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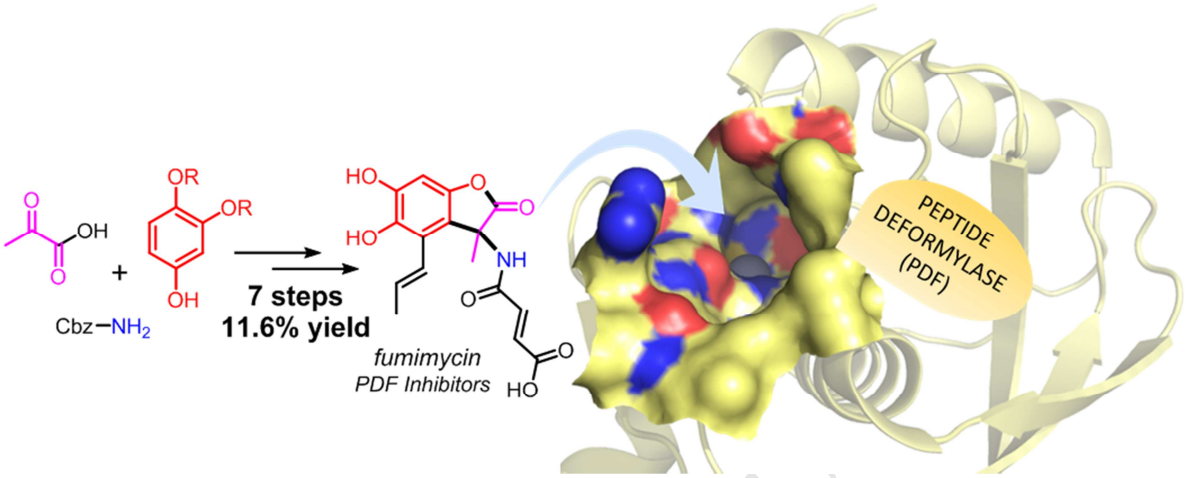

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Total synthesis of (±)-fumimycin and analogues for biological evaluation as peptide deformylase inhibitors

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ABSTRACT

A concise 7-step total synthesis of (±)-fumimycin in 11.6 % overall yield is reported. An acid-catalyzed intramolecular aza-Friedel–Crafts cyclization was developed to construct the benzofuranone skeleton of the natural product bearing an α,α-disubstituted amino acid moiety in a single step. Regioselective chlorination followed by a Suzuki–Miyaura cross-coupling rapidly enabled the preparation of a library of analogues which were evaluated against peptide deformylase for antibacterial activity.

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1. Introduction

The mycotoxin (–)-fumimycin (**1**), isolated in 2007 from *Aspergillus fumisynnematus* F746, contains a benzofuranone core wrapped around a highly congested quaternary stereocenter from a α,α-disubstituted amino ester moiety (Figure 1).[1] More than structurally challenging, **1** was found to have significant antibacterial activity against two strains of methicillin-resistant *Staphylococcus aureus* (MRSA: MIC_{90} = 100 µg/mL), thus providing an exciting starting point for developing small-molecule antibiotics. Indeed, the ever increasing number of "superbugs" such as MRSA[2a] multidrug-resistant *Neisseria gonorrhoeae,*[2b] vancomycin-resistant *Enterococcus*,[2c] multidrug-resistant *Mycobacterium tuberculosis*,[2d] carbapenem-resistant *Enterobacteriaceae*,[2e] are due to bacterial adaptation and to biocide overuse, which calls for the urgent need to develop therapeutic agents with new modes of action and novel bacterial targets. In this context, the (–)-fumimycin antibacterial activity has been credited to inhibiting the peptide deformylase (PDF) protein, which is a metalloenzyme critical to the protein synthesis of prokaryotes (*Sa*PDF: IC_{50} = 4.1 µM).[3] Bacterial PDFs are metalloproteases (typical S1'-S3' pockets, see Figure 1) that bind to ribosomes and deformylate the amino-terminal formyl group of nascent proteins emerging from the ribosomal exit tunnel.[4,5] The ribosome-nascent-chain complexes (RNCs) are co-translationally deformylated at the N-terminal methionine residue upon reaching the PDF catalytic site (RNCs: ~50-60 residues in length with 15-25 amino acids exposed outside of the ribosomal exit tunnel).[6] The eukaryotic human homolog of PDF (*Hs*PDF) has been exclusively located in the mitochondria and was shown to exhibit only poor catalytic activity,[7] suggesting the bacterial PDF to be an attractive target for developing some novel broad-spectrum antibiotics with low toxicity.[8] Consistent with this idea, and following the discovery of the first potent natural product PDF inhibitor, actinonin (**2**),[9] several inhibitors have been rationally designed to mimic a tripeptide model substrate for deformylation: fMet-Ala-Ser (MAS **3**) [11] and achieve a slow tight-binding activity.[10] As shown by the selection of inhibitors in Figure 1, a number of peptide-based drugs (*e.g.* **4-7**) have been developed over the past 15 years by several pharmaceutical companies and advanced as preclinical and clinical candidates.[12],[13]

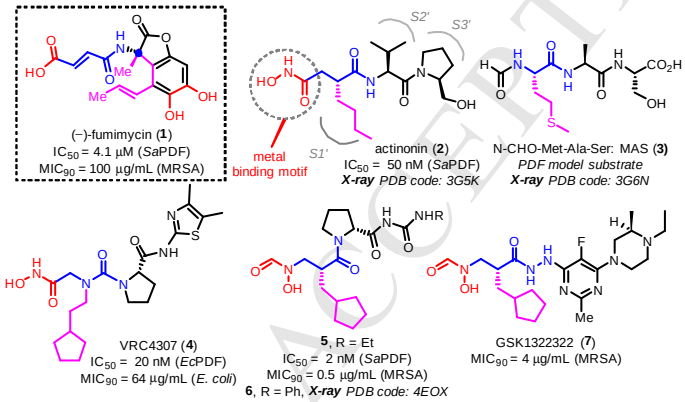


Figure 1. (–)-fumimycin (**1**) and other reported PDF inhibitors with the possible metal binding motifs in red, the backbone running through the PDF pocket in blue and the side chain mimic of methionine for the S1' pocket in pink.

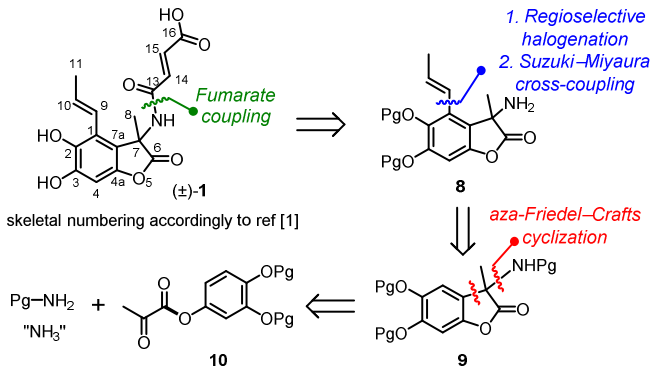
Because of the limited examples of non-peptidic small-molecule inhibitors of PDF have been reported,[14] we were drawn to synthesize fumimycin (**1**) and some potentially accessible analogues for biological evaluation. Despite its reported inhibitory effect against *Sa*PDF, **1** lacked antibacterial activity, therefore we hypothesized that some simple functional manipulations of the fumarate and styrenyl moieties may unveil some more potent analogues, and also shed some light into the latent role of the catechol fragment in the PDF binding. Here, we report a convergent, concise and high-yielding synthesis of (±)-**1** (7 steps), as well as a small library of analogues which have been evaluated for PDF inhibition.

2. Results and discussion

The first reported total synthesis of (±)-fumimycin and the mono-methoxy fumimycin analogue in a racemic fashion[15] afforded invaluable insights on the difficulties to install the quaternary stereocenter α,α-disubstituted amino acid moiety and to deprotect the catechol moiety at a late stage. These efforts from the Bräse group culminated in the asymmetric synthesis of the fumimycin (+)-antipode (18 steps, 90% ee and 1.6% overall yield), highlighted by an enantioselective 1,2-alkylation of ketimine (59% ee) and a styrenyl installation promoted by a Claisen rearrangement–isomerization sequence.[16] Inspired by the more recent report from Zhou towards fumimycin[17] and by our work on direct aza-Friedel–Crafts arylation of α-iminoglycinates to synthesize arylated non-proteinogenic α-amino esters,[18] we hypothesized that a similar strategy applied to an α-iminopyruvate substrate derived from **10** would drastically reduce the number of steps required to synthesize fumimycin and enable scaffold diversification the access numerous analogues.

2.1 Retrosynthetic Considerations

Therefore a rational retrosynthetic analysis consisted of preparing (±)-**1** from a late-stage deprotection and an amide coupling between a fumarate derivative and the free-amino lactone core **8** (Scheme 1). The styrenyl moiety of **8** would be introduced through a regioselective halogenation followed by a palladium-catalyzed cross-coupling reaction from lactone **9**, which skeleton could potentially be obtained in a single step from an aza-Friedel–Crafts cyclization. The intramolecular aza-Friedel–Crafts approach was chosen as the pivotal step because it would enable the condensation between a protected amine, surrogate of ammonia, and a pyruvate derivative **10**, and the formation of the central C7–C7a bond in a domino fashion.



Scheme 1. Proposed retrosynthetic analysis towards (±)-fumimycin (**1**)

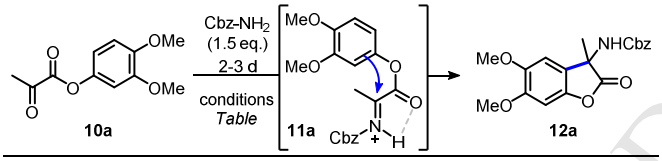
2.2 Total Synthesis of (±)-Fumimycin (*1*) and a Library of Analogues *17a-g*.

We began our journey with a Steglich esterification of pyruvic acid with the 3,4-dimethoxyphenol (see Scheme 3).[19] Surprisingly, the typical carbodiimide EDCi and the more recent uronium-type (COMU) coupling reagents proved inefficient for this particular esterification, whereas (aza)benzotriazole-based reagents (PyBOP, HATU, HBTU) afforded the desired pyruvate **10a** with only moderate efficiency (< 58% yield). Eventually, the reaction proceeded smoothly with DCC (1.5 eq.) and was achieved on a multigram scale to prepare **10a** in a robust and reproducible manner (82% yield). Due to the presence of the vicinal carbonyls in the α-ketoester product, **10a** was found to be

sensitive to hydrolysis under basic conditions or on silica gel, therefore the chromatography purification was achieved on a neutralized and oven-dried silica. Our laboratory recently developed a one-pot domino methodology of condensation/aza-Friedel–Crafts arylation for the synthesis of α-arylated amino esters,[18] thus expanding the method for the synthesis of the more challenging α,α-disubstituted α-amino ester was deemed appropriate.

Despite the recent interest and progresses in advancing asymmetric methods for the synthesis of the highly congested α,α-disubstituted α-amino acids, no general method has yet been reported.[20] Only a few groups have recently described the use of enamides (as iminium precursor) to achieve direct aza-Friedel–Crafts/lactonization domino reactions.[17,21] Displacing the iminium/enamide tautomerization equilibrium which typically favors the later isomer remains challenging. Therefore, a sequence of amine condensation followed by an *in situ* aza-Friedel–Crafts cyclization catalyzed by a Brønsted acid was evaluated as the key step toward building the fumimycin skeleton in a domino fashion (Table 1). Of all the carbamates tested (Boc, Fmoc, or Cbz) and the Ellman's sulfinamide, only benzyl carbamate (Cbz-NH_2) could be successfully condensed with pyruvate **10a**. In this regard, dichloromethane was found to give overall better results compared to other solvents (toluene, $CHCl_3$, Et_2O). We then investigated the effect of the Brønsted acid catalysts and found no distinct correlation between reaction conversions and the acids' pKas. When weak to moderately strong Brønsted acids (BzOH, HCl and phosphoric acids **14**-**15**) were tested (Table 1, entries 1-4), the acid-catalyzed aza-Friedel–Crafts reaction did not occur, nor did the initial condensation.

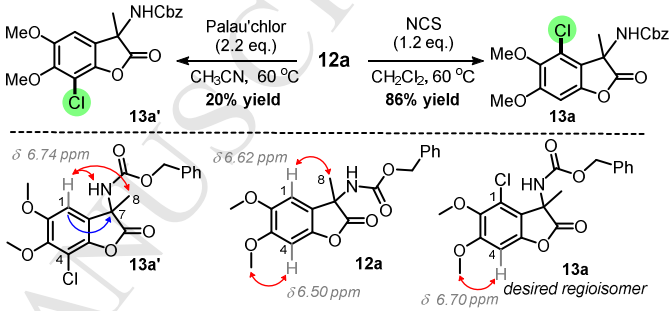
Table 1. Optimization of the key step: protio-aza-Friedel–Crafts cyclization



Entry	Catalyst	Cat. pKa (DMSO)	Cat. Loading (mol %)	Solvent [0.1 M]	T (°C)	% Yield
1	BzOH	11.1	15	CH_2Cl_2	60	traces
2	**14**	2.6	10	CH_2Cl_2	60	NR
3	**15**	3.1-3.4	10	$CHCl_3$	60	NR
4	HCl	1.8	15	CH_2Cl_2	60	NR
5	**15** / $SOCl_2$	–	10 / 150	Toluene	60	11[a]
6	**15** / $SOCl_2$	–	10 / 150	CH_2Cl_2	60	34[a]
7	PTSA	–	15	CH_2Cl_2	60	16
8	(+)-CSA	5.6	100	CH_2Cl_2	60	23[a]
9	**16**	0.1	15	CH_2Cl_2	60	NR
10	Tf_2NH	-10.4	15	CH_2Cl_2	RT	24[a]
11	**17** / TfOH	–	15 / 10	CH_2Cl_2	RT	38
12	**TfOH**	**-14.7**	**30**	**CH_2Cl_2**	**RT**	**56**
13	TfOH	-14.7	30	CH_2Cl_2	60	–[b]

[a] Enamide and bis-Cbz protected aminal intermediates were isolated as side-products.
CAS: **14**: 791616-62-1; **15**: 39648-67-4; **16**: 1174193-01-1; **17**: 1092934-19-4;
[b] Full decomposition was observed for the reaction carried at 60 °C

To our delight, when the phosphoric acid catalyst **15** was used with stoichiometric amounts of $SOCl_2$ (entries 5-6), the aza-Friedel–Crafts product **12a** was obtained albeit in low yields (11% and 34% yields, respectively). Despite the mechanism remaining uncertain, $SOCl_2$ likely facilitates the dehydration of an (hemi)aminal intermediate to generate the reactive iminium **11a** and promote the final 5-*exo*-trig cyclization. Unfortunately, the reactions proceeded without any enantiodiscrimination. While the reaction catalyzed by PTSA proceeded to a certain extent (16% yield), only traces of product **12a** were obtained upon catalysis with CSA, requiring the use of stoichiometric amounts of acid to achieve the reaction in 23% yield (entries 7-8). As revealed by the high quantities of intermediates obtained (enamide and bis-Cbz aminal), the reaction conversion was poor even after 3 days. Additionally, stronger Brønsted acid catalysts have then been tested (sulfonimides **16** and Tf_2NH, and sulfonic acids **17** and TfOH). Although the sulfonamide catalyst **16** was inefficient,[22] the reaction catalyzed by Tf_2NH afforded **12a** in 24% yield (entries 9-10). As shown in entries 11-12, the aza-Friedel–Crafts cyclization was achieved more efficiently at room temperature upon catalysis with BINSA-K **17**[23] and TfOH to obtain the lactone scaffold **12a** in 38% and 56% yields respectively. Interestingly, most of the sulfonimide- and sulfonate-derived catalysts tested in the reaction provided product **12a** (entries 7-12), in comparison to other Brønsted acid catalysts with lower pKas, suggesting a possible counteranion effect on the aza-Friedel–Crafts reactivity, potentially due to a stabilization of iminium **11a**.

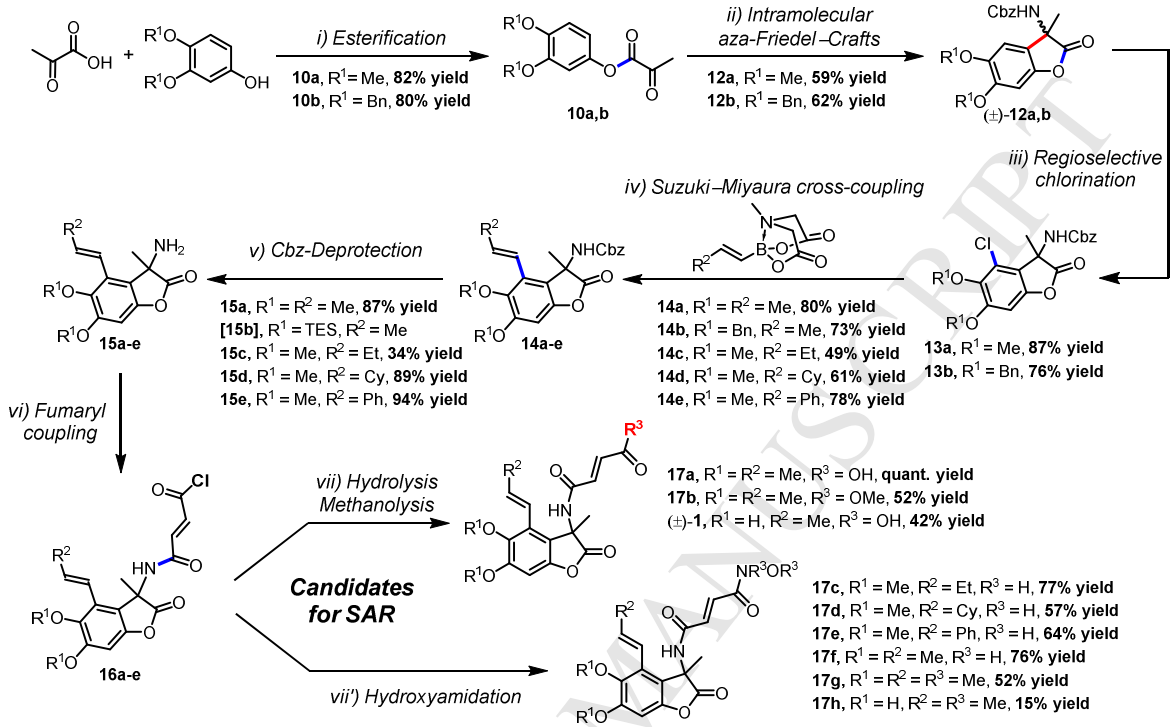


Scheme 2. Reagent-controlled regioselective chlorination of the fumimycin core **12a**. ^1H–^{13}C HMBC (→) and ^1H–^1H NOESY (↔) correlations observed.

With the prospect of a Suzuki–Miyaura cross-coupling in mind, the benzofuranone core **12a** needed to be functionalized regioselectively. Electing bromine for its higher reactivity,[24] we soon realized that bromination could not be achieved on **12a** under the most typical conditions (Br_2,[25] NBS,[26] TBCO,[27] TBAB),[28] and that only a smaller chlorine could be installed. Using NCS in CH_2Cl_2, the chlorination did not occur at room temperature, but the desired reaction proceeded with high regioselectivity at 60 °C in a sealed tube to deliver chlorobenzofuranone **13a** in 86% yield as a single regioisomer (Scheme 2). The chlorination could also be achieved in similar yields with the addition of catalytic amounts of Palau'chlor albeit with lower regioselectivity as characterized by the presence of the other regioisomer **13a'** in ^1H NMR (91% yield, rr **13a**:**13a'** = 87:4). By increasing the amount of Palau'chlor (2.2 eq.), the regioselectivity was switched and regioisomer **13a'** was obtained exclusively in 20% yield.[29] Overall, the regioselectivity of the chlorination was found to be highly dependent on the reaction conditions and solvent. Both protons H-4 and H-1 of the benzofuranone regioisomers **13a** and **13a'** have similar chemical shifts ($\delta_{H\text{-}4}$ 6.70 and $\delta_{H\text{-}1}$ 6.74 ppm) which precluded a direct assignment by direct comparison with the starting benzofuranone **12a** (Scheme 2). The combination of HMBC and nOe correlations enabled us to unambiguously determine the regioselectivity of chlorination. Indeed, the correlations observed in **13a'** (H-1 correlation to N-H and H-8) suggested that Palau'chlor preferentially chlorinated the less hindered position (C-4) away from the congested quaternary stereocenter.

Having established the two pivotal steps of the synthesis, the total synthesis fumimycin and other analogues were undertaken (Scheme 3). Aware of the potential difficulties of late-stage deprotection of the dimethoxy catechol moiety, a dibenzyl-protected pyruvate **10b** was prepared in 80% yield. Under the optimal conditions for the intramolecular aza-Friedel–Crafts, both **10a** and **10b** were converted into the corresponding 3,3-disubstituted benzofuranones **12a-b** in 59%

and 62% yields respectively. Interestingly, the later reaction with **10b** required only 15 mol% of TfOH, as higher catalyst loading was detrimental to the reaction. Regioselective chlorination was then performed at 50-60℃ (Scheme 3), to synthesize **13a-b** in 87% and 76% yields respectively. The Suzuki–Miyaura cross-coupling was readily achieved following Burke's procedure with commercially available MIDA-boronates.[30] This step was instrumental in the construction of a small library of analogues bearing variously decorated styrenes **14a-e**. Despite the noticeable oxygen sensitivity of the palladium-catalyzed cross-coupling and the relatively elevated



Scheme 3. Overall synthesis of (±)-fumimycin (**1**) and analogues **17a-g**. Reactions' conditions: i) DCC (1.5 eq.), DMAP, CH_2Cl_2, -15 ℃ to RT, 4 h; ii) Cbz-NH_2 (1.5 eq.), TfOH (15-30 mol%), CH_2Cl_2, RT, 2-3 d; iii) NCS (1.2 eq.), Palau'Chlor (5 mol%), CH_2Cl_2, 50-60 ℃,18 h; iv) $Pd(OAc)_2$, SPhos, K_3PO_4, THF/H_2O (5:1), 60 ℃, 30 h; v) $Pd(OAc)_2$, Et_3SiH, Et_3N, CH_2Cl_2, RT, 18 h; vi) fumaryl chloride, $CHCl_3$, 0 ℃, 30 min; vii/vii') SiO_2/(H_2O or MeOH or CH_3NHOCH_3·HCl) or TMSO-NH_2, THF, RT, 4 h.

temperature required (100 °C), Burke's method was robust and highly reproducible, affording styrenes **14a-e** with a complete *E*-selectivity (49-80% yields). The chemoselective removal of the benzyl carbamate proved more delicate than anticipated under hydrogenation conditions (Pd-C / H_2).[31] Indeed, the free-amine product generated from **15a** likely acted as a palladium inhibitor,[32] thus leading ineluctably to poor conversions and a mixture of reduced compounds. Other conditions with TMSI[33] or BBr_3[34] simply degraded the starting material **15a**. After considerable screening efforts, a less reactive palladium(0) catalyst generated *in situ* from $Pd(OAc)_2$ / Et_3SiH and Et_3N was found to accomplish the chemoselective carbamate cleavage at room temperature.[32a] The reaction time could be decreased to 3 h by heating at 60 °C without affecting the chemoselectivity, thus generating the series of free amines **15a-e** in 34-94% yields. Addition of the free amines **15a-e** to an excess of fumaryl chloride delivered the corresponding amides **16a-e** in a nearly quantitative manner (Scheme 3). These acyl chlorides were surprisingly stable to hydrolysis under neutral or acidic aqueous conditions and pure enough to be engaged in the next step without further purification. Fortuitously, the final step of hydrolysis/methanolysis or hydroxyamidation of **16a-e** could be promoted by the addition of silica into the reaction mixtures. Under these conditions, carboxylic acid **17a** and ester **17b** were obtained in a quantitative and 52% yields respectively. In a similar fashion, hydroxamic acid analogues **17c-e** and Weinreb amide derivatives **17f-g** were prepared in moderate to good yields (15-77% yields over 2-3 steps). Starting from **14b**, the Cbz-carbamate and benzyl protecting groups were cleaved collectively leading to **15b** (not isolated), which was directly acylated and further hydrolyzed at the C-terminal to conclude the total synthesis of (±)-**1** in 42% yield over 3 steps.

As expected from the seminal work from Bräse on fumimycin[15], the catechol *O*-demethylation step tested on **14a**, **15a** or **17a** remained elusive. Most reagents (TMSI, iodocyclohexane, HBr/AcOH, *L*-Met/MsOH) yielded decomposition of the starting materials **17a-f** or in some cases to the cleavage of the fumaroyl group (with BBr_3 or $B(C_6F_5)_3/Et_3SiH$). The deprotection of **17a** was also attempted with BI_3 at –20 °C unfortunately affording a single methyl deprotection of the catechol at the expense of the fumaroyl cleavage (76% yield). Oxidation of **17a-c** were also attempted with CAN, $Ce(SO_4)_2/HClO_4$ or PIFA, but no desired *o*-quinone was observed. Collectively, these results demonstrated that a dibenzyl protected catechol (*e.g.* **14b**) was strategically valuable to secure the concise total synthesis of (±)-**1** which was ultimately achieved in 7 steps and 11.6% overall yield.

2.3 Docking Studies and Biological Evaluation of the Library of PDF Inhibitors.

In 2000, Yuan described for the first time actinonin (**2**) as a potent PDF inhibitor of *E. coli* (K_i ~0.3 nM) and *S. Aureus*.[13a] Later, the crystal structure of *Ec*PDF in complex with **2** was obtained (PDB code: 3G5K), thus confirming that the hydroxamate moiety was responsible for inhibiting the hydrolytic ability of PDF by chelation to the enzyme metal center (Figure 2).[35,3a]

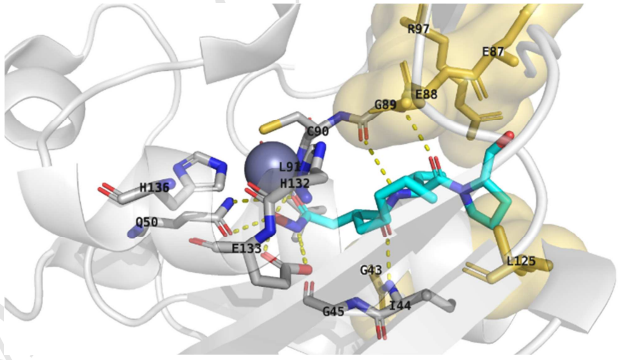


Figure 2. Summary of actinonin (**2**) interactions in the three sub-pockets (S1', S2', S3') of the *Ec*PDF catalytic site (PDB code: 1LRU)

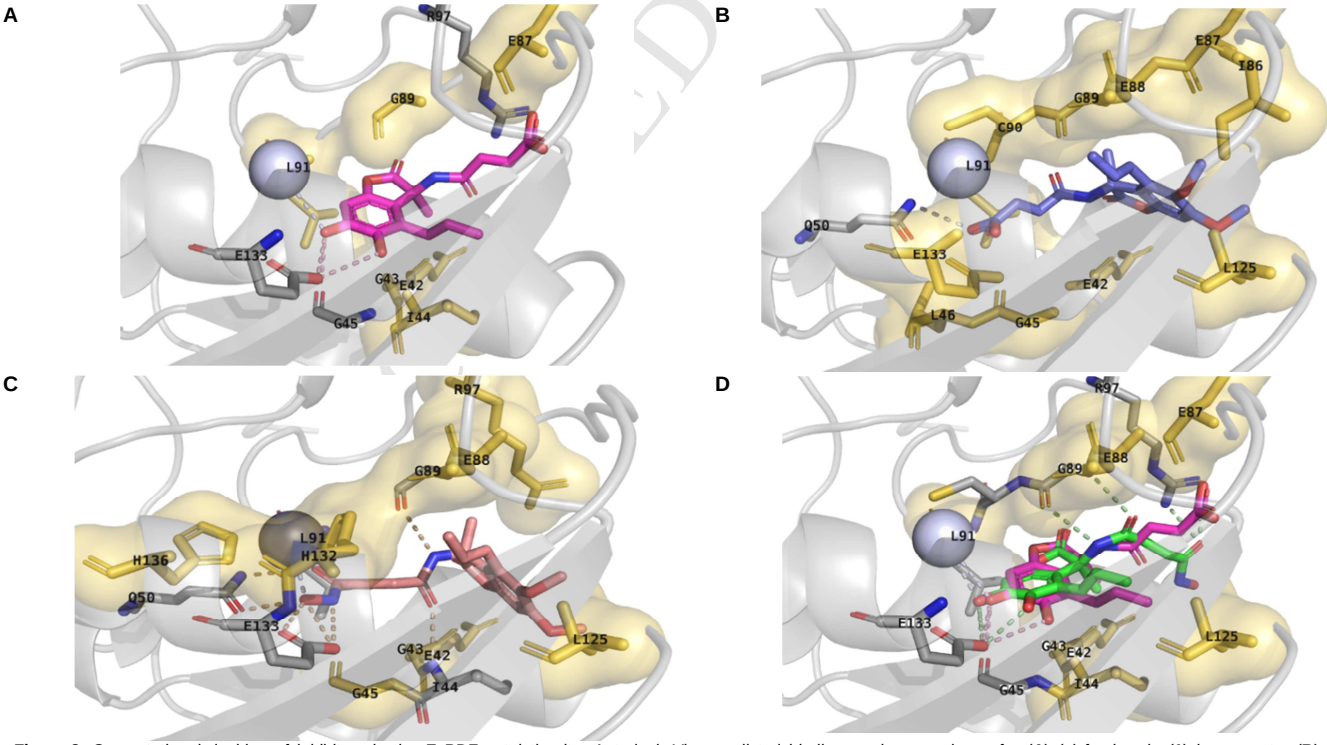
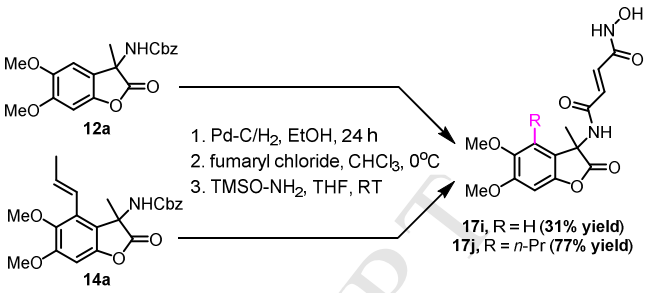


Figure 3. Computational docking of inhibitors in the *Ec*PDF catalytic site. Autodock Vina-predicted binding modes are shown for (**A**) (–)-fumimycin (**1**) in magenta, (**B**) dimethoxy fumimycin **17a** in aquamarine, (**C**) dimethoxy fumimycin hydroxamate **17f** in red, and (**D**) the overlay of (–)-**1** and its hydroxamate analogue in green. For the *Ec*PDF, key hydrogen-

bonded residues are represented in gray and hydrophobic residues and surfaces are in yellow. Salt-bridge and hydrogen-bond interactions are shown by dashed lines. Images were produced using PyRx

Due to a two-point binding aptitude of the hydroxamate group, **2** closely mimics the transition state of natural substrates bound to iron, thus extruding the proximate water molecule typically responsible for the deformylation at the catalytic site.[36, 3b,10c] Furthermore, like most hydrolase and metaloprotease enzymes, the PDF catalytic site is structured around three subsites (Figure 2: S1', S2' and S3').[37] To structurally accommodate any peptidic sequences of nascent proteins, the PDF binding pocket is conformationally flexible to allow the substrate entry in the S1' subsite and further assist binding from the S2' and S3' subsites. Therefore, the *n*-pentyl chain of **2** was shown to reach deep into the lipophilic S1' pocket (G^{45}, L^{125}, E^{133}, I44, G^{43}, G^{89}, E^{88}) at the vicinity of the metal binding site, while the leucine residue occupied the S2' cavity (R^{97}, G^{89}, E^{88}) and the prolinol motif the S3' cleft (E^{87}). Since this binding model from *Ec*PDF permitted to rationally prepare several peptide-based PDF-inhibitors, it was logically exploited to design fumimycin analogues.[38]

Due to the presence of two potential iron-binding motifs in fumimycin (fumaric acid side chain and catechol, see red motifs in Figure 1), a docking study of compounds **1**, **17a** and **17f** was performed using Autodock Vina in PyRx.[39] First, the docking was used to computationally predict the possible binding modes of **1** (Figure 3A). To this aim, inhibitor **2** was excised from the co-crystal of the *Ec*PDF–**2** complex (PDB code: 1LRU) and replaced with (–)-**1** at the binding site to achieve an induced-fit docking.[40] Examining the relative docking scores of fumimycin, expressed as free energies of the different calculated binding states, revealed that the catechol moiety, a well-known siderophore group,[41] might indeed bind the PDF metal center (Figure 3A). In this proposed model, the styrenyl side chain of **1** seems to reach into the large hydrophobic S1' cavity. The position of **1** in the binding pocket revealed that the catechol binds to the metal center while forming hydrogen bond contacts with E^{133} and G^{45} of *Ec*PDF and a salt bridge at the cavity's edge with R^{97}. To test for the potential importance of the catechol group in binding, two fumimycin analogues **17a** and **17f** bearing a dimethoxy catechol fragment were docked (Figure 3B & 3C). The lowest energy binding state of **17a** (docking score: -7.2 Kcal/mol) exhibited metal binding through the lactone ring, but interestingly a higher energy state (docking score: -5.2 Kcal/mol) revealed that binding of **17a** might occur from the fumaric acid side chain in proximity to the metal center with hydrogen bond to Q^{50} (Figure 3B). Similarly, for the hydroxamic acid analogue **17f**, the hydroxamate group was predicted to bind deep into the cavity to Q^{50}, E^{133} and I^{144}, but more importantly to the metal center thus positioning the inhibitor to enable more hydrogen bonds along the molecule scaffold with G^{89} and I^{144} (Figure 3C). In contrast, the docking of the hydroxamate analogue of **1** suggested a binding mode similar to **1**, indicating that the catechol fragment bound to both the metal center and the E^{133} residue (Figure 3D). Taken together, the docking results suggested that the catechol motif might be more suitable for the fumimycin analogues to bind the metal center of the PDF enzyme while positioning the allylic chain of the styrene into the hydrophobic S1' pocket.



Scheme 4. Intermediates functionalization to diversify the fumimycin analogues **17h-i**

With this model in mind, we decided to modify fumimycin to increase its innate affinity to the bacterial *Ec*PDF by altering the styrene which would mimic the *n*-pentyl of actinonin and secondly modifying the metal-binding motif from the carboxylate into and hydroxamate. Fortunately, the Suzuki–Miyaura cross-coupling and the later silica-mediated acyl substitution allowed for those two-point synthetic diversifications to prepare two other analogues **17i-j** with the modified benzene rings (Scheme 4). With the synthetic natural product (±)-**1** and a series of analogues (±)-**17a-j** in hand, a structure-activity relationship study (SAR) was initiated to evaluate the inhibitory effects of these novel compounds against *Ec*PDF. To determine the *in vitro* activity of the different racemic compounds, a coupled colorimetric assay (*Ec*PDF and aminopeptidase) was applied, with the deformylation by PDF being the rate-limiting reaction.[6] As an enzymatic substrate, the formylated dipeptide fMet-Leu-*p*NA (formyl-methionine-leucine-*p*-nitroaniline), similar to the model MAS **3** (see Figure 1), was used in a concentration equal to the previously determined K_M value (180 µM), since these conditions are sensitive to competitive inhibition. To observe differences in the inhibitory effect between the compounds, the inhibitor concentration was chosen to equal the reported IC_{50} value of (–)-fumimycin (**1**) for *S. aureus* PDF (4 µM).[1] The effect of each synthetic compound on *Ec*PDF activity is reported in Figure 4 as an initial reaction velocity relative to a control with DMSO ($v_o/v_{o(ctr)}$).

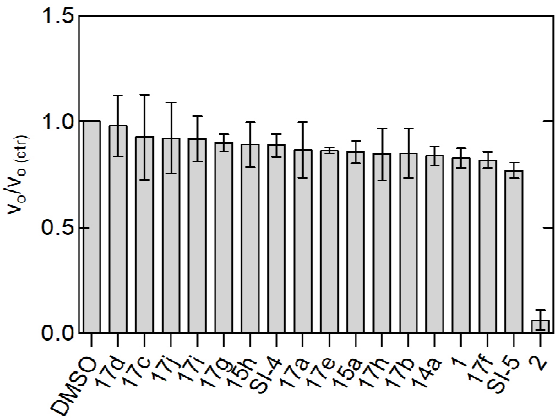


Figure 4. PDF inhibitor screen. fMet-Leu-*p*NA was deformylated by PDF in the presence of potential PDF inhibitors. The initial velocity of deformylation v_0 was standardized on the initial velocity of the DMSO only control. The error bars represent the standard deviation (n = 3).

As shown in this summary of enzymatic activity, most hydroxamate tested **17c-d**, **17g** and **17i-j** exhibited either very weak or no inhibition of *Ec*PDF. The hydroxamate derivative **17e** inhibited about 15% of the enzyme initial velocity which could suggest that the S1' cavity could accept the larger styrenyl fragment. The lack of activity of the

hydroxamate analogues also suggests that the metal-binding motif is unlikely to be located onto the fumaroyl side chain. Furthermore, three out of the six most active compounds (**17h**, **17b**, **14a**, **1**, **17f** and **SI-5** ~20% inhibition) possess a free-catechol moiety, which further support the catechol-binding model proposed in our docking studies (Figure 3A & 3D). This hypothesis is also supported by the direct comparison of inhibition activity between **17g** (~10%) and the free catechol **17h** (~16%). Down this line, the SAR study also revealed that the most active compounds **14a**, **17f** and **1** (~20% inhibition) possess the allylic side chain flanking the benzene ring. Gratifyingly, hydroxamate **17f** which was proposed to have a number of hydrogen bond interactions with *Ec*PDF in the docking study (Figure 3C), was found to exhibit slightly more inhibition than the fumimycin natural product. Interestingly the truncated scaffold **SI-5** (see supporting information, Figure SI-1) showed the largest inhibition activity (~25%). Overall, the new analogues **17a-j** and the natural product **1** offered only a weak inhibitory activity against *Ec*PDF in comparison to the model inhibitor actinonin (**2**) which was found to inhibit ~95% of the enzyme activity at 4 µM concentration. The low enzymatic activity for the synthetic natural product and its analogues was further confirmed by a phenotypic assay of bacterial growth on 2 clinical strains of pathogens. The library of analogues (±)-**17a-j** and (±)-**1** were tested in minimum inhibitory concentration (MIC) assays against the ATCC MRSA BAA-1707, a methicillin-resistant *Staphylococcus aureus*, and a clinical isolate of *Escherichia coli* (UAEC-1, University of Arkansas for Medical Sciences). Unfortunately no bacterial growth inhibition was detected on either pathogen at concentrations of the drugs up to 100 µM.[42]

3. Conclusions

We have developed a concise 7-step total synthesis of the natural product (±)-fumimycin in 11.6% overall yield and established a blueprint for the synthesis of analogues with several points of diversification. The key steps in our strategy include a triflic acid catalyzed aza-Friedel–Crafts cyclization to construct the benzofuranone skeleton wrapped around the α,α-disubstituted α-amino acid moiety in a single step, and a regioselective chlorination followed by a Suzuki–Miyaura cross-coupling for fragment diversification. As a result of the intramolecular aza-Friedel–Crafts optimization, the highly congested quaternary α-amino acid stereocenter was synthesized in 56% yield. In an attempt to improve the natural product inhibitory activity on peptide deformylase (PDF) and its antibacterial activity, a series of analogues **17a-j** were synthesized and evaluated on *Ec*PDF. A molecular docking study on selected compounds together with the results of inhibitory activity on *Ec*PDF revealed that the fumimycin scaffold is likely to bind the PDF iron-center in the enzyme catalytic site *via* the free-catechol moiety. Furthermore, the biological evaluation revealed that fumimycin and most analogues exhibited a weak inhibition (<25%) of *Ec*PDF at 4 µM concentrations, suggesting that this class of compounds may have only modest antibacterial activity (if any). The application of this highly convergent strategy to the synthesis of novel catechol analogues of fumimycin and their full biological evaluation on various bacterial PDFs are of ongoing interest in our group.

4. Experimental

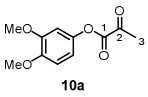
4.1 General information

Reagents were commercially available and used without further purification. All reactions were carried out in oven-dried vessels under an atmosphere of argon and in anhydrous solvents unless otherwise stated. Dry solvents: THF was distilled over sodium in the presence of benzophenone before use; CH_2Cl_2 and $CHCl_3$ were distilled over calcium hydride. Dry silica was obtained after successive acetone and *n*-hexane washes, followed by storage in an oven (~ 130 ℃) for at least two days. Reactions were monitored by TLC on Silicycle silica gel 60-F_{254} glass backed (ref. TLG-R10011B-323, lot 210418), using UV absorption and further revealed with either cerium-ammonium-molybdate (2.5% $(NH_4)_2MoO_4$ + 1% $Ce(SO_4)_2$ + 10% H_2SO_4 in water), basic permanganate (1% $KMnSO_4$ + 15% Na_2CO_3 in water) or ninhydrin (0.25% ninhydrin in ethanol + 3% AcOH) as staining system. The products were purified over silica gel column chromatography (Silicycle silica gel, 40-63 µm, ref. R10030B, lot 110814). NMR spectra were recorded on a Bruker 400 MHz Avance III spectrometer. Chemical shifts (δ) are quoted in ppm with internal calibration from residual solvent peak ($CDCl_3$:7.26, 77.0 ppm; DMSO-d_6: 2.50, 39.5 ppm; CD_3CN: 1.94, 1.32 ppm for ^1H and ^{13}C NMR, respectively). All coupling constants (*J*) are reported in Hertz. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (*m/z*) and High resolution spectra (HRMS) were obtained from the University of Florida using an Agilent 6210 TOF instrument, using electrospray ionization (ESI) or direct analysis in real time (DART). Infrared spectra were recorded on a Nicolet IS5 FT-IR spectrophotometer. The following abbreviations are used to designate band intensities: s = strong, m = medium, w = weak. Melting points were measured on a MPA 160 digital apparatus.

4.2 General procedure for the Steglich esterification

To a solution of selected phenol derivative (1.0 eq.), pyruvic acid (1.5 eq.) and DMAP (0.2 eq.) stirred in CH_2Cl_2 (0.13 M rel. to phenol) at -15 °C was added dropwise a solution of DCC (1.5 eq.) in CH_2Cl_2 (0.4 M rel. to phenol). After completion of the addition, the reaction mixture was allowed to warm to RT over 3 h. The reaction mixture was cooled down to 0 °C and most of the urea by-product precipitate was filtered through a pad of celite® by washing with small portions of cold CH_2Cl_2. Aq. $KHSO_4$ (5%) was added to the organic layer and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 and the combined organic layers were dried with Na_2SO_4, then evaporated *in vacuo*.

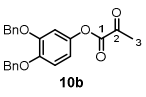
4.2.1 3,4-dimethoxyphenyl 2-oxopropanoate 10a



10a

Compound **10a** was synthesized accordingly to the general procedure above from commercially available 3,4-dimethoxyphenol. Silica gel chromatography (dry silica) with *n*-hexane, CH_2Cl_2 and acetone (7:2.5:0.5) as eluents afforded pure compound **10a** as a yellow oil (9.16 g, 82% yield). $\mathbf{R_f}$ (DCM) = 0.37; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.86 (d, *J* = 8.7 Hz, 1H, *m*-H_{Ar}), 6.74 (dd, *J* = 8.7, 2.2 Hz, 1H, *o*-H_{Ar}), 6.71 (d, *J* = 1.9 Hz, 1H, *o*-H_{Ar}), 3.88 (s, 3H, O-C$\underline{H}_3$), 3.86 (s, 3H, O-C$\underline{H}_3$), 2.59 (s, 3H, 3-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 191.3 (C-2), 159.4 (C-1), 149.6 (*m*-C_{Ar}), 147.5 (*i*-C_{Ar}), 143.7 (*p*-C_{Ar}), 112.4 (*o*-C_{Ar}), 111.2 (*m*-C_{Ar}), 105.1 (*o*-C_{Ar}), 56.3 (O-$\underline{C}H_3$), 56.2 (O-$\underline{C}H_3$), 26.9 (C-3); **HRMS** (DART) *m/z* calcd. for $C_{11}H_{16}O_5N$ $[M+NH_4]^+$ 242.1023, found 242.1022.

4.2.2 3,4-bis(benzyloxy)phenyl 2-oxopropanoate 10b



10b

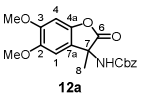
Compound **10b** was synthesized accordingly to the general procedure above from **SI-3**. Successive trituration with Et_2O and filtration over fritted glass afforded compound **10b** as a light brown powder (3.0 g, 80% yield). $\mathbf{R_f}$ (DCM) = 0.62; **m.p.** 79.8 ± 1.4 ℃; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49 – 7.28 (m, 2 x 5H, H_{Ph}), 6.95 (d, *J* = 8.8 Hz, 1H, *m*-H_{Ar}), 6.83 (d, *J* =

2.7 Hz, 1H, *o*-H_{Ar}), 6.73 (dd, *J* = 8.8, 2.7 Hz, 1H, *o*-H_{Ar}), 5.15 (s, 2H, O-CH_2), 5.14 (s, 2H, O-CH_2), 2.57 (s, 3H, 3-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 191.1 (C-2), 159.2 (C-1), 149.7, 147.4, 144.2, 137.1 (*i*-C_{Ph}), 136.6 (*i*-C_{Ph}), 128.64 (*m*-C_{Ph}), 128.59 (*m*-C_{Ph}), 128.1 (*p*-C_{Ph}), 128.0 (*p*-C_{Ph}), 127.4 (*o*-C_{Ph}), 115.4 (*o*-C_{Ar}), 113.2 (*m*-C_{Ar}), 108.3 (*o*-C_{Ar}), 71.8 (O-CH_2), 71.4 (O-CH_2), 26.8 (C-3); **IR (film)** 1730 (s), 1595 (w), 1510 (m), 1453 (m), 1423 (m), 1393 (m), 1290 (m), 1267 (m), 1222 (s), 1163 (s), 1131 (s), 1121 (s), 1078 (w), 1037 (m), 1028 (s), 983 (m), 951 (w), 899 (w), 886 (m), 847 (w), 830 (w), 803 (m), 752 (w), 730 (s), 691 (s) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{23}H_{24}O_5N$ $[M+NH_4]^+$ 394.1649, found 394.1660.

4.3 General procedure for the aza-Friedel-Crafts cyclization

To a solution of **10a,b** (1.0 eq.) and benzyl carbamate (1.5 eq.) in CH_2Cl_2 (0.12 M rel. to **10a** or **10b**) at 0 °C was added a solution of TfOH (*x* mol %) in CH_2Cl_2 (0.53 M rel. to **10**). After 3 days at RT, an aq. sat. solution of NH_4Cl was added and the crude reaction mixture was extracted several times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo*.

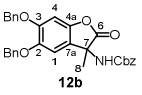
4.3.1 Dihydrobenzofuranone 12a



12a

Compound **12a** was synthesized accordingly to the general procedure above from **10a** with *x* = 30: Silica gel chromatography with *n*-hexane and EtOAc (gradient elution 8:2 to 6:4) as eluents afforded pure product **12a** as a white solid (887 mg, 56% yield). **R_f** (*n*-hexane:EtOAc, 7:3) = 0.22; **m.p.** 141 ± 0.2 °C; **^{1}H NMR** (400 MHz, Benzene-d_6) δ 7.16 – 7.07 (m, 5 H_{Ph}), 6.62 (s, 1H, 1-H), 6.50 (s, 1H, 4-H), 5.26 (s, NH), 5.00 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.89 (d, *J* = 12.4 Hz, 1H, CH_2-Ph), 3.44 (s, 3H, 2-O-CH_3), 3.27 (s, 3H, 3-O-CH_3), 1.20 (s, 3H, 8-H); **^{1}H NMR** (401 MHz, $CDCl_3$) δ 7.31 (s, 5H_{Ph}), 6.76 (s, 1H, 1-H), 6.72 (s, 1H, 4-H), 5.47 (s, 1H, NH), 5.05 (m, 1H, CH_2-Ph), 5.01 – 4.88 (m, 1H, CH_2-Ph), 3.88 (s, 3H, 2-O-CH_3), 3.86 (s, 3H, 3-O-CH_3), 1.62 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.0 (C-6), 154.5 (NH-C=O), 150.6 (C-3), 147.0 (C-4a), 146.5 (C-2), 135.5 (*i*-C_{Ar}), 128.6 (2 *m*-C_{Ph}) 128.5 (*p*-C_{Ph}) 128.4 (2 *o*-C_{Ph}), 119.5 (C-7a), 105.8 (C-1), 96.3 (C-4), 67.7 (CH_2-Ph), 58.1 (C-7), 56.7 (3-O-CH_3), 56.4 (2-O-CH_3), 24.8 (C-8); **IR (film)** 3315 (w), 2838 (w), 1802 (m), 1693 (s), 1682 (s), 1630 (w), 1603 (w), 1584 (w), 1526 (m), 1498 (m), 1471 (w), 1453 (m), 1421 (m), 1373 (w), 1325 (m), 1299 (s), 1262 (s), 1217 (s), 1192 (m), 1133 (s), 1094 (m), 1066(m), 1039 (s), 1028 (m), 990 (m), 934 (m), 903 (m), 864 (m), 827 (w), 809 (w), 782 (m), 754 (m), 739 (m), 705 (s), 667 (m) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{19}H_{20}NO_6$ $[M+H]^+$ 358.1285, found 358.1294.

4.3.2 Dihydrobenzofuranone 12b



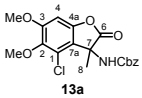
12b

Compound **12b** was synthesized accordingly to the general procedure above from **10b** with *x* = 15: Successive precipitations with petroleum ether and Et_2O (7:3) afforded pure compound **12b** as a yellow solid (2.05 g, 50% yield). **R_f** (*n*-hexane: CH_2Cl_2:acetone, 5:4.75:0.25) = 0.27; **m.p.** 140.8 ± 1.4 °C; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.56 – 7.19 (m, 15H), 7.01 (s, 1H, 1-H), 6.90 (s, 1H, 4-H), 6.71 (s, NH), 5.11 (s, 2H, 3-O-CH_2-Ph), 5.04 (s, 2H, 2-O-CH_2-Ph), 4.99 (d, *J* = 12.4 Hz, 1H, C(O)-O-CH_2-Ph), 4.92 (d, *J* = 13.0 Hz, 1H, C(O)-O-CH_2-Ph), 1.53 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 178.0 (C-6), 155.8 (NH-C(O)-CH_2-Ph), 151.3 (C-3), 148.2 (C-4a), 146.8 (C-2), 138.2 (2- O-CH_2-*i*-C_{Ph}), 137.8 (3- O-CH_2-*i*-C_{Ph}), 137.5 (*i*-C_{Ph}), 129.54 (C_{Ph}), 129.46 (C_{Ph}), 129.4 (C_{Ph}), 129.11 (C_{Ph}), 129.07 (C_{Ph}), 129.02 (C_{Ph}), 128.98 (C_{Ph}), 128.9 (C_{Ph}), 128.8 (C_{Ph}), 122.1 (C-7a), 111.2 (C-1), 99.2 (C-4), 72.9 (2- O-CH_2-Ph), 71.9 (3- O-CH_2-Ph), 67.6 (NH-C(O)-CH_2-Ph), 59.0 (C-7), 24.5 (C-8); **IR (film)** 3345 (br, w), 3032 (w), 2930 (w), 1810 (m), 1716 (m), 1627 (w), 1495 (s), 1454 (m), 1432 (m), 1375 (w), 1321 (m), 1294 (m), 1248 (m), 1206 (m), 1135 (s), 1083 (m), 1039 (s), 905 (m), 848 (w), 786 (w), 736 (s), 696 (s) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{31}H_{27}NO_6$ $[M+H]^+$ 510.1910, found 510.1926.

4.4 General procedure for arene chlorination

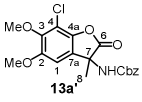
A solution of **12a,b** (1.0 eq.), NCS (*x* eq.) and Palau'chlor (5 mol %) in CH_2Cl_2 (0.25 M rel. to **12a** or **12b**) was heated to a specific temperature T (°C) in a sealed vial. After 1 day, the reaction mixture was cooled to RT and the solvent was evaporated to obtain the crude product **13a,b**.

4.4.1 1-Chloro-dihydrobenzofuranone 13a



13a

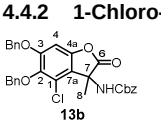
Compounds **13a/13a'** were synthesized accordingly to the general procedure above from **12a** with *x* = 1.2; T = 60 °C: Silica gel chromatography of the crude with *n*-hexane and EtOAc (gradient elution 90:10 to 75:25) as eluents afforded the major regioisomer **13a** as an orange solid (479 mg, 87% yield), and further increasing polarity with *n*-hexane and EtOAC (6:4) afforded the minor regioisomer **13a'** as a yellow solid (19 mg, 4% yield). **R_f 13a** (*n*-hexane:EtOAc, 7:3) = 0.41; **m.p.** 127.7 ± 0.5 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.24 (m, 5H, C_6H_5), 6.70 (s, 1H, 4-H), 5.62 (s, 1H, NH), 5.07 (dd, *J* = 12.1, 2.6 Hz, 1H, CH_2), 4.98 (d, *J* = 12.3 Hz, 1H, CH_2), 3.88 (s, 3H, 3-O-CH_3), 3.82 (s, 3H, 2-O-CH_3), 1.74 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.4 (C-6), 155.2 (C-3), 154.6 (NH-C=O), 149.7 (C-4a), 142.5 (C-2), 135.6 (*i*-C_{Ph}), 128.7 (2 *m*-C_{Ph}), 128.5 (*p*-C_{Ph}), 128.2 (2 *o*-C_{Ph}), 124.4 (C-1), 117.0 (C-7a), 95.5 (C-4), 67.7 (CH_2-Ph), 61.2 (2-O-CH_3), 58.7 (C-7), 56.5 (3-O-CH_3), 22.2 (C-8); **IR (film)** 3332 (br, w), 2939 (br, w), 1818 (m), 1714 (m), 1619 (m), 1517 (m), 1480.24 (m), 1454.40 (m), 1410.47 (m), 1375.35 (w), 1331.63 (m), 1292 (m), 1251 (m), 1217 (w), 1192 (m), 1154 (s), 1100 (w), 1047 (s), 988 (s), 919 (m), 825 (w), 785 (m), 735 (s), 697 (s), 661 (w) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{19}H_{19}ClNO_6$ $[M+H]^+$ 392.0895, found 392.0897.



13a'

R_f 13a' (*n*-hexane:EtOAc, 7:3) = 0.17; **m.p.** 60.6 ± 0.4 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30 (s, 5H, C_6H_5), 6.74 (s, 1H, 1-H), 5.76 (s, NH), 5.13 – 5.02 (m, 1H, O-CH_2-Ph), 4.94 (s, 1H, O-CH_2-Ph), 3.88 (s, 3H, 3-O-CH_3), 3.85 (s, 3H, 2-O-CH_3), 1.60 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.3 (C-6), 154.4 (NH-C=O), 151.1 (C-2), 146.8 (C-3), 143.4 (C-4a), 135.2 (*i*-C_{Ph}), 128.6 (2 *m*-C_{Ph}), 128.5 (*p*-C_{Ph}), 128.3 (2 *o*-C_{Ph}), 124.4 (C-7a), 112.6 (C-4), 104.8 (C-1), 67.8 (CH_2-Ph), 61.1 (3-O-CH_3), 58.7 (C-7), 56.7 (2-O-CH_3), 29.7 (C-8); **IR (film)** 3336 (br, w), 2939 (br, w), 1819 (s), 1717 (m), 1600.61 (w), 1517 (m), 1467 (s), 1435 (m), 1377 (w), 1339 (m), 1283 (m), 1254 (m), 1224 (m), 1147 (m), 1087 (m), 1038 (s), 975 (w), 907 (w), 862 (w), 782 (w), 746 (w), 698 (m), 667 (w) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{19}H_{19}ClNO_6$ $[M+H]^+$ 392.0895, found 392.0906.

4.4.2 1-Chloro-dihydrobenzofuranone 13b

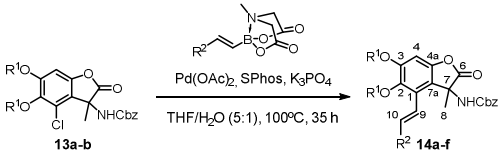


13b

Compound **13b** was synthesized accordingly to the general procedure above from **12b** with *x* = 1.6; T = 50 °C: Silica gel chromatography with *n*-hexane, EtOAc and $CHCl_3$ (80:15:5) as eluents afforded **13b** (single regioisomer) as a yellow solid (1.58 g, 73% yield). **R_f** (*n*-hexane:EtOAc:$CHCl_3$, 80:15:5) = 0.23; **m.p.** 116.5 ± 2.2 °C; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.54 – 7.48 (m, 2H_{Ph}), 7.46 – 7.21 (m, 15H_{Ph}), 6.96 (s, 1H, 4-H), 6.92 (s, NH), 5.16 (s, 2H, 3-O-CH_2-Ph), 5.00 (s, 2H, 2-O-CH_2-Ph), 4.97 (d, *J* = 10.5 Hz, 1H, C(O)-O-CH_2-Ph), 4.93 (d, *J* = 10.5 Hz, 1H, C(O)-O-CH_2-Ph), 1.67 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 176.7 (C-6), 155.8 (C-2), 155.1 (C-3), 150.5 (C-4a), 142.2 (NH-C(O)-CH_2-Ph), 138.0 (2- O-CH_2-*i*-C_{Ph}), 137.7 (*i*-C_{Ph}), 137.3 (3- O-

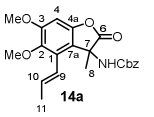
CH_2-*i*-C$_{Ph}$), 129.7 (C$_{Ph}$), 129.5 (C$_{Ph}$), 129.4 (C$_{Ph}$), 129.3 (C$_{Ph}$), 129.2 (C$_{Ph}$), 129.1 (C$_{Ph}$), 128.6 (C$_{Ph}$), 125.4 (C-1), 118.9 (C-7a), 97.8 (C-4), 76.0 (NH-C(O)-CH_2-Ph), 72.2 (3- O-CH_2-Ph), 67.6 (2- O-CH_2-Ph), 59.8 (C-7), 21.9 (C-8); **IR (film)** 3341 (br w), 2934 (br w), 1820 (s), 1715 (m), 1621 (w), 1498 (m), 1477 (w), 1454 (m), 1431 (m), 1374 (w), 1330 (m), 1293 (m), 1253 (m), 1215 (w), 1156 (s), 1098 (w), 1045 (s), 978 (m), 930 (w), 825 (w), 739 (m), 697 (s); **HRMS** (ESI) *m/z* calcd. for $C_{31}H_{26}ClNO_6Na$ $[M+Na]^+$ 566.1341, found 566.1361.

4.5 General Suzuki-Miyaura cross-coupling procedure



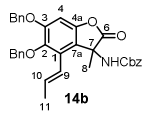
Reactions were performed in sealed tubes. A solution of **13a,b** (1.0 eq.), the commercially available MIDA ester (*x* eq.), $Pd(OAc)_2$ (10 mol %) and SPhos (20 mol %) in THF (0.085 M rel. to **13a** or **13b**) was stirred for 15 mins at RT. Then, an aqueous solution of K_3PO_4 (1.5 M, 3.8 eq.) was added and the resulting dark red biphasic solution was degassed thoroughly for 1 hour (frozen with liquid nitrogen, left under vacuum for 1 h before purged with argon and temperature slowly raised back to RT). The reaction mixture was then heated to 100 ℃ for 35 hours to achieve the cross-coupling. The solvent was evaporated and the residue taken back in an $NaHCO_3$ aq. sol., extracted several times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo* to dryness.

4.5.1 Propenyl-benzofuranone 14a



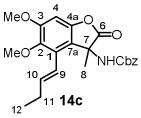
Compound **14a** was synthesized accordingly to the general procedure above from **13a**. R^1 = R^2 = Me; *x* = 1.5: Silica gel chromatography of the crude with CH_2Cl_2 and Et_2O as eluents (gradient elution 100:0 to 98:2) afforded pure product **14a** as a white solid (153 mg, 77% yield). **R$_f$** (CH_2Cl_2) = 0.29; **m.p.** 53.1 ± 0.2 ℃; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30 (br, 5H, Ph), 6.61 (s, 1H, 4-H), 6.49 (dq, *J* = 15.9, 6.5 Hz, 1H, 10-H), 6.35 (dq, *J* = 15.9, 1.6 Hz, 1H, 9-H), 5.52 (s, NH), 5.13 – 5.03 (m, 1H, O-CH_2-Ph), 4.98 (br, 1H, O-CH_2-Ph), 3.85 (s, 3H, 3-O-CH_3), 3.69 (s, 3H, 2-O-CH_3), 1.90 (dd, *J* = 6.5, 1.6 Hz, 3H, 11-H), 1.63 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 177.0 (C-6), 154.5 (C-3), 154.3 (7-NH-C=O), 149.2 (C-4), 143.9 (C-2), 134.0 (C-10), 128.9 (C-1), 128.5 (C$_{Ph}$), 128.3 (C$_{Ph}$), 128.2 (C$_{Ph}$), 127.0 (*i*-C$_{Ph}$), 121.3 (C-9), 95.0 (C-4), 67.5 (CH_2-Ph), 60.0 (2-O-CH_3), 58.6 (C-7), 56.1 (3-O-CH_3), 23.5 (C-8), 19.7 (C-11); **IR (film)** 3344 (br, w), 2940 (br, w), 1816 (s), 1716 (s), 1620 (m), 1590 (m), 1520 (m), 1453 (s), 1419 (m), 1379 (w), 1334 (s), 1301 (m), 1253 (s), 1227 (s), 1190 (m), 1156 (s), 1115 (m), 1090 (s), 1060 (m), 1030 (s), 1012 (s), 978 (m), 912 (w), 826 (w), 782 (w), 741 (m), 697 (m), 671 (w) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{22}H_{24}NO_6$ $[M+H]^+$ 398.1598, found 398.1616.

4.5.2 Propenyl-benzofuranone 14b



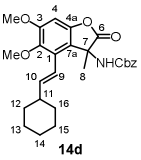
Compound **14b** was synthesized accordingly to the general procedure above from **13b**. R^1 = Bn; R^2 = Me; *x* = 2.0: Silica gel chromatography of the crude with CH_2Cl_2 and Et_2O (gradient elution 100:0 to 98.5:1.5) as eluents afforded pure **14b** as an orange powder (218 mg, 74% yield). **R$_f$** (*n*-hexane:EtOAc, 8:2) = 0.25; **m.p.** 49.9 ± 5.8 ℃; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.54 – 7.47 (m, 2H$_{Ph}$), 7.44 – 7.29 (m, 13H$_{Ph}$), 6.91 (s, NH), 6.85 (s, 1H, 4-H), 6.51 (dq, *J* = 15.9, 6.2 Hz, 1H, 10-H), 6.42 (dd, *J* = 15.9, 1.3 Hz, 1H, 9-H), 5.11 (s, 2H, 3-O-CH_2), 5.01 (s, 2H, 2-O-CH_2), 4.85 (d, *J* = 10.4 Hz, 1H, NH-C(O)-CH_2-Ph), 4.78 (d, *J* = 10.4 Hz, 1H, NH-C(O)-CH_2-Ph), 1.88 (dd, *J* = 6.2, 1.3 Hz, 3H, 11-H), 1.59 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 177.8 (C-6), 155.7 (NH-C(O)-CH_2-Ph), 154.3 (C-3), 150.1 (C-4a), 143.7 (C-2), 138.6 (*i*-C$_{Ph}$), 137.7 (*i*-C$_{Ph}$), 135.1 (C-10), 130.4 (C-1), 129.6 (C$_{Ph}$), 129.50 (C$_{Ph}$), 129.45 (C$_{Ph}$), 129.23 (C$_{Ph}$), 129.19 (C$_{Ph}$), 129.0 (C$_{Ph}$), 128.9 (C$_{Ph}$), 128.6 (C$_{Ph}$), 122.3 (C-9), 118.9 (C-7a), 97.2 (C-4), 75.1 (NH-C(O)-CH_2-Ph), 71.8 (3-O-CH_2), 67.5 (2-O-CH_2), 59.5 (C-7), 23.5 (C-8), 19.6 (C-11**); IR (film)** 3341 (br w), 3031 (w), 2934 (br w), 1810 (m), 1715 (m), 1618 (w), 1586 (w), 1497 (m), 1453 (m), 1442 (m), 1374 (w), 1331 (m), 1296 (m), 1248 (m), 1214 (m), 1157 (m), 1077 (m), 1058 (m), 1028 (s), 973 (m), 919 (m), 879 (w), 786 (w), 734 (s), 695 (s) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{34}H_{31}NO_6Na$ $[M+Na]^+$ 572.2044, found 572.2062.

4.5.3 Butenyl-benzofuranone 14c



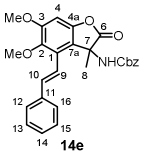
Compound **14c** was synthesized accordingly to the general procedure above from **13a**. R^1 = Me; R^2 = Et; *x* = 1.5: Silica gel chromatography of the crude with CH_2Cl_2 and Et_2O (100:0 to 97:3) as eluents afforded pure **14c** as an yellow solid (170 mg, 82% yield). **R$_f$** (CH_2Cl_2) = 0.19; **m.p.** 47.5 ± 0.1 ℃; **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.40 – 7.12 (br s, 5H, H$_{Ph}$), 6.75 (s, 1H, 4-H), 6.58 (dt, *J* = 15.9, 6.6 Hz, 1H, 10-H), 6.42 (dt, *J* = 15.9, 1.5 Hz, 1H, 9-H), 5.06 – 4.92 (m, 2H, O-CH_2-Ph), 3.83 (s, 3H, 2-O-CH_3), 3.64 (s, 3H, 3-O-CH_3), 2.24 (qdd, *J* = 7.5, 6.5, 1.5 Hz, 2H, 11-H), 1.58 (s, 3H, 8-H), 1.08 (t, *J* = 7.5 Hz, 3H, 12-H); **^{13}C NMR** (100 MHz, MeOD) δ 178.9 (C-6), 156, 8 (CO-CH_2-Ph), 155.6 (C-3), 150.6 (C-4a), 145.1 (C-2), 141.5 (C-10), 129.8 (*i*-C$_{Ph}$), 129.4 (*m*-C$_{Ph}$), 129.0 (*p*-C$_{Ph}$), 128.7 (*o*-C$_{Ph}$), 120.4 (C-9), 119.0 (C-7a), 96.2 (C-4), 67.7 (O-CH_2-Ph), 60.3 (2-O-CH_3), 59.8 (C-7), 56.7 (3-O-CH_3), 28.0 (C-11), 23.4 (C-8), 13.9 (C-12); **IR (film)** 3342 (br, w), 2963 (w), 1810 (m), 1715 (m), 1618 (w), 1586 (w), 1516 (m), 1454 (m), 1419 (w), 1375 (w), 1332 (m), 1297 (m), 1127 (m), 1222 (m), 1192 (m), 1157 (m), 1127 (w), 1092 (m), 1059 (m), 1033 (s), 999 (s), 914 (w), 895 (w), 820 (w), 783 (w), 734 (s), 697 (s), 668 (w) cm^{-1}; **HRMS** (DART) *m/z* calcd. for $C_{23}H_{25}NO_6$ $[M+H]^+$ 412.1755, found 412.1767.

4.5.4 Cyclohexylvinyl-benzofuranone 14d



Compound **14d** was synthesized accordingly to the general procedure above from **13a**. R^1 = Me; R^2 = C_6H_{11}; *x* = 1.5: Silica gel chromatography of the crude with *n*-hexane and EtOAc (9:1 to 85:15) as eluents afforded pure **14d** as an oil (143 mg, 61% yield). **R$_f$** (*n*-hexane:EtOAc) = 0.28; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.40 – 7.18 (m, 5H, Ph), 6.82 (s, NH), 6.72 (s, 1H, 4-H), 6.41 (dd, *J* = 16.2, 6.5 Hz, 1H, 10), 6.33 (d, *J* = 16.5 Hz, 1H, 9), 5.02 (d, *J* = 12.9 Hz, 1H, O-CH_2-Ph), 4.95 (d, *J* = 12.4 Hz, 1H, O-CH_2-Ph), 3.83 (s, 3H, 3-O-CH_3), 3.62 (s, 3H, 2-O-CH_3), 2.16 – 2.08 (m, 1H, 11-H), 1.84 – 1.63 (m, 4H, 12-H, 13-H, 15-H, 16-H), 1.57 (s, 3H, 8-H), 1.41 – 1.14 (m, 6H, 12-H, 13-H, 14-H, 15-H, 16-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 178.0 (C-6), 155.6 (NH-C(O)-CH_2-Ph), 155.3 (C-3), 150.1 (C-4a), 145.3 (C-10), 144.7 (C-2), 130.0 (C-1), 129.4 (C$_{Ph}$), 129.0 (C$_{Ph}$), 128.6 (C$_{Ph}$), 118.7 (C-9), 118.3 (C-7a), 96.0 (C-4), 67.4 (NH-C(O)-CH_2-Ph), 60.2 (2-O-CH_3), 59.6 (C-7), 56.9 (3-O-CH_3), 42.9 (C-11), 33.5 (C-12, C-16), 26.9 (C-14), 26.7 (C-13, C-15), 23.5 (C-8); **IR (film)** 3345 (br w), 2925 (s), 2850 (m), 1814 (s), 1719 (s), 1618 (m), 1586 (m), 1516 (m), 1454 (s), 1418 (m), 1375 (w), 1337 (m), 1298 (m), 1249 (m), 1225 (m), 1193 (m), 1159 (w), 1134 (w), 1087 (m), 1061 (m), 1027 (m), 1008 (s), 971 (w), 910 (w), 820 (w), 782 (w), 742 (w), 698 (w) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{32}NO_6$ $[M+H]^+$ 466.2224, found 466.2227.

4.5.5 Styryl-benzofuranone 14e

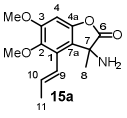

14e

Compound **14e** was synthesized accordingly to the general procedure above from **13a**. R^1 = Me; R^2 = C_6H_5; *x* = 1.5: Silica gel chromatography of the crude with *n*-hexane and EtOAc (9:1 to 8:2) as eluents afforded pure **14e** as a white solid (180 mg, 78% yield); $\mathbf{R_f}$ (CH_2Cl_2) = 0.29; **m.p.** 71.2 ± 0.5 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 – 7.27 (m, 10H + 1H, 10 Ph, 9-H), 7.10 (d, *J* = 16.4 Hz, 1H, 10-H), 6.66 (s, 1H, 4-H), 5.62 (s, NH), 5.09 (d, *J* = 12.0 Hz, 1H, O-C$\underline{H}_2$-Ph), 5.01 (d, *J* = 11.0 Hz, 1H, O-C$\underline{H}_2$-Ph), 3.89 (s, 3H, 3-O-C$\underline{H}_3$), 3.75 (s, 3H, 2-O-C$\underline{H}_3$), 1.66 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.8 (C-6), 154.63 (NH-$\underline{C}$=O), 154.56 (C-3), 149.4 (C-4a), 144.3 (C-2), 137.4 (C-11), 136.3 (C-9), 135.6 (*i*-C_{Ph}), 128.9, 128.7, 128.5, 128.4, 128.3, 126.8, 119.1 (C-10), 117.4 (C-7a), 95.7 (C-4), 67.8 ($\underline{C}H_2$-Ph), 60.3 (2-O-$\underline{C}H_3$), 58.8 (C-7), 56.3 (3-O-$\underline{C}H_3$), 24.0 (C-8); **IR (film)** 3335 (br, w), 2936 (w), 1809 (m), 1714 (m), 1614 (w), 1585 (m), 1497 (m), 1471 (m), 1454 (m), 1418 (m), 1376 (w), 1339 (m), 1293 (m), 1249 (m), 1227 (m), 1192 (m), 1154 (s), 1090 (s), 1057 (s), 1027 (s), 1005 (s), 970 (m), 911 (m), 822 (m), 784 (m), 734 (s), 693 (s) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{26}NO_6$ $[M+H]^+$ 460.1755, found 460.1760.

4.6 General carbamate deprotection procedure

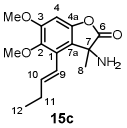
To a solution of $Pd(OAc)_2$ (0.1 eq.) in CH_2Cl_2 (0.2 M rel. to **14**) were added successively Et_3N (0.2 eq.) and Et_3SiH (2.2 eq.) at RT. The dark mixture was stirred for 30 mins before a solution of **14a-e** (1.0 eq.) in CH_2Cl_2 (0.2 M rel. to **14**) was added. The vial was sealed and after 18 hours of reaction, the black precipitate was filtered off over a pad of celite® and the filtrate washed with an aq. sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 several times (3 x 2 mL), dried over Na_2SO_4 and concentrated *in vacuo*.

4.6.1 Propenyl-benzofuranone free amine 15a


15a

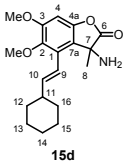
Compound **15a** was synthesized accordingly to the general procedure above from **14a**. Silica gel chromatography of the crude with CH_2Cl_2 and Et_2O (gradient elution 100:0 to 95:5) as eluents afforded the pure amine **15a** as a white solid (152 mg, 87% yield). $\mathbf{R_f}$ (*n*-hexane:EtOAc, 8:2) = 0.24; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.86 (dq, *J* = 16.0, 6.5 Hz, 1H, 10-H), 6.73 (dq, *J* = 16.0, 1.6 Hz, 1H, 9-H), 6.58 (s, 1H, 4-H), 3.87 (s, 3H, 3-O-C$\underline{H}_3$), 3.71 (s, 3H, 2-O-C$\underline{H}_3$), 1.95 (dd, *J* = 6.5, 1.6 Hz, 3H, 11-H), 1.82 (br, NH_2), 1.57 (s, 3H, 8-C$\underline{H}_3$); **HRMS** (ESI) *m/z* calcd. for $C_{14}H_{17}NO_4Na$ $[M+Na]^+$ 286.1050, found 286.1064.

4.6.2 Butenyl-benzofuranone free amine 15c


15c

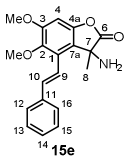
Compound **15c** was synthesized accordingly to the general procedure above from **14c**. Silica gel chromatography of the crude with *n*-hexane and EtOAc (9:1) as eluents afforded the pure amine **15c** as a solid (62 mg, 54% yield), $\mathbf{R_f}$ (*n*-hexane:EtOAc, 4:1) = 0.36 ; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.86 (dt, *J* = 16.1, 6.6 Hz, 1H, 10-H), 6.69 (dt, *J* = 16.1, 1.5 Hz, 1H, 9-H), 6.56 (s, 1H, 4-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.69 (s, 3H, 2-O-C$\underline{H}_3$), 2.41 – 2.19 (m, 2H, 11-H), 1.83 (br s, NH_2), 1.55 (s, 3H, 8-H), 1.10 (t, *J* = 7.4 Hz, 3H, 12-H); **HRMS** (ESI) *m/z* calcd. for $C_{15}H_{19}NO_4Na$ $[M+Na]^+$ 300.1206, found 300.122.

4.6.3 Cyclohexylvinyl-benzofuranone free amine 15d


15d

Compound **15a** was synthesized accordingly to the general procedure above from **14d**. Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (100:0 to 95:5) as eluents afforded the pure amine **15d** as a solid (88 mg, 89% yield). $\mathbf{R_f}$ (CH_2Cl_2) = 0.18; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 16.2, 7.0 Hz, 1H, 10-H), 6.65 (dd, *J* = 16.3, 1.0 Hz, 1H, 9-H), 6.55 (s, 1H, 4-H), 3.83 (s, 3H, 3-O-C$\underline{H}_3$), 3.67 (s, 3H, 2-O-C$\underline{H}_3$), 2.16 (tdt, *J* = 10.7, 6.9, 3.5 Hz, 1H, 11-H), 1.84 – 1.61 (m, 6H, NH2, 12-H, 13-H, 15-H, 16-H), 1.54 (s, 3H, 8-H), 1.39 – 1.08 (m, 6H, 12-H', 13-H', 14-H, 15-H', 16-H'); **HRMS** (ESI) *m/z* calcd. for $C_{19}H_{25}NO_4Na$ $[M+Na]^+$ 354.1676, found 354.1689.

4.6.4 Styryl-benzofuranone free amine 15e

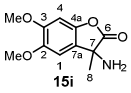

15e

Compound **15a** was synthesized accordingly to the general procedure above from **14e**. Silica gel chromatography of the crude with *n*-hexane and EtOAc (9:1) as eluents afforded the pure amine **15e** as a white solid (106 mg, 94% yield); $\mathbf{R_f}$ (CH_2Cl_2:Et_2O, 99:1) = 0.41; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 16.6 Hz, 1H, 9-H), 7.59 (d, *J* = 16.6 Hz, 1H, 10-H), 7.58 – 7.55 (m, 2H, *o*-H_{Ph}), 7.43 – 7.35 (m, 2H, *m*-H_{Ph}), 7.32 – 7.27 (m, 1H, *p*-H_{Ph}), 6.65 (s, 1H, 4-H), 3.90 (s, 3H, 3-O-C$\underline{H}_3$), 3.79 (s, 3H, 2-O-C$\underline{H}_3$), 1.90 (s, NH_2), 1.61 (s, 3H, 8-H); **HRMS** (ESI) *m/z* calcd. for $C_{19}H_{19}NO_4Na$ $[M+Na]^+$ 348.1206, found 348.122.

4.7 General procedure for tandem Cbz-carbamate hydrogenolysis and alkene reduction

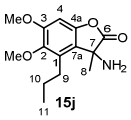
Hydrogen (1 atm balloon) was bubbled into a mixture of the protected amine **12a** or **14a** (1.0 eq.) and Pd/C (10% w/w) in EtOH (0.1 M rel. to **12a** or **14a**) for 24 hours at RT. Upon completion, the reaction mixture was directly filtered on a pad of celite® which was further washed with EtOH. The solvent was then evaporated *in vacuo* and the crude residue purified by silica gel chromatography.

4.7.1 Benzofuranone free amine (15i)


15i

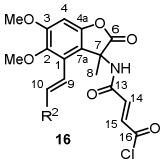
Compound **15i** was synthesized accordingly to the general procedure above from **12a**. (113 mg, 91% yield). $\mathbf{R_f}$ (*n*-hexane:EtOAc, 4:1) = 0.36; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.87 (s, 1H, 1-H), 6.65 (s, 1H, 4-H), 3.83 (s, 3H, 3-O-C$\underline{H}_3$), 3.82 (s, 3H, 2-O-C$\underline{H}_3$), 2.05 (br s, NH_2), 1.48 (s, 3H, 8-H); **HRMS** (ESI) *m/z* calcd. for $C_{11}H_{13}NO_4Na$ $[M+Na]^+$ 246.0737, found 246.0741.

4.7.2 Propyl-benzofuranone free amine 15j


15j

Compound **15j** was synthesized accordingly to the general procedure above from **14a**. (72 mg, quant. yield). $\mathbf{R_f}$ (CH_2Cl_2:MeOH) = 0.73; **^{1}H NMR** (400 MHz, CD_3CN) δ 6.71 (s, 1H, 4-H), 3.83 (s, 3H), 3.74 (s, 3H), 2.81 (ddd, *J* = 12.4, 11.4, 4.9 Hz, 1H, 9-H), 2.66 (ddd, *J* = 12.5, 11.3, 5.3 Hz, 1H, 9-H), 1.74 – 1.58 (m, 1H, 10-H), 1.51 (s, 3H, 8-H), 1.58 – 1.41 (m, 1H, 10-H), 1.01 (t, *J* = 7.4 Hz, 3H, 11-H); **HRMS** (ESI) *m/z* calcd. for $C_{14}H_{19}NO_4Na$ $[M+Na]^+$ 288.1206, found 288.1215.

*4.8 General fumaryl coupling procedure to **16** followed by the final nucleophilic acyl substitution*

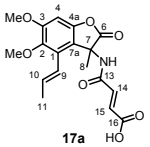

16

To a solution of fumaryl chloride (2.0 eq.) in $CHCl_3$ (0.2 M rel. to **15**) at 0 °C was added dropwise a solution of **15a,c-e,i,j** (1.0 eq.) in $CHCl_3$ (0.2 M rel. to **15**). The reaction mixture was then stirred at 0

°C for another 30 mins after which an aq. sat. solution of NH_4Cl was added to quench the reaction. The mixture was then extracted several times with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4 and evaporated to yield the corresponding acyl chlorides **16** as solids. **16a**: R^2 = Me: **^{1}H NMR** (400 MHz, CD_3CN) δ 8.20 (s, NH), 7.13 (d, *J* = 15.2 Hz, 1H, 15-H), 6.84 (d, *J* = 15.1 Hz, 1H, 14-H), 6.76 (s, 1H, 4-H), 6.48 (dq, *J* = 16.0, 6.4 Hz, 1H, 10-H), 6.36 (dq, *J* = 15.8, 1.4 Hz, 1H, 9-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 1.92 (dd, *J* = 6.4, 1.6 Hz, 3H, 11-H), 1.66 (s, 3H, 8-H). **16c**: R^2 = Et: **^{1}H NMR** (400 MHz, CD_3CN) δ 8.20 (s, NH), 7.13 (d, *J* = 15.1 Hz, 1H, 15-H), 6.84 (d, *J* = 15.1 Hz, 1H, 14-H), 6.77 (s, 1H, 4-H), 6.49 (dt, *J* = 16.0, 6.6 Hz, 1H, 10-H), 6.32 (dt, *J* = 16.0, 1.5 Hz, 1H, 9-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 2.33 – 2.19 (m, 2H, 11-H), 1.66 (s, 3H, 8-H), 1.10 (t, *J* = 7.5 Hz, 3H, 12-H).

The crude acyl chlorides obtained were engaged directly into the nucleophilic acyl substitution step without further purification. To a solution of crude **16** (1.0 eq.) in THF (0.1 M rel. to **16**) at RT were added the appropriate nucleophile (x eq.) and dry silica powder (if needed 3 mg/mg of **16**). The resulting slurry was then stirred for 4 hours, filtered and evaporated to dryness.

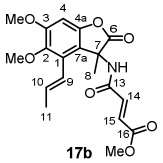
4.8.1 Dimethoxy-fumimycin 17a



17a

Compound **17a** was synthesized accordingly to the general procedure above from **15a**. Nucleophile: H_2O (x = 50 eq.): The crude residue was triturated with MeCN and filtered over Na_2SO_4. The filtrate was evaporated to quantitatively yield **17a** as a white solid which necessitated no further purification (133 mg, quant. Yield over 2 steps). **R_f** (CH_2Cl_2:MeOH, 95:5) = 0.28; **m.p.** 227.5 ± 1.4 °C; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.97 (s, NH), 6.90 (d, *J* = 15.5 Hz, 1H, 14-H), 6.76 (s, 1H, 4-H), 6.59 (d, *J* = 15.5 Hz, 1H, 15-H), 6.47 (dq, *J* = 15.9, 6.4 Hz, 1H, 10-H), 6.36 (dq, *J* = 15.7, 1.4 Hz, 1H, 9-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 1.91 (dd, *J* = 6.4, 1.5 Hz, 3H, 11-H), 1.64 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 176.8 (C-6), 166.3 (d, *J* = 1.6 Hz, C-16), 163.6 (C-13), 155.3 (C-3), 150.3 (C-5a), 144.6 (C-2), 135.5 (C-14), 134.8 (C-10), 131.8 (C-15), 129.4 (C-1), 122.3 (C-9), 117.8 (C-7a), 95.9 (C-4), 60.2 (2-O-$\underline{C}H_3$), 58.9 (C-7), 56.8 (3-O-$\underline{C}H_3$), 23.0 (C-8), 19.6 (C-11); **IR (film)** 3305 (br w), 2929 (br m), 2852 (w), 1812 (s), 1710 (m), 1636 (s), 1618 (m), 1586 (m), 1537 (m), 1458 (m), 1421 (m), 1374 (w), 1333 (s), 1296 (s), 1222 (m), 1194 (s), 1151 (s), 1115 (m), 1088 (s), 1031 (s), 1013 (s), 977 (s), 907 (w), 832 (w), 785 (w), 737 (w), 673 (w); **HRMS** (DART) *m/z* calcd. for $C_{18}H_{20}NO_7$ $[M+H]^+$ 362.1234, found 362.1250

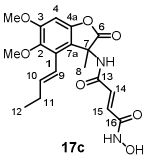
4.8.2 Dimethoxy-fumimycin methyl ester 17b



17b

Compound **17b** was synthesized accordingly to the general procedure above from **15b**. Nucleophile: MeOH. Silica gel chromatography with CH_2Cl_2 and MeOH (998:2) as eluents afforded pure **17b** as a yellow solid (15 mg, 52% yield over 2 steps). **R_f** (CH_2Cl_2:EtOH, 95:5) = 0.62; **m.p.** 232.8 ± 0.3 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.39 (s, NH), 7.02 (d, *J* = 15.4 Hz, 1H, 15-H), 6.79 (d, *J* = 15.4 Hz, 1H, 14-H), 6.68 (s, 1H, 4-H), 6.49 (dq, *J* = 15.8, 6.5 Hz, 1H, 10-H), 6.30 (dq, *J* = 15.8, 1.6 Hz, 1H, 9-H), 3.86 (s, 3H, 3-O-C$\underline{H}_3$), 3.79 (s, 3H, 2-O-C$\underline{H}_3$), 3.67 (s, 3H, COOC$\underline{H}_3$), 1.89 (dd, *J* = 6.6, 1.6 Hz, 3H, 11-H), 1.73 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.2 (C-6), 166.3 (C-16), 162.7 (C-13), 154.6 (C-3), 149.7 (C-5a), 144.1 (C-2), 134.5 (C-2), 134.1 (C-14), 131.8 (C-10), 128.8 (C-15), 121.5 (C-1), 116.4 (C-7a), 95.2 (C-4), 60.1 (2-O-$\underline{C}H_3$), 58.4 (C-7), 56.3 (3-O-$\underline{C}H_3$), 52.6 (COO$\underline{C}H_3$), 23.2 (C-8), 19.8 (C-11); **IR (film)** 3304 (br w), 2926 (m), 2854 (w), 1813 (s), 1731 (m), 1706 (m), 1665 (s), 1621 (m), 1589 (m), 1532 (m), 1457 (m), 1437 (m), 1378 (w), 1334 (s), 1305 (m), 1232 (w), 1218 (w), 1193 (m), 1157 (s), 1112 (m), 1089 (m), 1073 (w), 1034 (m), 1012 (s), 974 (m), 907 (w), 850 (w), 821 (w), 782 (m), 680 (w) ; **HRMS** (DART) *m/z* calcd. for $C_{19}H_{25}NO_8$ $[M+NH_4]^+$ 393.1656, found 393.1660.

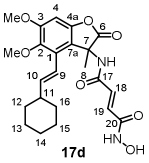
4.8.3 Dimethoxy-butenyl-benzofuranone hydroxamic acid 17c



17c

Compound **17c** was synthesized accordingly to the general procedure above from **15c**. No silica (SiO_2) required. Nucleophile: TMSO-NH_2 (x = 15 eq.). Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (100:0 to 93:7) as eluents afforded pure hydroxamic acid **17c** as a yellow wax (21 mg, 77% yield over 2 steps). **R_f** (CH_2Cl_2:MeOH, 9:1) = 0.31; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.73 (s, NH), 6.91 (s, 1H, 4-H), 6.90 (d, *J* = 15.6 Hz, 1H, 14-H), 6.51 (dt, *J* = 16.0, 6.7 Hz, 1H, -10-H), 6.46 (d, *J* = 15.5 Hz, 1H, 15-H), 6.33 (dt, *J* = 15.9, 1.5 Hz, 1H, 9-H), 3.82 (s, 3H, 3-O-C$\underline{H}_3$), 3.58 (s, 3H, 2-O-C$\underline{H}_3$), 2.33 – 2.09 (m, 2H, 11-H), 1.58 (s, 3H, 8-H), 1.05 (t, *J* = 7.4 Hz, 3H, 12-H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 175.8 (C-6), 166.2 (C-13), 162.5 (C-16), 153.8 (C-3), 148.8 (C-4a), 143.2 (C-2), 139.7 (C-10), 134.0 (C-14), 131.8 (C-15), 127.4 (C-1), 119.3 (C-9), 117.1 (C-7a), 95.4 (C-4), 59.3 (2-O-$\underline{C}H_3$), 57.3 (C-7), 56.2 (3-O-$\underline{C}H_3$), 26.5 (C-11), 22.5 (C-8), 13.5 (C-12); **IR (film)** 3253 (br w), 2963 (w), 1808 (m), 1713 (w), 1670 (m), 1620 (w), 1533 (m), 1456 (m), 1419 (w), 1377 (w), 1332 (m), 1295 (m), 1221 (w), 1193 (m), 1155 (m), 1093 (m), 1024 (s), 1001 (s), 824 (w); **HRMS** (ESI) major fragment observed as the corresponding carboxylic acid, *m/z* calcd. for $C_{19}H_{22}NO_7$ $[M+H]^+$ 376.1396, found 376.1408.

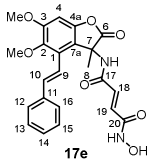
4.8.4 Dimethoxy-cyclohexylvinyl-benzofuranone hydroxamic acid 17d



17d

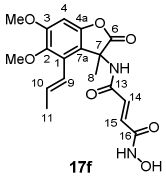
Compound **17d** was synthesized accordingly to the general procedure above from **15d**. No silica (SiO_2) required. Nucleophile: TMSO-NH_2 (x = 15 eq.). Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (100:0 to 93:7) as eluents afforded the pure hydroxamic acid **17d** as a light yellow solid (10 mg, 57% yield over 2 steps). **R_f** (CH_2Cl_2:MeOH, 97:3) = 0.4; **m.p.** 241.5 ± 0.3 °C; **^{1}H NMR** (400 MHz, $CDCl_3$:MeOD, 1:1) δ 6.94 (d, *J* = 15.5 Hz, 1H, 19-H), 6.67 (s, 1H, 4-H), 6.65 (d, *J* = 16.4 Hz, 1H, 18-H), 6.42 (dd, *J* = 16.0, 7.3 Hz, 1H, 10-H), 6.30 – 6.21 (dd, *J* = 16.0, 0.8 Hz, 1H, 9-H), 3.85 (s, 3H, 3-O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 2.20 – 2.06 (m, 1H, 11-H), 1.83 – 1.67 (m, 4H, 12-H, 13-H, 15-H, 16-H), 1.65 (s, 3H, 8-H), 1.36 – 1.13 (m, 6H, 12-H, 13-H, 14-H, 15-H, 16-H); **^{13}C NMR** (100 MHz, $CDCl_3$:MeOD, 1:1) δ 177.4 (C-6), 168.1 (C-20), 164.6 (C-17), 155.0 (C-3), 150.1 (C-4a), 145.5 (C-10), 144.3 (C-2), 134.7 (C-19), 132.9 (C-18), 129.5 (C-1), 118.1 (C-9), 117.8 (C-7a), 95.5 (C-4), 60.2 (2-O-$\underline{C}H_3$), 58.8 (C-7), 56.5 (3-O-$\underline{C}H_3$), 42.9 (C-11), 33.33 (C-12 or C-16), 33.29 (C-12 or C-16), 26.6 (C-14), 26.5 (C-13 or C-15), 26.4 (C-13 or C-15), 22.8 (C-8); **IR (film)** 3305 (br w), 2926 (s), 2851 (m), 1812 (s), 1710 (m), 1651 (s), 1620 (m), 1586 (m), 1535 (m), 1452 (s), 1419 (m), 1378 (w), 1335 (s), 1280 (m), 1222 (m), 1193 (m), 1156 (s), 1089 (m), 1009 (s), 976 (m), 837 (w); **HRMS** (DART) major fragment observed as the corresponding carboxylic acid, *m/z* calcd. for $C_{23}H_{28}NO_7$ $[M+H]^+$ 430.1866, found 430.1882.

4.8.5 Dimethoxy-styryl-benzofuranone hydroxamic acid 17e


17e

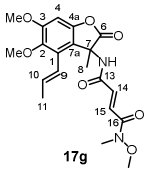
Compound **17e** was synthesized accordingly to the general procedure above from **15e**. No silica (SiO_2) required. Nucleophile: TMSO-NH_2 (x = 15 eq.): Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (99:1 to 90:10) as eluents afforded the pure hydroxamic acid **17e** as a white solid (32 mg, 64% yield over 2 steps). $\mathbf{R_f}$ (CH_2Cl_2:MeOH, 9:1) = 0.41; **m.p.** 211.8 ± 0.1 ℃; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 9.99 (s, NH), 7.68 – 7.63 (m, 2H, 12-H, 16-H), 7.49 (d, *J* = 16.5 Hz, 1H, 9-H), 7.43 (t, *J* = 7.6 Hz, 2H, 13-H, 15-H), 7.36 – 7.29 (m, 1H, 14-H), 7.10 (d, *J* = 16.4 Hz, 1H, 10-H), 7.01 (s, 1H, 4-H), 6.96 (d, *J* = 15.6 Hz, 1H, 18-H), 6.49 (d, *J* = 15.6 Hz, 1H, 19-H), 3.86 (s, 3H, 3-O-C$\underline{H}_3$), 3.68 (s, 3H, 2-O-C$\underline{H}_3$), 1.65 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, DMSO) δ 176.2 (C-6), 166.6 (C-17), 163.0 (C-20), 154.4 (C-3), 149.4 (C-4a), 144.1 (C-2), 137.5 (C-11), 135.6 (C-9), 134.6 (d, *J* = 15.3 Hz, C-18), 132.2 (C-19), 129.2 (C-13, C-15), 128.8 (t, *J* = 7.9 Hz, C-14), 127.4 (C-12, C-16), 127.1 (C-1), 119.4 (C-10), 118.2 (C-7a), 96.6 (C-4), 60.0 (2-O-$\underline{C}H_3$), 58.0 (C-7), 56.9 (3-O-$\underline{C}H_3$), 23.3 (C-8); **IR (film)** 3301 (br w), 2938 (br w), 1811 (s), 1715 (m), 1669 (m), 1585 (w), 1534 (w), 1497 (m), 1455 (m), 1420 (w), 1337 (m), 1297 (m), 1222 (m), 1192 (m), 1152 (s), 1090 (m), 1024 (m), 1007 (s), 974 (m), 918 (w), 823 (w), 785 (w), 749 (w), 693 (w); **HRMS** (ESI) major fragment observed as the corresponding carboxylic acid, *m/z* calcd. for $C_{23}H_{22}NO_7$ $[M+H]^+$ 424.1396, found 424.1399.

4.8.6 Dimethoxy-fumimycin hydroxamic acid 17f


17f

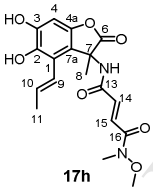
Compound **15f** was synthesized accordingly to the general procedure above from **15f**. No silica (SiO_2) required. Nucleophile: TMSO-NH_2 (x = 15 eq.). Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (100:0 to 95:5) as eluents afforded the pure hydroxamic acid **17f** as a light yellow solid (29 mg, 76% yield over 2 steps). $\mathbf{R_f}$ (CH_2Cl_2:MeOH, 9:1) = 0.17; **m.p** 205 ± 0.3 ℃; **^{1}H NMR** (400 MHz, Acetonitrile-d_3) δ 8.01 (s, NH), 6.89 (d, *J* = 15.5 Hz, 1H, 14-H), 6.76 (s, 1H, 4-H), 6.59 (d, *J* = 15.6 Hz, 1H, 15-H), 6.48 (dq, *J* = 15.9, 6.4 Hz, 1H, 10-H), 6.36 (dq, *J* = 15.9, 1.5 Hz, 1H, 9-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 1.91 (dd, *J* = 6.4, 1.5 Hz, 3H, 11-H), 1.63 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 176.9 (C-6), 166.5 (C-16), 163.7 (C-13), 155.4 (C-3), 150.4 (C-4a), 144.7 (C-2), 135.4 (C-10), 134.9 (C-15), 132.1 (C-14), 129.5 (C-1), 122.4 (C-9), 118.0 (C-7a), 96.0 (C-4), 60.3 (2-O-$\underline{C}H_3$), 59.0 (C-7), 56.9 (3-O-$\underline{C}H_3$), 23.1 (C-8), 19.7 (C-11); **IR (film)** 3288 (br w), 2922 (m), 2851 (w), 1812 (s), 1714 (m), 1651 (s), 1587 (w), 1536 (m), 1457 (m), 1420 (w), 1378 (w), 1333 (s), 1296 (m), 1221 (m), 1194 (m), 1154 (s), 1114 (w), 1088 (m), 1033 (m), 1012 (s), 973 (m), 909 (w), 820 (w); **HRMS** (DART) *m/z* calcd. for $C_{18}H_{24}O_7N_2$ $[M+NH_4]^+$ 394.1609, found 394.1603.

4.8.7 Dimethoxy-fumimycin Weinreb amide 17g


17g

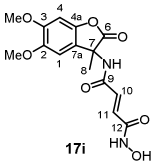
Compound **17g** was synthesized accordingly to the general procedure above from **15g**. Nucleophile: MeNHOMe·HCl (x = 15 eq.). Silica gel chromatography of the crude with *n*-hexane and EtOAc (7:3 to 3:7) as eluents afforded the pure hydroxamic acid **17g** as an oil (6 mg, 52% yield over 2 steps). $\mathbf{R_f}$ (*n*-hexane:EtOAc, 1:1) = 0.17; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.97 (s, NH), 7.25 (d, *J* = 15.3 Hz, 1H, 14-H), 6.87 (d, *J* = 15.3 Hz, 1H, 15-H), 6.76 (s, 1H, 4-H), 6.48 (dq, *J* = 15.9, 6.4 Hz, 1H, 10-H), 6.37 (dq, *J* = 15.9, 1.5 Hz, 1H, 9-H), 3.84 (s, 3H, 3-O-C$\underline{H}_3$), 3.68 (s, 3H, O-C$\underline{H}_3$), 3.63 (s, 3H, 2-O-C$\underline{H}_3$), 3.19 (s, 3H, N-C$\underline{H}_3$), 1.91 (dd, *J* = 6.4, 1.5 Hz, 3H, 11-H), 1.64 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, CD_3CN) δ 177.0 (C-6), 165.4 (C-16), 164.2 (C-13), 155.4 (C-3), 150.4 (C-4a), 144.7 (C-2), 134.8 (C-10), 133.3 (C-15), 130.7 (C-14), 129.5 (C-1), 122.4 (C-9), 118.1 (C-7a), 96.0 (C-4), 62.9 (O-$\underline{C}H_3$), 60.3 (2-O-$\underline{C}H_3$), 59.0 (C-7), 56.9 (3-O-$\underline{C}H_3$), 32.3 (N-$\underline{C}H_3$), 23.1 (C-8), 19.7 (C-11); **IR (film)** 3295 (br w), 2926 (br m), 2853 (w), 1811 (s), 1681 (w), 1647 (s), 1621 (s), 1588 (m), 1530 (m), 1456 (s), 1420 (m), 1379 (m), 1332 (s), 1296 (w), 1275 (w), 1222 (m), 1193 (m), 1154 (s), 1114 (m), 1033 (m), 1012 (s), 974 (m), 909 (w), 919 (w), 735 (w) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{20}H_{24}N_2O_7Na$ $[M+Na]^+$ 427.1476, found 427.1494.

4.8.8 Fumimycin Weinreb amide 17h


17h

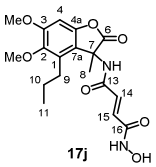
Compound **17h** was synthesized accordingly to the general procedure above from **15b**. Nucleophile: MeNHOMe·HCl (x = 40 eq.). Relatively unstable product. Silica gel chromatography with *n*-hexane and EtOAc (1:1) as eluents afforded **17h** as a brown oil. (4.8 mg, 15% yield over 3 steps). $\mathbf{R_f}$ (CH_2Cl_2:EtOH, 95:5) = 0.47; **^{1}H NMR** (400 MHz, CD_3CN) δ 7.96 (s, NH), 7.27 (d, *J* = 15.3 Hz, 1H, 14-H), 6.89 (d, *J* = 15.3 Hz, 1H, 15-H), 6.62 (s, 1H, 4-H), 6.64 – 6.51 (dq, *J* = 16.0, 6.5 Hz**,** 1H, 10-H), 6.41 (dq, *J* = 16.0, 1.5 Hz, 1H, 9-H), 3.70 (s, 3H, O-C$\underline{H}_3$), 3.22 (s, 3H, NH-C$\underline{H}_3$), 1.94 (dd, *J* = 6.6, 1.6 Hz, 3H, 11-H), 1.66 (s, 3H, 8-H); **IR (film)** 2935 (br w), 1736 (s), 1712 (w), 1650 (w), 1606 (w), 1508 (s), 1452 (m), 1417 (w), 1360 (w), 1263 (m), 1228 (s), 1185 (m), 1149 (s), 1111 (s), 1024 (s), 977 (m), 935 (w), 880 (m), 797 (w), 767 (m), 733 (w) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{18}H_{20}N_2O_7Na$ $[M+Na]^+$ 399.1163, found 399.1171.

4.8.9 Dimethoxy-benzofuranone hydroxamic acid 17i


17i

Compound **17i** was synthesized accordingly to the general procedure above from **15i**. The crude product was precipitated with CH_2Cl_2, filtered through celite® and washed successively with cold CH_2Cl_2 and petroleum ether to obtain **17i** as a brown solid (30 mg, 31% yield over 2 steps). $\mathbf{R_f}$ (CH_2Cl_2:MeOH) = 0.14; **m.p** 124.4 ± 1.1 ℃; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 7.30 (br, 1H), 6.96 (s, 1-H), 6.94 (d, *J* = 15.6 Hz, 1H, 10-H), 6.88 (s, 1H, 4-H), 6.45 (d, *J* = 15.5 Hz, 1H, 11-H), 3.78 (s, 3H, 3-O-C$\underline{H}_3$), 3.72 (s, 3H, 2-O-C$\underline{H}_3$), 1.56 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 176.1 (C-6), 166.2 (C-9), 162.6 (C-12), 150.0 (C-3), 146.5 (C-4a), 146.0 (C-2), 134.4 (C-15), 131.6 (C-14), 119.9 (C-7a), 106.6 (C-1), 96.5 (C-4), 57.0 (C-7), 56.2 (3-O-$\underline{C}H_3$), 56.1 (2-O-$\underline{C}H_3$), 23.5 (C-8); **IR (film)** 3252 (br w), 2978 (br w), 1804 (m), 1712 (m), 1667 (m), 1630 (m), 1538 (m), 1499 (s), 1445 (m), 1420 (w), 1378 (w), 1336 (m), 1306 (m), 1214 (m), 1192 (m), 1136 (s), 1087 (m), 1045 (s), 1025 (m), 980 (s), 901 (w), 827 (w), 779 (w) cm^{-1}; **HRMS** (ESI) major fragment observed as the corresponding carboxylic acid, *m/z* calcd. for $C_{15}H_{19}N_2O_7$ $[M+NH_4]^+$ 339.1192, found 339.1191.

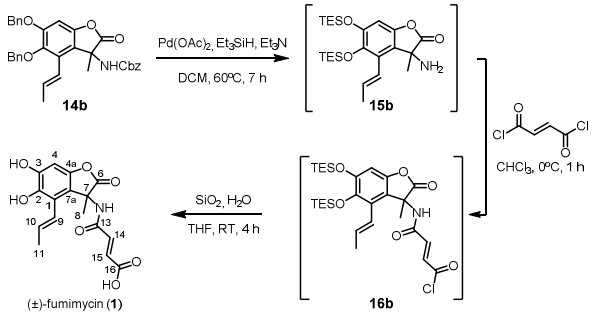
4.8.10 Dimethoxy-propyl-benzofuranone hydroxamic acid 17j


17j

Compound **17j** was synthesized accordingly to the general procedure above from **15j**. The crude product was precipitated with CH_2Cl_2, filtered through celite® and washed successively with cold CH_2Cl_2 and petroleum ether to obtain **17j** as a yellow solid (8.3 mg, 77% yield over 2 steps). $\mathbf{R_f}$ (CH_2Cl_2:EtOH, 95:5) = 0.48; **m.p.** 254.3 ± 0.2 ℃; **^{1}H NMR** (400 MHz,

DMSO-d_6) δ 9.77 (s, NH), 6.90 (d, *J* = 15.5 Hz, 1H, 14-H), 6.89 (s, 1H, 4-H), 6.46 (d, *J* = 15.5 Hz, 1H, 15-H), 3.81 (s, 3H, 3-O-C$\underline{H}_3$), 3.67 (s, 3H, 2-O-C$\underline{H}_3$), 2.56 (ddd, *J* = 12.7, 11.3, 5.2 Hz, 1H, 9-H), 2.44 (ddd, *J* = 12.6, 11.0, 5.3 Hz, 1H, 9-H), 1.60 (s, 3H, 8-H), 1.48 – 1.32 (m, 1H, 10-H), 1.32 – 1.17 (m, 1H, 10-H), 0.93 (t, *J* = 7.3 Hz, 3H, 11-H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 176.4 (C-6), 166.7 (C-13), 162.99 (C-16), 153.7 (C-3), 149.1 (C-4a), 144.1 (C-2), 134.5 (C-14), 132.8 (C-1), 132.3 (C-15), 118.5 (C-7a), 95.5 (C-4), 60.8 (2-O-$\underline{C}H_3$), 57.7 (C-7), 56.6 (3-O-$\underline{C}H_3$), 28.5 (C-9), 24.2 (C-10), 23.7 (C-8), 14.8 (C-11); **IR (film)** 2963 (w), 1809 (m), 1710 (m), 1626 (s), 1597 (w), 1537 (w), 1480 (w), 1455 (m), 1421 (m), 1378 (w), 1330 (s), 1289 (w), 1217 (m), 1191 (m), 1158 (s), 1109 (m), 1092 (m), 1073 (m), 1037 (w), 1003 (s), 977 (m), 905 (w), 779 (w), 746 (w) cm^{-1}.

*4.9 Procedure for the synthesis of (±)-fumimycin (**1**)*



To a solution of $Pd(OAc)_2$ (27 mg, 0.12 mmol, 30 mol %) in CH_2Cl_2 (2.1 mL) were successively added Et_3N (57 µL, 0.41 mmol, 1.0 eq.) and Et_3SiH (650 µL, 4.0 mmol, 10 eq.) at RT. The dark reaction mixture was stirred for 5 mins at RT before a solution of **14b** (234 mg, 0.41 mmol, 1.0 eq.) in CH_2Cl_2 (2 mL) was added dropwise. The vial was sealed and the solution heated at 60 ℃ for 7 h ours. The black precipitate was then filtered off over a pad of celite® and the filtrate was washed with an aq. sat. $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (3 x 1 mL), dried over Na_2SO_4 and concentrated *in vacuo*. A solution of the crude **15b** (0.33 mmol) in $CHCl_3$ (1.6 mL) was added to a solution of fumaryl chloride (70 µL, 0.65 mmol) in $CHCl_3$ (1.7 mL) at 0 ℃. After 1 hour at this temperature , an aq. sat. NH_4Cl solution was added and the resulting mixture was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic layers were dried over Na_2SO_4 and evaporated *in vacuo* to obtain crude **16b**. To a stirring solution of crude **16b** (0.16 mmol) from the previous step in THF (1.6 mL) were added successively H_2O (178 µL, 8.5 mmol, 60 eq.) and silica (3 mg/mg rel. to **16b**) at RT. After 4 hours, the slurry mixture was filtered over fritted glass, then washed with acetonitrile and evaporated to dryness. Silica gel chromatography of the crude with CH_2Cl_2 and MeOH (gradient elution 99:1 to 90:10) as eluents afforded the pure natural product (±)-fumimycin **1** as a brown solid (23 mg, 42% yield over 3 steps). **R_f** (CH_2Cl_2:MeOH, 95:5) = 0.05; **m.p.** 109.1 ℃; **^{1}H NMR** (400 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.92 (s, OH), 7.78 (s, OH), 6.62 (d, *J* = 15.4 Hz, 1H, 14-H), 6.56 (s, NH), 6.38 (d, *J* = 15.7 Hz, 1H, 15-H), 6.36 – 6.27 (m, 1H, 10-H), 6.19 (s, 1H, 4-H), 6.02 (dq, *J* = 15.6, 1.5 Hz, 1H, 9-H), 1.60 (dd, *J* = 6.6, 1.7 Hz, 3H, 11-H), 1.34 (s, 3H, 8-H); **^{13}C NMR** (100 MHz, $CDCl_3$:CD_3OD 1:1) δ 178.0 (C-6), 167.9 (C-16), 164.3 (C-13), 146.6 (C-4a), 146.3 (C-3), 141.0 (C-2), 135.2 (C-10), 134.1 (C-15), 132.2 (C-14), 122.4 (C-9), 121.9 (C-1), 116.4 (C-7a), 97.4 (C-4), 58.9 (C-7), 23.1 (C-8), 19.7 (C-11); **IR (film)** 3303 (br s) 1790 (s), 1710 (m), 1635 (s), 1595 (w), 1538 (w), 1475 (w), 1447 (m), 1377 (w), 1311 (s), 1183 (m), 1153 (s), 1102 (m), 1047 (m), 972 (m), 931 (m), 834 (w), 798 (w) cm^{-1}; **HRMS** (ESI) *m/z* calcd. for $C_{16}H_{15}NO_7Na$ $[M+Na]^+$ 356.0741, found 356.0742.

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Appendix A. Supplementary data

Supplementary data to this article summarizing the synthetic protocols to prepare the starting materials, the protocol for the PDF *in vitro* activity assay, as well as ^{1}H and ^{13}C NMR spectra for all new compounds and the spectral data comparison of the fumimycin natural product (isolated and synthetic) can be found online at https//doi…

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