Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 2133

www.rsc.org/obc

PAPER

Chemical synthesis and biological evaluation of gallidermin-siderophore conjugates

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Received 6th October 2010, Accepted 13th December 2010
DOI: 10.1039/c0ob00846j

The lantibiotic gallidermin was modified at lysine residues by regioselective attachment of derivatives of pyochelin, agrobactin and desferrioxamine B with the objective of having siderophore receptors of Gram-negative bacteria transport the antibiotic-iron chelator conjugate through the outer membrane. All of the conjugates retained activity against the Gram-positive indicator strain, *Lactococcus lactis* subsp. *cremoris* HP. However, testing of the conjugates against several Gram-negative strains yielded unexpected results. Bacteria treated with 100 μM of the conjugates complexed with Fe^{3+} grew better than bacteria grown in iron-free media but worse than bacteria grown in the same media supplemented with 10 μM $FeCl_3$. Although these findings indicate that the conjugates are unable to inhibit the growth of Gram-negative bacteria, they indicate penetration of the outer membrane and provide structure–activity information for design of other lantibiotic conjugates. The synthetic strategy is applicable for linking biomarkers or fluorescence probes to gallidermin for studies on its localization and mode of action. As there are many lantibiotics that operate with unknown mechanisms of action, this chemical approach provides a means to modify such peptides with biomarkers for biological investigations.

Introduction

Opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa* cause numerous life threatening infections in humans every year.[1] Although there are many antibiotic treatments available, some bacteria have found ways to develop resistance to almost all clinically used drugs. This provides a great challenge to treat infections caused by these resistant strains.[1,2] In recent years, lantibiotics have attracted much interest as a potential new class of antibiotics for clinical applications and food preservation.[3] Lantibiotics such as nisin and gallidermin (Fig. 1) are posttranslationally modified antimicrobial peptides with potent antibacterial activity against many pathogenic Gram-positive bacteria.[4,5] Moreover, some lantibiotics exhibit a dual mode of action, by inhibiting peptidoglycan biosynthesis and by forming cell membrane pores that lead to leakage and cell death.[4] Nisin has been used as a food preservative in dairy products for over 50 years, and bacteria have yet to develop significant resistance to nisin.[3] Although lantibiotics have great potential for clinical use, their inability to penetrate the outer membranes of Gram-negative bacteria greatly limits their use against such bacteria. As such, there is an increased interest in identifying chemical and biological approaches to overcome this limitation. Nature has already developed a strategy to transport antibiotics through the outer membranes of Gram-negative bacteria by taking advantage of a class of outer membrane receptors that recognize siderophores. Siderophores, which are small molecule iron chelators produced by bacteria for acquiring iron, are actively transported into the cell *via* dedicated receptors.[6]

Albomycins,[7] salmycins[8] and microcin E492m[9] are examples of natural antibiotics that incorporate siderophore-mimicking moieties within their strucures. The siderophore transporters recogize these moieties and transport the entire molecule into the cell, thus enabling the antibiotic to gain access to its intracellular targets.[9–12] Researchers have successfully employed a similar strategy by linking small molecule antibiotics to siderophores;[13–19] however, there is a considerable reduction in the antibacterial activity of these conjugates. A possible explanation may be that small molecule antibiotics are generally enzyme inhibitors whose binding affinities for their targets could be adversely affected by the covalent attachement of a large siderophore unit. Conversely, lantibiotics are shown to bind to lipid II, an external target found in the cell membrane.[20] As well, attaching siderophore moieties to lantibiotics may not have as serious consequences with regards to their mechanism of action, since lantibiotics are comparatively larger than siderophores. Moreover, lantibiotics are many orders of magnitude more potent than small molecule antibiotics against Gram-positive bacteria, so even if linking siderophores to lantibiotics causes a small reduction in activity, the conjugates could still be highly potent. Thus, we were interested in pursuing the possibility of rendering lantibiotics active against Gram-negative bacteria.

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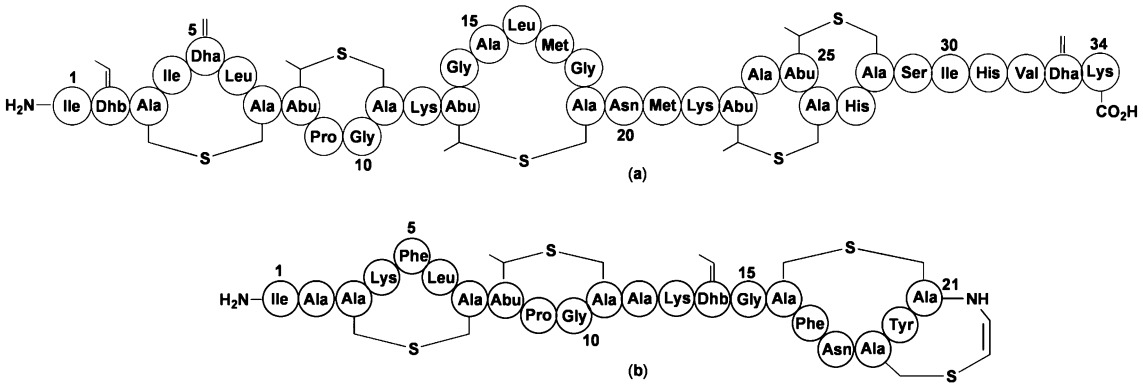


Fig. 1 Simplified structure of (a) nisin and (b) gallidermin.

Our research group as well as others have reported that increasing the permeability of the outer membrane sensitizes Gram-negative bacteria towards lantibiotics.[21–24] In particular, our recent work has shown that when gallidermin and a number of other bacteriocins from lactic acid bacteria are tested in combination with ethylenediaminetetraacetic acid (EDTA), they exhibit activity against Gram-negative bacteria. These findings indicate that if we find a way to transport lantibiotics through the outer membrane, they may be able to inhibit the growth of Gram-negative bacteria. Encouraged by our results from the EDTA testing, we decided to explore synthetic strategies to effectively link lantibiotics to siderophore analogues, such that these antimicrobial peptides could gain entry through the outer membrane. Our hypothesis drew support from the fact that microcin E492m is an 84 amino acid peptide with a siderophore moiety that is recognized by a bacterial outer membrane receptor that is large enough to allow the peptide's entry into the cell.[11,25,26] Therefore, we hoped that the size of the lantibiotic-siderophore conjugate would not be a limiting factor in its transport into the cell. More importantly, the chemical methodology developed in our study will expand the scope of modifications that can be made to lantibiotics for the purpose of increasing their spectrum of activity and for studying their mechanisms of action. For this study, three different siderophore analogues, including pyochelin, agrobactin and desferrioxamine B were selected. Pyochelin is secreted by *Pseudomonas aeruginosa* and *Burkholderia* species.[27,28] Agrobactin, a biscatechol containing siderophore, and desferrioxamine B, a hydroxamate containing siderophore, are utilized by either *E. coli* or *Salmonella* species.[6,29,30]

In undertaking the synthesis of gallidermin-siderophore conjugates, several difficulties must be addressed. Firstly, an effective linking strategy must be identified. It is important to use a suitable bifunctional linker that would allow us to regioselectively link siderophores to gallidermin. Here we decided to pursue 1,5-difluoro-2,4-dinitrobenzene (DFDNB) and dimethyl squarate as possible bifunctional linkers for this chemistry, since they can be reacted in a stepwise fashion to link two different molecules. Secondly, it is generally difficult to modify a natural peptide regioselectively without the use of protecting groups. Since gallidermin has no C-terminus and no side chain carboxyl groups, the remaining options are to modify one of two available lysine side chains or the N-terminus. Since modifications close to the N-terminus tend to compromise the antibacterial activity of lantibiotics, the best site for modification is the lysine at the 13th position. Finally, the reaction conditions used need to be compatible with the presence of thioether bridges, dehydro amino acid residues and the amino-vinylcysteine unit in gallidermin.

Aside from simply making use of the bifunctional linkers, two of the siderophores, pyochelin and agrobactin, needed to be structurally modified in order to react with DFDNB or dimethyl squarate. We envisioned synthesizing analogues that contain sidechains with either a hydroxyl or amine group for the linking chemistry. A pyochelin analogue reported by Rivault *et al.* was modified at the phenyl ring.[28,31] Although the authors provide no rationale for choosing to modify that specific position with respect to binding of pyochelin to its receptor, examination of the crystal structure of pyochelin bound to its receptor sheds light on why the position was a suitable choice (Fig. 2).[32] In the crystal structure, the phenyl ring of pyochelin extends outward from the receptor's binding pocket, making it the most exposed part of the molecule. This coupled with the fact that the phenyl ring is not directly involved in coordinating Fe^{3+} makes it a logical site to attach a side chain. Rivault *et al.*[16] have also used the pyochelin analogue to make a pyochelin-norfloxacin conjugate, and have shown that it is taken in by a *Pseudomonas* species, further supporting the hypothesis that this pyochelin analogue can be recognized by its receptor. In a similar fashion, we designed an agrobactin analogue with an amine side chain that extends from

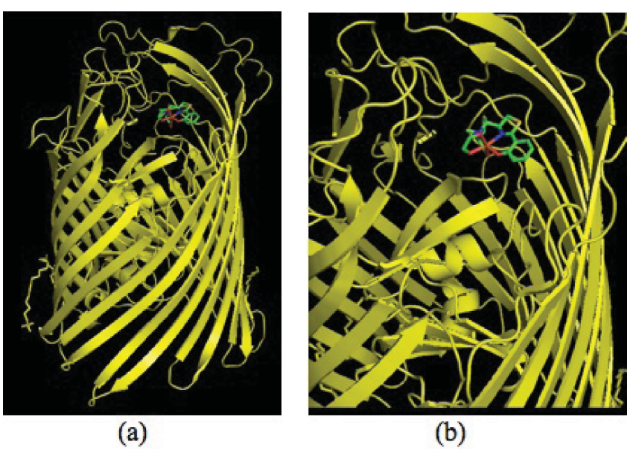
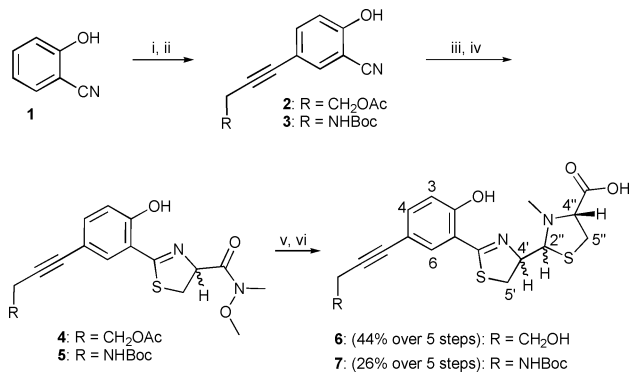


Fig. 2 Co-crystal structure of pyochelin outer membrane receptor, FptA from *Pseudomonas aeruginosa* (PDB ID : 1XKW)[32] (a) full view and (b) closer look at pyochelin binding site.

a site least likely to interfere with iron binding. Herein, we report the attachment of these siderophore analogues to gallidermin, and the biological evaluation of the resulting conjugates against Gram-negative bacteria.

Results and discussion

The first step of our project was to synthesize both pyochelin and agrobactin analogues. Pyochelin analogues were prepared, using a modified literature procedure,[28] as two sets of interconvertable diastereomers (4′*R*2″*R*4″*R*:4′*R*2″*S*4″*R*:4′*S*2″*R*4″*R*:4′*S*2″*S*4″*R*/1 : 1 : 3 : 2 by ^{1}H NMR) (Scheme 1). These pyochelin analogues contain either an amine or a hydroxyl functionality for use in our linking chemistry.



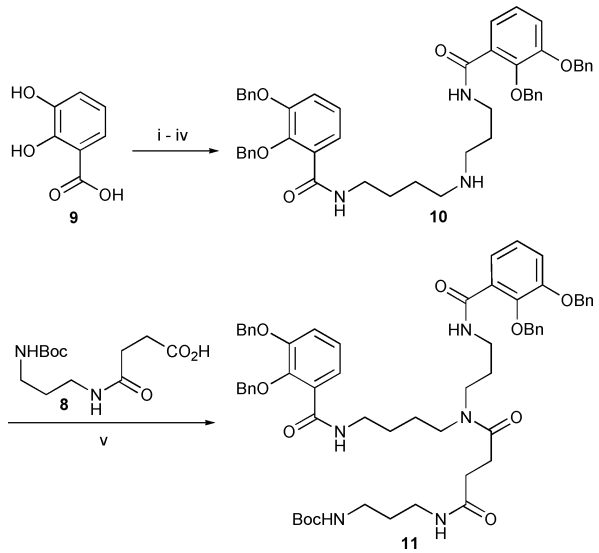
Scheme 1 *Reagents and conditions*: (i) $HBF_4{\cdot}Et_2O$, NIS, MeCN, –30 °C (ii) alkyne derivative, DIPEA, CuI, $Pd(PPh_3)_4$, DMF (iii) (*R*)-cysteine, PO_4-buffer/THF, 60 °C (iv) HN(OMe)Me, EDCI, HOBt, DIPEA, CH_2Cl_2 (v) $LiAlH_4$, THF, –40 °C (vi) (*R*)-*N*-methylcysteine, KOAc, EtOH/H_2O.

In addition, an agrobactin analogue with an amine-containing side chain was synthesized using the strategy shown in Scheme 2. The compound **11** belongs to a class of bis-catechol containing synthetic siderophores and is known as spermexatol, due to the presence of a spermidine unit.[30,33]

The third siderophore used in our investigation, the hydroxamate-based desferrioxamine B, already has an amine tail suitable for linking chemistry. Since it was commercially available, it was used directly in reactions with the bifunctional linkers.

Before proceeding with the linking chemistry, we confirmed that the selected siderophore analogues are recognized by outer membrane receptors. Both growth curve studies (Fig. 3)[34] and fluorescence microscopy (Fig. 4)[34] were used to evaluate the ability of these siderophore analogues to enter the cell. For the growth promotion studies and fluorescence evaluation, all of the siderophores were precomplexed with Fe^{3+} to give a final concentration of 10 μM and added to the test strains. All bacteria were grown in M9 minimal media for both of these studies. The results show that all three siderophores enter, and are therefore utilized by, Gram-negative bacteria under iron deficient conditions.

Following this, we went forward to explore an applicable linking strategy. Several attempts were made to identify a suitable bifunctional linker. In particular, DFDNB was thought to be useful for attaching molecules together *via* their hydroxyl moieties. In reality, DFDNB was found to be highly reactive but non-selective, causing it to link onto the phenol in addition to



Scheme 2 *Reagents and conditions*: (i) $SOCl_2$, MeOH, reflux, 85% (ii) BnBr, K_2CO_3, NaI, DMF, 63% (iii) $NaOH_{(aq)}$/THF, 93%; (iv) CDI, spermidine, CH_2Cl_2, 59% (v) PyBOP, HOBt, DIPEA, CH_2Cl_2, 91%.

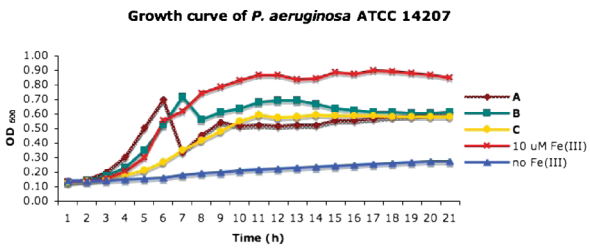


Fig. 3 Growth promotion of *P. aeruginosa* by the siderophore derivatives (A: pyochelin analogue; B: agrobactin analogue; C: desferrioxamine B).

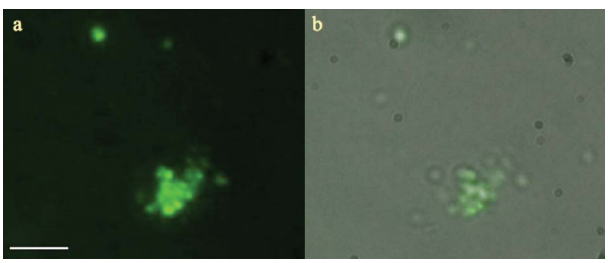
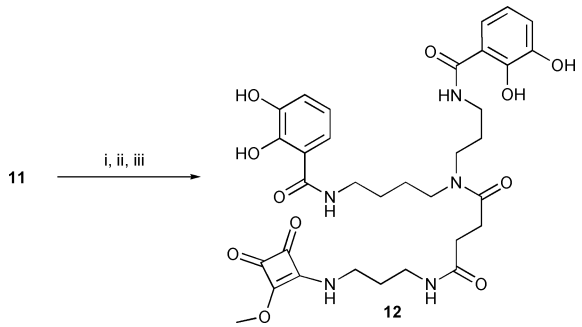


Fig. 4 Images of *P. aeruginosa* cells treated with fluorescein labeled agrobactin analogue (a) fluorescence image and (b) overlay of fluorescence and white-field images (scale bar, 10 μm).

the alcohol sidechain. Hence, we turned our focus to using dimethyl squarate, which ultimately proved to be the best linker for our studies. Controlling the pH of the reaction allows the squarate to react with an amine-containing siderophore first, while avoiding the formation of dimerized side products. Purification of the squarate functionalized siderophore, and then subsequent reaction with gallidermin affords the desired conjugates. As such, dimethyl squarate can react in a stepwise fashion with two different molecules that each contain an amine handle. Since this linker reacts selectively with amino groups, the presence of other reactive functional groups in the molecules is compatible with this synthesis.

To employ the chosen linking strategy, siderophore analogue **11** was fully deprotected and then reacted with dimethyl squarate to yield the monofunctional derivative **12** (Scheme 3).



Scheme 3 *Reagents and conditions*: (i) TFA, CH_2Cl_2, 99% (ii) H_2, Pd/C, MeOH, 98% (iii) dimethyl squarate, MeOH, DIPEA, 86%.

In an analogous fashion, the other two siderophores were reacted with dimethyl squarate to give the monofunctional siderophore derivatives **13** and **14** (Fig. 5).

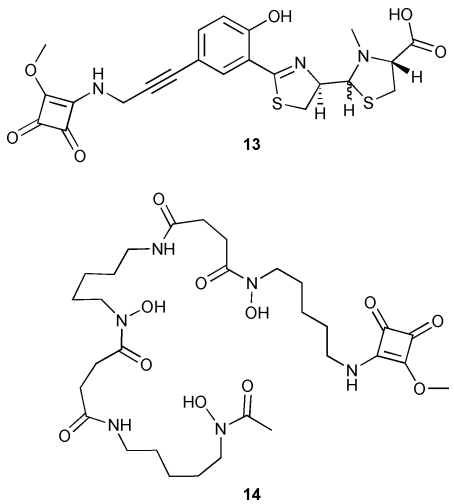


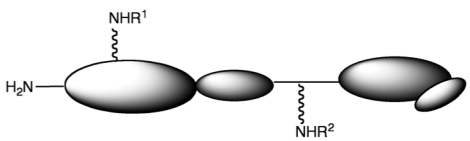
Fig. 5 Siderophore-monomethylsquarate esters.

These derivatives were then linked to gallidermin. The second addition to the squarate is highly dependent on the pH of the reaction mixture (pH~9). Therefore, the reaction was done in borate buffer. Each of the siderophore derivatives (**12**, **13** and **14**) was reacted with gallidermin in a mixture of methanol and borate buffer (pH~9). We found that **13** successfully reacted with gallidermin at 23 °C to give the desired monotagged product. However, both **12** and **14** produced multiply tagged conjugates at 23 °C. By lowering the reaction temperature to 4 °C, **12** could be used to produce monotagged conjugates (~1 : 1 mixture of regioisomers) with gallidermin. On the other hand, when the reaction with **14** was lowered to 4 °C, the reaction rates were too slow to produce any conjugates. Ultimately, ditagged conjugates were isolated from the reaction of **14** with gallidermin, and these products were used for the antibacterial testing. After extensive HPLC purification and MS/MS fragmentation analysis, we identified that the two lysine residues were the most reactive, giving rise to conjugates tagged at either (or both) of the two positions. Once we successfully attached all three siderophore conjugates to gallidermin regioselectively, the next step was to evaluate the biological activity of these conjugates.

All of the conjugates retained at least 1/10 of gallidermin's potency in terms of antibacterial activity against the indicator strain, *Lactococcus lactis* subsp. *cremoris* HP on a spot-on-lawn assay. This suggests that modified gallidermin still behaves in a similar fashion to the natural peptide. This result is encouraging since our chemical strategy is the first successful example of attachment of siderophore analogues to natural lantibiotics.

After completing the tests against the indicator strain, the same spot-on-lawn overlay method was used to test conjugates **15–19** against several Gram-negative bacteria, including *Pseudomonas aeruginosa* ATCC 14207, *Burkholderia cepacia* ATCC 25416, *E. coli* DH5α and *Salmonella enterica* Typhimurium ATCC 13311. All of the conjugates were complexed to Fe^{3+} prior to biological evaluation. All of the conjugates showed no antibacterial activity in both iron sufficient LB media and iron deficient M9 or succinic media at 100 μM concentrations. To gain more insight into the conjugates' activity profiles, additional testing using a 64-well plate assay in iron deficient M9 media was done. For this assay, all of the compounds complexed to Fe^{3+} were tested at 100 μM concentrations and the strains were grown in M9 minimal media at 37 °C.

Fig. 7 shows the results of the well plate assay from the testing of conjugate **18** against *P. aeruginosa* and *B. cepacia* (for other growth curves, see Supporting Information). Conjugates **15–19** slightly promoted the growth of *P. aeruginosa*. Conjugates **15–18** showed a similar activity profile against *B. cepacia*, but compound **19** showed no effect. Both *E. coli* and *Salmonella* strains showed no significant effect when treated with all five conjugates. From these observations, we speculate that the siderophore-Fe(III) complexes do enter the cell, however, there may be a very small local concentration of the conjugates or the conjugates fail to reach the inner membrane to show a complete killing effect. The early growth promotion is possibly due to a small amount of ferric ion being supplied by the conjugates. We believe that this indicates the



15 : R^1 = H, R^2 = pyochelin derivative; **16** : R^1 = pyochelin derivative, R^2 = H; **17** : R^1 = H, R^2 biscatechol derivative; **18** : R^1 = biscatechol derivative, R^2 = H; **19** : $R^1 = R^2$ = desferrioxamine B

Fig. 6 Gallidermin-siderophore conjugates.

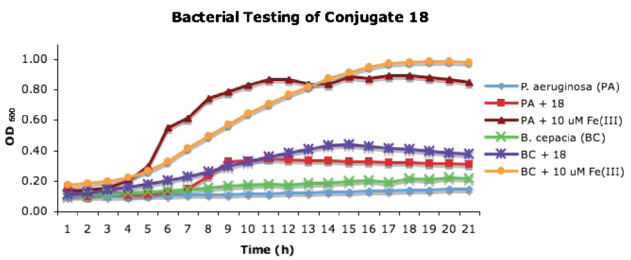


Fig. 7 Biological evaluation of conjugate **18** against *P. aeruginosa* and *B. cepacia* using a well plate assay.

conjugates are imported into the Gram-negative bacteria, but it is possible that they provide a form of solubilized iron that can be utilized by extracellular ferric ion exchange processes. Further research is needed to explain the observed results. Although unexpected, this iron delivery and growth promotion are encouraging in that they show evidence of the conjugates being recognized by bacterial membrane receptors. This preliminary evaluation serves as the ground work for investigating the ability of lantibiotics to cross the outer membrane of Gram-negative bacteria. Further investigations by attaching siderophores with better recognition and iron binding affinity (*e.g.* enterobactin and salmochelins) to gallidermin could provide a better conjugate. From this work, we have developed an efficient synthetic strategy for attaching small molecules to gallidermin. In turn, this approach can be extended to synthesize other lantibiotic-siderophore conjugates.

Conclusions

An efficient synthetic approach was developed to attach siderophores to gallidermin. The conjugates retained respectable bioactivity against the indicator organism. However, these conjugates showed unexpected, slight growth promotion in ferric ion deficient media. The antibacterial effect may be too low to observe a complete inhibition of bacteria. This synthetic strategy can be extended to modify other lantibiotics with various siderophores. With the identification of numerous novel lantibiotics, there is little known about the mechanism of action of these peptides in literature. Therefore, it would be advantageous to use this chemical methodology to modify such lantibiotics with biomarkers or fluorescence probes to investigate their mechanism of action.

Experimental

General

All reactions inlvolving anhydrous reaction conditions were done in flame-dried glassware under an argon atmosphere. All commercially available reagents were purchased and used without further purification. All the solvents used for reactions were distilled over appropriate drying reagents prior to use. Commercially available ACS grade solvents (> 99.0%) were used for column chromatography without any further purification. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates coated with silica gel 60 F_{254} (EMD Chemicals Inc.). Flash chromatography was performed using 230–400 mesh silica gel (Silicycle). HPLC purification and analysis were performed on a Varian ProStar instrument, monitored using a dual wavelength detector (220 and 280 nm). For HPLC, water (Solvent A) and acetonitrile (Solvent B), both containing 0.1% trifluoroacetic acid were used as eluent. NMR spectra were recorded on an Inova 600, Inova 500, Unity 500, Inova 400 or Inova 300 spectrometer. For ^{1}H (300, 400, 500 or 600 MHz) spectra, δ values were referenced to $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm) or $(CD_3)_2CO$ (2.04 ppm). For ^{13}C (100, 125 or 150 MHz) spectra, δ values were referenced to $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or $(CD_3)_2CO$ (29.8 ppm) as the solvents. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Mass spectra (MS) were recorded on a Kratos AEIMS-50 mass spectrometer (high resolution, HRMS).

HPLC purification methods

Method A. Purification was done using a C18 semi-preparative column (Vydac 5 μm, 10 mm × 250 mm). Flow rate: 3.0 mL/min. Gradient: Starting from 5% CH_3CN for 7 min and 1st ramp to 60% over 23 min, 2nd ramp to 90% CH_3CN over 2 min, followed by ramping down to 10% CH_3CN over 1 min, then 10% CH_3CN for 4 min. **Method B**. Purification was done using a C18 semi-preparative column (Vydac 5 μm, 10 mm × 250 mm). Flow rate: 3.0 mL/min. Gradient: Starting from 10% CH_3CN for 3 min and 1st ramp to 30% over 4 min, 2nd ramp to 90% CH_3CN over 23 min, followed by ramping down to 10% CH_3CN over 1 min, then 10% CH_3CN for 4 min. **Method C**. Purification was done using a C8 preparative column (ZORBAX Rx 7 μm, 21.2 mm × 250 mm). Flow rate: 10.0 mL/min. Gradient: Starting from 10% CH_3CN for 3 min and 1st ramp to 40% over 27 min, 2nd ramp to 90% CH_3CN over 3 min and then 90% CH_3CN for 5 min, followed by ramping down to 10% CH_3CN over 1 min, then 10% CH_3CN for 4 min. **Method D**. Purification was done using a C18 semi-preparative column (Vydac 5 μm, 10 mm × 250 mm). Flow rate: 3.0 mL/min. Gradient: Starting from 10% CH_3CN for 3 min and 1st ramp to 30% over 4 min, 2nd ramp to 50% CH_3CN over 26 min, 3rd ramp to 90% CH_3CN over 2 mininutes, followed by ramping down to 10% CH_3CN over 1 min, then 10% CH_3CN for 4 min.

General procedure for the synthesis of functionalized 2-hydroxybenzonitriles (2) and (3)

A solution of 5-iodo-2-hydroxybenzonitrile (1.0 equiv.), alkyne derivative (1.5 equiv.) and DIPEA (4.5 equiv.) in DMF (6 mL $mmol^{-1}$) was degassed for 20 min by bubbling argon through the solution. The solution was cooled to 0 °C and was added with $Pd(PPh_3)_4$ (0.05 equiv.) and CuI (0.1 equiv.). The reaction mixture was again degassed for another 20 min and allowed to stir at 23 °C for 10 h under argon atmosphere. Then the reaction mixture was concentrated *in vacuo* and product was purified by silica column chromatography (Et_2O–hexanes, 1 : 2) to obtain the compounds **2** or **3**.

4-(3-Cyano-4-hydroxyphenyl)but-3-ynyl acetate (2). Isolated as a yellow solid (2.6 g, 96%). ν_{max}(cast)/cm^{-1} 3400–3000 (br), 2962, 2233, 1740, 1708, 1607 and 1508; δ_H(300 MHz; $CDCl_3$) 7.54 (d, 1H, *J* 2.1, H-6), 7.48 (dd, 1H, *J* 8.7 and 2.1, H-4), 6.91 (d, 1H, *J* 8.7, H-3), 4.25 (t, 2H, *J* 6.9, H-10), 3.51 (s, 1H, Ar-OH), 2.73 (t, 2H, *J* 6.9, H-9) and 2.11 (s, 3H, CH_3); δ_C(100 MHz; $CDCl_3$) 171.8, 158.5, 137.7, 136.0, 116.7, 116.1, 115.7, 99.8, 85.6, 79.7, 62.5, 20.9 and 19.8; *m/z*(ES+) calcd for $C_{13}H_{11}NO_3Na$ 252.0631, found 252.0629 [MNa^+].

***tert*-Butyl-3-(3-cyano-4-hydroxyphenyl)prop-2-ynylcarbamate (3).** Isolated as a yellow oil (3.12 g, 85%). ν_{max}(cast)/cm^{-1} 3324–3050 (br), 2979, 2934, 2231, 1681, 1606 and 1510; δ_H(400 MHz; CD_3OD) 7.53 (d, 1H, *J* 2.0, H-6), 7.45 (dd, 1H, *J* 2.1 and 8.7, H-4), 6.88 (d, 1H, *J* 8.7, H-3), 4.00 (s, 2H, N-CH_2) and 1.44 (s, 9H, $(CH_3)_3$); δ_C(100 MHz; CD_3OD) 161.5, 158.0, 138.7, 137.4, 117.4, 116.9, 115.9, 101.1, 81.1, 80.6, 79.4, 31.3 and 28.7; *m/z*(ES+) calcd for $C_{15}H_{17}N_2O_3$ 273.1239, found [MH^+].

General procedure for the synthesis of Weinreb amides (4) and (5)

To a mixture of functionalized 2-hydroxybenzonitriles **2** or **3** (1.0 equiv.) and (*R*)-cysteine (2.0 equiv.) in THF (7 mL mmol^{-1}) was added PO_4-buffer (7 mL mmol^{-1}). The mixture was heated under reflux at 60 °C for 2 days and then THF was removed *in vacuo*. The suspention was diluted with water (300 mL), acidified with citric acid (pH~2) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to yield the corresponding thiazolines as a yellow solid. The thiazolines were used in the next step without further purification. To a solution of thiazoline in CH_2Cl_2 (20 mL mmol^{-1}) at 0 °C was added EDCI (1.2 equiv.), HOBt (1.2 equiv.) and DIPEA (1.2 equiv.), immediately followed by a solution of HN(OMe)Me·HCl (1.5 equiv.) and DIPEA (1.5 equiv.) in CH_2Cl_2 (20 mL mmol^{-1}). The reaction mixture was allowed to warm to 23 °C and after stirred for 2.5 h. The crude product was purified uisng column chromatography (Et_2O–hexanes, 1 : 1 to 2 : 1) to obtain the Weinreb amides **4** or **5**.

4-(4-Hydroxy-3-(4-(methoxy(methyl)carbamoyl)-4,5-dihydrothiazol-2-yl)phenyl)but-3-ynyl acetate (4). Isolated as a off-white solid (3.02 g, 79%). ν_{max}(microscope)/cm^{-1} 3300–2900 (br), 2940, 1739, 1672, and 1619; δ_H(400 MHz; $CDCl_3$) 7.46 (d, 1H, *J* 2.1, H-6), 7.36 (dd, 1H, *J* 8.8 and 2.1, H-4), 6.90 (d, 1H, *J* 8.8, H-3), 5.64 (t, 1H, *J* 8.7, H-4′), 4.23 (t, 2H, *J* 6.9, H-10), 3.80 (s, 3H, OCH_3), 3.75 (t, 1H, *J* 9.4, H-5′), 3.48 (dd, 1H, *J* 10.9 and 9.2, H-5′), 3.27 (s, 3H, N-CH_3), 2.72 (t, 2H, *J* 6.9, H-9) and 2.08 (s, 3H, CH_3); δ_C(125 MHz; $CDCl_3$) 173.6, 170.8, 169.5, 158.8, 136.4, 134.0, 117.3, 116.0, 113.9, 84.1, 80.9, 74.6, 62.3, 61.7, 33.0, 32.5, 20.8 and 19.8; *m*/*z*(ES+) calcd for $C_{18}H_{21}N_2O_5S$ 377.1166, found 377.1168 [MH$^+$].

***tert*-Butyl-3-(4-hydroxy-3-(4-(methoxy(methyl) carbamoyl)-4,5-dihydrothiazol-2-yl)phenyl)prop-2-ynylcarbamate (5).** Isolated as a yellow oil (1.30 g, 49%). ν_{max}(microscope)/cm^{-1} 3335, 2976, 2935, 2229, 1697, 1619, 1568 and 1488; δ_H(300 MHz; $CDCl_3$) 7.49 (d, 1H, *J* 2.0, H-6), 7.38 (dd, 1H, *J* 2.0 and 8.6, H-4), 6.91 (d, 1H, *J* 8.6, H-3), 5.68 (t, 1H, *J* 8.6, H-4′), 4.79 (s, 1H, NH), 4.12 (d, 2H, *J* 5.5, H-9), 3.81 (s, 3H, OCH_3), 3.76 (t, 1H, *J* 8.0, H-5′), 3.50 (m, 1H, H-5′), 3.28 (s, 3H, OCH_3) and 1.46 (s, 9H, $(CH_3)_3$); δ_C(100 MHz; $CDCl_3$) 173.7, 159.2, 155.3, 136.6, 134.3, 117.5, 116.1, 113.4, 84.3, 82.2, 74.7, 61.8, 33.1, 32.6, 31.3 and 28.5; *m*/*z*(ES+) calcd for $C_{20}H_{26}N_3O_5S$ 420.1593, found 420.1588 [MH$^+$].

General procedure for the synthesis of pyochelin analogues (6) and (7)

The Weinreb amides **4** or **5** (1.0 equiv.) were dissolved in dry THF (16 mL mmol^{-1}) and cooled to –40 °C. To the resulting solution, $LiAlH_4$ (1.0 M in THF) (2.0 equiv.) was added drop wise. The reaction mixture was stirred at –20 °C for 1 h and then quenched with MeOH (1 mL mmol^{-1} of $LiAlH_4$), saturated NH_4Cl (3 mL mmol^{-1} of $LiAlH_4$), and 5% H_2SO_4 (3 mL mmol^{-1} of $LiAlH_4$). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield the corresponding aldehyde, which was used in the next step immediately without further purification. To a solution of the aldehyde (1.0 equiv.) in ethanol (20 mL mmol^{-1}) was added (*R*)-*N*-methylcysteine (2.0 equiv.), KOAc (5.0 equiv.) and water (8 mL mmol^{-1} of aldehyde). The reaction mixture was stirred in the dark for 24 h. The reaction mixture was diluted with water (150 mL) and washed with hexanes (2 × 100 mL). The aqueous layer was acidified with solid citric acid to pH~4 and extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield the pyochelin analogues **6** or **7** in pure form. The products exist as a mixture of four diastereomers **I** : **II** : **III** : **IV**/1 : 1 : 3 : 2.

Pyochelin analogue 6. Isolated as a yellow solid (0.291 g, 66% over two steps). ν_{max}(microscope)/cm^{-1} 3322, 3055, 2954, 2230, 1704, 1618, 1572 and 1490; *m*/*z*(ES+) calcd for $C_{18}H_{21}N_2O_4S_2$ 393.0937, found 393.0940 [MH$^+$]. Isomer **I** (4′*R*,2″*R*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.40 (m, 2H, H-6, H-4), 6.91 (m, 1H, H-3), 5.21 (td, 1H, *J* 5.5 and 9.0, H-4′), 4.61 (d, 1H, *J* 5.5, H-2″), 3.66-3.74 (m, 4H, H-4″, H-10), 3.49-3.64 (m, 2H, H-5′), 3.21-3.23 (m, 2H, H-5″), 2.64 (s, 3H, N-CH_3) and 2.58 (t, 2H, *J* 6.5, H-9); δ_C(125 MHz; Acetone-d_6) 172.9, 172.0, 159.5, 137.1, 134.1, 118.2, 117.0, 115.5, 87.2, 81.1, 80.1, 77.2, 73.3, 61.4, 41.6, 33.2, 33.0 and 24.3; Isomer **II** (4′*R*,2″*S*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.40 (m, 2H, H-6, H-4), 6.91 (m, 1H, H-3), 5.02 (q, 1H, *J* 8.4, H-4′), 4.55 (d, 1H, *J* 8.2, H-2″), 4.25 (app t, 1H, *J* 6.5, H-4″), 3.66-3.74 (m, 3H, H-5′, H-10), 3.42-3.46 (m, 2H, H-5′), 3.21-3.23 (m, 2H, H-5″), 2.58 (t, 2H, *J* 6.5, H-9) and 2.50 (s, 3H, N-CH_3); δ_C(125 MHz; Acetone-d_6) 172.9, 172.0, 159.5, 137.1, 134.1, 118.2, 117.0, 115.5, 87.2, 81.1, 80.9, 77.8, 71.0, 61.4, 37.8, 35.1, 32.1 and 24.3; Isomer **III** (4′*S*,2″*R*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.40 (m, 2H, H-6, H-4), 6.91 (m, 1H, H-3), 4.84 (q, 1H, *J* 8.3, H-4″), 4.34 (d, 1H, *J* 8.3, H-2′), 4.02 (dd, 1H, *J* 6.9 and 5.3, H-4″), 3.66-3.69 (m, 1H, H-5′), 3.53-3.64 (m, 1H, H-5″), 3.42-3.46 (m, 1H, H-5′), 3.23-3.30 (m, 1H, H-5′), 2.63 (s, 3H, N-CH_3) and 2.58 (t, 2H, *J* 6.9, H-9); δ_C(125 MHz; Acetone-d_6) 172.9, 172.0, 159.5, 137.1, 134.1, 118.2, 117.0, 115.5, 87.2, 83.3, 81.1, 79.3, 61.4, 44.8, 35.1, 33.6 and 24.3; Isomer **IV** (4′*S*,2″*S*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.40 (m, 2H, H-6, H-4), 6.91 (m, 1H, H-3), 5.28-5.34 (m, 1H, H-4′), 5.05 (d, 1H, *J* 4.8, H-2″), 4.25 (app t, 1H, *J* 6.5, H-4″), 3.70-3.74 (m, 2H, H-10),3.49-3.64 (m, 1H, H-5′), 3.42-3.46 (m, 1H, H-5′), 3.30-3.33 (m, 1H, H-5″), 3.21-3.23 (m, 1H, H-5″), 2.70 (s, 3H, N-CH_3) and 2.58 (t, 2H, *J* 6.9, H-9); δ_C(125 MHz; Acetone-d_6) 172.9, 172.0, 159.5, 137.1, 134.1, 118.2, 117.0, 115.5, 87.2, 81.1, 79.3, 73.5, 70.4, 61.4, 36.2, 32.4, 32.3 and 24.3.

Pyochelin analogue 7. Isolated as a yellow solid (0.950 g, 82% over two steps). ν_{max}(microscope)/cm^{-1} 3374, 3053, 2979, 2934, 2230, 1709, 1619, 1572 and 1489; *m*/*z*(ES-) calcd for $C_{22}H_{26}N_3O_5S_2$ 476.1314, found 476.1319 [MH$^-$]. Isomer **I** (4′*R*,2″*R*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.42 (m, 2H, H-6, H-4) 6.93 (m, 1H, H-3), 5.20 (m, 1H, H-4′), 4.61 (d, 1H *J* 5.5, H-2″), 4.08 (m, 2H, H-9), 3.72 (m, 1H, H-4″), 3.51 (m, 2H, H-5′), 3.22 (m, 2H, H-5″), 2.64 (s, 3H, N-CH_3) and 1.42 (s, 9H, $Si(CH_3)_3$); δ_C(125 MHz; Acetone-d_6) 172.9, 160.0, 156.2, 137.0, 134.2, 118.3, 117.0, 114.4, 86.4, 83.2, 81.5, 80.2, 77.1, 73.1, 41.6, 33.2, 32.6, 31.4 and 28.6; Isomer **II** (4′*R*,2″*S*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.42 (m, 2H, H-6, H-4) 6.93 (m, 1H, H-3), 5.02 (q, 1H, *J* 8.4, H-4′), 4.55 (d, 1H, *J* 8.2, H-2″), 4.08 (m, 2H, H-9), 3.70 (m, 1H, H-5′), 3.52 (m, 1H, H-5′), 3.22 (m, 2H, H-5″), 2.50 (s, 3H, N-CH_3) and 1.42 (s, 9H, $Si(CH_3)_3$); δ_C(125 MHz; Acetone-d_6) 172.9, 160.0, 156.2, 137.0, 134.2, 118.3, 117.0, 114.4, 86.4, 83.2, 81.5, 77.8, 70.9, 37.8, 34.9, 32.1, 31.4 and 28.6; Isomer **III** (4′*S*,2″*S*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.42 (m, 2H, H-6, H-4) 6.93 (m, 1H, H-3), 5.30 (m,

1H, H-4′), 5.05 (d, 1H, *J* 4.7, H-2″), 4.25 (m, 1H, H-4″), 4.08 (m, 2H, H-9), 3.54 (m, 1H, H-5′), 3.44 (m, 1H, H-5′), 3.32 (m, 1H, H-5″), 3.22 (m, 1H, H-5″), 2.70 (s, 3H, N-CH_3) and 1.42 (s, 9H, $Si(CH_3)_3$); δ_C(125 MHz; Acetone-d_6) 172.9, 160.0, 156.2, 137.0, 134.2, 118.3, 117.0, 114.4, 86.4, 83.2, 81.5, 79.3, 73.3, 70.4, 36.2, 32.4, 32.3, 31.4 and 28.6; Isomer **IV** (4′*S*,2″*R*,4″*R*): δ_H(500 MHz; Acetone-d_6) 7.42 (m, 2H, H-6, H-4) 6.93 (m, 1H, H-3), 4.84 (q, 1H, *J* 8.4, H-4′), 4.34 (d, 1H, *J* 8.3, H-2″), 4.08 (m, 2H, H-9), 4.02 (dd, 1H, *J* 6.9 and 5.3, H-4″), 3.67 (m, 1H, H-5′), 3.52 (m, 1H, H-5″), 3.44 (m, 1H, H-5″), 3.31 (m, 1H, H-5′), 2.63 (s, 3H, N-CH_3) and 1.42 (s, 9H, $Si(CH_3)_3$); δ_C(125 MHz; Acetone-d_6) 172.9, 160.0, 156.2, 137.0, 134.2, 118.3, 117.0, 114.4, 86.4, 83.2, 81.5, 79.3, 73.8, 44.8, 35.1, 33.6, 31.4 and 28.6.

4-(3-(*tert*-Butoxycarbonylamino)propylamino)-4-oxobutanoic acid (8)

A solution of *tert*-butyl-3-aminopropylcarbamate (1.0 g, 5.74 mmol) and succinic anhydride (0.69 g, 6.89 mmol) in THF (25 mL) was heated to reflux for 16 h. The reaction mixture was then concentrated and redissolved in CH_2Cl_2 (100 mL). The organic layer was then washed with water (50 mL) and brine (50 mL), followed by evaporation of CH_2Cl_2 *in vacuo* to yield **8** (1.2 g, 76%) as a white solid as pure compound. ν_{max}(microscope)/cm^{-1} 3500–2900 (br), 2978, 1716, 1659, 1535 and 1440; δ_H(400 MHz; $CDCl_3$) 9.45 (s, br, 1H, CO_2H), 7.05 (s, br, 1H, NH), 5.22 (s, br, 1H, NH), 3.24 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 2.64 (m, 2H, CH_2), 2.49 (m, 2H, CH_2), 1.59 (m, 2H, CH_2) and 1.40 (s, 9H, $C(CH_3)_3$); δ_C(125 MHz; $CDCl_3$) 175.8, 172.9, 156.8, 79.5, 37.3, 36.4, 30.8, 29.8, 28.4 and 28.4; *m*/*z*(EI) calcd for $C_{12}H_{22}N_2O_5$ 274.1529, found 274.1362 [M^+].

Methyl 2,3-dihydroxybenzoate

To a stirring solution of 2,3-dihydroxybenzoic acid (5.0 g, 32.4 mmol) in CH_3OH (25 mL), $SOCl_2$ (2.6 mL, 35.6 mmol) was added drop wise at 23 °C. The reaction mixture was heated to reflux for 10 h, after which it was concentrated and redissolved in ethyl acetate (200 mL). The organic layer was washed with saturated $NaHCO_3$ (2 × 100 mL), water (100 mL) and brine, dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo* to yield methyl-2,3-dihydroxybenzoate (3.9 g, 85%) as a white solid. ν_{max}(microscope)/cm^{-1} 3600–3150 (br), 1675, 1597 and 1428; δ_H(500 MHz; $CDCl_3$) 10.91 (s, 1H, CO_2H), 7.35 (dd, 1H, *J* 8.1 and 1.5, Ar-H), 7.11 (dd, 1H, *J* 7.9 and 1.5, Ar-H), 6.78 (t, 1H, *J* 7.9, Ar-H), and 3.94 (s, 3H, OCH_3); δ_C(125 MHz; $CDCl_3$) 170.8, 148.8, 145.0, 120.6, 119.9, 119.2, 112.4 and 52.4; *m*/*z*(EI) calcd for $C_8H_8O_4$ 168.0423, found 168.0422 [M^+].

Methyl 2,3-bis(benzyloxy)benzoate

In a flask, methyl-2,3-dihydroxybenzoate (3.90 g, 23.2 mmol), K_2CO_3 (12.8 g, 92.8 mmol), and NaI (0.300 g) were dissolved in DMF (30 mL). To the resulting mixture was added a solution of benzyl bromide (6.06 mL, 51.0 mmol) in DMF (10 mL). The reaction mixture was stirred at 23 °C for 18 h and then filtered to remove insoluble materials. The filtrate was concentrated *in vacuo* and the residue was dissolved in ether (100 mL). The organic layer was washed with 2% $NaOH_{(aq)}$ (30 mL) and water (30 mL). It was then dried over Na_2SO_4, filtered, and concentrated *in vacuo* to about 20 mL. Upon addition of hexanes the product precipitated in its pure form (5.1 g, 63%) as a pale yellow solid. ν_{max}(cast)/cm^{-1} 3065, 3032, 2950, 2880, 1728, 1580, 1498 and 1474; δ_H(500 MHz; $CDCl_3$) 7.50 (m, 4H, Ar-H), 7.34-7.44 (m, 7H, Ar-H), 7.18 (dd, 1H, *J* 1.7 and 8.1, Ar-H), 7.11 (t, 1H, *J* 8.2, Ar-H), 5.18 (s, 2H, OCH_2), 5.16 (s, 2H, OCH_2) and 3.89 (s, 3H, OCH_3); δ_C(125 MHz; $CDCl_3$) 166.7, 152.7, 148.2, 137.4, 128.5, 128.2, 127.9, 126.8, 123.9, 122.8, 117.9, 71.2 and 52.0; *m*/*z*(EI) calcd for $C_{22}H_{20}O_4$ 348.1362, found 348.1362 [M^+].

2,3-Bis(benzyloxy)benzoic acid

A solution of methyl-2,3-bis(benzyloxy)benzoate (2.48 g, 7.12 mmol) in THF (5 mL) was diluted with THF/H_2O (1 : 1, 100 mL), to which was added 1.0 M $KOH_{(aq)}$ (15 mL). The reaction was allowed to proceed at 23 °C for 10 h. The reaction mixture was then concentrated to remove volatile components and acidified to pH ~ 2.0 with 1 M $HCl_{(aq)}$. The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layer was washed with water (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the pure product (2.21 g, 93%) as a white solid. ν_{max}(microscope)/cm^{-1} 2945, 2667, 2576, 1689, 1598, 1577 and 1497; δ_H(400 MHz; $CDCl_3$) 7.66 (dd, 1H, *J* 1.7 and 7.9, Ar-H), 7.22-7.46 (m, 10H, Ar-H), 7.18 (dd, *J* 1.8 and 8.1), 7.10 (t, 1H, *J* 7.9, Ar-H), 5.18 (s, 2H, CH_2) and 5.12 (s, 2H, CH_2); δ_C(100 MHz; $CDCl_3$) 165.3, 151.4, 147.2, 135.9, 134.7, 129.3, 128.9, 128.6, 127.8, 125.0, 124.5, 123.1, 119.1 and 71.6; *m*/*z*(ES+) calcd for $C_{21}H_{18}O_4Na$ 357.1097, found 357.1098 [MNa^+].

2,3-Bis(benzyloxy)-*N*-(3-(4-(2,3-bis(benzyloxy)benzamido) butylamino)propyl)benzamide (10)

To a solution of 2,3-bis(benzyloxy)benzoic acid (1.59 g, 4.76 mmol) in CH_2Cl_2 (100 mL) was added 1,1′-carbonyldiimidazole (0.81 g, 5.0 mmol) and the reaction mixture was allowed to stir at 23 °C for 90 min. Then, a solution of spermidine (392 μL, 2.5 mmol) in CH_2Cl_2 (20 mL) was added to the flask. After 14 h, the reaction mixture was concentrated, redissolved in ethyl acetate (200 mL) and washed with 0.5 M $NaOH_{(aq)}$ (2 × 100 mL), water (100 mL) and brine (50 mL). The organic phase was then dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a pale brown oil. The crude product was purified by flash chromatography (5% to 10% CH_3OH in CH_2Cl_2) to obtain **10** (1.1 g, 59% yield) as a clear oil. ν_{max}(microscope)/cm^{-1} 3384, 3065, 2935, 1653, 1576 and 1559; δ_H(500 MHz; $CDCl_3$) 8.07 (t, 1H, *J* 5.4, NH), 7.93 (t, 1H, *J* 5.5, NH), 7.71 (m, 2H, Ar–H), 7.46 (m, 4H, Ar–H), 7.28–7.41 (m, 16H, Ar–H), 7.14 (m, 4H, Ar–H), 5.15 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 5.06 (s, 2H, CH_2), 3.34 (q, 2H, *J* 6.3, CH_2), 3.26 (q, 2H, *J* 6.1, CH_2), 2.48 (t, 2H, *J* 6.9, CH_2), 2.41 (t, 2H, *J* 6.7, CH_2), 1.52 (quint., 2H, *J* 6.8, CH_2) and 1.34 (m, 4H, CH_2); δ_C(125 MHz; $CDCl_3$) 165.2, 165.0, 151.75, 151.72, 146.76, 146.70, 136.49, 136.45, 128.76, 128.73, 128.67, 128.63, 128.24, 127.70, 127.67, 127.61, 124.42, 124.40, 123.3, 123.2, 116.85, 116.82, 77.3, 76.3, 71.33, 71.27, 49.4, 47.4, 39.5, 37.8, 29.5, 27.4 and 27.0; *m*/*z*(ES+) calcd for $C_{49}H_{52}N_3O_6$ 778.3851, found 778.3841 [MH^+].

tert-Butyl-3-(4-((4-(2,3-bis(benzyloxy)benzamido)butyl)(3-(2,3-bis(benzyloxy)benzamido)propyl)amino)-4-oxobutanamido)propylcarbamate (11)

A solution of **10** (610 mg, 0.78 mmol), **8** (258 mg, 0.94 mmol) and DIPEA (273 μL, 1.57 mmol) in CH_2Cl_2 (50 mL) was cooled in ice-water bath. HOBt (159 mg, 1.18 mmol) and PyBOP (614 mg, 1.18 mmol) was then added to the solution. After stirring at 0 °C for 20 min, the reaction mixture was warmed to 23 °C and stirred for another 14 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organic phase was washed with 5% $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to yield a yellow oil. The crude product was purified by flash chromatography (2% to 5% CH_3OH in CH_2Cl_2) to obtain **11** (738 mg, 91% yield) as a yellow oil. ν_{max}(microscope)/cm^{-1} 3384, 3065, 2935, 1653, 1576 and 1559; δ_H(500 MHz; $CDCl_3$) 8.07 (t, 1H, *J* 5.4, NH), 7.93 (t, 1H, *J* 5.5, NH), 7.71 (m, 2H, Ar–H), 7.46 (m, 4H, Ar–H), 7.28–7.41 (m, 16H, Ar–H), 7.14 (m, 4H, Ar–H), 5.15 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 5.06 (s, 2H, CH_2), 3.34 (q, 2H, *J* 6.3, CH_2), 3.26 (q, 2H, *J* 6.1, CH_2), 2.48 (t, 2H, *J* 6.9, CH_2), 2.41 (t, 2H, *J* 6.7, CH_2), 1.52 (quint., 2H, *J* 6.8, CH_2) and 1.34 (m, 4H, CH_2), δ_C(125 MHz; $CDCl_3$) 172.7, 171.6, 165.2, 165.0, 156.2, 151.61, 151.55, 146.7, 146.5, 136.32, 136.26, 128.64, 128.59, 128.54, 128.41, 128.12, 127.53, 127.51, 127.50, 124.30, 124.28, 123.0, 122.8, 116.8, 116.6, 78.8, 77.3, 76.4, 71.13, 71.06, 47.2, 45.4, 43.1, 38.8, 36.9, 36.1, 31.4, 29.9, 28.3, 27.5, 26.6, 25.9 and 24.9; *m/z*(ES+) calcd for $C_{61}H_{71}N_5O_{10}Na$ 1056.5093, found 1056.5085 [MNa^+].

General procedure for the reaction of siderophore with dimethyl squarate

Et_3N (2 equiv.) and dimethyl squarate (1.5 equiv.) was added to the siderophore (1 equiv.) dissolved in CH_3OH. The reaction mixture was allowed to stir at 23 °C for 6 h and was then diluted with water to get a 1 : 1 (v/v) mixture of CH_3OH/H_2O. The crude product was purified by HPLC (eluent: CH_3CN/H_2O (with 0.1% TFA)). The product exists as a mixture of a pair of tautomers.

Agrobactin analogue-squarate (12). Isolated as a pale brown residue (20 mg, 86%). δ_H(500 MHz; CD_3OD) 7.20 (m, 2H, Ar–H), 6.91 (m, 2H, Ar–H), 6.69 (m, 2H, Ar–H), 4.32 and 4.31 (s, 3H, OCH_3), 3.60–3.31 (m, 10H, CH_2), 3.20 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 2.48 (m, 2H, CH_2) and 1.98–1.56 (m, 8H, CH_2); δ_C(175 MHz; CD_3OD) 190.0, 189.8, 185.0, 184.7, 178.5, 177.9, 174.5, 173.8, 171.7, 171.5, 171.4, 163.2, 163.0, 162.8, 150.5, 150.3, 147.4, 147.3, 118.6, 118.0, 116.7, 116.0, 61.1, 61.0, 46.7, 44.3, 43.5, 43.0, 42,4, 40.0, 39.8, 38.0, 37.5, 37.3, 36.9, 32.0, 31.8, 31.4, 31.2, 29.6, 29.3, 29.1, 28.5, 27.6, 26.9 and 26.0; *m/z*(ES+) calcd for $C_{33}H_{41}N_5O_{11}$ 683.2803, found 683.2801 [MH^+]; HPLC retention time (method A): 25.1 min.

Pyochelin analogue-squarate (13). Isolated as a pale yellow residue (8 mg, 24%). (4′*R*,2″*R*,4″*R*): δ_H(600 MHz; CD_3COCD_3) 7.46 (m, 2H, Ar–H), 6.95 (m, 1H, Ar–H), 5.23 (q, 1H, *J* 6.9, H4′), 4.61 (d, 1H, *J* 5.4, H2″), 4.38 (m, 5H, NCH_2 and OCH_3), 4.02 (dd, 1H, *J* 6.9, 5.3 Hz, H4″), 3.67 (m, 1H, H5′), 3.52 (m, 1H, H5′), 3.44 (m, 1H, H5″), 3.31 (m, 1H, H5″), 2.63 (s, 3H, N–CH_3); δ_C(125 MHz; CD_3COCD_3) 190.0, 184.7, 175.1, 174.1, 172.9, 172.0, 159.5, 137.0, 134.4, 118.3, 117.0, 115.6, 87.2, 81.1, 80.1, 77.2, 73.3, 61.1, 44.5, 41.6, 33.2 and 33.0; *m/z*(ES-) calcd for $C_{22}H_{20}N_3O_6S_2$ 486.0799, found 486.0796 [MH^-]; HPLC retention time (method B): 15.5 min.

Desferrioxamine-squarate (14). Isolated as an off-white solid (15 mg, 88%). δ_H(600 MHz, CD_3OD) 4.35 (s, 3H, OCH_3), 3.59 (m, 7H, NCH_2- and N-OH), 3.38 (t, 2H, *J* 6.7, CH_2), 3.16 (t, 4H, *J* 7.0, CH_2), 2.74 (m, 4H, CH_2), 2.43 (t, 4H, *J* 7.2, CH_2), 2.08 (s, 3H, CH_3), 1.63 (m, 8H, CH_2) and 1.54–1.31 (m, 12H, CH_2); δ_C(100 MHz; CD_3OD) 189.9, 185.0, 178.0, 176.2, 174.9, 174.5, 173.5, 61.1, 52.2, 52.0, 45.2, 40.3, 39.8, 31.6, 31.5, 31.0, 30.3, 30.0, 28.9, 28.8, 27.3, 27.1, 24.9, 24.5, 24.3, 24.1 and 20.2; *m/z*(ES+) calcd for $C_{30}H_{51}N_6O_{11}$ 671.3610, found 671.3607 [MH^+]; HPLC retention time (method C): 24.5 min.

General procedure for the reaction of gallidermin with siderophore-squarate

To a solution of gallidermin (1.0 mM) in borate buffer (pH = 9.0) was added a solution of siderophore-squarate (1.5 equiv.) in THF. The reaction mixture was allowed to stir at either 4 °C (agrobactin conjugates) or 23 °C (pyochelin and desferrioxamine conjugates) for 14 h and then diluted with water. The crude product was purified by HPLC.

Gallidermin-pyochelin conjugates (15) and (16). Isolated as an off-white residue (2.3 mg, 40%, two regioisomers). Conjugate **15**: HPLC retention time (method D): 19.8 min. Conjugate **16**: HPLC retention time (method D): 21.1 min.; *m/z*(MALDI-TOF) calcd for $C_{119}H_{159}N_{28}O_{28}S_6$ 2619.0, found 2620.0 [MH^+].

Gallidermin-agrobactin conjugates (17) and (18). Isolated as an off-white residue (3.1 mg, 58%, two regioisomers). Conjugate **17**: HPLC retention time (method A): 26.6 min. Conjugate **18**: HPLC retention time (method A): 27.0 min. *m/z*(MALDI-TOF) calcd for $C_{130}H_{179}N_{30}O_{33}S_4$ 2815.2, found 2815.6 [MH^+].

Gallidermin-desferrioxamine B conjugate (19). Isolated as an off-white residue (3.3 mg, 45%, two regioisomers). Conjugate **19**: HPLC retention time (method D): 16.9 min.; *m/z*(MALDI-TOF) calcd for $C_{156}H_{234}N_{37}O_{43}S_4$ 3440.6, found 3440.9 [MH^+].

Antibacterial assay

All test samples were dissolved in H_2O:CH_3CN (4 : 1). For the spot-on-lawn overlay assay, the indicator strain, *Lactococcus lactis* subsp. *cremoris* HP was grown in all purpose tween (APT) media at 25 °C. The gram negative bacteria were grown in Luria broth at 37 °C with shaking at 200 rpm. For testing under iron sufficient conditions, Gram-negative bacterial cultures grown in Luria broth overnight were used. For testing under iron deficient conditions, Gram-negative bacterial cultures were first grown in LB broth overnight, subcultured (2% innoculum) into either M9 or succinic minimal media and grown to an OD_{600} ~ 0.1 at 37 °C, shaking at 200 rpm. M9 media (500 mL) was prepared as follows: 100 mL of 5 × M9 salts (0.25 M $Na_2HPO_4{\cdot}7H_2O$, 0.11 M KH_2PO_4 and 0.043 M NaCl) was diluted with 400 mL of milli-Q water and sterilized. Then, filter sterilized solutions of 20% glucose (5 mL), 20% $(NH_4)_2SO_4$ (1.25 mL), 1 M $MgSO_4$ (1 mL), 0.1 M $CaCl_2$ (0.5 mL) and 10 mg mL^{-1} thiamine (50 μL) were added to the

media. Succinic minimal media (500 mL) consists of K_2HPO_4 (3 g), KH_2PO_4 (1.5 g), $(NH_4)_2SO_4$ (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.1 g) and succinic acid (2 g) dissolved in milli-Q water. The pH of the media was adjusted to 7.0 and sterilized before use. All samples tested against Gram-negative organisms were mixed with equimolar ferric iron prior to testing.

In the spot on lawn assays, all agar was mixed with media (APT, LB, M9 or succinic minimal media), dissolved in water, and sterilized before use. For each test organism, 100 μL of grown culture was added to a tube containing 10 mL of melted soft (0.7% W V^{-1}) agar (40 °C) before being gently vortexed and poured onto a hard (1.5% W V^{-1}) agar plate. After the soft agar layer solidified, the test samples (10 μL) were pipetted on to the soft agar, allowed to air dry and incubated overnight at appropriate temperatures (25 °C or 37 °C). Activity was detected by the appearance of a circular zone of growth inhibition in and around the area where the samples were spotted.

The 64-well plate assay was done under iron deficient conditions, using M9 minimal media. In each well, 50 μL of bacterial culture and 50 μL of test sample were added. The 64-well plate was then incubated in an automatic reader at 37 °C and optical densities were recorded at 600 nm.

Fluorescent microscopy imaging

All test organims were first grown in LB broth overnight, subcultured (2% innoculum) in M9 minimal media and grown to an OD_{600} ~ 0.1. The sampls were centrifuged and resuspended in Tris-buffer. Cell culture (50 μL) and fluorescently labeled siderophore-Fe(III) complexes (50 μL) were mixed, vortexed and incubated for 1 h. Cells were isolated by centrifugation (5 mins, 13,000 rpm), washed with Tris-buffer three times, resuspended in Tris-buffer (100 μL) and spotted on glass slides. The cells were immobilized with poly-lysine treated coverslips. Bacterial cells were imaged using a Nikon TE2000-E microscope, equiped with an epifluorescence optic, using a 100× objective. Images were captured using a Fastcam Super 10 K camera (Photron USA, Inc., San Diego, CA).

Acknowledgements

We thank Professor Jonathan J. Dennis (University of Alberta) for providing us with a sample of the *B. cepacia* strain and Dr Caishun Li for assistance with fluorescence microscopy. We thank Dr Marco van Belkum for helpful discussions, Victor Dong for assistance with HPLC purifications and Dr Randy Whittal and Jing Zheng for extensive MS/MS analyses. We are grateful for financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chair in Bioorganic and Medicinal Chemistry.

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34 *Please see the supporting document for evaluation of other bacterial strains.*