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Novel hamamelitannin analogues for the treatment of biofilm related MRSA infections – a scaffold hopping approach

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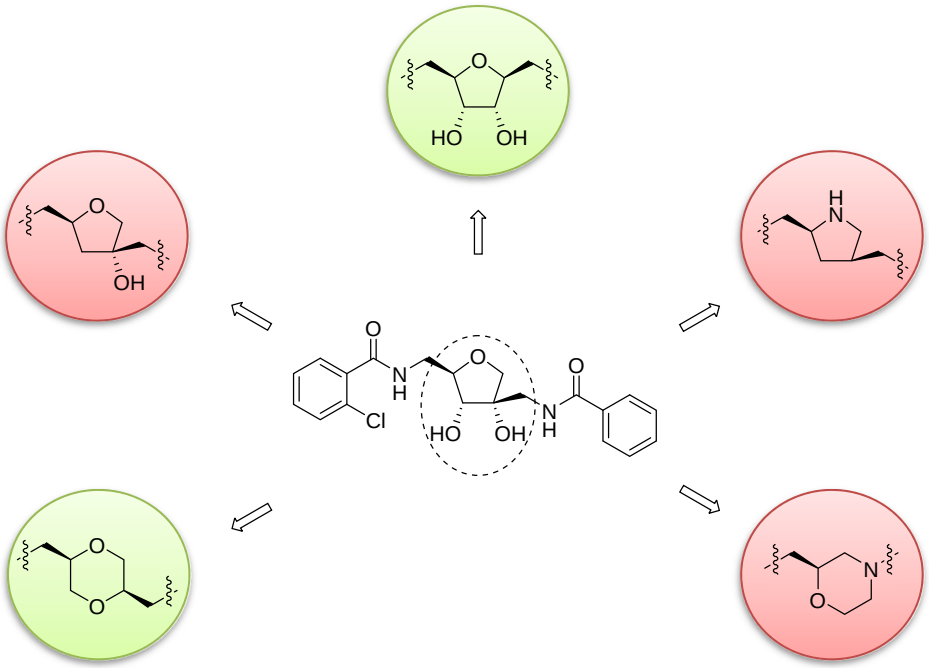
Keywords

Hamamelitannin analogues
Scaffold hopping
MRSA
Antibiotic potentiators
Quorum sensing

Graphical abstract



Abstract

Antimicrobial research is increasingly being focused on the problem of resistance and biofilm formation. Hamamelitannin (HAM) was recently identified as an antimicrobial potentiator for conventional antibiotics towards *Staphylococcus aureus*. This paper describes the synthesis and biological evaluation of novel hamamelitannin analogues with alternative central scaffolds. Via a ligand-based approach, several interesting compounds with improved synthetic accessibility were identified as potentiators for vancomycin in the treatment of MRSA infections.

1. Introduction

The utility of conventional antibiotics has become compromised by the emergence of antimicrobial resistance (AMR). In a recently published report, it is estimated that by 2050 10 million lives a year will be at risk due to the rise of drug-resistant infections.[1] Antibiotic resistance is not only a future threat, but poses a serious global problem to patients and healthcare providers right now. Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for nosocomial as well as community-acquired infections and represents a serious health threat.[2] Compounding the problem even further is the fact that *Staphylococcus aureus* (*S. aureus*) is notorious for its ability to form biofilms, surface-associated communities of cells in a polymer-based matrix. Biofilm formation provides a multi-level protection to microbes against antibiotics. It alters growth rate and metabolism, raises the fraction of dormant persister cells and hampers the penetration of antimicrobial agents.[3] The global problem of AMR is further aggravated by a lack of novel antibiotic development, which is fraught with scientific risk and business challenges.[4] The fact that resistance development is unavoidable with agents that target bacterial viability, prompted the scientific community to explore alternative approaches, such as neutralizing virulence mechanisms. Therapeutic strategies to reduce the expression of bacterial virulence factors would render pathogens more susceptible to natural host defenses while maintaining the normal microbiota. In *S. aureus*, biofilm formation and virulence in general are regulated via quorum sensing (QS), an intercellular communication system that controls gene expression.

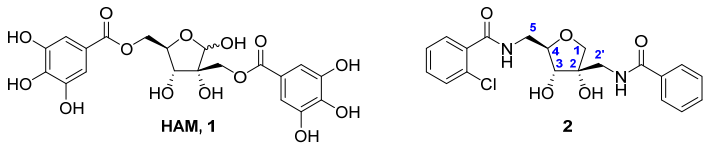


Figure 1 Structure of hamamelitannin (HAM, **1**) and optimized lead **2**.

Recently, the natural product hamamelitannin (HAM, **1**, Figure 1) was identified as a quorum sensing inhibitor (QSI) in *S. aureus*.[5] We previously demonstrated that HAM and optimized derivative **2** interfere with the RAP-TRAP (RAP-targeting of the RNAIII-activating peptide) signal transduction system, one of the regulators that is believed to control the production of toxic shock syndrome toxin-1, enterotoxins, proteases and δ-hemolysins, which allow *S. aureus* to survive, disseminate, and establish an infection.[6] HAM and the metabolically stable lead compound **2** were also able to potentiate the effect of several classes of antibiotics *in vitro* and to increase the effect of cefalexin in a murine mastitis model of *S. aureus* infection.[7],[8] Previous studies of our group have focused on the structural optimization of the substituents at both the 5- and the 2'-position of the molecule.[8-10] Several HAM analogues were shown to be active potentiators of vancomycin (VAN), a drug of last resort for the treatment of MRSA infections. After having investigated the structure-activity relationship (SAR) of both 'wings' of HAM, we explored alternatives for the central hamamelose-like scaffold in **2,** in the search for derivatives with improved synthetic accessibility and/or potentiator activity.

2. Ligand-based virtual screening

HAM and optimized derivatives interfere with the RAP/TRAP QS system in *S. aureus*. Prior to the discovery of these small-molecule modulators of TRAP, the only TRAP inhibitors reported were RIP, a heptapeptide (YSPXTNF, where X can be a Cys, a Trp or a modified amino acid) originally isolated from culture supernatants of *S. xylosis*, and synthetic variants.[11-13] Although the structure of the membrane-associated signal transduction protein TRAP was reported,[14] in the absence of X-ray data of TRAP–inhibitor complexes, structure-based drug design remains elusive. Hence, in the present study, we followed a ligand-based approach to discover TRAP inhibitors with alternative scaffolds. The open source platform KNIME was used as interface and a publically available ZINC[15] database of more than 9 million compounds was used for virtual screening. A combined substructure search and 2D fingerprint similarity search protocol was used (Figure 2). 2D fingerprints are binary vectors in which the individual bits (1 or 0) encode the presence (1) or absence (0) of certain fragments. The degree of resemblance between the reference and a database molecule can be calculated, which allows to arrange the database in descending order of similarity with the target structure. Unfortunately,

however, 2D fingerprints tend to lose information about the connectivity of atoms. This means that although a database compound may contain all of the fragments present in the reference structure, the fragments are not necessarily connected in the 'correct' way. Therefore, prior to the 2D fingerprint similarity searching, we subjected the database to a substructure search using 2-chloro-*N*-(2-methoxyethyl)benzamide as reference fragment. The latter seems a privileged substructure according to our previous findings.[9] With this more focused library of 2564 molecules, we started the 2D fingerprint based similarity search using lead compound **2** as a probe. MACCS fingerprints were generated and for each fingerprint a Tanimoto coefficient (T_C) was determined. A score of 1 means identity, 0 means no similarity at all. The obtained hit list from this combined procedure was screened manually: the most appealing structures and ideas extracted from this virtual screening approach were used to guide us in the design of novel HAM analogues with alternative core structure. Thus, in the present study, we were not interested in the library compounds as such; we wanted to use the hit list merely as a means and as a source of inspiration for the design of HAM analogues in order to get insight into the requirements for optimal potentiating activity. An important factor that was taken into account was the synthetic feasibility of the scaffold (and ultimately the final compound). An overview of the selected scaffolds, together with a selection of database compounds that inspired us, is given in Figure 2. Inspired by compounds **a** and **b** (Figure 2), a first scaffold was obtained by transposing the 2-benzamidomethyl moiety to position 1 of the tetrahydrofuran core (I). A second central scaffold was achieved by deleting the 3-hydroxyl group of **2** (II). Based on compounds **c** and **d**, a 2,5-substituted 1,4-dioxane core (III) was put forward as possible bio-isostere of the tetrahydrofuran ring present in **2**. Also 1,3-substituted morpholine (IV) and 2,4-substituted pyrrolidine (V) emerged as alternative scaffolds.

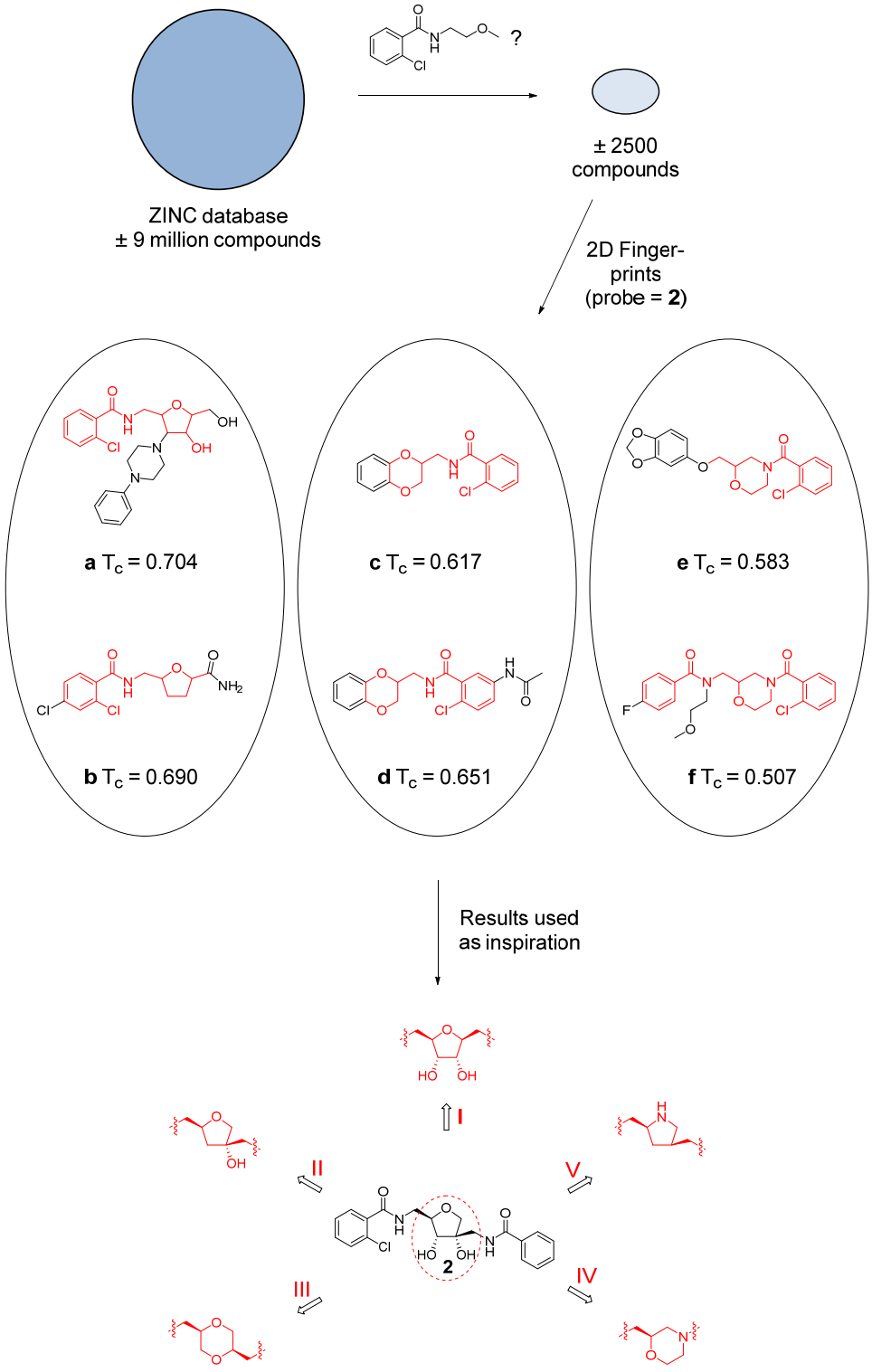
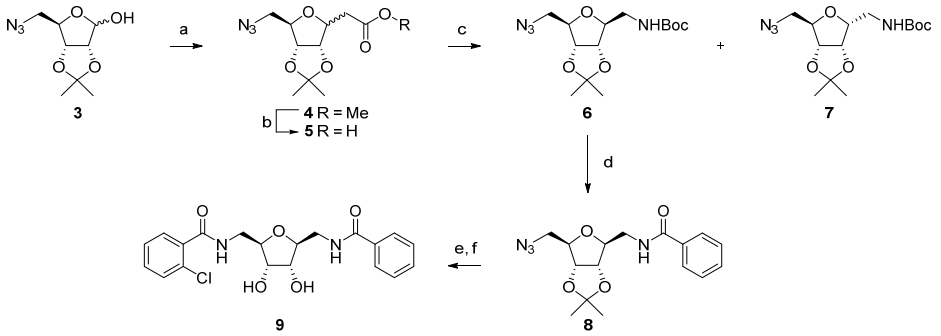


Figure 2 Overview of scaffold hopping strategies.

3. Chemistry

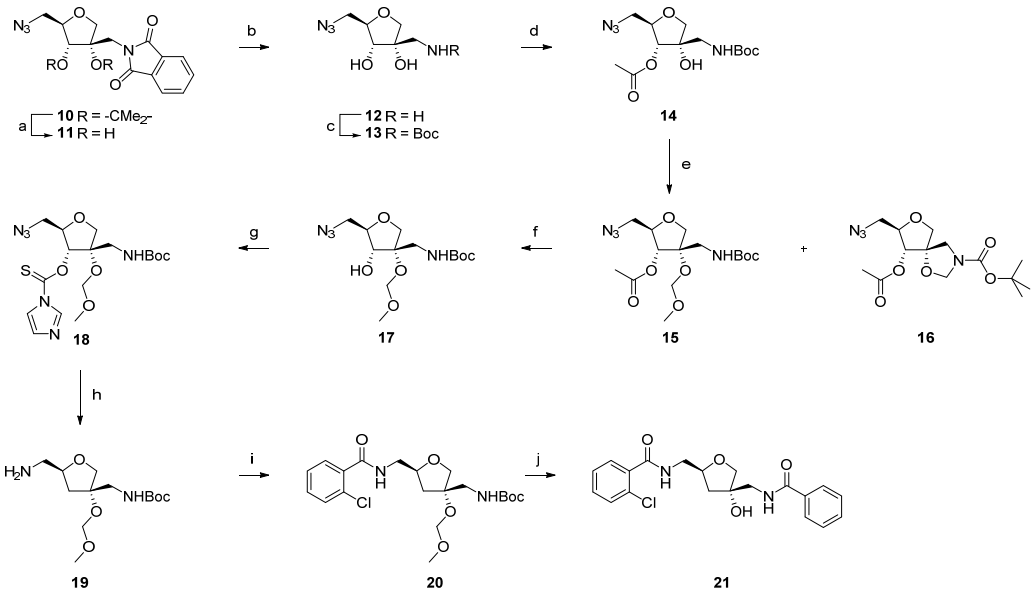
Antimicrobial potentiator **2** consists of a 5-*ortho*-chlorobenzamidomethyl and a 2'-benzamidomethyl moiety, linked to a central (3*S*,4*R*)-tetrahydrofuran-3,4-diol scaffold (Figure 1). While maintaining both aromatic moieties intact, we first investigated the impact of transposing the 2-benzamidomethyl fragment to position 1. The synthesis of **9**, a rather conservative scaffold hop (reflected in a T_c of 0.909), starts from the known

azidolactole **3**[16] (Scheme 1). Horner-Wadsworth-Emmons olefination of **3** with methyl (triphenylphosphoranylidene)acetate and treatment of the resulting α,β-unsaturated carbonyl intermediate with a methanolic sodium methoxide solution afforded methyl ester **4** as an inseparable mixture of *cis* and *trans* epimers.[17] Saponification yielded a mixture of epimeric carboxylic acids **5**, which were converted into the separable Boc-protected amines by Curtius rearrangement. TFA-mediated Boc-deprotection of **6** and subsequent EDC-mediated acylation with benzoic acid gave benzamide **8**. The structure and stereochemistry of the latter was confirmed by a 2D ^{1}H-^{1}H NOESY experiment (Supporting information). The benzamide was subjected to Staudinger reduction and the resulting amine was converted into an *ortho*-chlorobenzamide. Acidic hydrolysis of the acetonide gave **9**, a regioisomer of **2** (Scheme 1).



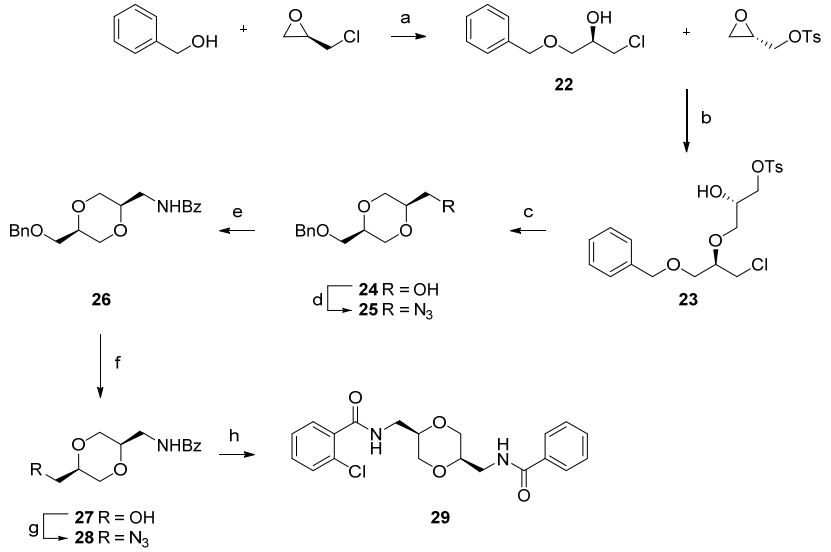
Scheme 1. Reagents and conditions: (a) [i] $Ph_3P{=}CHCO_2Me$, MeCN, 85 °C, [ii] NaOMe, MeOH, 46%; (b) NaOH, H_2O/dioxane, q.; (c) DPPA, Et_3N, tBuOH, 100 °C, 46% for **6**, 13% for **7**; (d) [i] TFA, 1,2-DCE, [ii] PhCOOH, EDC·HCl, DIPEA, HOBt, DMF, 80%; (e) [i] PMe_3, THF, 3h, [ii] H_2O, 45 min, [iii] 2-chlorobenzoic acid, EDC·HCl, DIPEA, HOBt, DMF, 80%; (f) 35% TFA in H_2O, q.

Phthalimide **10**[8] served as an orthogonally protected intermediate for the synthesis of analogue **21**, the 3-deoxy analogue of **2** (T_c = 0.982). Acidic deprotection of the acetonide, treatment with ethanolic hydrazine and Boc-protection of the resulting amine afforded **13** (Scheme 2). Selective acetylation of the secondary hydroxyl group of **13** gave acetate **14**. To circumvent the use of toxic MOM-Cl, methoxymethylation of the tertiary alcohol group was performed by treatment of **14** with dimethoxymethane and phosphorus pentoxide.[18] Interestingly, this reaction also yielded a heterocyclic spiro compound (**16**) in which the Boc-protected amine and the tertiary alcohol are connected via a methylene group to form an oxazolidine ring. Although ^{1}H NMR and HRMS confirmed the structure of **16**, further structural proof was given by ^{13}C NMR and a 2D ^{13}C-^{1}H HSQC experiment, in which a cross peak was observed between the protons at the hemiaminal carbon and their proximate ^{13}C (Supporting information). Removal of the acetate in **15** under standard conditions gave alcohol **17**. The latter was acylated with thiocarbonyldiimidazole to yield azidothiocarbamate **18**, which was the substrate for the simultaneous radical deoxygenation and azide reduction with tributyltin hydride and a catalytic amount of azoisobutyronitrile in refluxing toluene. Final 3-deoxy derivative **21** was obtained via standard transformations of amine **19**.



Scheme 2. Reagents and conditions: (a) 35% TFA in H_2O; (b) $N_2H_2 \cdot H_2O$, toluene/MeOH, 60 °C; (c) $(Boc)_2O$, K_2CO_3, H_2O/dioxane, 85% from **10**; (d) Ac_2O, pyr, 76%; (e) P_2O_5, dimethoxymethane, $CHCl_3$, 24h, 27% for **15**, 40% for **16**; (f) NaOMe, MeOH, q.; (g) TCDI, toluene, 125 °C, 65%; (h) Bu_3SnH, AIBN, toluene, 85 °C, 69%; (i) 2-chlorobenzoic acid, EDC·HCl, DIPEA, HOBt, DMF, 54%; (j) [i] 35% TFA in H_2O, [ii] BzCl, Et_3N, DCM, 71%.

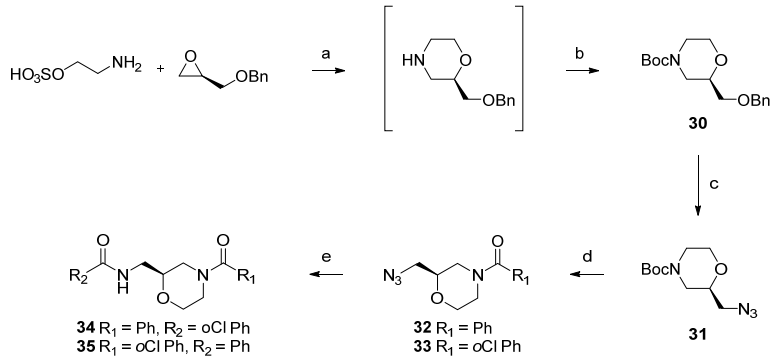
Inspired by the results from the substructure search and 2D fingerprint-based screening, a 1,4-dioxane motif was explored as central scaffold. Derivative **29** contains a similar atom connectivity as **2** and shows a T_c of 0.754. The synthesis of **29** starts with the reaction of benzyl alcohol with (*R*)-epichlorohydrin (Scheme 3). The resulting (*R*)-1-(benzyloxy)-3-chloropropan-2-ol was treated with (*S*)-glycidyl tosylate to give intermediate **23**. Treatment of the latter with aqueous NaOH solution lead to intramolecular ring closure in a 6-exo-tet process to give monoprotected *cis*-dioxanedimethanol intermediate **24**. Subsequent transformation of **24** into heterobisbenzamide **29** occurred via known procedures (Scheme 3).



Scheme 3. Reagents and conditions: (a) [i] $BF_3 \cdot OEt_2$, 1,2-DCE, 16h, [ii] reflux, 2h, 48%; (b) $BF_3 \cdot OEt_2$, 1,2-DCE, 16h, 50%; (c) [i] 1.0 M NaOH, 2.5h, [ii] 90 °C, 4h, [iii] rt, 16h, [iv] 90 °C, 2h, 66%; (d) [i] MsCl, Et_3N, DCM, [ii] NaN_3, DMF, 60 °C, 91%; (e) [i] PMe_3, THF, 3h, [ii] H_2O, 45 min, [iii] PhCOOH, EDC·HCl, DIPEA, HOBt, DMF, 90%; (f) H_2, Pd/C, MeOH, q.; (g) [i] MsCl, Et_3N, DCM, [ii] NaN_3, DMF, 60 °C, 69%; (h) [i] PMe_3, THF, 3h, [ii] H_2O, 45 min, [iii] 2-chlorobenzoic acid, EDC·HCl, DIPEA, HOBt, DMF, 74%.

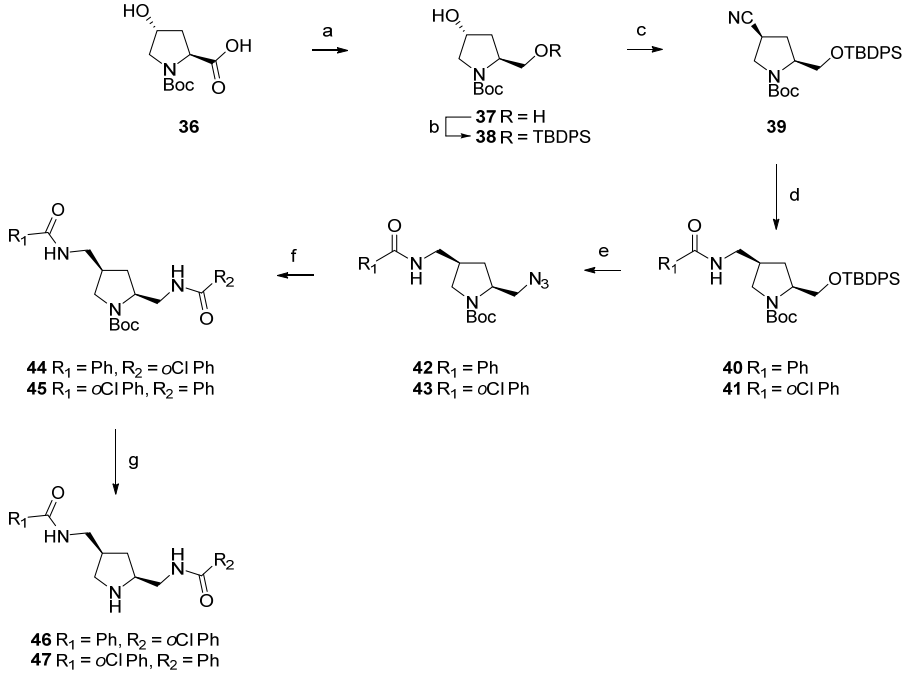
Next, we also synthesized two analogues with a morpholine scaffold (Scheme 4). (*R*)-benzylglycidyl ether was treated with 2-aminoethylsulfate in the presence of aqueous NaOH. Intramolecular ring closure via a 6-exo-tet process gave an intermediate morpholine, the amino function of which was subsequently Boc-protected to give

30. Hydrogenolysis of the benzyl protecting group and conversion of the resulting primary alcohol into the corresponding azide gave intermediate **31** with two orthogonally protected amino groups. Installation of an *ortho*-chlorobenzamide and a benzamide on either side of the molecule gave final compounds **34** and **35** (Scheme 4), both with a T_c of 0.597.



Scheme 4. Reagents and conditions: (a) [i] NaOH, H_2O, MeOH, 50 °C, 1h, [ii] NaOH, toluene, 65 °C, 16h; (b) $(Boc)_2O$, K_2CO_3, acetone/H_2O, 2h, 43%; (c) [i] H_2, Pd/C, MeOH, [ii] MsCl, Et_3N, DCM, [iii] NaN_3, DMF, 60 °C, 81%; (d) [i] TFA, DCM, [ii] RCOOH, EDC·HCl, DIPEA, HOBt, DMF, 82% for **32**, 79% for **33**; (e) [i] PMe_3, THF, 3h, [ii] H_2O, 45 min, [iii] RCOOH, EDC·HCl, DIPEA, HOBt, DMF, 62% for **34**, 91% for **35**.

Nitrile **39** was a key intermediate in the synthesis of the pyrrolidine-containing derivatives (Scheme 5). It was synthesized according to the method described by Zlatopolskiy *et al.*[19] Borane-mediated reduction of the carboxylic acid in Boc-protected hydroxyproline **36** yielded the corresponding primary alcohol **37**. Selective protection of the latter as a silyl ether gave **38**. The secondary hydroxyl group was mesylated and subsequent displacement by cyanide anion provided nitrile **39**. Reduction of this nitrile with $NaBH_4$ / $CoCl_2$ gave the corresponding primary amine, which was converted into a benzamide via EDC-mediated acylation with the appropriate benzoic acid (**40** and **41**). TBAF-mediated liberation of the primary alcohol and transformation of the latter into the corresponding azide gave **42** and **43**. Reduction of this azide functionality with trimethylphosphine and carbodiimide-mediated acylation of the resulting amine with the appropriate benzoic acid, furnished derivatives **46** and **47** after final deprotection (T_c = 0.661).

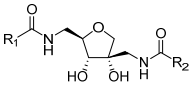
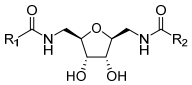
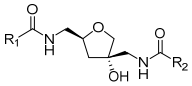
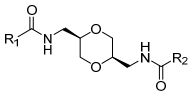
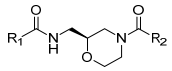
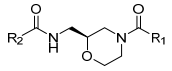
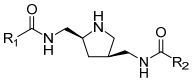
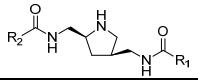


Scheme 5. Reagents and conditions: (a) BH_3·THF, 96%; (b) TBDPSCl, imidazole, DMF, 63%; (c) [i] MsCl, Et_3N, DCM, [ii] $nBu_4N^+CN^-$, MeCN, 65 °C, 19%; (d) [i] $NaBH_4$, $CoCl_2$, MeOH, [ii] RCOOH, EDC·HCl, DIPEA, HOBt, DMF, 68% for **40**, 60% for **41**; (e) [i] TBAF, THF, [ii] MsCl, Et_3N, DCM, [iii] NaN_3, DMF, 60 °C, 55% for **42**, 55% for **43**; (f) [i] PMe_3, THF, 3h, [ii] H_2O, 45 min, [iii] RCOOH, EDC·HCl, DIPEA, HOBt, DMF, 38% for **44**, 66% for **45**; (g) TFA, DCM, q. for **46**, q. for **47**.

4. Results and discussion

For all final compounds, minimum inhibitory concentrations (MIC) against *S. aureus* Mu50 were determined to rule out a direct effect on growth. In all cases, MIC values were higher than 500 µM, being the highest concentration tested (data not shown). Subsequently, the HAM derivatives were tested for their *in vitro* effect on *S. aureus* biofilm susceptibility to VAN, both under pretreatment and combination treatment regimens. To evaluate the effect of pretreatment, *S. aureus* Mu50 was allowed to form a biofilm in the presence of the HAM analogues, after which the biofilm was treated with VAN (20 µg/ml). In the combination treatment setup, the bacteria were allowed to form a mature biofilm after which a HAM analogue and VAN were administered simultaneously. When used alone, VAN resulted only in a minor reduction of the number of *S. aureus* sessile cells (30 ± 14% compared to an untreated control, Table 1). In contrast, combined treatment of VAN with **2** resulted in significantly more killing of bacterial biofilm cells, both under pretreatment and under combined treatment regimens (Table 1). Initially, all of the compounds with alternative scaffolds were tested in a concentration of 100 µM. For the most active derivatives, the effect on biofilm susceptibility towards VAN was tested in lower concentrations, which allowed us to determine an EC_{50} value. The latter is defined as the concentration of the analogue needed to double the activity of VAN, as measured by the number of surviving cells.

Table 1 Microbiological evaluation of HAM analogues with different scaffold. [a]: Percentage reduction in Colony Forming Units (CFU's) per biofilm when biofilms are treated with VAN alone (20 µg/ml) or in combination with HAM or a HAM-analogue (100 µM) compared to the untreated (negative) control. * significantly different from treatment with HAM + VAN ($p < 0.05$).

Compound	R_1 = *o*Cl Ph R_2 = Ph	Reduction in CFU's compared to control (%)[a]		EC_{50} (µM)	
		Pretreatment	Combination treatment	Pretreatment	Combination treatment
VAN alone	-	30 ± 14*	30 ± 14*	-	-
HAM, 1	-	57 ± 13	57 ± 22	145.5	165.1
2		88 ± 2*	92 ± 4*	0.3890	7.976
9		93 ± 6*	95 ± 2*	2.598	3.708
21		21 ± 11*	65 ± 14	n.d.	n.d.
29		95 ± 3*	91 ± 1*	5.568	13.12
34		-6 ± 42*	51 ± 5	n.d.	n.d.
35		-7 ± 31*	41 ± 37	n.d.	n.d.
46		54 ± 8	50 ± 7	n.d.	n.d.
47		62 ± 24	47 ± 11	n.d.	n.d.

When comparing the potentiating activity of lead compound **2** with its isomer **9**, it can be concluded that this scaffold rearrangement is well-tolerated (Table 1). Bisbenzamide **9** shows very good potentiating activity in the combination treatment setup (EC_{50} = 3.708 µM, compared to 7.976 µM for **2**). This is encouraging, as clinicians in daily practice are often confronted with already infected and biofilm-related wound infections. Moreover, the improved synthetic accessibility (compared to a longer route towards **2**) makes compound **9** even more interesting. Deletion of the 3-hydroxyl function in **2** completely abolishes activity, which suggests that the 3-OH group exhibits signature interactions with the target. In the combination treatment regimen, dioxane derivative **29** caused little change in activity compared to **2** and **9**. This compound contains to a large extend the same

connectivity of atoms relative to **2** and **9**. With **29** being almost equipotent in the combination treatment setup, this scaffold hop proves that it is possible to replace the central hamamelose-like central scaffold. Morpholine derivatives **34** and **35** were completely devoid of activity. Finally, pyrrolidines **46** and **47** lacked activity as well. The absence of both hydroxyl groups may account for the poor potentiating properties, as well as the fact that the protonated molecules might interact with the negatively charged extracellular DNA in the biofilm matrix, as previously demonstrated for positively-charged nanoparticles.[20]

5. Conclusion

Several practical pathways towards the synthesis of novel HAM analogues with an alternative central scaffold were successfully pioneered. The resulting compounds were tested for their ability to potentiate the activity of VAN in *S. aureus* biofilms *in vitro*. The readily accessible 2,5-anhydro-D-allitol derivative **9** and dioxane derivative **29** showed comparable activity to that of lead compound **2** in the combination treatment setup. These findings imply that there is room for structural improvement in the central part of the molecule and several analogues represent attractive starting points for further lead optimization of the potentiating activity.

6. Experimental

6.1. Chemistry

All reactions described were performed under an argon atmosphere and at ambient temperature unless stated otherwise. All reagents and solvents were purchased from Sigma-Aldrich (Diegem, Belgium), Acros Organics (Geel Belgium), TCI Europe (Zwijndrecht, Belgium) or Carbosynth Ltd (Compton Berkshire, United Kingdom) and used as received. NMR solvents were purchased from Eurisotop (Saint-Aubin, France). Reactions were monitored by TLC analysis using TLC aluminium sheets (Macherey-Nagel, Alugram Sil G/UV_{254}) with detection by UV or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in H_2SO_4 (10 %) followed by charring or an aqueous solution of $KMnO_7$ (20 g/L) and K_2CO_3 (10 g/L) or an ethanolic solution of ninhydrin (2 g/L) and acetic acid (1% v/v) followed by charring. Silica gel column chromatography was performed manually using Grace Davisil 60Å silica gel (40-63 μm) or automated using a Grace Reveleris X2 system and the corresponding flash cartridges. High resolution spectra were recorded with a Waters LCT Premier XE Mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Mercury-300BB (300/75 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard (^{1}H NMR) or the NMR solvent (^{13}C NMR). In ^{19}F NMR, signals have been referred to $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm. Coupling constants are given in Hz. Preparative HPLC purifications were carried out using a Laprep preparative HPLC system equipped with a Xbridge Prep C18 column (19×250 mm, 5 micron) using a water/acetonitrile/formic acid gradient system.

6.1.1. General procedure 1: EDC-mediated amide formation.

To a solution of compound **19** or the crude amine obtained from **6** or **39**, in DMF (25 mL/mmol) were added the appropriate organic acid (1.5 equiv per amine), EDC.HCl (2 equiv per amine), diisopropylethylamine (4 equiv per amine) and a catalytic amount of 1-hydroxybenzotriazole. The reaction mixture was stirred overnight at rt. The mixture was concentrated and partioned between water and EtOAc. The organic layer was dried over sodium sulphate, filtered and concentrated *in vacuo*. The products were purified by column chromatography with appropriate eluents.

6.1.2. General procedure 2: Staudinger reduction and subsequent EDC-mediated acylation of the resulting amine with the appropriate benzoic acid.

A solution of compound **8**, **25**, **28**, **32**, **33**, **42** or **43** (0.4 to 1.4 mmol) in THF (10 mL/mmol) was treated with Me_3P (1 M solution, 5 equiv) and the reaction mixture was stirred for 3h. Water (13 equiv) was added and the solution was stirred for another hour, after which it was concentrated. The residue was co-evaporated with

toluene. The obtained crude amine was used without further purification. To a solution of this crude amine in DMF (25 mL/mmol) were added the appropriate organic acid (1.5 equiv), EDC.HCl (2 equiv), diisopropylethylamine (4 equiv) and a catalytic amount of 1-hydroxybenzotriazole and the reaction mixture was stirred overnight at rt. The reaction mixture was concentrated and partioned between water and EtOAc. The organic layer was dried over sodium sulphate, filtered and evaporated. The products were then purified by column chromatography with appropriate eluents.

6.1.3. General procedure 3: TFA in water – mediated deprotection.

A known amount of the isopropylidene protected compound **20** or the crude obtained from **8** was treated with a 35% aq. CF_3COOH solution (30 mL/mmol) overnight at room temperature. When TLC indicated that the deprotection was complete, the reaction mixture was concentrated and, if required, purified by column chromatography.

6.1.4. General procedure 4: Boc deprotection and subsequent EDC-mediated acylation of the resulting amine with the appropriate benzoic acid.

A solution of compound **31** (248 mg, 1.02 mmol) in 20% trifluoroacetic acid in CH_2Cl_2 (10 mL/mmol) was stirred for 3 hours. When finished, volatile organics were evaporated under reduced pressure and the resulting crude amine was taken up in DMF (25 mL/mmol). The appropriate organic acid (1.5 equiv per amine), EDC.HCl (2 equiv per amine), diisopropylethylamine (4 equiv per amine) and a catalytic amount of 1-hydroxybenzotriazole were added. The reaction mixture was stirred overnight at rt. The mixture was concentrated and partioned between water and EtOAc. The organic layer was dried over sodium sulphate, filtered and concentrated *in vacuo*. The products were purified by column chromatography with appropriate eluents.

*6.1.5. (3aR,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol (**3**)*

See reference 8 and 15. Spectroscopy data for **3** are consistent with those published previously.[8, 16]

*6.1.6. methyl 2-((3aS,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)acetate (**4**)*

A solution of lactol **3** (4.00 g, 18.6 mmol) in acetonitrile (60 mL) was flushed with N_2 gas. Methyl (triphenylphosphoranylidene)acetate (6.84 g, 20.5 mmol) was added and the reaction mixture was heated to reflux for 90 minutes. Then, the mixture was concentrated *in vacuo* and the residue was taken up in MeOH (40 mL), after which a methanolic sodium methoxide solution (5.4 M, 370 µL) was added. This mixture was stirred for another 90 minutes and then neutralized with Amberlite (IR 120 H-form). The suspension was filtered and the filtrate was concentrated. FCC of the residue (toluene/EtOAc 1:1) gave methyl esters **4** as a pale oil in 46% yield. Epimeric ratio = 94:6. **Major epimer:** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34 (s, 3 H) 1.54 (s, 3 H) 2.65 (dd, *J* = 15.8, 7.0 Hz, 1 H) 2.74 (dd, *J* = 15.8, 5.4 Hz, 1 H) 3.34 (dd, *J* = 13.2, 4.7 Hz, 1 H) 3.54 (dd, *J* = 13.2, 4.0 Hz, 1 H) 3.71 (s, 3 H) 4.05 - 4.12 (m, 1 H) 4.27 - 4.33 (m, 1 H) 4.52 - 4.63 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.6, 27.5, 38.1, 52.0, 52.4, 81.0, 82.2, 83.2, 84.3, 115.1, 170.9. **Minor epimer:** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.33 (s, 3 H) 1.49 (s, 3 H) 2.76 - 2.79 (m, 2 H) 3.24 - 3.47 (m, 2 H) 3.71 (s, 3 H) 4.18 - 4.24 (m, 1 H) 4.41 (td, *J* = 6.8, 4.0 Hz, 1 H) 4.64 (dd, *J* = 6.2, 1.2 Hz, 1 H) 4.80 (dd, *J* = 6.2, 4.1 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.1, 26.4, 34.4, 51.7, 51.9, 77.5, 81.4, 82.8, 83.4, 115.1, 170.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{18}N_3O_5^+$ 272.12410; Found 272.1240.

*6.1.7. 2-((3aS,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)acetic acid (**5**)*

To a solution of methyl esters **4** (0.950 g, 3.50 mmol) in dioxane (10 mL), was added a 1 M NaOH solution (4.20 mL). After 150 minutes of stirring, TLC analysis (toluene/EtOAc 4:1) showed complete consumption of starting material and presence of a lower-running spot. The pH of the reaction mixture was adjusted to approximately 2 by addition of HCl (1 M solution). The reaction mixture was extracted with EtOAc. The organic layers were pooled, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product was obtained as a transparent oil without further purification (q.). Epimeric ratio = 76:24. **Major epimer:** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm

1.35 (s, 3 H) 1.55 (s, 3 H) 2.63 - 2.74 (dd, *J* = 16.1, 7.3 Hz, 1 H) 2.74 - 2.82 (m, 1 H) 3.35 (dd, *J* = 13.1, 4.5 Hz, 1 H) 3.56 (dd, *J* = 13.1, 3.8 Hz, 1 H) 4.08 - 4.13 (m, 1 H) 4.26 - 4.34 (m, 1 H) 4.52 - 4.58 (dd, *J* = 6.7, 4.4 Hz, 1 H) 4.58 - 4.63 (dd, *J* = 6.7, 4.1 Hz, 1 H) 10.82 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.5, 27.4, 38.1, 52.3, 80.6, 82.0, 83.1, 84.1, 115.2, 176.1. **Minor epimer:** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34 (s, 3 H) 1.50 (s, 3 H) 2.72 - 2.89 (m, 2 H) 3.32 (dd, *J* = 12.9, 4.7 Hz, 1 H) 3.44 (d, *J* = 12.9, 6.2 Hz, 1 H) 4.18 - 4.24 (m, 1 H) 4.37 - 4.44 (m, 1 H) 4.66 (dd, *J* = 6.2, 1.2 Hz, 1 H) 4.81 (dd, *J* = 6.2, 4.1 Hz, 1 H) 10.82 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.0, 26.2, 34.4, 51.8, 77.3, 81.3, 82.7, 83.3, 113.2, 176.8. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{10}H_{14}N_3O_5^-$ 256.09389; Found 256.0939.

*6.1.8. tert-butyl (((3aS,4S,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)carbamate (**6**)*

A flask containing carboxylic acids **5** (1.20 g, 4.66 mmol) and molecular sieves in *tert*-butanol (90 mL) was purged with N_2 gas. Tiethylamine (0.715 mL, 5.13 mmol) and diphenylphosphoryl azide (1.10 mL, 5.13 mmol) were added and the reaction mixture was heated to reflux for 20 hours. TLC analysis (hexane/EtOAc 85:15) showed complete consumption of starting material. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. This residue was adsorbed onto celite and purified via FCC (hexane/EtOAc 10:0 → 8:2). Both compound **6** (46% yield) and its *trans* epimer **7** (13% yield) were obtained as transparent oils. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.33 (s, 3 H) 1.45 (s, 9 H) 1.53 (s, 3 H) 3.29 - 3.49 (m, 3 H) 3.60 (dd, *J* = 13.0, 3.7 Hz, 1 H) 3.98 - 4.09 (m, 2 H) 4.46 - 4.57 (m, 2 H) 4.91 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.5, 27.5, 28.4, 42.5, 52.4, 79.6, 81.8, 82.4, 83.1, 83.5, 114.9, 156.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{25}N_4O_5^+$ 329.18195; Found 329.1829.

*6.1.9. tert-butyl (((3aS,4R,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)carbamate (**7**)*

Compound **7** was obtained according to the procedure described for compound **6**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.33 (s, 3 H) 1.45 (s, 9 H) 1.49 (s, 3 H) 3.23 - 3.48 (m, 4 H) 4.07 - 4.15 (m, 1 H) 4.17 - 4.25 (m, 1 H) 4.62 (dd, *J* = 6.2, 1.5 Hz, 1 H) 4.74 (dd, *J* = 6.2, 4.2 Hz, 1 H) 4.94 (br. s., 1 H). HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{25}N_4O_5^+$ 329.18195; Found 329.1831.

*6.1.10. N-(((3aS,4S,6R,6aR)-6-(azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)benzamide (**8**)*

Boc-protected amine **6** (100 mg, 0.305 mmol) was taken up in dry 1,2-dichloroethane (2 mL) and molecular sieves (3 Å rods) were added. The flask was purged with N_2 gas and cooled on ice to 0 °C. Trifluoroacetic acid (0.179 mL, 2.34 mmol) was added and the reaction mixture was stirred for 3 hours. After filtration, the filtrate was taken to dryness and the residue was adsorbed onto celite. FCC (CH_2Cl_2/MeOH/NH_4OH 100:0:0.1 → 90:10:0.1) gave the corresponding primary amine, which was subjected to general procedure 1. Transparent oil, 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (s, 3 H) 1.53 (s, 3 H) 3.44 (dd, *J* = 13.0, 4.0 Hz, 1 H) 3.64 - 3.76 (m, 2 H) 3.85 (ddd, *J* = 14.1, 6.6, 5.1 Hz, 1 H) 4.06 - 4.14 (m, 1 H) 4.16 - 4.23 (m, 1 H) 4.51 - 4.60 (m, 2 H) 6.69 (br. s., 1 H) 7.37 - 7.55 (m, 3 H) 7.75 - 7.87 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.5, 27.5, 41.6, 52.4, 81.6, 82.5, 83.1, 83.3, 115.0, 127.1, 128.7, 131.7, 134.3, 167.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}N_4O_4^+$ 333.15573; Found 333.1563.

*6.1.11. N-(((2R,3S,4R,5S)-5-(benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-chlorobenzamide (**9**)*

Compound **8** was subjected to general procedure 2. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (s, 3 H) 1.53 (s, 3 H) 3.52 - 3.86 (m, 4 H) 4.10 - 4.21 (m, 2 H) 4.49 - 4.60 (m, 2 H) 6.88 (br. s., 1 H) 7.00 (br. s., 1 H) 7.21 - 7.41 (m, 5 H) 7.42 - 7.51 (m, 1 H) 7.55 - 7.65 (m, 1 H) 7.75 - 7.81 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.5, 27.4, 42.16, 42.22, 82.79, 82.84, 83.6, 83.8, 114.7, 127.19, 127.23, 128.6, 130.1, 130.4, 130.7, 131.5, 131.6, 134.1, 135.1, 167.3, 167.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{25}ClN_2O_5^+$ 445.15248; Found 445.1523. The isopropylidene protected derivative was subjected to general procedure 3. White foam, 80% from **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.21 - 3.55 (m, 4 H) 3.73 - 3.90 (m, 4 H) 4.86 (br. s., 2 H) 7.29 - 7.58 (m, 7 H) 7.77 - 7.88 (m, 2 H) 8.37 - 8.52 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 41.3, 41.8, 72.1, 72.3, 81.4,

81.6, 127.0, 127.2, 128.2, 128.9, 129.5, 129.9, 130.7, 131.1, 134.4, 137.0, 166.5, 166.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{22}ClN_2O_5^+$ 405.12118; Found 405.1213.

*6.1.12. 2-(((3aS,6R,6aR)-6-(azidomethyl)-2,2-dimethyldihydrofuro[3,4-d][1,3]dioxol-3a(4H)-yl)methyl)isoindoline-1,3-dione (**10**)*

See reference 8. Spectroscopy data for **10** are consistent with those published previously.[8]

*6.1.13. 2-(((3S,4R,5R)-5-(azidomethyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)isoindoline-1,3-dione (**11**)*

Compound **10** (920 mg, 2.57 mmol) was dissolved in a 35% TFA in H_2O solution (30 mL) and put at sonication overnight. MS analysis showed complete consumption of starting material. Reaction mixture was concentrated *in vacuo* and the remaining nacre-looking solid (*i.e.* the title compound) was used without further purification in the next step. Yield was determined over several steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.82 (d, *J* = 7.0 Hz, 1 H) 3.38 (dd, *J* = 13.2, 4.7 Hz, 1 H) 3.58 - 3.67 (dd, *J* = 13.2, 2.9 Hz, 1 H) 3.77 - 4.09 (m, 7 H) 7.75 - 7.82 (m, 2 H) 7.86 - 7.93 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 43.5, 52.1, 74.6, 75.5, 78.6, 81.9, 124.0, 131.7, 134.8, 169.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{15}N_4O_5^+$ 319.10370; Found 319.1024.

*6.1.14. (2R,3R,4S)-4-(aminomethyl)-2-(azidomethyl)tetrahydrofuran-3,4-diol (**12**)*

To a solution of compound **11** (obtained in the previous step) in toluene/MeOH 1:1 (26 mL) was added hydrazine monohydrate (0.312 mL, 6.43 mmol). The reaction mixture was heated to 60 °C for 4h. Floculation was seen and when the reaction was complete, the suspension was filtered and the filtrate concentrated *in vacuo*. Crude amine **12** (pale oil) was used in the next step without further purification. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_6H_{13}N_4O_3^+$ 189.09822; Found 189.0983.

*6.1.15. tert-butyl (((3S,4R,5R)-5-(azidomethyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)carbamate (**13**)*

Diol-amine **12** (obtained in the previous step) was dissolved in a dioxane/water 3:1 mixture (28 mL in total). K_2CO_3 (1.07 g) was added, followed by di-*tert*-butyl dicarbonate (0.589 g) and the reaction mixture was stirred vigorously for 4h. The dioxane was evaporated and the remaining water layer was extracted with CH_2Cl_2 (4 x 40 mL). The organic layers were pooled, dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC afforded title compound **13** as a pale yellow oil (85% over 3 steps from **10**). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 3.19 - 3.41 (m, 3 H) 3.55 - 3.94 (m, 6 H) 4.42 (br. s., 1 H) 5.39 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.3, 46.0, 52.0, 74.0, 75.1, 78.6, 80.8, 82.1, 158.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{15}N_4O_5^+$ 319.10370; Found 319.1024. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{21}N_4O_5^+$ 289.15065; Found 289.1512.

*6.1.16. (2R,3R,4S)-2-(azidomethyl)-4-(((tert-butoxycarbonyl)amino)methyl)-4-hydroxytetrahydrofuran-3-yl acetate (**14**)*

To an ice-cold solution of diol **13** (340 mg, 1.18 mmol) in pyridine (12 mL) was added acetic anhydride. The reaction mixture was stirred for 16h. Volatile organics were removed under reduced pressure and the residue was taken up in EtOAc and washed with HCl (0.1 N solution), $NaHCO_3$ (sat. aq. soln.) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC (hexane/EtOAc 10:0 → 4:6) afforded pure acetate **14** as a colorless oil in 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 2.14 (s, 3 H) 3.27 - 3.47 (m, 3 H) 3.62 (dd, *J* = 13.2, 3.2 Hz, 1 H) 3.69 (d, *J* = 9.7 Hz, 1 H) 3.91 (d, *J* = 9.7 Hz, 1 H) 3.99 - 4.10 (m, 2 H) 4.83 (d, *J* = 6.2 Hz, 1 H) 5.33 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.8, 28.3, 46.1, 52.2, 74.6, 74.7, 79.4, 80.4, 81.3, 157.8, 170.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{23}N_4O_6^+$ 331.16121; Found 331.1604.

*6.1.17. (2R,3R,4S)-2-(azidomethyl)-4-(((tert-butoxycarbonyl)amino)methyl)-4-(methoxymethoxy)tetrahydrofuran-3-yl acetate (**15**)*

A solution of compound **14** (0.280 mg, 0.848 mmol) and molecular sieves in $CHCl_3$ (8.5 mL) was purged with N_2 gas. P_2O_5 (1.55 g; 10.9 mmol) was added as well as dimethoxymethane (3.86 mL, 43.6 mmol) and the reaction mixture was flushed again. After 24 hours of stirring, the suspension was filtered with $CHCl_3$ and the filtrate was washed with Na_2CO_3 (sat. aq. soln.). The organic layer was dried over Na_2SO_4, filtered and

concentrated *in vacuo*. Flash silica-gel chromatography (toluene/EtOAc 10:0 → 6:4) afforded title compound **15** as a colorless oil in 27% yield, along with some left over starting material **14** (colorless oil, 8%) and spiro compound **16** (colorless oil, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 2.13 (s, 3 H) 3.32 - 3.48 (m, 6 H) 3.58 (dd, *J* = 13.2, 3.8 Hz, 1 H) 3.92 (d, *J* = 10.3 Hz, 1 H) 3.98 (d, *J* = 10.3 Hz, 1 H) 4.08 (app. td, *J* = 6.1, 3.7 Hz, 1 H) 4.76 (app. s, 2 H) 4.95 (d, *J* = 6.4 Hz, 1 H) 5.38 (t, *J* = 5.1 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.9, 28.4, 43.2, 52.3, 55.9, 72.2, 74.9, 79.7, 81.0, 83.3, 92.5, 156.3, 170.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{27}N_4O_7^+$ 375.18743; Found 375.1887.

*6.1.18. tert-butyl (5S,8R,9R)-9-acetoxy-8-(azidomethyl)-1,7-dioxa-3-azaspiro[4.4]nonane-3-carboxylate (**16**)*

Compound **16** was obtained as a side product in the synthesis of compound **15**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.48 (s, 9 H) 2.12 (s, 3 H) 3.38 (dd, *J* = 13.3, 4.5 Hz, 1 H) 3.52 (d, *J* = 10.8 Hz, 1 H) 3.60 (dd, *J* = 13.5, 3.2 Hz, 1 H) 3.77 (d, *J* = 10.8 Hz, 1 H) 3.89 (d, *J* = 9.7 Hz, 1 H) 3.96 (d, *J* = 9.7 Hz, 1 H) 4.12 (ddd, *J* = 6.3, 4.5, 3.5 Hz, 1 H) 4.91 (br. s., 2 H) 5.03 (d, *J* = 6.4 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.8, 28.4, 50.5, 52.0, 73.9, 75.9, 79.3, 80.9, 81.0, 86.0, 152.7, 170.3. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{23}N_4O_6^+$ 343.16121; Found 343.1611.

*6.1.19. tert-butyl (((3S,4R,5R)-5-(azidomethyl)-4-hydroxy-3-(methoxymethoxy)tetrahydrofuran-3-yl)methyl)carbamate (**17**)*

By addition of a methanolic solution of NaOMe (30% wt), the pH of a solution of compound **16** (86.0 mg, 0.230 mmol) in MeOH (5 mL) was adjusted to 9 – 10. The reaction mixture was stirred overnight. TLC analysis (toluene/EtOAc 6:4) showed presence of 1 lower-running spot. Amberlite (IR 120 H-form) was added until pH was neutral. The suspension was filtered and the filtrate concentrated *in vacuo*. The pure title compound was obtained as a colorless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 3.02 (br. s., 1 H) 3.33 - 3.56 (m, 6 H) 3.58 - 3.66 (m, 1 H) 3.84 - 3.91 (m, 3 H) 4.06 (d, *J* = 10.5 Hz, 1 H) 4.82 - 4.91 (m, 2 H) 5.13 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.4, 43.2, 52.3, 56.1, 71.9, 75.9, 80.0, 82.3, 83.3, 92.2, 156.4. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{25}N_4O_6^+$ 333.17686; Found 333.1779.

*6.1.20. O-((2R,3R,4S)-2-(azidomethyl)-4-(((tert-butoxycarbonyl)amino)methyl)-4-(methoxymethoxy)tetrahydrofuran-3-yl) 1H-imidazole-1-carbothioate (**18**)*

To a solution of alcohol **17** (76.0 mg, 0.229 mmol) in toluene (2.5 mL) was added thiocarbonyldiimidazole (61.3 mg, 0.344 mmol). The solution was heated to reflux overnight. Reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue obtained was purified by silica-gel chromatography (toluene/EtOAc 10:0 → 5:5) to give thiocarbamate **18** as a pale yellow oil (65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 3.34 - 3.48 (m, 4 H) 3.56 - 3.77 (m, 3 H) 3.94 - 4.03 (m, 2 H) 4.18 - 4.25 (m, 1 H) 4.71 (d, *J* = 7.6 Hz, 1 H) 4.74 (d, *J* = 7.6 Hz, 1 H) 5.25 (t, *J* = 5.9 Hz, 1 H) 5.82 (d, *J* = 5.0 Hz, 1 H) 7.03 - 7.10 (t, *J* = 0.9 Hz, 1 H) 7.65 (t, *J* = 1.3 Hz, 1 H) 8.36 (app. s, 1 H). HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{27}N_6O_6S^+$ 443.17073; Found 443.1707.

*6.1.21. tert-butyl (((3S,5S)-5-(aminomethyl)-3-(methoxymethoxy)tetrahydrofuran-3-yl)methyl)carbamate (**19**)*

Thiocarbamate **18** (66.0 mg, 0.149 mmol) was dissolved in toluene (5 mL) and purged with N_2 gas. Azoisobutyronitrile (catalytic quantity, ± 5 mg) was added and the solution was heated to 85 °C. Tributyltinhydride (0.201 mL, 0.745 mmol) was added and the reaction mixture was stirred for 3h at 85 °C, after which volatile organics were removed under reduced pressure. FCC afforded **19** as a colorless oil in 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 9 H) 1.53 - 1.69 (m, 3 H) 2.14 (dd, *J* = 13.3, 6.6 Hz, 1 H) 2.73 (dd, *J* = 13.2, 6.4 Hz, 1 H) 2.85 (dd, *J* = 13.2, 3.8 Hz, 1 H) 3.34 - 3.47 (m, 5 H) 3.78 (d, *J* = 10.0 Hz, 1 H) 3.86 (d, *J* = 9.7 Hz, 1 H) 4.08 - 4.19 (m, 1 H) 4.67 - 4.78 (m, 2 H) 5.15 (br. s., 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.5, 38.0, 45.6, 46.1, 55.8, 74.6, 80.7, 92.3, 110.1, 156.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{27}N_2O_5^+$ 291.19145; Found 291.1919.

*6.1.22. tert-butyl (((3S,5S)-5-((2-chlorobenzamido)methyl)-3-(methoxymethoxy)tetrahydrofuran-3-yl)methyl)carbamate (**20**)*

Compound **19** was subjected to general procedure 1. Colorless oil, 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.43 (s, 9 H) 1.71 (dd, *J* = 13.5, 9.7 Hz, 1 H) 2.21 (dd, *J* = 13.5, 6.2 Hz, 1 H) 3.35 - 3.55 (m, 6 H) 3.73 - 3.89 (m, 3 H) 4.35 (dtd, *J* = 9.5, 6.2, 6.2, 3.5 Hz, 1 H) 4.72 (d, *J* = 7.6 Hz, 1 H) 4.75 (d, *J* = 7.6 Hz, 1 H) 5.12 (br. s., 1 H) 6.59 (br. s., 1 H) 7.28 - 7.43 (m, 3 H) 7.60 - 7.68 (m, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.5, 37.9, 43.0, 45.4, 55.9, 75.0, 77.8, 79.7, 87.4, 92.3, 127.2, 130.1, 130.3, 130.8, 131.4, 135.3, 156.3, 167.0. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{30}ClN_2O_6^+$ 429.17869; Found 429.1797.

*6.1.23. N-(((2S,4S)-4-(benzamidomethyl)-4-hydroxytetrahydrofuran-2-yl)methyl)-2-chlorobenzamide (**21**)*

Compound **20** (23.0 mg, 0.054 mmol) was subjected to general procedure 3, after which the crude aminoalcohol obtained was taken up in DCM (1 mL). Triethylamine (22.6 µL, 0.162 mmol) and benzoyl chloride (6.62 µL, 0.057 mmol) were added and the reaction mixture was stirred overnight at room temperature. Then, the mixture was washed with HCl (0.1 N solution), $NaHCO_3$ (sat. aq. soln.) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC (CH_2Cl_2/MeOH 10:0 → 9:1) afforded **21** as a white foam in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.85 (dd, *J* = 13.2, 9.1 Hz, 1 H) 2.09 (dd, *J* = 13.0, 6.3 Hz, 1 H) 3.22 (br. s., 1 H) 3.43 - 3.62 (m, 2 H) 3.68 - 3.81 (m, 3 H) 3.87 (d, *J* = 9.7 Hz, 1 H) 4.33 - 4.43 (m, 1 H) 6.67 (t, *J* = 5.7 Hz, 1 H) 7.13 (t, *J* = 5.7 Hz, 1 H) 7.27 - 7.43 (m, 5 H) 7.46 - 7.53 (m, 1 H) 7.55 - 7.62 (m, 1 H) 7.74 - 7.82 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 40.9, 43.2, 48.0, 77.3, 77.7, 81.9, 127.29 (2 C), 128.7, 130.0, 130.4, 130.7, 131.6, 132.1, 133.7, 135.0, 167.3, 169.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{22}ClN_2O_4^+$ 389.12626; Found 389.1264.

*6.1.24. (R)-1-(benzyloxy)-3-chloropropan-2-ol (**22**)*

To a flame-dried, three neck round bottomed flask was added dry 1,2-dichloroethane (45 mL). *R*-(-)-epichlorohydrin (3.92 mL, 50.0 mmol) and benzyl alcohol (10.4 mL, 100 mmol) were added and the flask was purged with N_2 gas. The solution was cooled on ice to 0 °C under N_2. The stirring solution was then treated with boron trifluoro etherate (0.272 mL, 2.20 mmol) and again purged with N_2. The reaction mixture was allowed to attain room temperature overnight (16h). Subsequently, the mixture was heated to reflux for 2h and then allowed to cool and washed with $NaHCO_3$ (sat. aq. soln.). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Excess benzyl alcohol was removed under reduced pressure overnight in an oil bath at 50 °C. FCC (hexane/EtOAc 10:0 → 7:3) of the residue afforded the title compound as a colorless liquid in 48% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.54 (d, *J* = 5.9 Hz, 1 H) 3.55 - 3.69 (m, 4 H) 3.94 - 4.06 (m, 1 H) 4.56 (s, 2 H) 7.26 - 7.40 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 46.2, 70.4, 70.9, 73.7, 127.9, 128.1, 128.6, 137.7.

*6.1.25. (S)-3-(((R)-1-(benzyloxy)-3-chloropropan-2-yl)oxy)-2-hydroxypropyl 4-methylbenzenesulfonate (**23**)*

A solution of compound **22** (1.87 g, 9.32 mmol) in dry 1,2-dichloroethane (40 mL) was purged with N_2. *S*-glycidyl tosylate (0.710 g, 3.11 mmol) was added and the resulting solution was back-flushed and cooled on ice to 0 °C. Boron trifluoro etherate (catalytic quantity, 100 µL) was added and the reaction mixture was allowed to attain room temperature overnight. The mixture was washed with $NaHCO_3$ (sat. aq. soln.) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. FCC (hexane/EtOAc 10:0 → 4:6) afforded compound **23** as a colorless liquid in 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.44 (s, 3 H) 2.94 (d, *J* = 5.0 Hz, 1 H) 3.47 - 3.65 (m, 5 H) 3.67 - 3.76 (m, 2 H) 3.92 - 4.12 (m, 3 H) 4.53 (s, 2 H) 7.27 - 7.40 (m, 7 H) 7.74 - 7.83 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 21.8, 44.0, 68.7, 69.8, 70.3, 71.3, 73.7, 79.9, 127.9, 128.09, 128.15, 128.7, 130.1, 132.8, 137.6, 145.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{26}ClO_6S^+$ 429.11331; Found 429.1139.

*6.1.26. ((2R,5R)-5-((benzyloxy)methyl)-1,4-dioxan-2-yl)methanol (**24**)*

To a flask containing compound **23** (0.740 g, 1.73 mmol) was added an aqueous NaOH solution (1 M, 6 mL, 6 mmol) and the resulting biphasic mixture was stirred vigorously at room temperature. After 150 minutes, the reaction mixture was heated to 90 °C for 4h and then cooled to room temperature again for reaction overnight (16h). Then, the mixture was heated to 90 °C and stirred for 2h. After that, the mixture was neutralized with HCl (1 M) and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were rewashed with a saturated aqueous solution of $NaHCO_3$ and brine, then dried over Na_2SO_4, filtered and concentrated *in vacuo*. Silica-gel

chromatography (CH_2Cl_2/MeOH 100:0 → 96:4) afforded the title compound as a colorless oil in 66% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.36 (br. s., 1 H) 3.50 - 3.86 (m, 10 H) 4.51 - 4.59 (m, 2 H) 7.23 - 7.39 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 61.0, 63.9, 64.3, 68.2, 72.2, 73.5, 73.8, 127.77, 127.81, 128.5, 137.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{19}O_4^+$ 239.12779; Found 239.1279.

*6.1.27. (2R,5R)-2-(azidomethyl)-5-((benzyloxy)methyl)-1,4-dioxane (**25**)*

To a solution of compound **24** (530 mg, 2.22 mmol) and triethylamine (0.618 mL, 4.44 mmol) in CH_2Cl_2 (12 mL) stirred at 0 °C, methanesulfonyl chloride (0.205 mL, 2.66 mmol) was added dropwise. The reaction mixture was allowed to attain ambient temperature. After 3 hours, TLC analysis (hexane/EtOAc 1:1) showed complete consumption of the starting material. The reaction mixture was washed with saturated sodium bicarbonate solution and water. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the mesylate as a yellow to orange colored oil. To this crude mesylate, dissolved in DMF (12 mL), was added sodium azide (0.722 g, 11.1 mmol). After overnight reaction at 60 °C, TLC analysis (hexane/EtOAc 1:1) showed the presence of one major product. The solvent was evaporated and the residue was taken up in EtOAc. The resulting solution was washed with saturated $NaHCO_3$ solution and water. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. This crude material was purified by flash column chromatography (hexane/EtOAc 10:0 → 5:5) to afford azide **25** as a colorless liquid (91% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.26 (dd, J = 12.9, 5.0 Hz, 1 H) 3.47 - 3.85 (m, 9 H) 4.55 (s, 2 H) 7.21 - 7.39 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 49.9, 64.0, 64.7, 68.2, 71.9, 72.5, 73.4, 127.71, 127.74, 128.4, 137.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{18}N_3O_4^+$ 264.13427; Found 264.1349.

*6.1.28. N-(((2R,5R)-5-((benzyloxy)methyl)-1,4-dioxan-2-yl)methyl)benzamide (**26**)*

Compound **25** was subjected to general procedure 2. Colorless liquid, 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.52 - 3.88 (m, 10 H) 4.56 (s, 2 H) 6.53 (br. s., 1 H) 7.27 - 7.55 (m, 8 H) 7.72 - 7.82 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 39.5, 64.2, 65.2, 68.4, 72.0, 72.4, 73.6, 127.1, 127.87, 127.93, 128.6, 128.7, 131.7, 134.5, 138.0, 167.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{24}NO_4^+$ 342.16998; Found 342.1703.

*6.1.29. N-(((2R,5R)-5-(hydroxymethyl)-1,4-dioxan-2-yl)methyl)benzamide (**27**)*

A solution of benzamide **26** (440 mg, 1.29 mmol) in MeOH (50 mL) was placed under an N_2 atmosphere. Palladium black (catalytic amount) was added and the reaction vessel was purged again with N_2. Hydrogen gas was bubbled through the solution for 3h (MS analysis for conversion). The vessel was purged with nitrogen gas and the reaction mixture was filtered over a Whatman fiberglass filter. The filtrate was concentrated *in vacuo* and the residue (*i.e.* the product) was obtained as a colorless oil, quantitatively. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.45 - 3.84 (m, 11 H) 7.17 (br. s., 1 H) 7.32 - 7.53 (m, 3 H) 7.70 - 7.86 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 39.2, 60.7, 63.3, 64.9, 71.6, 73.9, 127.0, 128.5, 131.6, 134.1, 168.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{18}NO_4^+$ 252.12303; Found 252.1236.

*6.1.30. N-(((2R,5R)-5-(azidomethyl)-1,4-dioxan-2-yl)methyl)benzamide (**28**)*

Following a similar procedure described for compound **25**, compound **27** (325 mg, 1.29 mmol) gave azide **28** as a pale oil in 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.27 - 3.35 (m, 1 H) 3.48 - 3.59 (m, 1 H) 3.60 - 3.89 (m, 8 H) 6.76 (t, J = 4.8 Hz, 1 H) 7.37 - 7.54 (m, 3 H) 7.75 - 7.84 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 39.4, 50.0, 64.2, 64.7, 72.0, 72.1, 127.0, 128.6, 131.6, 134.2, 167.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{17}N_4O_3^+$ 277.12952; Found 277.1299.

*6.1.31. N-(((2R,5R)-5-(benzamidomethyl)-1,4-dioxan-2-yl)methyl)-2-chlorobenzamide (**29**)*

Compound **28** was subjected to general procedure 2. White foam, 74%. ^{1}H NMR (300 MHz, MeOD) δ ppm 3.47 - 3.58 (m, 2 H) 3.64 - 3.89 (m, 8 H) 7.33 - 7.56 (m, 7 H) 7.79 - 7.85 (m, 2 H). ^{13}C NMR (75 MHz, MeOD) δ ppm 40.5, 40.6, 65.8, 66.0, 73.3, 73.4, 128.1, 128.3, 129.6, 129.9, 131.0, 131.9, 132.2, 132.7, 135.6, 137.6, 170.3, 170.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{22}ClN_2O_4^+$ 389.12626; Found 389.1264.

*6.1.32. tert-butyl (R)-2-((benzyloxy)methyl)morpholine-4-carboxylate (**30**)*

Benzyl-(*R*)-glycidyl ether (2.00 g, 12.2 mmol) and NaOH (4.00 g, 100 mmol) in H_2O (9.2 mL) and MeOH (3.6 mL) were treated with 2-aminoethyl hydrogen sulfate (7.00 g, 49.59 mmol). The reaction mixture was stirred for

90 minutes at 40 °C. The mixture was allowed to cool to room temperature, toluene (14 mL) and NaOH (2.00 g, 50.0 mmol) were added and then it was stirred overnight at 65 °C. Toluene (5 mL) and H_2O (20 mL) were added and the organic layer was separated. The water layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give crude amine which was taken up in acetone (20 mL) and H_2O (6 mL) at 0 °C. Di-*tert*-butyl dicarbonate (2.60 g, 11.9 mmol) was added and the resulting mixture was stirred vigorously for 2h. The acetone was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC (hexane/EtOAc 10:0 → 7:3) afforded the title compound as a colorless oil in 43% (over 2 steps). ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 1.41 (s, 9 H) 2.69 (dd, *J* = 12.9, 9.4 Hz, 1 H) 2.87 (ddd, *J* = 13.2, 11.4, 3.5 Hz, 1 H) 3.28 - 3.56 (m, 4 H) 3.68 (ddt, *J* = 13.2, 3.0, 1.6 Hz, 1 H) 3.75 - 3.86 (m, 2 H) 4.50 (s, 2 H) 7.22 - 7.38 (m, 5 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 27.7, 43.0, 45.3, 65.1, 70.2, 72.2, 73.6, 78.7, 126.9, 127.0, 127.7, 138.0, 153.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{26}NO_4^+$ 308.18563; Found 308.1867.

*6.1.33. tert-butyl (R)-2-(azidomethyl)morpholine-4-carboxylate (**31**)*

A solution of compound **30** (1.56 g, 5.07 mmol) in MeOH (50 mL) was placed under an N_2 atmosphere. Palladium black (catalytic amount) was added and the reaction vessel was purged again with N_2. Hydrogen gas was bubbled through the solution for 3h. The vessel was purged with nitrogen gas and the reaction mixture was filtered over a Whatman fiberglass filter. The filtrate was concentrated *in vacuo* and the residue was subjected to a procedure that is similar to the one described for **25** and **28**. Colorless oil, 81% from **30**. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 1.41 (s, 9 H) 2.69 (dd, *J* = 12.9, 10.3 Hz, 1 H) 2.89 (ddd, *J* = 13.3, 11.3, 3.7 Hz, 1 H) 3.28 - 3.37 (m, 2 H) 3.37 - 3.56 (m, 2 H) 3.72 (ddt, *J* = 13.3, 3.0, 1.7 Hz, 1 H) 3.74 - 3.87 (m, 2 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 27.7, 42.8, 45.1, 51.6, 65.2, 73.4, 78.8, 153.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{10}H_{19}N_4O_3^+$ 243.14517; Found 243.1452.

*6.1.34. (R)-(2-(azidomethyl)morpholino)(phenyl)methanone (**32**)*

Compound **31** was subjected to general procedure 4. Pale yellow oil, 82%. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{15}N_4O_2^+$ 247.11895; Found 247.1201.

*6.1.35. (R)-(2-(azidomethyl)morpholino)(2-chlorophenyl)methanone (**33**)*

Compound **31** was subjected to general procedure 4. Pale yellow oil, 79%. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{14}ClN_4O_2^+$ 281.07998; Found 281.0803.

*6.1.36. (S)-N-((4-benzoylmorpholin-2-yl)methyl)-2-chlorobenzamide (**34**)*

Compound **32** was subjected to general procedure 2. White foam, 62%. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 2.89 (dd, *J* = 12.9, 10.5 Hz, 1 H) 3.01 - 3.15 (m, 1 H) 3.23 - 3.43 (m, 2 H) 3.44 - 3.64 (m, 2 H) 3.78 - 3.93 (m, 2 H) 3.96 - 4.23 (br. s., 1 H) 7.17 - 7.53 (m, 9 H) 8.24 (br. s., 1 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 40.9, 45.0 (weak), 48.2 (weak), 65.4, 73.6, 126.47, 126.52, 128.0, 128.4, 129.08, 129.13, 129.6, 130.2, 135.4, 136.4, 166.1, 168.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{20}ClN_2O_3^+$ 359.11570; Found 359.1168.

*6.1.37. (S)-N-((4-(2-chlorobenzoyl)morpholin-2-yl)methyl)benzamide (**35**)*

Compound **33** was subjected to general procedure 2. White foam, 91%. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 2.69 - 4.00 (m, 8 H) 4.26 - 5.53 (m, 1 H) 7.27 - 7.95 (m, 9 H) 8.10 - 8.44 (m, 1 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 41.1, 44.0, 48.1 (weak), 65.3, 73.9, 126.7, 127.1, 127.6, 127.7, 128.9, 129.0, 130.0, 130.6, 134.2, 135.2, 165.4, 166.3. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{20}ClN_2O_3^+$ 359.11570; Found 359.1158.

*6.1.38. tert-butyl (2S,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidine-1-carboxylate (**37**)*

4-hydroxyproline **36** (5.00 g, 21.6 mmol) was purged with N_2 and cooled on ice to 0°C. BH_3·THF (1 M solution, 64.9 mL, 64.9 mmol) was added dropwise and the solution was stirred for 1h at 0 °C. When complete, the excess BH_3·THF was quenched by careful addition of water (60 mL). The mixture was extracted with brine and EtOAc (3 x 100 mL), the combined organic layers were dried over Na_2SO_4, filtered an concentrated *in vacuo*. FCC

(CH_2Cl_2/MeOH 92:8) gave the title compound as a thick colorless oil in 96% yield. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 1.40 (s, 9 H) 1.74 - 1.87 (m, 1 H) 1.89 - 2.01 (m, 1 H) 3.09 (br. s, 1 H) 3.17 - 3.31 (m, 2 H) 3.40 (app. dd, *J* = 10.5, 5.9 Hz, 1 H) 3.48 (app. dd, *J* = 10.5, 3.8 Hz, 1 H) 3.74 - 3.84 (m, 1 H) 4.22 (app. dt, *J* = 9.2, 4.5 Hz, 1 H) 4.46 (br. s, 1 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 27.9, 36.7, 54.6, 57.2, 62.2, 67.7, 77.8, carbonyl peak missing, even at 80 °C. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{10}H_{20}NO_4^+$ 218.13868; Found 218.1390.

*6.1.39. tert-butyl (2S,4R)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-4-hydroxypyrrolidine-1-carboxylate (**38**)*

A solution of hydroxyprolinol **37** (3.00 g, 13.8 mmol) in DMF (40 mL) was cooled on ice to 0 °C and purged with N_2. Imidazole (1.88 g, 27.6 mmol) and *tert*-butyl(chloro)diphenyl silane (3.95 mL, 15.2 mmol) were added and the resulting solution was flushed again. After 16 hours, volatile organics were removed under reduced pressure, the residue was taken up in EtOAc and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC (toluene/EtOAc 1:0 → 0:1) yielded the title product as a colorless oil in 63% yield. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) δ ppm 1.02 (s, 9 H) 1.33 (s, 9 H) 1.88 - 2.02 (m, 1 H) 2.08 - 2.22 (m, 1 H) 3.33 (app. d, *J* = 4.1 Hz, 2 H) 3.66 - 4.00 (m, 3 H) 4.32 (m, 1 H) 4.66 (d, *J* = 4.1 Hz, 1 H) 7.33 - 7.67 (m, 10 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 18.4, 26.3, 27.8, 36.7 (weak), 54.7, 56.7, 64.2 (weak), 67.7, 77.8, 127.28, 127.31, 129.3 (2 C), 132.9, 133.0, 134.5, 134.6, 153.4. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{26}H_{38}NO_4Si^+$ 456.25646; Found 456.2375.

*6.1.40. tert-butyl (2S,4S)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-4-cyanopyrrolidine-1-carboxylate (**39**)*

To an ice-cold solution of alcohol **38** (0.630 g, 1.38 mmol) in CH_2Cl_2 (14 mL) was added triethylamine (0.385 mL, 2.76 mmol) and mesyl chloride (0.128 mL, 1.66 mmol). The reaction mixture was allowed to attain room temperature overnight and when finished, it was washed with a saturated solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The obtained crude mesylate was taken up in acetonitrile (14 mL) and tetrabultylammonium cyanide (1.85 g, 6.90 mmol) was added carefully. The reaction mixture was heated to 65 °C overnight and then poured into a separating funnel that contained an EtOAc/hexane 1:4 mixture. This organic phase was washed with a saturated aquatic solution of $NaHCO_3$ and brine, then dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC of the residue (hexane/EtOAc 1:0 → 1:1) gave nitrile **39** as a pale yellow oil in 26% yield. ^{1}H NMR (300 MHz, 80 °C, DMSO-*d*$_6$) δ ppm 1.03 (s, 9 H) 1.32 (s, 9 H) 2.23 - 2.34 (m, 1 H) 2.50 - 2.58 (m, 1 H) 3.27 - 3.39 (m, 2 H) 3.76 - 3.95 (m, 4 H) 7.36 - 7.49 (m, 6 H) 7.58 - 7.64 (m, 4 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 18.4, 25.2, 26.3, 27.6, 31.3, 49.3, 57.2, 63.5, 78.8, 120.4, 127.4, 129.4, 132.7, 134.6, 152.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{27}H_{37}N_2O_3Si^+$ 465.25680; Found 465.2574.

*6.1.41. tert-butyl (2S,4R)-4-(benzamidomethyl)-2-(((tert-butyldiphenylsilyl)oxy)methyl)pyrrolidine-1-carboxylate (**40**)*

To a cooled (0 °C) solution of nitrile **39** (280 mg, 0.603 mmol) in MeOH (6 mL) was added cobalt(II) chloride (78.0 mg, 0.603 mmol) and the resulting pink solution was stirred for 10 minutes. To this mixture was added $NaBH_4$ (57.0 mg, 1.51 mmol) in portions as to maintain a black precipitate that appeared following each addition. After overnight reaction, a saturated solution of NH_4Cl (3.5 mL) was added to quench the reaction and this mixture was stirred for another hour. The mixture was made alkaline by the addition of $NaHCO_3$ (sat. aq. soln.), after which the black precipitate was filtered off. A clear pink solution was obtained, which was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude amine was subjected to general procedure 1. Colorless oil, 68%. ^{1}H NMR (300 MHz, 80 °C, DMSO-*d*$_6$) δ ppm 1.00 (s, 9 H) 1.31 (s, 9 H) 1.84 (ddd, *J* = 12.4, 10.9, 7.8 Hz, 1 H) 2.11 - 2.44 (m, 2 H) 2.93 (app. t, *J* = 10.3 Hz, 1 H) 3.24 - 3.47 (m, 2 H) 3.62 - 3.90 (m, 4 H) 7.24 - 7.53 (m, 9 H) 7.55 - 7.65 (m, 4 H) 7.80 - 7.87 (m, 2 H) 8.30 (t, *J* = 5.3 Hz, 1 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 18.4, 26.3, 27.7, 32.0, 37.4, 41.3, 50.7, 57.8, 63.3, 77.9, 126.7, 127.26, 127.31, 127.7, 129.2, 130.4, 132.9, 134.6, 153.0, 159.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{34}H_{45}N_2O_4Si^+$ 573.31431; Found 573.3157.

*6.1.42. tert-butyl (2S,4R)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-4-((2-chlorobenzamido)methyl)pyrrolidine-1-carboxylate (**41**)*

Following a similar procedure described for compound **40**, compound **39** (0.153 g, 0.329 mmol) gave benzamide **41** as a colorless oil in 60% yield. ^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) d ppm 1.03 (s, 9 H) 1.32 (s, 9 H) 1.72 - 1.89 (m, 1 H) 2.16 - 2.43 (m, 2 H) 2.92 (app. t, *J* = 10.3 Hz, 1 H) 3.19 - 3.41 (m, 2 H) 3.72 - 3.90 (m, 4 H) 7.28 - 7.52 (m, 10 H) 7.56 - 7.67 (m, 4 H) 8.27 (t, *J* = 5.3 Hz, 1 H). ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) δ ppm 18.4, 26.3, 27.8, 32.0, 37.3, 41.3, 50.7, 57.8, 63.5, 77.9, 126.5, 127.32, 127.34, 128.4, 129.1, 129.3, 130.1, 132.95, 133.02, 134.6, 153.0, 159.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{34}H_{44}ClN_2O_4Si^+$ 607.27534; Found 607.2766.

*6.1.43. tert-butyl (2S,4R)-2-(azidomethyl)-4-(benzamidomethyl)pyrrolidine-1-carboxylate (**42**)*

To a solution of protected alcohol **40** (0.211 g, 0.368 mmol) in THF (3.5 mL) was added tetrabutylammonium fluoride solution (1 M in THF, 0.405 mL, 0.405 mmol). After 3 hours, the reaction mixture was taken up in EtOAc and washed with water (2 x 10 mL) and the combined water layers were re-extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (3.5 mL) and cooled on ice to 0 °C. Triethylamine (0.103 mL, 0.736 mmol) and mesyl chloride (34.2 µL, 0.442 mmol) were added. After 3 hours, the reaction mixture was washed with $NaHCO_3$ (sat. aq. soln.) and brine, then dried over Na_2SO_4, filtered and concentrated *in vacuo*. This residue was taken up in DMF (3.5 mL) and sodium azide (0.120 g, 1.84 mmol) was added. The reaction mixture was stirred overnight at 60 °C. Volatile organics were evaporated under reduced pressure and the residue was taken up in EtOAc, which was washed with $NaHCO_3$ (sat. aq. soln.) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. FCC (toluene/EtOAc 1:0 → 0:1) gave the title azide as a colorless oil in 55% yield. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{26}N_5O_3^+$ 360.20302; Found 360.2024.

*6.1.44. tert-butyl (2S,4R)-2-(azidomethyl)-4-((2-chlorobenzamido)methyl)pyrrolidine-1-carboxylate (**43**)*

Following a similar procedure described for compound **42**, compound **41** (95.0 g, 0.156 mmol) gave benzamide **43** as a colorless oil in 55% yield. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{25}ClN_5O_3^+$ 394.16404; Found 394.1632.

*6.1.45. tert-butyl (2S,4R)-4-(benzamidomethyl)-2-((2-chlorobenzamido)methyl)pyrrolidine-1-carboxylate (**44**)*

Compound **42** was subjected to general procedure 2. White foam, 38%. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{25}H_{31}ClN_3O_4^+$ 472.19976; Found 472.1992.

*6.1.46. tert-butyl (2S,4R)-2-(benzamidomethyl)-4-((2-chlorobenzamido)methyl)pyrrolidine-1-carboxylate (**45**)*

Compound **43** was subjected to general procedure 2. White foam, 66%. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{25}H_{31}ClN_3O_4^+$ 472.19976; Found 472.1996.

*6.1.47. N-(((2S,4S)-4-(benzamidomethyl)pyrrolidin-2-yl)methyl)-2-chlorobenzamide (**46**)*

Compound **44** (29.0 mg, 0.0610 mmol) was dissolved in a 20% trifluoroacetic acid in CH_2Cl_2 mixture (2.5 mL) and cooled on ice to 0 °C. The mixture was stirred for 3 hours and allowed to attain room temperature. Volatile organics were evaporated under reduced pressure and the residue was purified via FCC (CH_2Cl_2/MeOH/NH_4OH 100:0:0.1 → 90:10:0.1). White foam, q. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.19 - 1.41 (m, 1 H) 2.15 (app. dt, *J* = 13.1, 6.5 Hz, 1 H) 2.42 - 2.60 (m, 2 H) 2.88 (dd, *J* = 11.0, 7.5 Hz, 1 H) 3.14 (dd, *J* = 11.0, 8.1 Hz, 1 H) 3.25 - 3.58 (m, 5 H) 7.33 - 7.57 (m, 7 H) 7.80 - 7.88 (m, 2 H) 8.54 - 8.65 (m, 2 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 33.1, 38.3, 42.0, 42.1, 48.8, 58.7, 127.1, 127.2, 128.2, 129.0, 129.6, 129.9, 130.9, 131.1, 134.5, 136.6, 166.5, 166.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{23}ClN_3O_2^+$ 372.14733; Found 372.1471.

*6.1.48. N-(((3S,5S)-5-(benzamidomethyl)pyrrolidin-3-yl)methyl)-2-chlorobenzamide (**47**)*

Following a similar procedure described for compound **46**, compound **45** (24.0 g, 0.0508 mmol) gave benzamide **47** as a colorless oil in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 - 1.60 (m, 1 H) 2.25 (app. dt, *J* = 13.0, 6.6 Hz, 1 H) 2.54 - 2.69 (m, 1 H) 3.06 (dd, *J* = 11.4, 8.8 Hz, 1 H) 3.28 - 3.49 (m, 4 H) 3.55 - 3.66 (m, 2 H) 3.70 - 3.85 (m, 1 H) 7.30 - 7.64 (m, 7 H) 7.84 - 7.94 (m, 2 H) 8.64 (t, *J* = 5.7 Hz, 1 H) 8.89 (t, *J* = 5.7 Hz, 1 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 32.6, 37.8, 40.8, 41.4, 48.1, 60.1, 127.5, 127.8, 128.8, 129.2,

130.0, 130.2, 131.2, 132.0, 134.3, 136.6, 167.60 (2 C). HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{23}ClN_3O_2^+$ 372.14733; Found 372.1473.

6.2. Virtual screening

The open source platform KNIME (version 2.11.0) was used as interface. The SDF files from the ZINC database Drugs Now were downloaded in February 2014 and used: http://zinc.docking.org/subsets/drugs-now.
A substructure search was performed using 2-chloro-*N*-(2-methoxyethyl)benzamide as reference fragment. The CDK Substructure search Community Node was used.
2D-fingerprints were generated with the CDK Fingerprints community node in KNIME. Lead compound **2** was used as reference molecule. MACCS Fingerprints were generated and for each fingerprint, a Tanimoto coefficient (T_C) was generated. A T_C cut-off value of 0.750 was applied.

6.3. Microbiology

6.3.1. Reagents used

Hamamelitannin (HAM) and vancomycin (VAN) were purchased from Sigma Aldrich (Bornem, Belgium).
HAM was stored in DMSO at -20°C. VAN was dissolved in ultrapure water and stored at 4°C.

6.3.2. Strains and culture conditions

Methicillin-resistant *Staphylococcus aureus* Mu50 (MRSA Mu50) was cultured in Mueller-Hinton broth (MH, Oxoid, Basingstoke, England) at 37°C under aerobic conditions.

6.3.3. Determination of the MIC

MICs of HAM analogues used against *S. aureus* Mu50 were determined in triplicate using flat-bottom 96-well microtiter plates (TPP, Trasadingen, Switzerland) as previously described.[21]

6.3.4. Effect of pretreatment and co-treatment on biofilm susceptibility

S. aureus Mu50 biofilms were formed and HAM analogues were evaluated as previously described.[7-9] In brief, overnight cultures in MH were centrifuged, the pellet was resuspended in double-concentrated MH (2 x MH) and diluted to an $OD_{590\,nm}$ of 0.2. Fifty microliter of the diluted bacterial suspension was transferred to the wells of a round-bottom 96-well microtiter plate (TPP). Control wells received 50 µl MilliQ. Wells used to evaluate pre-treatment received 50 µl of HAM-analogue solution. Bacteria were allowed to adhere and grow without agitation for 4 h at 37 °C. After 4 h, medium was removed, and the adhered cells were washed with sterile physiological saline (0.9% NaCl; PS). After this washing step, control wells were filled with 50 µl 2 × MH and 50 µl MilliQ. Other wells were filled with 50 µl 2 × MH and 50 µl of HAM analogue solution, and the plate was incubated for 20 h at 37 °C. To evaluate the effect of co-treatment on mature biofilms, control biofilms were formed in the absence of HAM analogues, as described above. After 24 h of biofilm formation, the medium was removed and the wells were rinsed with PS. Control wells were either filled with 100 µl PS (untreated controls) or with 50 µl PS and 50 µl antibiotic solution. Wells used to evaluate the effect of pre-treatment were also filled with 50 µl PS and 50 µl antibiotic solution while wells used to evaluate combination treatment were filled with 50µl of a HAM analogue solution and 50 µl antibiotic solution. The plates were then incubated for an additional 24 h at 37 °C. After biofilm formation and treatment of the biofilms, the number colony forming units (CFU) per biofilm were determined by conventional plating. To collect the cells for plating, plates were rinsed with PS, sessile cells were removed from the microtiter plate by two cycles of vortexing (5 min) and sonication (5 min) and the number of CFU/biofilm was determined by plating the resulting suspensions. The number of CFU/biofilm (for plating) of the control biofilms was set to 100% and the results of the treated biofilms were compared to this. Each condition was tested in at least three wells in each assay, and each assay was carried out at least in triplicate ($n \geq 9$).

6.3.5. Statistical evaluation

The normal distribution of the data was checked by using the Shapiro-Wilk test. Normally distributed data were analyzed using a one-way ANOVA. Non-normally distributed data were analyzed using the Kruskal-Wallis test. Statistics were determined using SPSS software, version 22.0.

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