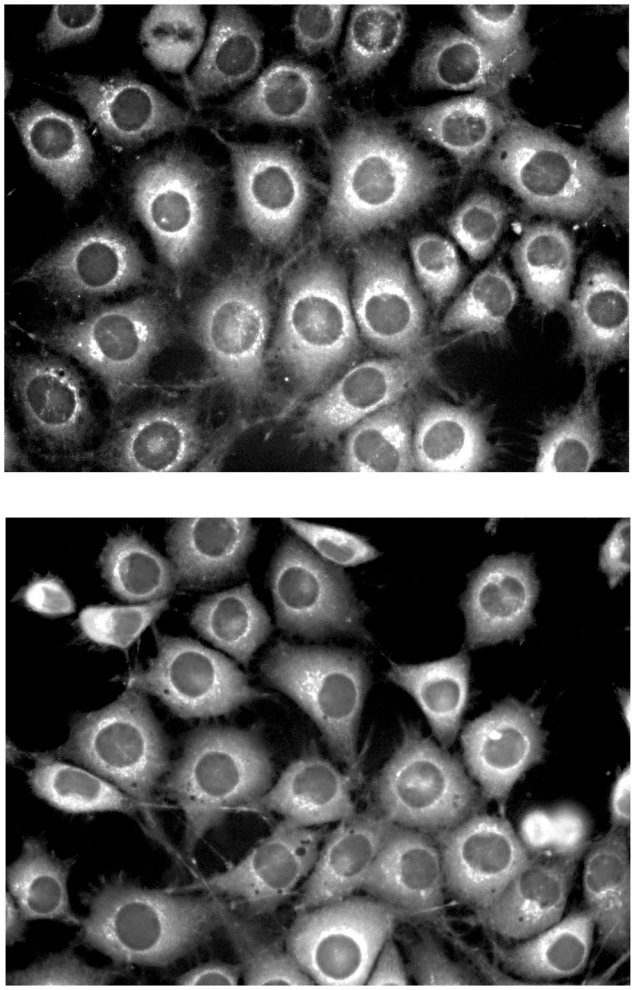


Fig. 2 Live cell imaging of fluorescent compounds **25** (above) and **26** (below) in L-929 mouse fibroblast cells.

Conclusion

In summary, we report the synthesis of the first fluorescent psammaplin A (**1**) derivatives by replacement of one of the tyrosine-derived α-(hydroxyimino)acyl units by a 4-coumarinacetyl moiety. Interestingly, the cytotoxicity of psammaplin A (**1**) against the mouse fibroblast cell line L-929 (IC_{50} 0.42 μg mL^{-1}) is about two-fold higher than that of the fluorescent hybrid compounds **25** and **26**, whereas the disulfide containing two 4-coumarinylacetyl moieties is inactive.

Activity of the natural product against Gram-positive bacteria in the single-digit micromolar range is confirmed, for the first time against *Mycobacterium phlei*. However, we did not identify more potent antibacterial derivatives of psammaplin A (**1**).

We report the first live cell imaging study of fluorescent psammaplin derivatives. The strongest fluorescence is found in close proximity of the nuclear envelope, whereas only minor fluorescence is observed in the nucleus being the location of the thiol target enzyme HDAC1. We postulate that cleavage of the disulfide to the thiol takes place before entering the nucleus. For entering the cell, however, the disulfide appears to be necessary, because cytotoxicity is diminished for the thiol.

Consequently, we are currently working on the synthesis of psammaplin A analogs with coumarin-containing α-(hydroxyimino)acyl units which we expect to be enriched in the nucleus.

Experimental section

General methods

NMR spectra were taken with a Bruker DPX-200 (200.1 MHz for ^{1}H, 188.3 MHz for ^{19}F), a Bruker AV II-300 (300.1 MHz for ^{1}H, 75.5 MHz for ^{13}C), a Bruker DRX-400 (400.1 MHz for ^{1}H, 100.6 MHz for ^{13}C, 376.3 MHz for ^{19}F), a Bruker AV III-400 (400.1 MHz for ^{1}H, 100.6 MHz for ^{13}C) and a Bruker AV II-600 (600.1 MHz for ^{1}H; 150.9 MHz for ^{13}C), referenced to the solvent signal or TMS. All measurements were carried out at 300 K. Mass spectra were obtained with a ThermoFinnigan MAT (MAT95XL) spectrometer and a ThermoFisher Scientific

(LTQ-Orbitrap Velos) spectrometer. IR spectra were recorded with a Bruker Tensor 27 spectrometer. UV/Vis spectra were measured with a Varian Cary 100 Bio UV/Vis-spectrometer. Fluorescence spectra were measured with a Varian Cary Eclipse fluorescence spectrophotometer. Melting points were measured with a Büchi 530 melting point apparatus. Chemicals were purchased from commercial suppliers and used without further purification. Silica gel 60 (40–63 µm, Merck) and silica gel LiChroprep RP-18 (40–63 µm, Merck) were used for column chromatography. The used petroleum ether (PE) had a boiling range from 40 to 60 °C. HPLC separation was carried out with a Merck Hitachi intelligent pump, fitted with a Phenomenex Luna C18(2) 5 µ column.

Syntheses

4-(Benzyloxy)-3-bromobenzaldehyde (4) and 4-(benzyloxy)-3,5-dibromobenzaldehyde. *p*-Hydroxybenzaldehyde (2.00 g, 16.4 mmol, 1.00 equiv.) was dissolved in $CHCl_3$–MeOH (10 : 1, 22 mL). A solution of Br_2 (0.84 mL, 2.62 g, 16.4 mmol, 1.00 equiv.) in $CHCl_3$ (70 mL) was added slowly and the reaction mixture was stirred for 22 h at rt. It was washed with H_2O (20 mL), saturated aqueous $Na_2S_2O_3$ solution (20 mL), and H_2O (4 × 20 mL) until pH neutrality. The organic phase was dried over $MgSO_4$, filtered and the solvent was evaporated. The resulting mixture of 3-bromo and 3,5-dibromo aldehyde was used directly for benzyl protection without further purification. A mixture of 3-bromo aldehyde (1.00 g, 4.98 mmol, 0.75 equiv.) and 3,5-dibromo aldehyde (0.47 g, 1.68 mmol, 0.25 equiv.) was dissolved in DMF (7.3 mL) under an argon atmosphere. K_2CO_3 (0.79 g, 5.69 mmol, 0.85 equiv.) and BnCl (0.80 mL, 0.88 g, 6.95 mmol, 1.04 equiv.) were added and the reaction mixture was heated at reflux for 75 min. After cooling to rt, 40 mL H_2O was added and the precipitate was filtered off. The crude product was purified by column chromatography on silica [PE–EtOAc (30 : 1)] to obtain **4** (1.36 g, 4.67 mmol, 58% over 2 steps) and the 3,5-dibromobenzaldehyde (0.23 g, 0.62 mmol, 7% over 2 steps) as colorless solids; **4**: TLC [silica, PE–EtOAc (30 : 1)]: R_f = 0.08; m.p.: 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.83 (s, 1H, C*H*O), 8.10 (d, ^4J = 2.0 Hz, 1H, 2-*H*), 7.77 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H, 6-*H*), 7.48–7.45 (m, 2H, *o*-Ph-*H*), 7.43–7.38 (m, 2H, *m*-Ph-*H*), 7.37–7.32 (m, 1H, *p*-Ph-*H*), 7.04 (d, ^3J = 8.5 Hz, 1H, 5-*H*), 5.25 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.5 (1C, *C*HO), 159.7 (1C, *C*q-4), 135.4 (1C, *C*qPh), 134.7 (1C, *C*-2), 131.0 (1C, *C*-6), 130.9 (*C*q-1), 128.7 (2C, *m*-Ph-*C*), 128.3 (1C, *p*-Ph-*C*), 126.9 (2C, *o*-Ph-*C*), 113.2 (1C, *C*-5), 113.0 (1C, *C*qBr), 71.0 (1C, *C*H_2); IR (ATR): $\tilde{\nu}$ = 1676 cm^{-1} (vs), 1589 (s), 1277 (s), 1255 (s), 1189 (s), 980 (s), 810 (s), 737 (s), 695 (s), 665 (s), 646 (s); UV-Vis (MeOH): λ_{max} (log ε) = 272 nm (4.21), 225 (4.26), 208 (4.40); MS (EI, 70 eV): *m*/*z* (%) = 292/290 (24/26) [M$^+$], 209 (7), 201 (5), 143 (3), 92 (9), 91 (100), 89 (3), 65 (11), 51 (2).

4-(Benzyloxy)-3,5-dibromobenzaldehyde. TLC [silica, PE–EtOAc (30 : 1)]: R_f = 0.25; m.p.: 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.87 (s, 1H, C*H*O), 8.06 (s, 2H, *H*-2, *H*-6), 7.60–7.57 (m, 2H, *o*-Ph-*H*), 7.48–7.36 (m, 3H, *m*-Ph-*H*, *p*-Ph-*H*), 5.12 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.7 (1C, *C*HO), 158.1 (1C, *C*q-4), 135.9 (1C, *C*qPh), 134.7 (1C, *C*q-1), 134.3 (2C, *C*-2, *C*-6), 129.0 (2C, *o*-Ph-*C*), 128.9 (3C, *m*-Ph-*C*, *p*-Ph-*C*), 120.1 (1C, *C*qBr), 75.4 (1C, *C*H_2); IR (ATR): $\tilde{\nu}$ = 1690 cm^{-1} (vs), 1362 (s), 1254 (vs), 1186 (s), 948 (s), 923 (s), 741 (s), 721 (s), 692 (s), 663 (s); UV-Vis (MeOH): λ_{max} (log ε) = 263 nm (3.81), 207 (4.61); MS (EI, 70 eV): *m*/*z* (%) = 373/370/368 (2/5/2) [M$^+$], 289 (7), 287 (7), 281 (12), 279 (22), 277 (10), 261 (4), 259 (3), 253 (15), 251 (36), 249 (16), 223 (7), 181 (3), 152 (3), 143 (4), 141 (4), 92 (21), 91 (100), 65 (30), 63 (13).

(*E*/*Z*)-Methyl-3-(4-(benzyloxy)-3-bromophenyl)-2-(*tert*-butyldimethylsilyloxy)acrylate (7). Diisopropylamine (0.71 mL, 5.03 mmol, 1.50 equiv.) was dissolved in dry THF (3.5 mL) under an argon atmosphere and cooled to 0 °C. Then *n*-BuLi (1.6 N solution in hexane, 3.14 mL, 5.03 mmol, 1.50 equiv.) was added dropwise and the mixture was stirred for 10 min at 0 °C and then for 30 min at −78 °C. A solution of phosphonate **6** (1.05 g, 3.35 mmol, 1.00 equiv.) in dry THF (2.5 mL) was added slowly and the reaction mixture was stirred for 30 min at −78 °C. A solution of aldehyde **4** (1.36 g, 4.67 mmol, 1.39 equiv.) in dry THF (4 mL) was added dropwise, the reaction mixture was stirred for 4 h at −78 °C and warmed to rt within 16 h. It was quenched with 15 mL of saturated aqueous NH_4Cl solution and diluted with 70 mL EtOAc. After washing with saturated aqueous NH_4Cl solution (3 × 15 mL), H_2O (3 × 15 mL) and saturated aqueous NaCl solution (3 × 15 mL), the organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica [PE–EA (30 : 1)] to give the *E*/*Z*-isomers **7** (1 : 1) as a colorless oil (0.82 g, 1.72 mmol, 51%); TLC [silica, PE–EtOAc (10 : 1)]: R_f = 0.46; ^{1}H NMR (400 MHz, $CDCl_3$): (*Z*)-**7**: δ = 8.12 (d, ^4J = 2.1 Hz, 1H, 2-*H*), 7.48–7.43 (m, 5H, Ph-*H*), 7.41–7.29 (m, 6H, Ph-*H* of *E*-**7** and 6-*H* of *Z*-**7**), 6.89 (d, ^3J = 8.6 Hz, 1H, 5-*H*), 6.73 (s, 1H, 7-*H*), 5.17 (s, 2H, CH_2Ph), 3.80 (s, 3H, COOCH_3), 0.98 (s, 9H, C(CH_3)$_3$), 0.16 (s, 6H, Si(CH_3)$_2$); (*E*)-**7**: δ = 7.49 (dd, $^6J_{H,Si}$ = 0.7 Hz, ^4J = 2.3 Hz, 1H, 2-*H*), 7.41–7.29 (m, 6H, Ph-*H* of *E*-**7** and 6-*H* of *Z*-**7**), 7.15 (ddd, $^6J_{H,Si}$ = 0.7 Hz, ^4J = 2.2 Hz, ^3J = 8.5 Hz, 1H, 6-*H*), 6.85 (d, ^3J = 8.5 Hz, 1H, 5-*H*), 6.29 (s, 1H, 7-*H*), 5.15 (s, 2H, CH_2Ph), 3.68 (s, 3H, COOCH_3), 0.97 (s, 9H, C(CH_3)$_3$), 0.21 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): (*Z*)-**7**: δ = 165.8 (1C, *C*qO), 154.6 (1C, *C*q-4), 139.8 (1C, *C*q-8), 136.3 (1C, *C*qPh), 134.4 (1C, *C*-2), 130.2 (1C, *C*-6), 128.6 (2C, 2× *m*-Ph-*C*), 128.5 (1C, Cq-1), 128.0 (1C, *p*-Ph-*C*), 127.0 (2C, 2× *o*-Ph-*C*), 117.3 (1C, *C*-7), 113.1 (1C, *C*-5), 112.3 (1C, *C*qBr), 70.8 (1C, *C*H$_2$Ph), 52.1 (1C, COO*C*H$_3$), 25.6 (3C, C(*C*H$_3$)$_3$), 18.6 (1C, *C*q(CH$_3$)$_3$), −3.6 (2C, Si(*C*H$_3$)$_2$); (*E*)-**7**: δ = 165.2 (1C, *C*qO), 154.2 (1C, *C*q-4), 141.6 (1C, *C*q-8), 136.4 (1C, *C*qPh), 133.7 (1C, C-2), 128.9 (1C, *C*-6), 128.6 (2C, 2× *m*-Ph-*C*), 128.5 (1C, Cq-1), 128.0 (1C, *p*-Ph-*C*), 127.0 (2C, 2× *o*-Ph-*C*), 119.2 (1C, *C*-7), 113.1 (1C, *C*-5), 111.9 (1C, *C*qBr), 70.8 (2C, *C*H$_2$Ph), 51.7 (1C, COO*C*H$_3$), 25.9 (3C, C(*C*H$_3$)$_3$), 18.3 (1C, *C*q(CH$_3$)$_3$), −4.8 (2C, Si(*C*H$_3$)$_2$); IR (ATR): $\tilde{\nu}$ = 1704 cm^{-1} (vs), 1244 (vs), 1127 (vs), 998 (s), 824 (s), 800 (s), 783 (s), 737 (s), 692 (vs); UV-Vis (MeOH): λ_{max} (log ε) = 296 nm (4.28), 203 (4.44); MS (EI, 70 eV): *m*/*z* (%) = 478/476 (<1/<1) [M$^+$], 340 (17), 330 (26), 328 (24), 315 (18), 313 (17), 234 (5), 213 (9), 211 (9), 91 (100), 89 (34), 75 (24), 73 (96), 59 (23), 45 (7); HREIMS: calcd for $C_{22}H_{26}BrO_4Si$ [M − 15]$^+$: 461.07782, found 461.07837.

(*E*)-Methyl-3-(4-(benzyloxy)-3-bromophenyl)-2-(hydroxyimino)-propanoate (9). Ester **7** (0.82 g, 1.72 mmol, 1.00 equiv.) was dissolved in 10 mL MeOH–$CHCl_3$ (4 : 1) under an argon atmosphere and $3HF{\cdot}NEt_3$ (4.76 mL, 4.71 g, 2.92 mmol, 1.70 equiv.) was added dropwise. After 30 min at rt was added $H_2NOH{\cdot}HCl$ (0.20 g, 2.92 mmol, 1.70 equiv.) in portions and the reaction mixture was stirred for another 19 h at rt. The solvent was evaporated and the residue was dissolved in 10 mL DCM and washed with H_2O (2 × 40 mL) and saturated aqueous $NaHCO_3$ solution (40 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The *E*-oxime **9** was obtained as a colorless solid (0.63 g, 1.66 mmol, 97%); TLC [silica, PE–EtOAc (5 : 1)]: R_f = 0.08; m.p.: 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.66 (sbr, 1H, NO*H*), 7.53 (d, ^4J = 2.2 Hz, 1H, 2-*H*), 7.46–7.44 (m, 2H, *o*-Ph-*H*), 7.39–7.35 (m, 2H, *m*-Ph-*H*), 7.33–7.29 (m, 1H, *p*-Ph-*H*), 7.18 (dd, ^4J = 2.2 Hz, ^3J = 8.4 Hz, 1H, 6-*H*), 6.83 (d, ^3J = 8.5 Hz, 1H, 5-*H*), 5.12 (s, 2H, OCH_2), 3.89 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.6 (1C, *C*qOOCH$_3$), 153.9 (1C, *C*q-4), 150.8 (1C, *C*═NOH), 136.5 (1C, *C*qPh), 134.0 (1C, *C*-2), 129.4 (1C, *C*q-1), 129.2 (1C, *C*-6), 128.6 (2C, *m*-Ph-*C*), 127.9 (1C, *p*-Ph-*C*), 127.0 (2C, *o*-Ph-*C*), 113.8 (1C, *C*-5), 112.4 (1C, *C*qBr), 70.9 (1C, OCH_2), 52.9 (1C, OCH_3), 29.3 (1C, CH_2); IR (ATR): $\tilde{\nu}$ = 1726 cm^{-1} (s), 1221 (s), 1018 (vs), 1001 (s), 805 (s), 730 (s), 696 (s), 676 (s); UV-Vis (MeOH): λ_{max} (log ε) = 281 nm (3.37), 204 (4.61); MS (EI, 70 eV): *m*/*z* (%) = 379/377 (7/7) [M$^+$], 363 (13), 361 (13), 272 (49), 270 (49), 212 (27), 210 (26), 132 (15), 105 (11), 103 (18), 91 (100), 65 (9); HREIMS: calcd for $C_{17}H_{16}BrNO_4$ [M]$^+$: 377.02572, found 377.02605.

(*E*/*Z*)-Methyl-3-(3-bromo-4-hydroxyphenyl)-2-(hydroxyimino)-propanoate. Benzyl ester **9** (0.55 g, 1.45 mmol, 1.00 equiv.) was dissolved in 44 mL dioxane–concentrated AcOH (1 : 1). To the solution was added Pd/C (0.15 g, 0.07 mmol, 0.04 equiv.) and the reaction mixture was hydrogenated for 41 h under 1 atm H_2 at rt. The reaction mixture was filtered by celite and the solvent was evaporated. The residue was dissolved in 70 mL EtOAc and washed with H_2O (3 × 12 mL) and saturated aqueous NaCl solution (20 mL). Then the organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica [PE–EtOAc (3 : 1)] afforded the *E*/*Z*-isomers (4 : 1) as a colorless solid (0.25 g, 0.74 mmol, 51%); TLC [silica, PE–EtOAc (10 : 1)]: R_f = 0.08; m.p.: 148–150 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): (*E*)-**7**: δ = 12.48 (sbr, 1H, NO*H*), 10.10 (sbr, 1H, O*H*), 7.28 (d, ^4J = 2.1 Hz, 1H, 2-*H*), 7.00 (dd, ^4J = 2.1 Hz, ^3J = 8.4 Hz, 1H, 6-*H*), 6.85 (d, ^3J = 8.3 Hz, 1H, 5-*H*), 3.72 (s, 3H, COOCH_3), 3.37 (s, 2H, CH_2); (*Z*)-**7**: δ = 12.37 (sbr, 1H, NO*H*), 9.23 (sbr, 1H, O*H*), 7.28 (d, ^4J = 2.1 Hz, 1H, 2-*H*), 6.97 (m, 1H, 6-*H*), 6.65 (m, 1H, 5-*H*), 3.72 (s, 3H, COOCH_3), 3.70 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$): (*E*)-**7**: δ = 164.1 (1C, *C*qOOCH$_3$), 152.5 (1C, *C*qOH), 149.5 (1C, *C*═NOH), 132.7 (1C, *C*-2), 128.9 (1C, *C*-6), 128.4 (1C, *C*q-1), 116.3 (1C, *C*-5), 108.9 (1C, *C*qBr), 52.2 (1C, COO*C*H$_3$), 28.8 (1C, *C*H$_2$); (*Z*)-**7**: δ = 164.2 (1C, *C*qOOCH$_3$), 155.8 (1C, *C*qOH), 149.9 (1C, *C*═NOH), 132.7 (1C, *C*-2), 129.6 (1C, *C*-6), 126.3 (1C, *C*q-1), 115.2 (1C, *C*-5), 108.9 (1C, *C*qBr), 52.1 (1C, COO*C*H$_3$), 29.1 (1C, *C*H$_2$); IR (ATR): $\tilde{\nu}$ = 1695 cm^{-1} (s), 1413 (s), 1250 (s), 1013 (vs), 798 (s), 726 (s); UV-Vis (MeOH): λ_{max} (log ε) = 283 nm (3.46), 203 (4.46); MS (EI, 70 eV): *m*/*z* (%) = 289/287 (14/14) [M$^+$], 273 (26), 271 (29), 258 (3), 256 (3), 239 (10), 237 (9), 227 (9), 225 (8), 212 (95), 210 (89), 193 (9), 191 (56), 187 (59), 185 (61), 159 (27), 147 (18), 132 (100), 118 (6), 107 (24), 105 (30), 91 (13), 77 (63), 69 (15), 59 (28), 51 (34), 44 (49); HREIMS: calcd for $C_{10}H_{10}BrNO_4$ [M$^+$]: 286.97877, found 286.97872.

(*E*/*Z*)-3-(3-Bromo-4-hydroxyphenyl)-2-(hydroxyimino)propanoic acid (12). To a solution of debenzylated methyl ester (0.18 g, 0.62 mmol, 1.00 equiv.) in 1.5 mL THF was added dropwise a solution of LiOH–H_2O (0.08 g, 1.85 mmol, 3.00 equiv.) in 5.5 mL H_2O. The reaction mixture was stirred for 48 h at rt. Then 2.40 mL 1 N aqueous HCl was added and the reaction mixture was stirred for another 10 min at rt before evaporating the solvent. The residue was dissolved in 20 mL EtOAc and washed with H_2O (3 × 20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The *E*/*Z*-acid **12** (4 : 1) was obtained as a colorless solid (0.17 g, 0.60 mmol, 97%); TLC [silica RP-18, MeOH–H_2O (1 : 1)]: R_f = 0.43; m.p.: 141–143 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): (*E*)-**12**: δ = 12.81 (sbr, 1H, COO*H*), 12.28 (sbr, 1H, NO*H*), 10.08 (sbr, 1H, O*H*), 7.28 (d, ^4J = 1.6 Hz, 1H, 2-*H*), 7.01–6.97 (m, 1H, 6-*H*), 6.85 (d, ^3J = 8.3 Hz, 1H, 5-*H*), 3.69 (s, 2H, CH_2); (*Z*)-**12**: δ = 12.81 (sbr, 1H, COO*H*), 12.16 (sbr, 1H, NO*H*), 9.19 (sbr, 1H, O*H*), 7.28 (d, ^4J = 1.6 Hz, 1H, 2-*H*), 7.01–6.97 (m, 1H, 6-*H*), 6.65 (d, ^3J = 8.5 Hz, 1H, 5-*H*), 3.69 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$): (*E*)-**12**: δ = 165.1 (1C, *C*qOOH), 152.4 (1C, *C*qOH), 150.2 (1C, *C*═NOH), 132.7 (1C, *C*-2), 128.9 (1C, *C*q-1), 128.7 (1C, *C*-6), 116.2 (1C, *C*-5), 108.9 (1C, *C*qBr), 28.6 (1C, *C*H$_2$). (*Z*)-**12**: δ = 165.3 (1C, *C*qOOH), 155.7 (1C, *C*qOH), 150.2 (1C, *C*═NOH), 132.7 (1C, *C*-2), 128.9 (1C, *C*q-1), 129.6 (1C, *C*-6), 115.1 (1C, *C*-5), 108.9 (1C, *C*qBr), 28.9 (1C, *C*H$_2$); IR (ATR): $\tilde{\nu}$ = 1693 cm^{-1} (s), 1201 (s), 1016 (vs), 801 (s), 695 (s); UV-Vis (MeOH): λ_{max} (log ε) = 283 nm (3.45), 203 (4.45); MS (EI, 70 eV): *m*/*z* (%) = 275/273 (<1/<1) [M$^+$], 213 (24), 211 (25), 201 (1), 199 (1), 187 (2), 185 (2), 133 (15), 132 (100), 106 (4), 104 (8), 102 (5), 78(6), 77 (15), 76 (7), 63 (4), 51 (9), 44 (23).

Psammaplin A (1). Acid **12** (0.14 g, 0.51 mmol, 1.00 equiv.) was dissolved in 10 mL DMF under an argon atmosphere. Then NHS (0.09 g, 0.77 mmol, 1.50 equiv.) and after 5 min DCC (0.16 g, 1.77 mmol, 1.50 equiv.) were added and the reaction mixture was stirred for 4 h at rt. After complete consumption of acid **12** (TLC [silica RP-18, H_2O–MeOH (1 : 1)]) cystamine dihydrochloride (**15**) (0.06 g, 0.26 mmol, 0.50 equiv.) and NEt_3 (0.14 mL, 0.10 g, 1.03 mmol, 2.00 equiv.) were added and the reaction mixture was stirred for another 40 h. After filtration from DCU the solvent was evaporated. The residue was purified by column chromatography on silica [$CHCl_3$–MeOH (20 : 1)] yielding psammaplin A (**1**, *E*,*E*-isomer) as a pale yellow oil (0.06 g, 0.09 mmol, 36%); TLC [silica, $CHCl_3$–MeOH (9 : 1)]: R_f = 0.37; ^{1}H NMR (400 MHz, acetone-d$_6$): δ = 10.33 (sbr, 2H, 2× O*H* or NO*H*), 7.67 (t, ^3J = 6.0 Hz, 2H, 2× N*H*), 7.47 (d, ^4J = 2.1 Hz, 2H, 2× 2-*H*), 7.16 (dd, ^4J = 2.1 Hz, ^3J = 8.3 Hz, 2H, 2× 6-*H*), 6.89 (d, ^3J = 8.3 Hz, 2H, 2× 5-*H*), 3.85 (s, 4H, 2× CH_2), 3.59 (dt, ^3J = 7.0 Hz, 4H, 2× NHCH_2), 2.88 (t, ^3J = 6.8 Hz, 4H, 2× SCH_2); ^{13}C NMR (100 MHz, acetone-d$_6$): δ = 164.1 (2C, 2× *C*qO), 153.2 (2C, 2× *C*q-OH), 153.2 (2C, 2× *C*═NOH), 134.3 (2C, 2× *C*-2), 130.5 (2C, 2× *C*q-1), 130.4 (2C, 2× *C*-6), 117.0

(2C, 2× *C*-5), 109.9 (2C, 2× *C*qBr), 39.2 (2C, 2× NH*C*H_2), 38.4 (2C, 2× S*C*H_2), 28.4 (2C, 2× *C*H_2); IR (ATR): $\tilde{\nu}$ = 1655 cm^{-1} (s), 1529 (s), 1493 (s), 1418 (s), 1207 (vs), 1012 (s), 981 (s), 543 (s); UV-Vis (MeOH): λ_{max} (log ε) = 282 nm (3.71), 204 (4.75); MS (ESI): *m*/*z* (%) = 689/687/685 (<2/<2/<2) [M + Na]$^+$, 561 (<2), 433 (<2), 370 (2), 334 (3), 316 (3), 299 (5), 281 (4), 249 (6), 218 (5), 216 (5), 213 (46), 211 (47), 201 (11), 199 (11), 187 (19), 185 (20), 143 (4), 132 (100), 115 (5), 105 (12), 103 (13), 77 (24), 61 (18), 51 (18), 44 (20); HRESIMS: calcd for $C_{22}H_{24}Br_2N_4NaO_6S_2$ [M + Na]$^+$: 684.93962, found 684.93809.

(*E*/*Z*)-Methyl-3-(3-bromophenyl)-2-(*tert*-butyldimethylsilyloxy)acrylate (8). Diisopropylamine (0.78 mL, 0.56 g, 5.53 mmol, 1.50 equiv.) was dissolved in 4 mL dry THF under an argon atmosphere and cooled to 0 °C. After 10 min *n*-BuLi (1.6 N solution in hexane, 3.45 mL, 5.53 mmol, 1.50 equiv.) was added dropwise to the solution and it was stirred for 10 min at 0 °C and then for 30 min at −78 °C. Dimethylphosphonate **6** (1.15 g, 3.69 mmol, 1.00 equiv.) in 1 mL dry THF was added dropwise and the reaction mixture was stirred for 30 min at −78 °C. To the solution was added 3-bromobenzaldehyde (**5**) (0.47 mL, 0.75 g, 4.05 mmol, 1.10 equiv.) in 1 mL dry THF dropwise and the reaction mixture was stirred for 19 h at −78 °C and warmed to rt within 30 min. It was quenched with 15 mL saturated aqueous NH_4Cl solution and stirred for 10 min at rt. The mixture was diluted with 75 mL EtOAc and washed with saturated aqueous NH_4Cl solution (3 × 30 mL), H_2O (3 × 30 mL) and saturated aqueous NaCl solution (30 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent evaporated. Purification by column chromatography on silica [PE–EtOAc (50 : 1 → 40 : 1 → 10 : 1 → 5 : 1)] afforded the *E*/*Z*-isomers **8** (3 : 1) as a colorless oil (1.14 g, 3.06 mmol, 83%); TLC [silica, PE–EtOAc (30 : 1)]: R_f = 0.35; ^{1}H NMR (400 MHz, DMSO-d_6): (*Z*)-**8**: δ = 8.00 (t, ^4J = 1.7 Hz, 1H, 2-*H*), 7.47 (d, ^3J = 7.8 Hz, 1H, 6-*H*), 7.39–7.34 (m, 1H, 5-*H*), 7.21 (d, ^3J = 7.9 Hz, 1H, 4-*H*), 6.76 (s, 1H, 7-*H*), 3.82 (s, 3H, COOCH_3), 0.96 (s, 9H, C(CH_3)$_3$), 0.16 (s, 6H, Si(CH_3)$_2$); (*E*)-**8**: δ = 7.40–7.37 (m, 1H, C*H*), 7.37–7.34 (m, 1H, C*H*), 7.18–7.15 (m, 1H, C*H*), 7.17–7.14 (m, 1H, C*H*), 6.32 (s, 1H, 7-*H*), 3.67 (s, 3H, COOCH_3), 0.98 (s, 9H, C(CH_3)$_3$), 0.22 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): (*Z*)-**8**: δ = 165.6 (1C, *C*qO), 141.32 (1C, *C*q-8), 136.2 (1C, *C*q-1), 132.3 (1C, *C*-2), 130.9 (1C, *C*-5), 129.6 (1C, *C*-4), 128.4 (1C, *C*-6), 122.3 (1C, *C*qBr), 117.2 (1C, *C*-7), 52.2 (1C, COO*C*H$_3$), 25.8 (3C, C(*C*H$_3$)$_3$), 18.6 (1C, *C*q(CH$_3$)$_3$), −3.9 (2C, Si(*C*H$_3$)$_2$); (*E*)-**8**: δ = 165.1 (1C, *C*qO), 142.83 (1C, *C*-8), 136.7 (1C, *C*q-1), 131.5 (1C, *C*H), 130.1 (1C, *C*H), 129.4 (1C, *C*H), 127.2 (1C, *C*H), 121.9 (1C, *C*qBr), 118.6 (1C, *C*-7), 51.8 (1C, COO*C*H$_3$), 25.5 (3C, C(*C*H$_3$)$_3$), 18.3 (1C, *C*q(CH$_3$)$_3$), −4.8 (2C, Si(*C*H$_3$)$_2$); IR (ATR): $\tilde{\nu}$ = 1248 cm^{-1} (s), 833 (vs), 779 (vs); UV-Vis (MeOH): λ_{max} (log ε) = 277 nm (4.08), 202 (4.24); MS (ESI): *m*/*z* (%) = 765 (7) [2M + Na]$^+$, 395/393 (100/96) [M + Na]$^+$, 373/371 (4/3) [M$^+$], 341 (3), 257 (1); HRESIMS: calcd for $C_{16}H_{23}BrNaO_3Si$ [M + Na]$^+$: 393.04920, found 393.04945.

(*E*)-Methyl-3-(3-bromophenyl)-2-(hydroxyimino)propanoate (10). The *E*/*Z*-olefin ester **8** (0.50 g, 1.35 mmol, 1.00 equiv.) was dissolved in 5 mL MeOH under an argon atmosphere and 3HF·NEt_3 (0.37 mL, 0.37 g, 2.29 mmol, 1.70 equiv.) was added dropwise. The reaction mixture was stirred for 30 min at rt. After finishing TBS deprotection (TLC [silica, PE–EtOAc (30 : 1)]) $HONH_2$·HCl (0.16 g, 2.29 mmol, 1.70 equiv.) was added in portions and the reaction mixture was stirred for another 15 h. After evaporation of the solvent the residue was dissolved in 20 mL DCM and washed with H_2O (2 × 15 mL) and saturated aqueous $NaHCO_3$ solution (15 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The *E*-oxime **10** was obtained as a colorless solid (0.35 g, 0.95 mmol, 96%); TLC [silica, PE–EtOAc (1 : 1)]: R_f = 0.56; m.p.: 98–103 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.60 (s, 1H, NO*H*), 7.42–7.37 (m, 2H, 2-*H*, 6-*H*), 7.25 (t, ^3J = 7.7 Hz, 1H, 5-*H*), 7.19 (d, ^3J = 1.2 Hz, ^4J = 7.6 Hz, 1H, 4-*H*), 3.84 (s, 2H, CH_2), 3.73 (s, 3H, COOCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.0 (1C, *C*qOOCH$_3$), 148.8 (1C, *C*═NOH), 139.2 (1C, *C*q-1), 131.2 (1C, *C*-2), 130.6 (1C, *C*-5), 129.2 (1C, *C*-6), 127.6 (1C, *C*-4), 121.5 (1C, *C*qBr), 52.2 (1C, COO*C*H$_3$), 29.7 (1C, *C*H$_2$); IR (ATR): $\tilde{\nu}$ = 1728 cm^{-1} (s), 1206 (s), 1126 (s), 1009 (vs), 723 (s); UV-Vis (MeOH): λ_{max} (log ε) = 202 nm (3.49); MS (EI, 70 eV): *m*/*z* (%) = 273/271 (10/10) [M$^+$], 257(17), 255 (17), 198 (15), 197 (27), 196 (35), 195 (27), 194 (23), 180 (31), 178 (100), 171 (20), 169 (22), 163 (5), 152 (7), 143 (23), 142 (16), 117 (16), 116 (58), 115 (19), 102 (7), 97 (6), 95 (8), 91 (16), 90 (20), 89 (32), 81 (8), 71 (11), 69 (11), 63 (13), 59 (14), 57 (14), 55 (12), 51 (7), 50 (8), 44 (41), 43 (15); HREIMS: calcd for $C_{10}H_{10}BrNO_3$ [M$^+$]: 270.98386, found 270.98375.

(*E*)-3-(3-Bromophenyl)-2-(hydroxyimino)propanoic acid (13). The methyl ester **10** (0.72 g, 2.64 mmol, 1.00 equiv.) was dissolved in 5.5 mL THF and cooled to 0 °C. A solution of LiOH–H_2O (0.22 g, 5.28 mmol, 2.00 equiv.) in 14 mL H_2O was added dropwise. The reaction mixture was stirred for 11 h at rt, then 7.2 mL 1 N aqueous HCl was added and the mixture was stirred for another 10 min at rt. After evaporation of THF the formed precipitate was filtered off and dried in an exsiccator over concentrated H_2SO_4. The *E*-isomer **13** was obtained as a colorless solid (0.60 g, 2.31 mmol, 87%); TLC [silica RP-18, MeOH–H_2O (1 : 1)]: R_f = 0.28; m.p.: 158–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.90 (sbr, 1H, COO*H*), 12.40 (sbr, 1H, NO*H*), 7.41–7.39 (m, 1H, 6-*H*), 7.37 (t, ^4J = 1.6 Hz, 1H, 2-*H*), 7.25 (t, ^3J = 7.7 Hz, 1H, 5-*H*), 7.20 (td, ^4J = 1.3 Hz, ^3J = 7.7 Hz, 1H, 4-*H*), 3.81 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 165.0 (1C, *C*qOOH), 149.6 (1C, *C*═NOH), 139.5 (1C, *C*q-1), 131.2 (1C, *C*-2), 130.5 (1C, *C*-5), 129.1 (1C, *C*-6), 127.7 (1C, *C*-4), 121.5 (1C, *C*qBr), 29.5 (1C, *C*H$_2$); IR (ATR): $\tilde{\nu}$ = 1691 cm^{-1} (s), 1022 (s), 774 (s), 697 (vs); UV-Vis (MeOH): λ_{max} (log ε) = 203 nm (4.37); MS (EI, 70 eV): *m*/*z* (%) = 259/257 (2/2) [M$^+$], 197 (37), 195 (37), 171 (2), 169 (2), 117 (11), 116 (100), 115 (5), 90 (7), 89 (26), 88 (5), 75 (5), 63 (8), 58 (5), 50 (7), 44 (53); HRESIMS: calcd for $C_9H_8BrNNaO_3$ [M + Na]$^+$: 279.95798, found 279.95819.

(2*E*,2′*E*)-*N*,*N*′-(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(3-(3-bromophenyl)-2-(hydroxyimino)propanamide) (16). To a solution of acid **13** (0.07 g, 0.27 mmol, 1.00 equiv.) in 1 mL dioxane were added NHPI (0.04 g, 0.27 mmol, 1.00 equiv.) and DCC (0.56 g, 0.27 mmol, 1.00 equiv.) at rt and the reaction mixture was stirred for 8 h. After complete consumption of acid **13** (TLC

[silica RP-18, H_2O–MeOH (1 : 1)]) a solution of cystamine dihydrochloride (**15**) (0.03 g, 0.13 mmol, 0.48 equiv.) and NEt_3 (0.38 mL, 0.28 g, 0.54 mmol, 2.00 equiv.) in 1 mL MeOH was added and the reaction mixture was stirred for another 48 h. The solvent was evaporated and purification by column chromatography on silica [$CHCl_3$–MeOH (40 : 1)] afforded the *E*,*E*-isomer **16** as a colorless oil (0.03 g, 0.05 mmol, 36%); TLC [silica, $CHCl_3$–MeOH (9 : 1)]: R_f = 0.47; ^{1}H NMR (400 MHz, acetone-d_6): δ = 7.52–7.51 (m, 2H, 2× 2-*H*), 7.35 (dd, ^4J = 1.4 Hz, ^3J = 7.9 Hz, 2H, 2× 4-*H*), 7.32 (d, ^3J = 7.8 Hz, 2H, 2× 6-*H*), 7.21 (t, ^3J = 7.8 Hz, 2H, 2× 5-*H*), 3.94 (s, 4H, 2× 7-CH_2), 3.58 (t, ^3J = 6.8 Hz, 4H, 2× NHCH_2), 2.89 (t, ^3J = 6.8 Hz, 4H, 2× SCH_2); ^{13}C NMR (100 MHz, acetone-d_6): δ = 163.9 (2C, 2× *C*qO), 152.5 (2C, 2× *C*═NOH), 140.6 (2C, 2× *C*q-1), 132.9 (2C, 2× *C*-2), 131.0 (2C, 2× *C*-5), 130.0 (2C, 2× *C*-4), 129.0 (2C, 2× *C*-6), 122.6 (2C, 2× *C*qBr), 39.1 (2C, 2× NH*C*H$_2$), 38.4 (2C, 2× S*C*H$_2$), 29.5 (2C, 2× *C*H$_2$-7); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.16 (sbr, 2H, 2× NO*H*), 7.45 (t, ^4J = 1.7 Hz, 2H, 2× 2-*H*), 7.28 (m, 2H, 2× 4-*H*), 7.25–7.24 (m, 2H, 2× 6-*H*), 7.21 (t, ^3J = 7.8 Hz, 2H, 2× N*H*), 7.07 (t, ^3J = 7.8 Hz, 2H, 2× 5-*H*), 3.92 (s, 4H, 2× 7-CH_2), 3.57 (q, ^3J = 6.1 Hz, 4H, 2× NHCH_2), 2.74 (t, ^3J = 6.2 Hz, 4H, 2× SCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.7 (2C, 2× *C*qO), 151.8 (2C, 2× *C*═NOH), 138.4 (2C, 2× *C*q-1), 132.1 (2C, 2× *C*-2), 129.9 (2C, 2× *C*-5), 129.6 (2C, 2× *C*-4), 128.1 (2C, 2× *C*-6), 122.4 (2C, 2× *C*qBr), 38.3 (2C, 2× NH*C*H$_2$), 37.7 (2C, 2× S*C*H$_2$), 28.9 (2C, 2× *C*H$_2$-7); IR (ATR): $\tilde{\nu}$ = 1655 cm^{-1} (vs), 1567 (vs), 768 (s); UV-Vis (MeOH): λ_{max} (log ε) = 673 nm (2.72), 203 (4.69); MS (ESI): *m*/*z* (%) = 1287 (2) [2M + Na]$^+$, 653/655/657 (46/84/39) [M + Na]$^+$, 631/633/635 (4/10/4) [M$^+$], 471 (11), 325 (11), 225 (100); HRESIMS: calcd for $C_{22}H_{24}Br_2N_4NaO_4S_2$ [M + Na]$^+$: 652.94979, found 652.95007.

(*E*)-*tert*-Butyl-2-((2-(2-(hydroxyimino)-3-(4-hydroxyphenyl)-propanamido)ethyl)disulfanyl)ethylcarbamate (22). The acid **21** (0.15 g, 0.75 mmol, 1.00 equiv.) was dissolved in 3 mL dry DMF under an argon atmosphere. NHS (0.13 g, 1.12 mmol, 1.50 equiv.) and after 5 min DCC (0.23 g, 1.12 mmol, 1.50 equiv.) were added in portions and the reaction mixture was stirred for 2 h at rt. After complete consumption of acid **21** (TLC [silica RP-18, H_2O–MeOH (1 : 1)]) was Boc protected cystamine **20** (0.21 g, 0.82 mmol, 1.10 equiv.) in 5 mL dry DMF added dropwise and the reaction mixture was stirred for 4 days at rt. The solvent was evaporated, dissolved in THF, filtered and the solvent evaporated again. Purification by column chromatography on silica [$CHCl_3$–MeOH (50 : 1)] afforded the *E*-isomer **21** as a pale yellow oil (0.24 g, 0.55 mmol, 74%); TLC [silica, $CHCl_3$–MeOH (40 : 1)]: R_f = 0.16; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.75 (s, 1H, NO*H*), 9.18 (s, 1H, O*H*), 8.04 (t, ^3J = 5.9 Hz, 1H, N*H*C-9), 6.99–6.98 (m, ^3J + ^5J = 8.6 Hz, 3H, 2-*H*, 6-*H*, N*H*C-14), 6.63–6.62 (m, ^3J + ^5J = 8.6 Hz, 2H, 3-*H*, 5-*H*), 3.68 (s, 2H, 7-CH_2), 3.41 (dt, ^3J = 6.3 Hz, ^3J = 13.5 Hz, 2H, 10-CH_2), 3.19 (dt, ^3J = 6.3 Hz, ^3J = 13.1 Hz, 2H, 13-CH_2), 2.81–2.79 (m, 2H, 11-CH_2), 2.74 (t, ^3J = 6.9 Hz, 2H, 12-CH_2), 1.37 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 163.3 (*C*q-9), 155.5 (1C, *C*qOH), 155.4 (1C, *C*q-14), 152.2 (1C, *C*═NOH), 129.7 (2C, *C*-2, *C*-6), 126.7 (1C, *C*q-1), 114.9 (2C, *C*-3, *C*-5), 77.7 (1C, *C*q(CH$_3$)$_3$), 39.2 (1C, *C*H$_2$-13), 38.1 (1C, *C*H$_2$-10), 37.5 (1C, *C*H$_2$-12), 36.8 (1C, *C*H$_2$-11), 28.1 (3C, C(*C*H$_3$)$_3$), 27.9 (1C, *C*H$_2$-7); IR (ATR): $\tilde{\nu}$ = 1511 cm^{-1} (vs), 1162 (s); UV-Vis (MeOH): λ_{max} (log ε) = 203 nm (4.20); MS (ESI): *m*/*z* (%) = 881 (63) [2M + Na]$^+$, 452 (100) [M + Na]$^+$, 396 (15), 330 (16), 299 (7); HRESIMS: calcd for $C_{18}H_{27}N_3NaO_5S_2$ [M + Na]$^+$: 452.12843, found 452.12857.

(*E*)-*tert*-Butyl-2-((2-(3-(3-bromo-4-hydroxyphenyl)-2-(hydroxyimino)propanamido)ethyl)disulfanyl)ethylcarbamate (23). The acid **12** (0.26 g, 0.96 mmol, 1.00 equiv.) was dissolved in 7 mL dry DMF under an argon atmosphere. NHS (0.17 g, 1.46 mmol, 1.50 equiv.) and after 5 min DCC (0.17 g, 1.45 mmol, 1.50 equiv.) were added in portions and the reaction mixture was stirred for 4 h at rt. After complete consumption of acid **12** (TLC [silica RP-18, H_2O–MeOH (1 : 1)]) was Boc-protected cystamine **20** (0.27 g, 1.06 mmol, 1.10 equiv.) in 2 mL dry DMF added dropwise and the reaction mixture was stirred for 15 h at rt and for 4 days at 30 °C. The solvent was evaporated, dissolved in THF, filtered and the solvent evaporated again. Purification by column chromatography on silica [$CHCl_3$–MeOH (70 : 1 → 40 : 1 → 5 : 1)] and on silica RP-18 [MeOH–H_2O (2 : 1)] afforded the *E*-isomer **23** as a pale yellow oil (0.14 g, 0.27 mmol, 28%); TLC [silica RP-18, MeOH–H_2O (2 : 1)]: R_f = 0.20; ^{1}H NMR (400 MHz, acetone-d_6): δ = 11.1 (sbr, 1H, NO*H*), 8.67 (sbr, 1H, O*H*), 7.65 (t, ^3J = 5.8 Hz, 1H, N*H*C-9), 7.47 (d, ^4J = 2.1 Hz, 1H, 2-*H*), 7.16 (dd, ^4J = 2.1 Hz, ^3J = 8.3 Hz, 1H, 6-*H*), 6.89 (d, ^3J = 8.3 Hz, 1H, 5-*H*), 6.20 (sbr, 1H, N*H*C-14), 3.84 (s, 2H, 7-CH_2), 3.60 (dt, ^3J = 6.7 Hz, ^3J = 13.1 Hz, 2H, 10-CH_2), 3.37 (dt, ^3J = 6.5 Hz, ^3J = 12.8 Hz, 2H, 13-CH_2), 2.91–2.88 (m, 2H, 11-CH_2), 2.87–2.82 (m, 2H, 12-CH_2), 1.40 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, acetone-d_6): δ = 164.1 (1C, *C*q-9), 156.7 (1C, *C*q-14), 153.3 (1C, *C*q-OH), 153.2 (1C, *C*═NOH), 134.3 (1C, *C*-2), 130.6 (1C, *C*-6), 130.5 (1C, *C*q-1), 117.0 (1C, *C*-5), 110.0 (1C, *C*qBr), 78.9 (1C, *C*q-15), 40.5 (1C, *C*H$_2$-13), 39.3 (1C, *C*H$_2$-12), 39.3 (1C, *C*H$_2$-10), 38.3 (1C, *C*H$_2$-11), 28.6 (3C, C(*C*H$_3$)$_3$), 28.4 (1C, *C*H$_2$-7); IR (ATR): $\tilde{\nu}$ = 1660 cm^{-1} (vs), 1161 (vs); UV-Vis (MeOH): λ_{max} (log ε) = 282 nm (3.46), 203 (4.50); MS (ESI): *m*/*z* (%) = 1039 (17) [2M + Na]$^+$, 532/530 (100/92) [M + Na]$^+$, 510/508 (19/18) [M$^+$], 410/408 (56/52), 168 (76); HRESIMS: calcd for $C_{18}H_{26}BrN_3NaO_5S_2$ [M + Na]$^+$: 530.03895, found 530.03911.

(*E*)-2-(Hydroxyimino)-3-(4-hydroxyphenyl)-*N*-(2-mercaptoethyl)propanamide (33). The Boc-amide **22** (0.26 g, 0.64 mmol, 1.00 equiv.) was dissolved in 10 mL MeOH and 1 M aqueous KOH solution (28 µL) was added. Dithiothreitol (**32**) (0.27 g, 1.92 mmol, 3.00 equiv.) was added and the reaction mixture was stirred for 15 h at rt. The reaction mixture was cooled to 0 °C and diluted with 0.5 M aqueous HCl solution (10 mL). After addition of saturated aqueous NaCl solution (10 mL) it was extracted with DCM (5 × 15 mL). The combined organic layer was washed with H_2O (100 mL) and saturated aqueous NaCl solution (100 mL), dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica RP-18 [H_2O–MeOH (2 : 1)] afforded the *E*-isomer **33** as a pale yellow oil (0.063 g, 0.25 mmol, 39%); TLC [silica RP-18, H_2O–MeOH (2 : 1)]: R_f = 0.17; ^{1}H NMR (300 MHz, CD_3OD): δ = 8.05 (m, 1H, N*H*), 7.08 (m, ^3J + ^5J = 8.7 Hz, 2H, 2-*H*, 6-*H*), 6.65 (m, ^3J + ^5J = 8.7 Hz, 2H, 3-*H*, 5-*H*), 3.80 (s, 2H, CH_2), 3.37 (dd, ^3J = 6.6 Hz, ^3J = 7.3 Hz, 2H, NCH_2), 2.58 (dd, ^3J =

6.5 Hz, ^3J = 7.4 Hz, 2H, SCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ = 166.1 (1C, *C*qO), 156.8 (1C, *C*qOH), 153.8 (1C, *C*=NOH), 131.1 (2C, *C*-2, *C*-6), 128.8 (1C, *C*q-1), 116.1 (2C, *C*-3, *C*-5), 43.7 (1C, NCH_2), 29.0 (1C, CH_2), 24.4 (1C, SCH_2); IR (ATR): $\tilde{\nu}$ = 1655 cm^{-1} (s), 1511 (vs), 1212 (s); UV-Vis (MeOH): λ_{max} (log ε) = 276 nm (3.32), 203 (4.18); MS (ESI): *m*/*z* (%) = 531 (86) [2M + Na]$^+$, 277 (100) [M + Na]$^+$, 255 (63) [M + H]$^+$; HRESIMS: calcd for $C_{11}H_{14}N_2NaO_3S$ [M + Na]$^+$: 277.06173, found 277.06176.

(2*E*,2′*E*)-*N*,*N*′-(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(2-(hydroxyimino)-3-(4-hydroxyphenyl)propanamide) (34). Compound **33** (0.02 g, 0.08 mmol, 1.00 equiv.) was stored in 0.6 mL acetone-d_6 at rt for 90 days. After complete oxidation to the corresponding *E*,*E*-isomer **34** (^{1}H NMR control) it was isolated by evaporation of the solvent as a colorless solid (0.02 g, 0.04 mmol, quant.); TLC [silica, $CHCl_3$–MeOH (10 : 1)]: R_f = 0.27; m.p.: 137–138 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.74 (s, 2H, 2× NO*H*), 9.18 (sbr, 2H, 2× O*H*), 8.03 (t, ^3J = 5.9 Hz, 2H, 2× N*H*), 7.00 (m, ^3J + ^5J = 8.6 Hz, 4H, 2× 2-*H*, 2× 6-*H*), 6.63 (m, ^3J + ^5J = 8.6 Hz, 4H, 2× 3-*H*, 2× 5-*H*), 3.68 (s, 4H, 2× CH_2-7), 3.41 (dt, ^3J = 6.3 Hz, ^3J = 13.6 Hz, 4H, 2× NHCH_2), 2.81–2 (m, 4H, 2× SCH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 163.3 (2C, 2× *C*qO-9), 155.5 (2C, 2× *C*qOH), 152.2 (2C, 2× *C*=NOH), 129.6 (4C, 2× *C*-2, 2× *C*-6), 126.7 (2C, 2× *C*q-1), 114.9 (2C, 2× *C*-3, 2× *C*-5), 38.0 (2C, 2× NHCH_2), 36.8 (2C, 2× SCH_2), 27.9 (2C, 2× CH_2-7); IR (ATR): $\tilde{\nu}$ = 1653 (s), 1511 (vs), 999 (s); UV-Vis (MeOH): λ_{max} (log ε) = 210 nm (4.44), 201 (4.53); MS (ESI): *m*/*z* (%) = 1035 (10) [2M + Na]$^+$, 529 (100) [M + Na]$^+$, 507 (5), 262 (5), 179 (7); HRESIMS: calcd for $C_{22}H_{26}N_4NaO_6S_2$ [M + Na]$^+$: 529.11860, found 529.11872.

(*E*)-2-(Hydroxyimino)-3-(4-hydroxyphenyl)-1-(pyrrolidin-1-yl)-propan-1-one (30). A solution of acid **21** (0.16 g, 0.82 mmol, 1.00 equiv.) in 7 mL dry DMF in a flask with a CaCl tube was cooled to 0 °C. NHS (0.14 g, 1.22 mmol, 1.50 equiv.) and after 5 min DCC (0.25 g, 1.22 mmol, 1.50 equiv.) were added and the reaction mixture was stirred for 6 h at rt. Pyrrolidine (**28**) (0.13 mL, 0.12 g, 1.63 mmol, 2.00 equiv.) was added and the reaction mixture was stirred for another 20 h. Under ice cooling was added 5% aqueous $KHSO_4$ solution (20 mL) and extracted with DCM (3 × 30 mL). The combined organic layer was washed in each case with 60 mL of H_2O, 5% aqueous $NaHCO_3$ solution, H_2O and saturated aqueous NaCl solution, dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in THF, filtered off from DCU and the solvent evaporated. Purification by column chromatography on silica [$CHCl_3$–MeOH (30 : 1) → (10 : 1)] obtained the *E*-isomer **30** as a colorless oil (0.08 g, 0.34 mmol, 42%); TLC [silica, $CHCl_3$–MeOH (20 : 1)]: R_f = 0.33; ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.68 (sbr, 1H, NO*H*), 8.27 (sbr, 1H, O*H*), 7.11 (m, ^3J + ^5J = 6.9 Hz, 2H, 3-*H*, 5-*H*), 6.74 (m, ^3J + ^5J = 6.6 Hz, 2H, 2-*H*, 6-*H*), 3.86 (s, 2H, 7-CH_2), 3.37 (dt, ^3J = 6.5 Hz, ^3J = 12.5 Hz, 4H, 2× NCH_2), 1.80–1.66 (m, 4H, 2× CH_2CH_2); ^{13}C NMR (100 MHz, acetone-d_6): δ = 164.7 (1C, *C*qO), 156.9 (1C, *C*q-4), 155.5 (1C, *C*=NOH), 131.0 (2C, *C*-3, *C*-5), 127.8 (1C, *C*q-1), 116.1 (2C, *C*-2, *C*-6), 49.0 (1C, NCH_2), 46.6 (1C, NCH_2), 30.7 (1C, CH_2-7), 26.6 (1C, CH_2CH_2), 24.5 (1C, CH_2CH_2); IR (ATR): $\tilde{\nu}$ = 1587 cm^{-1} (vs), 1512 (s), 1450 (s), 1224 (s); UV-Vis (MeOH): λ_{max} (log ε) = 278 nm (3.29), 202 (4.24); MS (ESI): *m*/*z* (%) = 519 (56) [2M + Na]$^+$, 271 (100) [M + Na]$^+$, 249 (66); HRESIMS: calcd for $C_{13}H_{16}N_2NaO_3$ [M + Na]$^+$: 271.10531, found 271.10528.

(*E*)-2-(Hydroxyimino)-3-(4-hydroxyphenyl)-1-(thiazolidin-3-yl)propan-1-one (31). A solution of acid **21** (0.21 g, 1.09 mmol, 1.00 equiv.) in 9 mL dry DMF in a flask with a CaCl tube was cooled to 0 °C. NHS (0.19 g, 1.64 mmol, 1.50 equiv.) and after 5 min DCC (0.34 g, 1.64 mmol, 1.50 equiv.) were added and the reaction mixture was stirred for 24 h at rt. Thiazolidine (**29**) (0.13 mL, 0.12 g, 1.63 mmol, 2.00 equiv.) was added and the mixture was stirred for another 20 h. Under ice cooling was added 5% aqueous $KHSO_4$ solution (25 mL) and extracted with DCM (7 × 30 mL). The combined organic layer was washed in each case with 60 mL of H_2O, 5% aqueous $NaHCO_3$ solution, H_2O and saturated aqueous NaCl solution, dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in THF, filtered off from DCU and evaporated again. After purification by column chromatography on silica [$CHCl_3$–MeOH (30 : 1) → (10 : 1)] was obtained the *E*-derivative **31** as a yellow oil of a (1 : 1) mixture of the two rotamers (0.12 g, 0.43 mmol, 40%); TLC [silica, $CHCl_3$–MeOH (10 : 1)]: R_f = 0.36; ^{1}H NMR (400 MHz, acetone-d_6): δ = 10.84 (sbr, 1H, NO*H*), 8.25 (sbr, 1H, O*H*), 7.10 (m, ^3J + ^5J = 8.0 Hz, 2H, 2-*H*, 6-*H*), 6.75/6.74 (m, ^3J + ^5J = 8.6 Hz, 2H, 3-*H*, 5-*H*), 4.50/4.50 (s, 2H, 10-CH_2), 3.87 (s, 2H, 7-CH_2), 3.73/3.70 (t, ^3J = 6.5/6.3 Hz, 2H, 12-CH_2), 2.97/2.79 (t, ^3J = 6.4/6.2 Hz, 2H, 11-CH_2); ^{13}C NMR (100 MHz, acetone-d_6): δ = 164.8/164.4 (1C, *C*q-9), 157.0/156.9 (1C, *C*qOH), 155.2/155.2 (1C, *C*=NOH), 131.0/131.0 (2C, *C*-2, *C*-6), 127.6/127.4 (1C, *C*q-1), 116.2 (2C, *C*-3, *C*-5), 51.5/49.5 (1C, CH_2-12), 50.6/48.3 (1C, CH_2-10), 31.4/29.6 (1C, CH_2-11), 30.8/30.7 (1C, CH_2-7); IR (ATR): $\tilde{\nu}$ = 1591 cm^{-1} (vs), 1451 (s), 556 (s); UV-Vis (MeOH): λ_{max} (log ε) = 277 nm (3.33), 203 (4.27); MS (EI, 70 eV): *m*/*z* (%) = 266 (18) [M$^+$], 249 (22), 221 (6), 146 (12), 132 (46), 116 (100), 107 (66), 90 (14), 88 (56), 77 (16), 70 (10), 61 (8), 56 (22), 41 (16); HREIMS: calcd for $C_{12}H_{14}N_2O_3S$ [M$^+$]: 266.07196, found 266.07208.

(*E*/*Z*)-Methyl-3-(3-bromophenyl)-2-(tetrahydro-2*H*-pyran-2-yloxyimino)propanoate (11). To a solution of TBS–ether **8** (0.40 g, 1.01 mmol, 1.00 equiv.) in 6 mL ethanol 3HF·NEt_3 (0.30 mL, 1.83 mmol, 1.70 equiv.) was added dropwise under an argon atmosphere. The reaction mixture was stirred for 30 min at rt. After finishing TBS deprotection (TLC [silica, PE–EtOAc (30 : 1)]) $THPONH_2$ (0.32 g, 2.69 mmol, 2.50 equiv.) was added in portions and the reaction mixture was stirred for another 48 h. After evaporation of the solvent the residue was dissolved in 60 mL DCM and washed with H_2O (2 × 30 mL) and saturated aqueous $NaHCO_3$ solution (30 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. After purification by column chromatography on silica [PE–EtOAc (15 : 1)] were obtained the *Z*-isomer **11** (0.03 g, 0.09 mmol, 9%) and the *E*-isomer **11** (0.34 g, 0.94 mmol, 88%) separated as colorless oils; (*Z*)-**11**: TLC [silica, PE–EtOAc (15 : 1)]: R_f = 0.19; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48–7.32 (m, 2H, 2-*H*, 5-*H*), 7.23–7.12 (m, 2H, 4-*H*, 6-*H*), 5.37 (t, ^3J = 3.1 Hz, 1H, 10-*H*),

3.92–3.86 (m, 1H, 14-OC*H*H), 3.76 (d, ^2J = 15.1 Hz, 1H, 7-C*H*H), 3.73 (s, 3H, OCH_3), 3.72 (d, ^2J = 15.0 Hz, 1H, 7-CH*H*), 3.68–3.63 (m, 1H, 14-OCH*H*), 1.83–1.53 (m, 6H, 11-CH_2, 12-CH_2, 13-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.0 (1C, *C*qOOCH$_3$), 151.0 (1C, *C*qN-8), 136.9 (1C, *C*q-1), 132.0 (1C, *C*-2), 130.3 (1C, *C*-5), 130.1 (1C, *C*-4), 127.6 (1C, *C*-6), 122.6 (1C, *C*q-Br), 100.6 (1C, *C*-10), 62.1 (1C, OCH_2-14), 52.0 (1C, OCH_3), 37.2 (1C, *C*H$_2$-7), 28.3 (1C, *C*H$_2$-11), 25.1 (1C, *C*H$_2$-13), 18.9 (1C, *C*H$_2$-12); (*E*)-**11**: TLC [silica, PE–EtOAc (15 : 1)]: R_f = 0.10; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (dd, ^4J = 1.7 Hz, 1H, 2-*H*), 7.34 (d, ^3J = 7.5 Hz, 1H, 4-*H*), 7.23 (d, ^3J = 7.7 Hz, 1H, 6-*H*), 7.14 (dd, ^3J = 7.8 Hz, 1H, 5-*H*), 5.50 (t, ^3J = 3.2 Hz, 1H, 10-*H*), 4.01 (d, ^2J = 13.5 Hz, 1H, 7-C*H*H), 3.89 (d, ^2J = 13.5 Hz, 1H, 7-CH*H*), 3.85 (s, 3H, OCH_3), 3.62–3.60 (m, 2H, 14-OCH_2), 1.84 (m, 2H, 11-CH_2), 1.82–1.67 (m, 2H, 12-CH_2), 1.66–1.54 (m, H, 13-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.9 (1C, *C*qOOCH$_3$), 151.0 (1C, *C*qN-8), 138.1 (1C, *C*q-1), 132.3 (1C, *C*-2), 130.0 (1C, *C*-5), 129.8 (1C, *C*-4), 127.7 (1C, *C*-6), 122.4 (1C, *C*q-Br), 101.9 (1C, *C*-10), 62.4 (1C, OCH_2-14), 53.0 (1C, OCH_3), 31.4 (1C, *C*H$_2$-7), 28.4 (1C, *C*H$_2$-11), 24.9 (1C, *C*H$_2$-13), 18.9 (1C, *C*H$_2$-12); IR (ATR): $\tilde{\nu}$ = 1720 cm^{-1} (s), 1202 (s), 1115 (s), 958 (vs), 773 (s); UV-Vis (MeOH): λ_{max} (log ε) = 204 nm (4.63); MS (ESI): *m*/*z* (%) = 735 (100) [2M + Na]$^+$, 396/394 (7/7) [M + K]$^+$, 380/378 (32/32) [M + Na]$^+$, 375/373 (19/19), 358/356 (12/12) [M$^+$], 274/272 (21/21), 171/169 (15/15); HRESIMS: calcd for $C_{15}H_{18}BrNNaO_4$ [M + Na]$^+$: 378.03114, found 378.03107.

(*E*)-3-(3-Bromophenyl)-2-(tetrahydro-2*H*-pyran-2-yloxyimino)-propanoic acid (14). To a solution of *E*-isomer **11** (0.27 g, 0.75 mmol, 1.00 equiv.) in 2 mL THF was added LiOH–H_2O (0.06 g, 1.51 mmol, 2.00 equiv.) in 5 mL H_2O dropwise at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and for another 14 h at rt. Then was added 1 N aqueous HCl solution (2.5 mL) and stirred for 10 min. The solvent was evaporated, the residue dissolved in EtOAc (20 mL) and washed with H_2O (2 × 15 mL) and saturated aqueous NaCl solution (15 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The *E*-isomer **14** was obtained as a pale yellow oil (0.23 g, 0.68 mmol, 90%); TLC [silica RP-18, MeOH–H_2O (1 : 1)]: R_f = 0.13; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 (dd, ^4J = 1.6 Hz, 1H, 2-*H*), 7.35 (d, ^3J = 7.8 Hz, 1H, 4-*H*), 7.25 (d, ^3J = 7.7 Hz, 1H, 6-*H*), 7.15 (dd, ^3J = 7.8 Hz, 1H, 5-*H*), 5.47 (m, 1H, 10-*H*), 3.98 (d, ^2J = 13.3 Hz, 1H, 7-C*H*H), 3.88 (d, ^2J = 13.3 Hz, 1H, 7-CH*H*), 3.63–3.52 (m, 2H, 14-OCH_2), 1.89–1.56 (m, 6H, 11-CH_2, 12-CH_2, 13-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.7 (1C, *C*qCOOH), 150.3 (1C, *C*qN-8), 137.4 (1C, *C*q-1), 132.4 (1C, *C*-2), 130.2 (1C, *C*-5), 130.0 (1C, *C*-4), 127.8 (1C, *C*-6), 122.5 (1C, *C*qBr), 102.1 (1C, *C*-10), 62.2 (1C, OCH_2-14), 30.3 (1C, *C*H$_2$-7), 28.1 (1C, *C*H$_2$-11), 24.8 (1C, *C*H$_2$-13), 18.45 (1C, *C*H$_2$-12); IR (ATR): $\tilde{\nu}$ = 2946 cm^{-1} (m), 1718 (m), 1592 (m), 1567 (m), 1427 (m), 1202 (m), 1114 (m), 1071 (m), 1036 (m), 963 (vs), 928 (m), 898 (m), 870 (m), 851 (m), 809 (m), 775 (s); UV-Vis (MeOH): λ_{max} (log ε) = 203 nm (4.41); MS (ESI): *m*/*z* (%) = 382/380 (9/9) [M + K]$^+$, 366/364 (92/100) [M + Na]$^+$, 361/359 (53/53), 344/342 (18/20) [M$^+$]; HRESIMS: calcd for $C_{14}H_{16}BrNNaO_4$ [M + Na]$^+$: 364.01549, found 364.01553.

(2*E*,2′*E*)-*N*,*N*′-(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(3-(3-bromophenyl)-2-(tetrahydro-2*H*-pyran-2-yloxyimino)propanamide) (17). To a solution of acid **14** (0.25 g, 0.72 mmol, 1.00 equiv.) in 3.5 mL dioxane were added NHPI (0.12 g, 0.72 mmol, 1.00 equiv.) and DCC (0.15 g, 0.72 mmol, 1.00 equiv.). The reaction mixture was stirred at rt for 4 h. A solution of cystamine dihydrochloride (**15**) (0.08 g, 0.35 mmol, 0.48 equiv.) and NEt_3 (0.20 mL, 0.15 g, 1.44 mmol, 2.00 equiv.) in 3.5 mL MeOH was added within 1.5 h and the reaction mixture was stirred for another 20 h. After evaporation of the solvent the crude product was isolated by column chromatography on silica [PE–EtOAc (9 : 1) → (2 : 1) → (1 : 2)] and purified by HPLC on RP-18 [MeOH–H_2O (9 : 1) → MeOH]. The *E*,*E*-isomer **17** was obtained as a colorless solid (0.13 g, 0.16 mmol, 45%); TLC [silica, PE–EtOAc (2 : 1)]: R_f = 0.35; m.p.: 95–97 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.33 (t, ^3J = 5.9 Hz, 2H, 2× N*H*), 7.47–7.47 (m, 2H, 2× 2-*H*), 7.40 (dd, ^4J = 2.3 Hz, ^3J = 6.8 Hz, 2H, 2× 4-*H*), 7.27–7.23 (m, 4H, 2× 5-*H*, 2× 6-*H*), 5.36 (t, ^3J = 2.7 Hz, 2H, 2× 10-*H*), 3.89 (d, ^2J = 13.3 Hz, 2H, 2× 7-C*H*H), 3.82 (d, ^2J = 13.3 Hz, 2H, 2× 7-CH*H*), 3.48–3.43 (m, 4H, 2× NHCH_2), 3.43–3.36 (m, 4H, 2× 14-OCH_2), 2.85 (t, ^3J = 7.0 Hz, 4H, 2× SCH_2), 1.74–1.70 (m, 4H, 2× 11-CH_2), 1.70–1.56 (m, 4H, 2× 12-CH_2), 1.59–1.42 (m, 4H, 2× 13-CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 162.3 (2C, 2× *C*q-9), 152.6 (1C, 2× *C*q-8), 139.0 (2C, 2× *C*q-1), 131.7 (2C, 2× *C*-2), 130.5 (2C, 2× *C*-5), 129.1 (2C, 2× *C*-4), 127.8 (2C, 2× *C*-6), 121.4 (2C, 2× *C*qBr), 100.5 (2C, 2× *C*-10), 60.9 (2C, 2× OCH_2-14), 38.3 (2C, 2× NH*C*H$_2$), 36.7 (2C, 2× S*C*H$_2$), 29.7 (2C, 2× *C*H$_2$-7), 28.0 (2C, 2× *C*H$_2$-11), 24.5 (2C, 2× *C*H$_2$-13), 18.2 (2C, 2× *C*H$_2$-12); IR (ATR): $\tilde{\nu}$ = 1651 cm^{-1} (s), 1532 (s), 1204 (s), 965 (vs), 706 (s), 687 (s); UV-Vis (MeOH): λ_{max} (log ε) = 202 nm (4.48); MS (ESI): *m*/*z* (%) = 823 (44) [M + Na]$^+$, 471 (21), 247 (100); HRESIMS: calcd for $C_{32}H_{40}Br_2N_4NaO_6S_2$ [M + Na]$^+$: 821.06482, found 821.06532.

***N*,*N*′-(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(2-(7-(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamide) (19).** 4-Coumarinacetic acid **18** (0.07 g, 0.30 mmol, 1.00 equiv.) was dissolved in 4 mL DCM and cooled to 0 °C. Then HOBt (0.04 g, 0.31 mmol, 1.05 equiv.) was added and after stirring for 10 min at 0 °C EDCI (0.06 mL, 0.05 g, 0.31 mmol, 1.05 equiv.) was added and the reaction mixture was stirred for another 30 min at 0 °C. Then cystamine dihydrochloride (**15**) (0.04 g, 0.17 mmol, 0.57 equiv.) and NEt_3 (0.05 mL, 0.03 g, 0.34 mmol, 1.14 equiv.) were added and the reaction mixture was stirred for 10 min at 0 °C and for 11 h at rt. The solvent was evaporated and the residue dissolved in 300 mL *n*-butanol and washed with 1 N aqueous HCl (3 × 100 mL), saturated aqueous $NaHCO_3$ solution (3 × 100 mL) and saturated aqueous NaCl solution (3 × 100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica [$CHCl_3$–MeOH (20 : 1)] afforded the bis(coumarinyl) compound **19** as a yellow solid (0.05 g, 0.08 mmol, 45%); TLC [silica, $CHCl_3$–MeOH (15 : 1)]: R_f = 0.37; m.p.: 146–148 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.43 (t, ^3J = 5.7 Hz, 2H, 2× N*H*), 7.51 (d, ^3J = 9.0 Hz, 2H, 2× 5-*H*), 6.68 (dd, ^4J = 2.5 Hz, ^3J = 9.0 Hz, 2H, 2× 6-*H*), 6.53 (d, ^4J = 2.5 Hz, 2H, 2× 8-*H*), 6.00 (s, 2H, 2× 3-*H*), 3.60 (s, 4H, 2× 9-CH_2), 3.36 (m, 4H, 2× NHCH_2), 3.00 (s, 12H, 2× (CH_3)$_2$), 2.78 (t, ^3J = 6.7 Hz, 4H, 2× SCH_2);

^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.0 (2C, 2× *C*qONH), 160.6 (2C, 2× *C*q-2), 155.3 (2C, 2× *C*q-8a), 152.7 (2C, 2× *C*q-7), 151.0 (2C, 2× *C*q-4a), 125.9 (2C, 2× *C*-5), 109.3 (2C, 2× *C*-3), 108.9 (2C, 2× *C*-6), 108.1 (2C, 2× *C*q-4), 97.3 (2C, 2× *C*-8), 39.6 (4C, 4× *C*H$_3$), 38.6 (2C, 2× *C*H$_2$-9), 38.0 (2C, 2× NH*C*H$_2$), 36.9 (2C, 2× S*C*H$_2$); IR (ATR): $\tilde{\nu}$ = 1595 cm^{-1} (vs), 1528 (s), 1400 (s), 1024 (s); UV-Vis (MeOH): λ_{max} (log ε) = 371 nm (4.44), 244 (4.38), 207 (4.71); fluorescence (MeOH): λ_{max} [Int. (a.u.), conc.] = 466 nm (980, 0.014 mg per 10 mL); MS (ESI): *m*/*z* (%) = 1243 (35) [2M + Na]$^+$, 633 (63) [M + Na]$^+$, 611 (7) [M + H]$^+$, 191 (100); HRESIMS: calcd for $C_{30}H_{34}N_4NaO_6S_2$ [M + Na]$^+$: 633.18120, found 633.18127.

***tert*-Butyl-2-((2-(2-(7-(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamido)ethyl)disulfanyl)ethylcarbamate (24).** 4-Coumarin-acetic acid **18** (0.33 g, 1.33 mmol, 1.00 equiv.) was dissolved in 15 mL dry DCM and cooled to 0 °C under an argon atmosphere. Then Boc protected cystamine **20** (0.37 g, 1.46 mmol, 1.10 equiv.) in 10 mL dry DCM was added dropwise. After 10 min at 0 °C were added EDCI (0.38 mL, 0.33 g, 2.13 mmol, 1.16 equiv.) and DMAP (57 mg, 0.47 mmol, 0.35 equiv.) and the reaction mixture was stirred for another 10 min at 0 °C and for 5 h at rt. The reaction mixture was diluted with 5% $KHSO_4$–ice solution (20 mL) and extracted with DCM (3 × 20 mL). The organic phase was washed in each case with 50 mL of H_2O, 5% aqueous $NaHCO_3$ solution, H_2O and saturated aqueous NaCl solution. It was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica [EtOAc–PE (2 : 1 → 4 : 1 → EtOAc)] afforded the coumarin carbamate **24** as a yellow solid (0.36 g, 0.75 mmol, 56%); TLC [silica, EtOAc–PE (2 : 1)]: R_f = 0.40; m.p.: 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (d, ^3J = 9.0 Hz, 1H, 5-*H*), 6.84 (sbr, 1H, 10-CON*H*), 6.61 (dd, ^4J = 2.6 Hz, ^3J = 9.0 Hz, 1H, 6-*H*), 6.49 (d, ^4J = 2.6 Hz, 1H, 8-*H*), 6.08 (s, 1H, 3-*H*), 4.99 (sbr, 1H, 15-CON*H*), 3.67 (s, 2H, 9-C*H*$_2$), 3.56 (dt, ^3J = 6.0 Hz, ^3J = 21.1 Hz, 2H, 11-C*H*$_2$), 3.42 (dt, ^3J = 6.4 Hz, ^3J = 13.9 Hz, 2H, 14-C*H*$_2$), 3.05 (s, 6H, N(C*H*$_3$)$_2$), 2.80 (t, ^3J = 6.0 Hz, 2H, 12-C*H*$_2$), 2.76–2.72 (m, 2H, 13-C*H*$_2$), 1.43 (s, 9H, C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.4 (1C, *C*q-10), 161.8 (1C, *C*q-2), 156.0 (2C, *C*q-8a, *C*q-15), 153.1 (1C, *C*q-7), 149.8 (1C, *C*q-4a), 125.7 (1C, *C*-5), 110.4 (1C, *C*-3), 109.2 (1C, *C*-6), 108.5 (1C, *C*q-4), 98.3 (1C, *C*-8), 79.5 (1C, *C*q(CH$_3$)$_3$), 40.4 (1C, *C*H$_2$-9), 40.1 (2C, N(*C*H$_3$)$_2$), 39.5 (1C, *C*H$_2$-14), 38.7 (1C, *C*H$_2$-11), 38.2 (1C, S*C*H$_2$-12), 37.6 (1C, S*C*H$_2$-13), 28.4 (3C, C(*C*H$_3$)$_3$); IR (ATR): $\tilde{\nu}$ = 1616 cm^{-1} (s), 1530 (vs), 1273 (s), 1162 (s), 1147 (s); UV-Vis (MeOH): λ_{max} (log ε) = 374 nm (4.29), 245 (4.22), 208 (4.55); fluorescence (MeOH): λ_{max} [Int. (a.u.), conc.] = 463 nm (302, 0.014 mg per 10 mL); MS (ESI): *m*/*z* (%) = 504 (100) [M + Na]$^+$, 482 (4) [M$^+$], 448 (13), 413 (64), 382 (15), 301 (37), 185 (49), 171 (78); HRESIMS: calcd for $C_{22}H_{31}N_3NaO_5S_2$ [M + Na]$^+$: 504.15973, found 504.15958.

(*E*)-3-(3-Bromo-4-hydroxyphenyl)-*N*-(2-((2-(2-(7-(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamido)ethyl)disulfanyl)ethyl)-2-(hydroxyimino)propanamide (25). Carbamate **24** (0.15 g, 0.31 mmol, 1.00 equiv.) was dissolved in 3 mL DCM under an argon atmosphere and cooled to 0 °C. Then 1 mL TFA was added dropwise and the reaction mixture was stirred for 10 min at 0 °C and for 60 min at rt. After finishing deprotection (TLC [silica, $CHCl_3$–MeOH (9 : 1)]) the solvent was evaporated and the residue vacuum dried for 2 h in a 40 °C water bath, washed with Et_2O (3 × 1 mL) and again vacuum dried. In a separate flask acid **12** (0.12 g, 0.44 mmol, 1.40 equiv.) was dissolved in 3 mL dry DMF under an argon atmosphere. Then NHS (0.07 g, 0.62 mmol, 2.00 equiv.) was added and after 5 min DCC (0.13 g, 0.62 mmol, 2.00 equiv.) was added and the reaction mixture was stirred for 2 h at rt. The deprotected coumarin amine was dissolved in 2 mL dry DMF and added dropwise. Afterwards was added dry NEt_3 (0.06 mL, 0.42 mmol, 1.30 equiv.) and the reaction mixture was stirred for 39 h at 30 °C and 4 h at 35 °C. After filtration from DCU cold H_2O (15 mL) was added, acidified under ice cooling with 1 N aqueous HCl solution to pH 3–4 and extracted with DCM (3 × 15 mL). The organic phase was washed with H_2O (15 mL) and saturated aqueous NaCl solution (15 mL), dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica [$CHCl_3$–MeOH (50 : 1)] yielded the *E*-heterodimer **25** as a yellow oil (0.11 g, 0.17 mmol, 55%). Under other conditions and work-up was also isolated the corresponding nitrile **27** as a yellow solid (0.02 g, 0.08 mmol, 18%); (*E*)-**25**: TLC [silica, $CHCl_3$–MeOH (9 : 1)]: R_f = 0.37; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.89 (sbr, 1H, NO*H*), 10.11 (sbr, 1H, O*H*), 8.41 (t, ^3J = 5.7 Hz, 1H, 10-CON*H*), 8.11 (t, ^3J = 5.9 Hz, 1H, 15-CON*H*), 7.54 (d, ^3J = 9.0 Hz, 1H, 5-*H*), 7.28 (d, ^4J = 2.1 Hz, 1H, 19-*H*), 7.00 (dd, ^4J = 2.1 Hz, ^3J = 8.3 Hz, 1H, 21-*H*), 6.82 (d, ^3J = 8.3 Hz, 1H, 20-*H*), 6.71 (dd, ^4J = 2.6 Hz, ^3J = 9.0 Hz, 1H, 6-*H*), 6.55 (d, ^4J = 2.5 Hz, 1H, 8-*H*), 6.01 (s, 1H, 3-*H*), 3.68 (s, 2H, 17-C*H*$_2$), 3.62 (s, 2H, 9-C*H*$_2$), 3.41 (dt, ^3J = 6.3 Hz, ^3J = 13.6 Hz, 2H, 14-C*H*$_2$), 3.37–3.35 (m, 2H, 11-C*H*$_2$), 3.01 (s, 6H, N(C*H*$_3$)$_2$), 2.82–2.79 (m, 2H, 13-C*H*$_2$), 2.79–2.77 (m, 2H, 12-C*H*$_2$); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.9 (1C, *C*q-10), 163.2 (1C, *C*q-15), 160.6 (1C, *C*q-2), 155.3 (1C, *C*q-8a), 152.7 (1C, *C*q-7), 152.3 (1C, *C*qOH), 151.7 (1C, *C*=NOH), 151.1 (1C, *C*q-4a), 132.7 (1C, *C*-19), 129.0 (1C, *C*-21), 128.6 (1C, *C*q-18), 125.9 (1C, *C*-5), 116.0 (1C, *C*-20), 109.4 (1C, *C*-3), 108.9 (1C, *C*-6), 108.8 (1C, *C*qBr), 108.1 (1C, *C*q-4), 97.4 (1C, *C*-8), 39.6 (2C, N(*C*H$_3$)$_2$), 38.7 (1C, *C*H$_2$-9), 38.1 (1C, *C*H$_2$-14), 37.9 (1C, *C*H$_2$-11), 37.0 (1C, *C*H$_2$-12), 36.7 (1C, *C*H$_2$-13), 27.6 (1C, *C*H$_2$-17); IR (ATR): $\tilde{\nu}$ = 1598 cm^{-1} (vs), 1526 (s), 1400 (s); UV-Vis (MeOH): λ_{max} (log ε) = 375 nm (4.21), 281 (3.59), 243 (4.26), 204 (4.72); fluorescence (MeOH): λ_{max} [Int. (a.u.), conc.] = 464 nm (337, 0.017 mg per 10 mL); MS (ESI): *m*/*z* (%) = 1297 (21) [2M + Na]$^+$, 661 (100) [M + Na]$^+$, 339 (5), 185 (7); HRESIMS: calcd for $C_{26}H_{29}BrN_4NaO_6S_2$ [M + Na]$^+$: 659.06041, found 659.06041.

2-(3-Bromo-4-hydroxyphenyl)acetonitrile (27). ^{1}H NMR (400 MHz, acetone-d_6): δ = 7.54 (d, ^4J = 2.2 Hz, 1H, 2-*H*), 7.24 (dd, ^4J = 2.2 Hz, ^3J = 8.4 Hz, 1H, 6-*H*), 7.03 (d, ^3J = 8.3 Hz, 1H, 5-*H*), 3.88 (s, 2H, 7-C*H*$_2$); ^{13}C NMR (100 MHz, acetone-d_6): δ = 154.6 (1C, *C*qOH), 133.6 (1C, *C*-2), 129.4 (1C, *C*-6), 124.8 (1C, *C*q-1), 119.2 (1C, *C*-5), 117.6 (1C, *C*qN), 110.5 (1C, *C*qBr), 22.2 (1C, 7-*C*H$_2$); MS (EI, 70 eV): *m*/*z* (%) = 213/211 (30/30) [M$^+$], 132 (100), 104 (7), 77 (12), 51 (7).

(*E*)-3-(3-Bromophenyl)-*N*-(2-((2-(2-(7-(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamido)ethyl)disulfanyl)ethyl)-2-(hydroxyimino)propanamide (26). Carbamate **24** (0.20 g, 0.42 mmol,

1.00 equiv.) was dissolved in 3 mL DCM under an argon atmosphere and cooled to 0 °C. Then 1 mL TFA was added and the reaction mixture was stirred for 10 min at 0 °C and for 45 min at rt. After finishing deprotection (TLC [silica, $CHCl_3$–MeOH (9 : 1)]) it was evaporated under vacuum, dried for 2 h, washed with Et_2O (3 × 1 mL) and again vacuum dried. In a separate flask acid **13** (0.11 g, 0.44 mmol, 1.05 equiv.) was dissolved in 2.4 mL dioxane at rt and NHPI (0.07 g, 0.42 mmol, 1.00 equiv.) and after 5 min DCC (0.09 g, 0.42 mmol, 1.00 equiv.) were added. After 2 h the deprotected amine was dissolved in 2 mL MeOH and added dropwise to the reaction mixture and NEM (0.05 mL, 0.05 g, 0.42 mmol, 1.00 equiv.) was added. The reaction mixture was stirred for 30 h at 30 °C under an argon atmosphere. After filtration from DCU it was diluted with cold H_2O (15 mL), acidified with 1 N aqueous HCl solution to pH 3–4 and extracted with DCM (3 × 15 mL). The organic phase was washed with H_2O (15 mL) and saturated NaCl solution (15 mL), dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography on silica [$CHCl_3$–MeOH (50 : 1)] afforded the *E*-heterodimer **26** as a yellow solid (0.16 g, 0.26 mmol, 62%); TLC [silica, $CHCl_3$–MeOH (9 : 1)]: R_f = 0.49; m.p.: 88–91 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ = 11.23 (sbr, 1H, NO*H*), 7.86–7.81 (m, 1H, N*H*), 7.75–7.71 (m, 1H, N*H*), 7.60 (d, ^3J = 9.0 Hz, 1H, 5-*H*), 7.50 (t, ^4J = 1.8 Hz, 1H, 19-*H*), 7.36–7.33 (m, 1H, 20-*H*), 7.32–7.29 (m, 1H, 22-*H*), 7.19 (t, ^3J = 7.8 Hz, ^3J = 9.0 Hz, 1H, 21-*H*), 6.69 (dd, ^4J = 2.6 Hz, ^3J = 9.0 Hz, 1H, 6-*H*), 6.49 (d, ^4J = 2.6 Hz, 1H, 8-*H*), 6.10 (s, 1H, 3-*H*), 3.93 (s, 2H, 17-CH_2), 3.71 (s, 2H, 9-CH_2), 3.60–3.55 (m, 2H, 14-CH_2), 3.54–3.49 (m, 2H, 11-CH_2), 3.01 (s, 6H, N(CH_3)$_2$), 2.89–2.84 (m, 2H, 13-CH_2), 2.87–2.81 (m, 2H, 12-CH_2); ^{13}C NMR (100 MHz, acetone-d_6): δ = 169.0 (1C, *C*q-10), 164.1 (1C, *C*q-15), 161.7 (1C, *C*q-2), 156.9 (1C, *C*q-8a), 154.1 (1C, *C*q-7), 152.6 (1C, *C*═NOH), 151.4 (1C, *C*q-4a), 140.6 (1C, *C*q-18), 132.8 (1C, *C*-19), 131.1 (1C, *C*-21), 130.1 (1C, *C*q-20), 129.0 (1C, *C*-22), 126.9 (1C, *C*-5), 122.7 (1C, *C*q-Br), 111.1 (1C, *C*-3), 109.8 (1C, *C*-6), 109.5 (1C, *C*q-4), 98.6 (1C, *C*-8), 40.2 (2C, N(*C*H_3)$_2$), 39.9 (1C, *C*H_2-9, from HSQC), 39.4 (1C, *C*H_2-11), 39.3 (1C, *C*H_2-14), 38.9 (1C, S*C*H_2-12), 38.1 (1C, S*C*H_2-13), 29.5 (1C, *C*H_2-17); IR (ATR): $\tilde{\nu}$ = 1595 cm^{-1} (vs), 1525 (s), 1400 (s); UV-Vis (MeOH): λ_{max} (log ε) = 374 nm (4.24), 244 (4.26), 204 (4.71); fluorescence (MeOH): λ_{max} [Int. (a.u.), conc.] = 465 nm (233, 0.015 mg per 10 mL); MS (ESI): *m*/*z* (%) = 1265 (5) [2M + Na]$^+$, 645/643 (100) [M + Na]$^+$, 623/621 (35) [M$^+$], 346 (9), 301 (20), 179 (13); HRESIMS: calcd for $C_{26}H_{29}BrN_4NaO_5S_2$ [M + Na]$^+$: 643.06550, found 643.06551.

Biological tests

MTT assays with mouse cell line L-929. An MTT assay was used to measure the influence of compounds on the propagation and viability of L-929 mouse fibroblasts (DSMZ ACC2) in 96-well plates. Cells are able to reduce MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) to a violet formazan product. The resulting purple colour gives a measure of the metabolic activity in each well. Cells were kept in a DME medium supplemented with 10% FBS. 60 µL of serial dilutions of the test compounds were added to 120 µL aliquots of a cell suspension (50 000 mL^{-1}) in each well. Blank and solvent controls were incubated under identical conditions. After 5 days 20 µL of MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL of isopropanol containing 0.4% hydrochloric acid. The microplates were gently shaken for 20 min to ensure complete dissolution of the formazan and finally measured at 590 nm using a plate reader. All experiments were carried out in two parallel experiments, the percentage of viable cells was calculated as the mean with respect to the controls set to 100%. An IC_{50} value was determined from the resulting dose–response curves.

Agar diffusion assays. Agar plates containing 15 mL of medium were inoculated with bacterial or yeast suspensions in a liquid broth to give a final OD of 0.01 (bacteria) or 0.1 (yeasts). In the case of molds, spores were collected from well-grown Petri dishes which were rinsed with 10 mL sterile aqua dest. 1 mL of the spore suspension was added to 100 mL of molten agar medium. 20 µL of test samples in methanol (1 mg mL^{-1}) were applied onto 6 mm cellulose discs. The methanol was allowed to evaporate and the discs were placed upon the inoculated agar plate. The diameters in mm of the resulting growth zones were determined after 24 h of incubation at 30 °C.

Serial dilution assay with bacteria. Antibiotic potential was estimated by measuring OD at 600 nm as a parameter of bacterial growth of bacteria in serial dilutions of a compound after 24 h of incubation. The IC_{50} was calculated from resulting inhibition curves.

HDAC assay. Histone deacetylase inhibition was measured using the Fluorometric Histone Deacetylase Assay Kit purchased from Sigma.

Acknowledgements

We thank Wera Collisi, Tatjana Hirsch and Bettina Hinkelmann for the excellent technical assistance with biological activity assays. This work has been supported in part by the "Evangelische Stiftung e.V. Villigst". We also thank Merck, BASF and Honeywell for their support with chemicals.

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