

Synthesis, biological evaluation, and molecular docking studies of aldotetronic acid-based LpxC inhibitors

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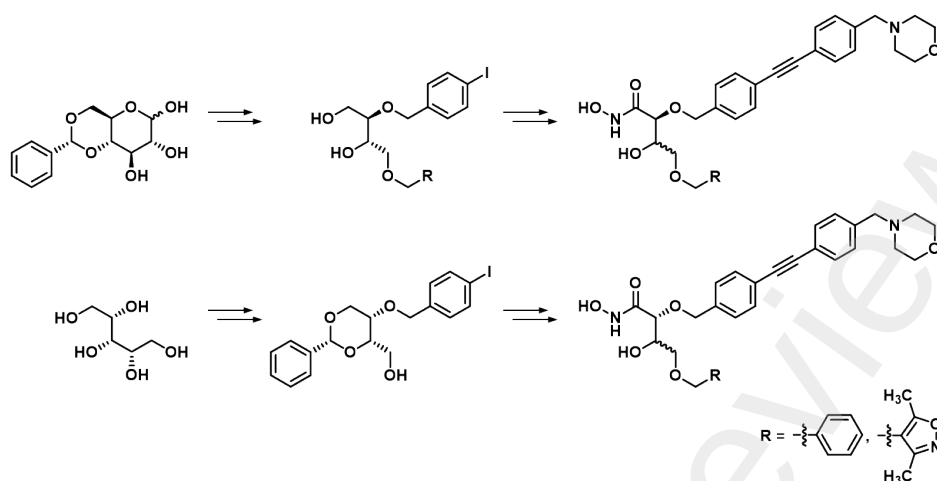
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Graphical abstract



Key words

antibiotics, LpxC inhibitors, aldotetronic acid derivatives, molecular-docking studies, bacterial uptake, LasB

Abstract

In order to develop novel inhibitors of the bacterial deacetylase LpxC bearing a substituent to target the UDP binding site of the enzyme, a series of aldotetronic acid-based hydroxamic acids was accessed in chiral pool syntheses starting from 4,6-O-benzylidene-D-glucose and L-arabinitol. The synthesized hydroxamic acids were tested for LpxC inhibitory activity *in vitro*, revealing benzyl ether **17a** ((2S,3S)-4-(benzyloxy)-N,3-dihydroxy-2-[(4-{4-(morpholinomethyl)phenyl}ethynyl)benzyl]oxy]butanamide) as the most potent LpxC inhibitor. This compound was additionally tested for antibacterial activity against a panel of clinically relevant Gram-negative bacteria, bacterial uptake, and susceptibility to efflux pumps. Molecular docking studies were performed to rationalize the observed structure–activity relationships.

Introduction

Over the past two decades, multi-drug resistant (MDR) Gram-negative bacilli (e.g., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) have emerged worldwide to cause a wide variety of severe infections.¹⁻² MDR phenotypes are particularly characterized by resistance against clinically relevant cell wall active β -lactam compounds and essentially depend on the expression of specific hydrolases referred to as β -lactamases. Fast spreading β -lactamase variants are able to efficiently break down and inactivate even 3rd generation cephalosporines and carbapenems, essentially limiting therapeutic options and leading to a significant morbidity and mortality associated with MDR infections.³⁻⁵ As a consequence of the ongoing world-wide spread of MDR organisms, recently novel β -lactamase inhibitors (BLIs) have been marketed, able to protect β -lactams and restore their antibiotic activity in the presence of β -lactamases, including KPC and Oxa-48.⁶ However, only shortly after their introduction into clinical use, resistance against novel BLIs (e.g., avibactam, relebactam) emerged.⁷ This obvious arms race, in combination with the tremendous abundance of different β -lactamases and the importance of non- β -lactamase mediated resistance mechanisms, underscores the need for the continuous development of novel antibacterial compounds.

In comparison to Gram-positive bacteria, the cell envelope of Gram-negative bacteria exhibits an additional outer membrane, acting as a permeability barrier, which shields the bacteria from noxious compounds.⁸ The outer monolayer of the outer membrane of most Gram-negative bacteria mainly consists of lipid A, which constitutes the hydrophobic membrane anchor of the lipopolysaccharides (LPS).⁹ Lipid A is required for the maintenance of an effective outer-membrane barrier, growth and viability of nearly all Gram-negative bacteria.¹⁰⁻¹² Thus, the inhibition of lipid A biosynthesis

represents an attractive strategy for the development of antibiotics targeting Gram-negative bacteria.¹³

The Zn^{2+} -dependent bacterial deacetylase LpxC catalyzes the committed step of lipid A biosynthesis.¹⁴⁻¹⁷ This essential enzyme, which irreversibly deacetylates UDP-3-O-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**, Figure 1a) in *E. coli*, is present in virtually all Gram-negative bacteria and highly conserved among them. It possesses no mammalian counterpart and is therefore an excellent but so far clinically unexploited target for the development of novel antibiotics.^{12, 18-20}

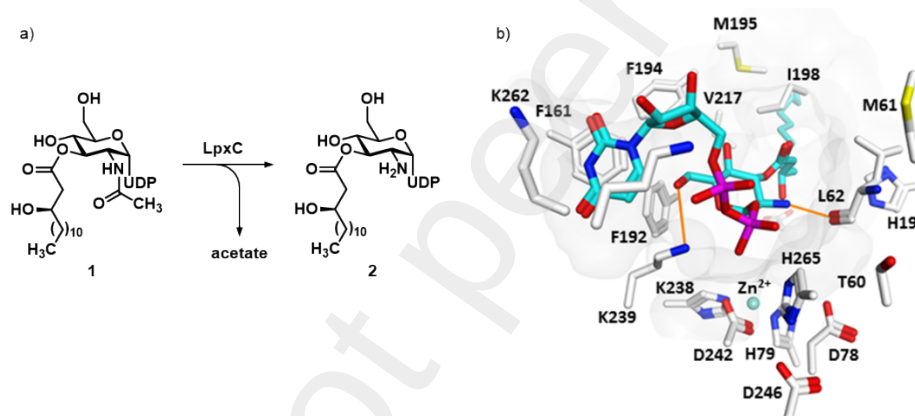


Figure 1: a) LpxC-catalyzed deacetylation of UDP-3-O-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**). b) Molecular surface of *E. coli* LpxC near the deacetylated natural product **2** (PDB 4MDT).²¹ Carbon atoms of **2** are shown in cyan color. The side chains of amino acid residues are shown in stick representation with white carbon atoms. The surface of the binding pocket is shown in white. The Zn^{2+} -ion is shown as a cyan sphere. Protein–ligand interactions are shown as orange lines.

The catalytic Zn^{2+} -ion of LpxC is located at the bottom of a conical active-site cleft, where it is complexed by two histidine residues and one aspartate residue (in case of

E. coli LpxC: H79, H238, and D242).^{19, 21} A hydrophobic tunnel to bind the 3-O-[(*R*)-3-hydroxyacyl] substituent of the enzyme's natural substrate **1** leads out of the active site pocket. Particular insight into substrate recognition by LpxC was gained from a crystal structure of *E. coli* LpxC in complex with the deacetylated reaction product, UDP-3-O-[(*R*)-3-hydroxymyristoyl]glucosamine (**2**, Figure 1b).²¹ Whereas the glucosamine moiety binds upon a hydrophobic patch formed by F192 and F194, it interacts with D242 *via* a water-mediated contact and undergoes direct hydrogen bonds between the 2-amino group and L62 as well as between the 6-OH group and K239. The diphosphate moiety of **2** directly interacts with the basic patch residue K239 and undergoes water-mediated contacts with K143 and D242. Whereas the ribose moiety does not form hydrogen bonds to the enzyme, the uridine moiety undergoes hydrogen bonds with D160 as well as hydrophobic and π -stacking interactions with K262 and F161.²¹

Various structural classes of LpxC inhibitors have been reported in the literature.^{19, 22} Like the *N*-aroyl-L-threonine derivative LPC-011 (**3a**, Figure 2), most of these compounds exhibit a Zn²⁺-binding hydroxamate moiety and a lipophilic side chain, which binds to the enzyme's hydrophobic tunnel.^{19, 22-26} However, only few LpxC inhibitors have been reported to date, which target the UDP binding site of LpxC, like the uridine-based compound 1-68A (**4**, Figure 2).^{19, 22, 27} A few attempts were undertaken to expand known inhibitors into the UDP binding site.²⁸⁻³¹ In case of LPC-011 (**3a**), a hydroxyphenyl group was attached to the β -carbon atom of the threonyl moiety that led to an improvement in inhibitory activity toward LpxC.²⁸ Also in case of ether **5a**, the attachment of suitable substituents caused an increase in inhibitory activity (Figure 2).²⁹

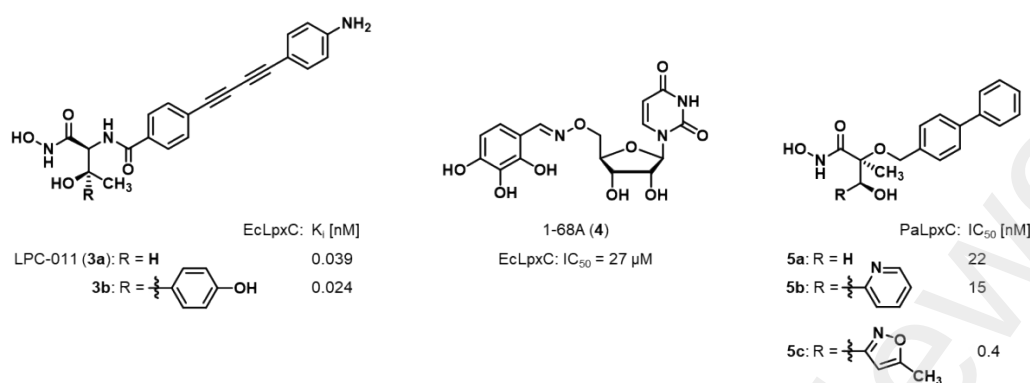


Figure 2: Structures of described LpxC inhibitors targeting the enzyme's UDP binding site and their reported inhibitory activities toward *E. coli* LpxC (EcLpxC) and *P. aeruginosa* LpxC (PaLpxC).

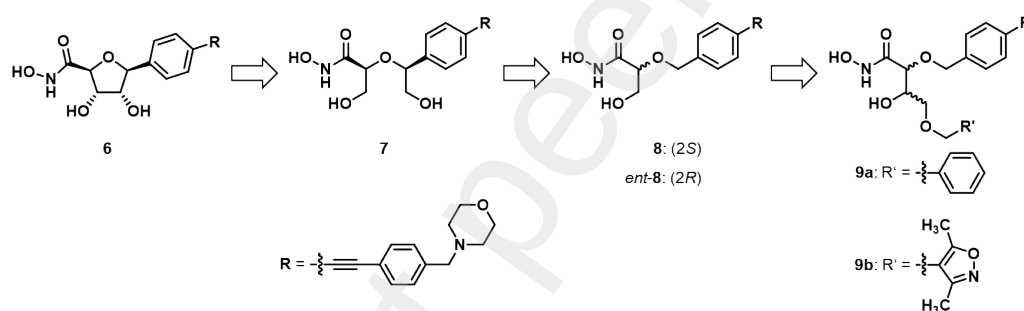


Figure 3: Structures of described and envisaged LpxC inhibitors.

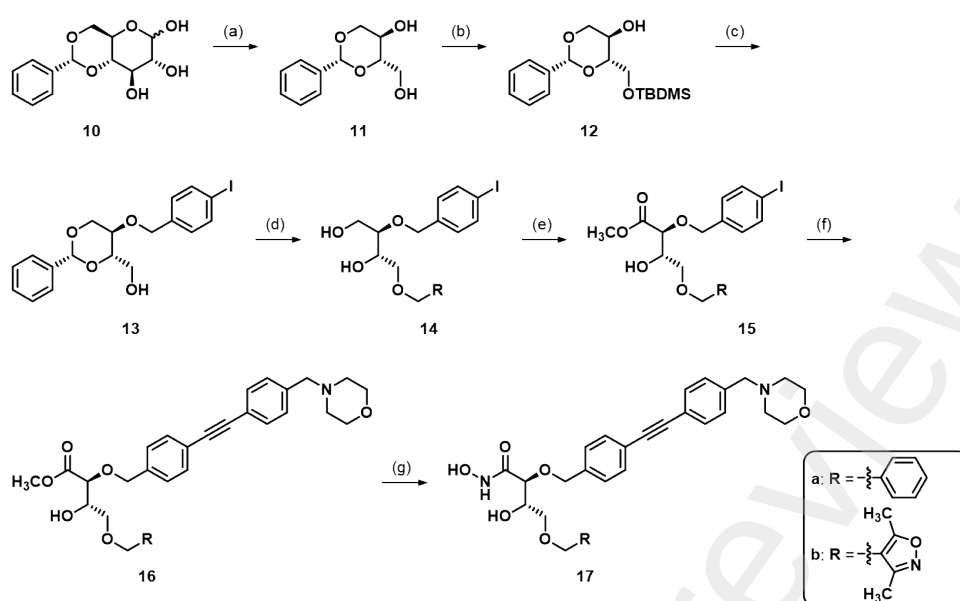
Previously, we reported on the design, synthesis, and biological evaluation of a series of C-furanosidic hydroxamic acids, among which stereoisomer **6** was found to be the most potent LpxC inhibitor (Figure 3).³²⁻³⁵ The cleavage of the tetrahydrofuran ring of these conformationally constrained C-furanosides led to open chain diols, like the (S,S)-configured hydroxamic acid **7**,^{33, 36} which were deconstructed to yield glyceric acid-derived hydroxamic acids **8** and *ent*-**8** (Figure 3).³⁷ As the glyceric acid derivatives showed promising LpxC inhibitory and antibacterial activities, the compounds should be developed further. To grow the inhibitors into the UDP binding site of LpxC, thereby

gaining further favorable interactions with the enzyme, a substituent was introduced at the β -carbon atom of these hydroxamic acids, leading to aldotetrionic acid derivatives **9**. On the one hand, we wanted to demonstrate the feasibility of this approach with a series of benzyl and (3,5-dimethylisoxazol-4-yl)methyl ethers. The latter substituent was chosen as in case of ethers **5**, the attachment of an isoxazole ring had led to a pronounced increase in inhibitory activity (cf. **5c**, Figure 2).²⁹ On the other hand, we wanted to elucidate the optimal stereochemistry of the aldotetrionic acid derivatives for LpxC inhibitory and antibacterial activity.

Results and discussion

Chemistry

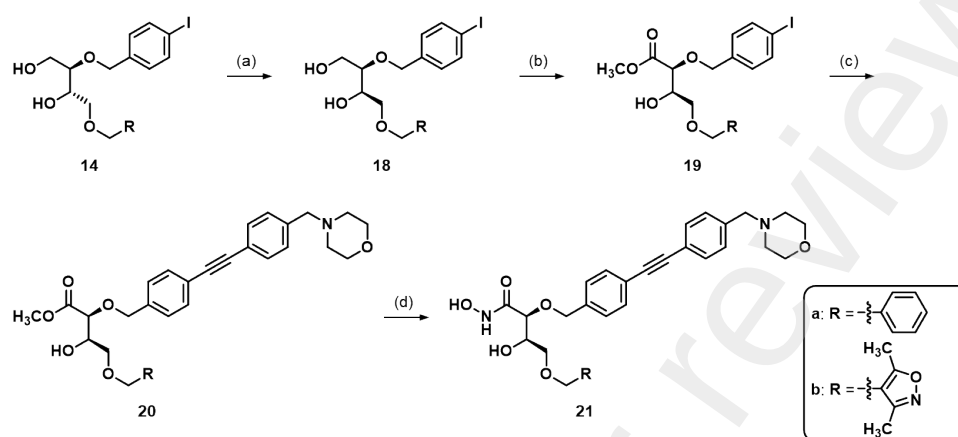
The envisaged stereoisomeric hydroxamic acids were accessed in chiral pool syntheses starting from 4,6-O-benzylidene-D-glucose and L-arabinitol, respectively. To obtain the (2*S*)-configured hydroxamic acids, 4,6-O-benzylidene-D-glucose (**10**) was subjected to a glycol cleavage with sodium metaperiodate, and the intermediate aldehyde was directly reduced with sodium borohydride to yield erythritol derivative **11** (Scheme 1).³⁸ Subsequently, the primary hydroxy group of diol **11** was protected with *tert*-butyldimethylsilyl chloride to afford silyl ether **12**.³⁹ After the alkylation of the remaining secondary hydroxy group of **12** with 4-iodobenzyl bromide, the silyl protective group was cleaved with tetrabutylammonium fluoride, yielding alcohol **13**. An additional Williamson ether synthesis with benzyl bromide or 4-chloromethyl-3,5-dimethylisoxazole, followed by an acid-catalyzed cleavage of the benzylidene acetal gave ethers **14a** and **14b**, respectively. Subsequently, the primary alcohol of diols **14a** and **14b** was selectively oxidized with TEMPO as catalyst and bis(acetoxy)iodobenzene as stoichiometric oxidant, and the thereby formed carboxylic acids were esterified with methanol in the presence of *p*-toluenesulfonic acid.⁴⁰ The esters **15a** and **15b** obtained were subjected to Sonogashira couplings with 4-(morpholinomethyl)phenylacetylene to yield diphenylacetylene derivatives **16a** and **16b**.^{32, 41} Finally, aminolyses with hydroxylamine were performed to convert esters **16a** and **16b** into hydroxamic acids **17a** and **17b**.



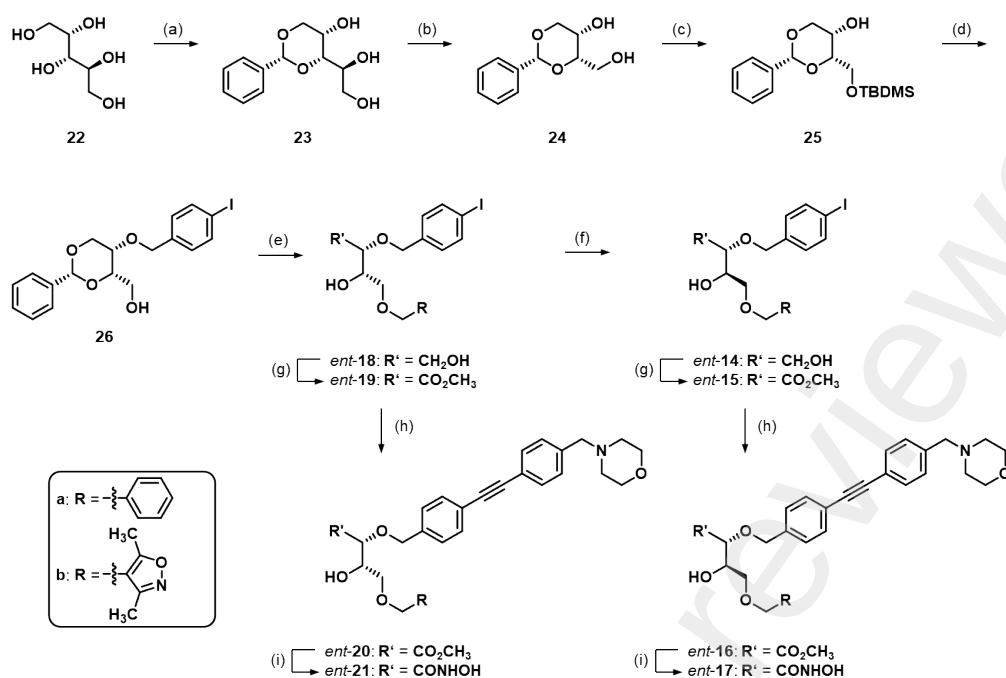
Scheme 1: Reagents and conditions: (a) 1. NaIO₄, NaHCO₃, H₂O, THF, rt, 2. NaBH₄, 0 °C → rt, 75%; (b) TBDMSCl, DMAP, NEt₃, THF, 0 °C → rt, 95%; (c) 1. NaH, 4-iodobenzyl bromide, DMF, 0 °C → rt, 2. TBAF, THF, rt, 80%; (d) 1. NaH, benzyl bromide or 4-chloromethyl-3,5-dimethylisoxazole, TBAI, DMF, 0 °C → rt, 2. *p*-TsOH, MeOH, THF, 75 °C, **14a** 76%, **14b** 61%; (e) 1. TEMPO, BAIB, ACN, H₂O, rt, 2. *p*-TsOH, MeOH, 75 °C, **15a** 54%, **15b** 47%; (f) 4-(4-ethynylbenzyl)morpholine, [Pd(PPh₃)₄], CuI, NEt₃, ACN, rt, **16a** 84%, **16b** 87%; (g) NH₂OH·HCl, NaOMe, MeOH, rt, **17a** 22%, **17b** 39%.

In order to access the respective (3*R*)-configured hydroxamic acids, the (3*S*)-configured diols **14a** and **14b** were subjected to Mitsunobu reactions with 4-nitrobenzoic acid, diisopropyl azodicarboxylate, and triphenylphosphine (Scheme 2).⁴² The thereby obtained diesters were subsequently cleaved to yield the (3*R*)-configured diols **18a** and **18b**. In principally the same way as already described for their diastereomers, diols **18a** and **18b** were transformed into the respective hydroxamic acids **21a** and **21b** via a selective oxidation and a subsequent esterification, a

Sonogashira coupling with 4-(morpholinomethyl)phenylacetylene and a final aminolysis with hydroxylamine.



Scheme 2: Reagents and conditions: (a) 1. DIAD, PPh_3 , *p*-nitrobenzoic acid, THF, 0 °C → rt, 2. K_2CO_3 , MeOH, rt, **18a** 48% or 1. DIAD, PPh_3 , *p*-nitrobenzoic acid, THF, 0 °C → rt, 2. aq. KOH, MeOH, 60 °C, **18b** 70%; (b) 1. TEMPO, NCS, aq. NaHCO_3 , ACN, 90 °C, 2. *p*-TsOH, MeOH, 75 °C, **19a** 39% or 1. TEMPO, BAIB, ACN, H_2O , rt, 2. *p*-TsOH, MeOH, 75 °C, **19b** 37%; (c) 4-(4-ethynylbenzyl)morpholine, $[\text{Pd}(\text{PPh}_3)_4]$, CuI, NEt_3 , ACN, rt, **20a** 68%, **20b** 85%; (d) aq. NH_2OH , KCN, MeOH, THF, rt, **21a** 67% or $\text{NH}_2\text{OH}\cdot\text{HCl}$, NaOMe, MeOH, 30 °C, **21b** 91%.



Scheme 3: Reagents and conditions: (a) benzaldehyde, HCl, rt, 71%; (b) 1. NaIO₄, NaHCO₃, H₂O, THF, rt, 2. NaBH₄, 0 °C → rt, 59%; (c) TBDMSCl, NEt₃, DMAP, THF, 0 °C → rt, 94%; (d) 1. NaH, 4-iodobenzyl bromide, DMF, 0 °C → rt, 2. TBAF, THF, rt, 61%; (e) 1. NaH, benzyl bromide, TBAI, THF, 0 °C → rt, 2. *p*-TsOH, MeOH, THF, 75 °C, *ent*-**18a** 68% or 1. NaH, 4-chloromethyl-3,5-dimethylisoxazole, TBAI, DMF, 0 °C → rt, 2. *p*-TsOH, MeOH, THF, 85 °C, *ent*-**18b** 90%; (f) 1. DIAD, PPh₃, *p*-nitrobenzoic acid, THF, 0 °C → rt, 2. K₂CO₃, MeOH, rt, *ent*-**14a** 55%, *ent*-**14b** 55%; (g) 1. TEMPO, NCS, aq NaHCO₃, ACN, 90 °C, 2. *p*-TsOH, MeOH, 75 °C, *ent*-**19a** 55%, *ent*-**19b** 91%, *ent*-**15a** 35%, *ent*-**15b** 60%; (h) 4-(4-ethynylbenzyl)morpholine, [Pd(PPh₃)₄], CuI, NEt₃, ACN, rt, *ent*-**20a** 64%, *ent*-**20b** 49%, *ent*-**16a** 66%, *ent*-**16b** 74%; (i) NH₂OH·HCl, NaOMe, MeOH, rt, *ent*-**21a** 58%, *ent*-**17a** 40% or aq NH₂OH, KCN, methanol, THF, rt, *ent*-**21b** 72%, *ent*-**17b** 100%.

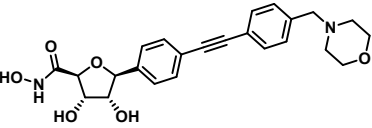
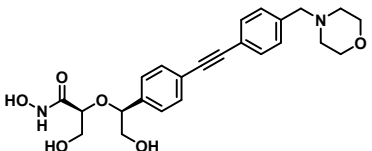
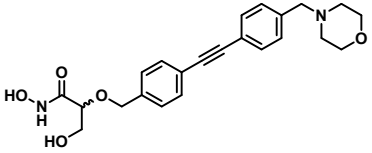
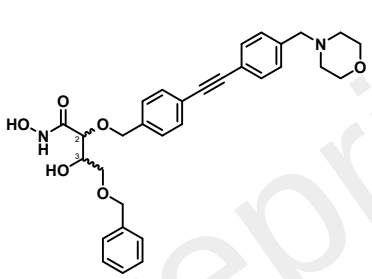
The enantiomeric hydroxamic acids exhibiting (*R*)-configuration in α-position of the hydroxamate moiety were synthesized from L-arabinitol (**22**, Scheme 3). After the acetalization of L-arabinitol (**22**) with benzaldehyde in the presence of HCl, the

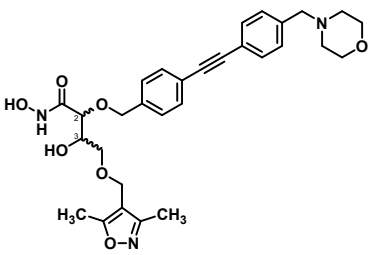
obtained 1,3-O-benzylidene derivative **23** was subjected to an oxidative cleavage with sodium periodate and a subsequent borohydride reduction, yielding L-threitol derivative **24**.⁴³⁻⁴⁴ Like its diastereomer **11**, diol **24** was selectively silylated at its primary hydroxy group to give silyl ether **25**, which was subjected to a Williamson ether synthesis with 4-iodobenzyl bromide and a subsequent desilylation affording 4-iodobenzyl ether **26**. After the establishment of a second ether moiety with benzyl bromide or 4-chloromethyl-3,5-dimethylisoxazole, the benzylidene acetal was cleaved to yield diols *ent*-**18a** and *ent*-**18b**, which were transformed into the respective (3*R*)-configured diastereomers *ent*-**14a** and *ent*-**14b** via a Mitsunobu reaction followed by an ester cleavage. Similarly to their enantiomers, the L-arabinitol-derived diols were selectively oxidized at their primary alcohol function and esterified, coupled with 4-(morpholinomethyl)phenylacetylene, and reacted with hydroxylamine to yield hydroxamic acids *ent*-**21a**, *ent*-**21b** and *ent*-**17a**, *ent*-**17b**.⁴⁵⁻⁴⁶

LpxC inhibitory and antibacterial activities

To evaluate the inhibitory activities of the newly synthesized hydroxamic acids toward LpxC, a fluorescence-based enzyme assay was performed. In this assay, *E. coli* LpxCC63A was employed, which exhibits a lower susceptibility to high Zn²⁺-concentrations compared to the wild-type enzyme.⁴⁷⁻⁴⁹ Additionally, antibacterial activities of the different hydroxamic acids were determined using cells of *E. coli* BL21(DE3) (*lpxC*⁺) and *E. coli* D22 (*lpxC101*),⁵⁰ a *lpxC* mutant showing a reduced LpxC activity. The antibacterial activities were determined as both, minimal inhibitory concentrations (MIC) in a broth dilution test and as inhibition zone diameters in a disc diffusion assay (Table 1).

Table 1: Antibacterial and LpxC inhibitory activities of reported and newly synthesized hydroxamic acids.

compound			Inhibition zone [mm]		MIC [$\mu\text{g/mL}$]		IC ₅₀ [μM]	K _i [μM]
			<i>E. coli</i> BL21(DE3)	<i>E. coli</i> D22	<i>E. coli</i> BL21(DE3)	<i>E. coli</i> D22	<i>E. coli</i> LpxCC63A	
	6³⁵	(2 <i>S</i> ,3 <i>S</i> ,4 <i>R</i> ,5 <i>S</i>)	22.3 $\pm$ 1.4	28.3 $\pm$ 1.4	8	0.5	3.2 $\pm$ 1.0	0.45 $\pm$ 0.13
	7³⁷	(<i>S</i> , <i>S</i>)	9.0 $\pm$ 0.5	20.8 $\pm$ 0.6	>64	4	2.6 $\pm$ 0.3	0.36 $\pm$ 0.04
	8³⁷	(2 <i>S</i>)	15.7 $\pm$ 0.6	25.8 $\pm$ 1.9	32	1	2.8 $\pm$ 0.5	0.39 $\pm$ 0.07
	<i>ent</i> - 8³⁷	(2 <i>R</i>)	12.3 $\pm$ 0.6	22.0 $\pm$ 1.3	64	2	1.7 $\pm$ 0.3	0.23 $\pm$ 0.04
	17a	(2 <i>S</i> ,3 <i>S</i>)	11.2 $\pm$ 0.8	19.5 $\pm$ 1.5	4	0.125	1.9 $\pm$ 1.4	0.26 $\pm$ 0.19
	21a	(2 <i>S</i> ,3 <i>R</i>)	7.7 $\pm$ 0.6	17.8 $\pm$ 0.3	>64	1	40 $\pm$ 37	5.5 $\pm$ 5.2
	<i>ent</i> - 21a	(2 <i>R</i> ,3 <i>S</i>)	8.5 $\pm$ 0.6	13.3 $\pm$ 1.7	>64	4	5.9 $\pm$ 2.0	0.81 $\pm$ 0.28
	<i>ent</i> - 17a	(2 <i>R</i> ,3 <i>R</i>)	7.7 $\pm$ 1.2	14.0 $\pm$ 1.0	>64	2	9.2 $\pm$ 0.9	1.3 $\pm$ 0.1

	17b	(2 <i>S</i> ,3 <i>S</i>)	14.9 ± 1.2	26.2 ± 1.6	16	1	6.9 ± 9.8	0.95 ± 1.4
	21b	(2 <i>S</i> ,3 <i>R</i>)	7.0 ± 0.8	20.2 ± 0.8	>64	8	110 ± 55	15 ± 7.5
	<i>ent</i> - 21b	(2 <i>R</i> ,3 <i>S</i>)	<6	19.0 ± 1.0	>64	16	27 ± 10	3.7 ± 1.4
	<i>ent</i> - 17b	(2 <i>R</i> ,3 <i>R</i>)	<6	19.7 ± 0.6	>64	8	44 ± 10	6.0 ± 1.4

In case of the previously described hydroxamic acids, the cleavage of the tetrahydrofuran ring of C-furanoside **6** only slightly affected LpxC inhibitory activity (Table 1).^{33, 35-36} However, the antibacterial activity of open chain diol **7** was found to be considerably reduced. The removal of the hydroxymethyl group in benzylic position of diol **7**, leading to glyceric acid derivative **8**, also did not strongly affect LpxC inhibitory activity, but increased antibacterial activity again.³⁷

Whereas in case of the glyceric acid-derived hydroxamic acids **8** and *ent*-**8** only a minor effect of the configuration in α -position on the antibacterial and LpxC inhibitory activities was observed,³⁷ the biological activities of the newly synthesized aldotetronic acid derivatives showed a pronounced dependency on the configuration of both stereocenters. In both series of compounds, the benzyl ethers as well as the isoxazolylmethyl ethers, concurrent trends could be observed. Whereas the (2*S*,3*S*)-configured stereoisomers **17a** and **17b** were found to exhibit the highest LpxC inhibitory and antibacterial activities of the respective series of ethers, particularly the inversion of configuration in β -position was detrimental to the inhibitory activity of the compounds. Thus, although showing promising antibacterial activities against *E. coli* D22, the (2*S*,3*R*)-configured stereoisomers **21a** and **21b** were found to be the least potent LpxC inhibitors, exhibiting more than 15-fold higher K_i -values compared to their (2*S*,3*S*)-configured diastereomers. The (2*R*)-configured stereoisomers showed intermediate inhibitory activities, with the (2*R*,3*S*)-configured compounds *ent*-**21a** and *ent*-**21b** being slightly more potent LpxC inhibitors than their (2*R*,3*R*)-configured diastereomers *ent*-**17a** and *ent*-**17b**.

When comparing the isoxazolylmethyl ethers with the respective benzyl ethers, the latter ones were found to exhibit higher inhibitory activities for the isolated enzyme *in vitro*. In agreement with this observation, the isoxazolylmethyl ethers show a higher

MIC for *E. coli* BL21(DE3) and *E. coli* D22 compared to the benzyl ethers, evidencing a higher antibacterial activity of the latter. The results of the disc diffusion assays, however, are contradictory as the isoxazolymethyl ethers cause larger zones of inhibition, indicating a higher antibacterial activity than the respective benzyl ethers. This might be due to different solubilities and diffusion rates in the agar medium.

The (2*S*,3*S*)-configured benzyl ether **17a**, the most potent LpxC inhibitor of the newly synthesized aldotetronic acid derivatives, showed the same LpxC inhibitory activity as glyceric acid derivative *ent*-**8** and was found to be a slightly more potent inhibitor than C-furanoside **6**. When comparing the MIC values against *E. coli* BL21(DE3) and *E. coli* D22, benzyl ether **17a** proved to exhibit higher antibacterial activity than the latter two compounds as well as the other synthesized aldotetronic acid derivatives.

Molecular-docking studies

To elucidate the structure–activity relationship of the compounds synthesized, their molecular docking into the *E. coli* LpxC structure (PDB ID: 3P3G) was performed.²⁶ A previously evaluated LpxC docking protocol was used for the novel developed compounds.^{35, 37} All compounds showed a similar binding mode, which resembled the commonly observed binding mode of known LpxC inhibitors, such as LPC-011 (**3a**, Figure 2) and CHIR-090. Generally, the hydroxamic acid part of all inhibitors synthesized chelates the Zn²⁺-ion in bidentate fashion. The hydroxamic acid group exhibits hydrogen bond interactions with the side chains of E78 and T191, respectively. The lipophilic distal parts of the compounds are located in the hydrophobic tunnel comprised of L18, F192, M195, I198, C207, A215, F212, and V217. The morpholine group is solvent-exposed at the exit of the tunnel. The substituents introduced at the β -carbon atom of the hydroxamic acid occupy the UDP-pocket of LpxC.

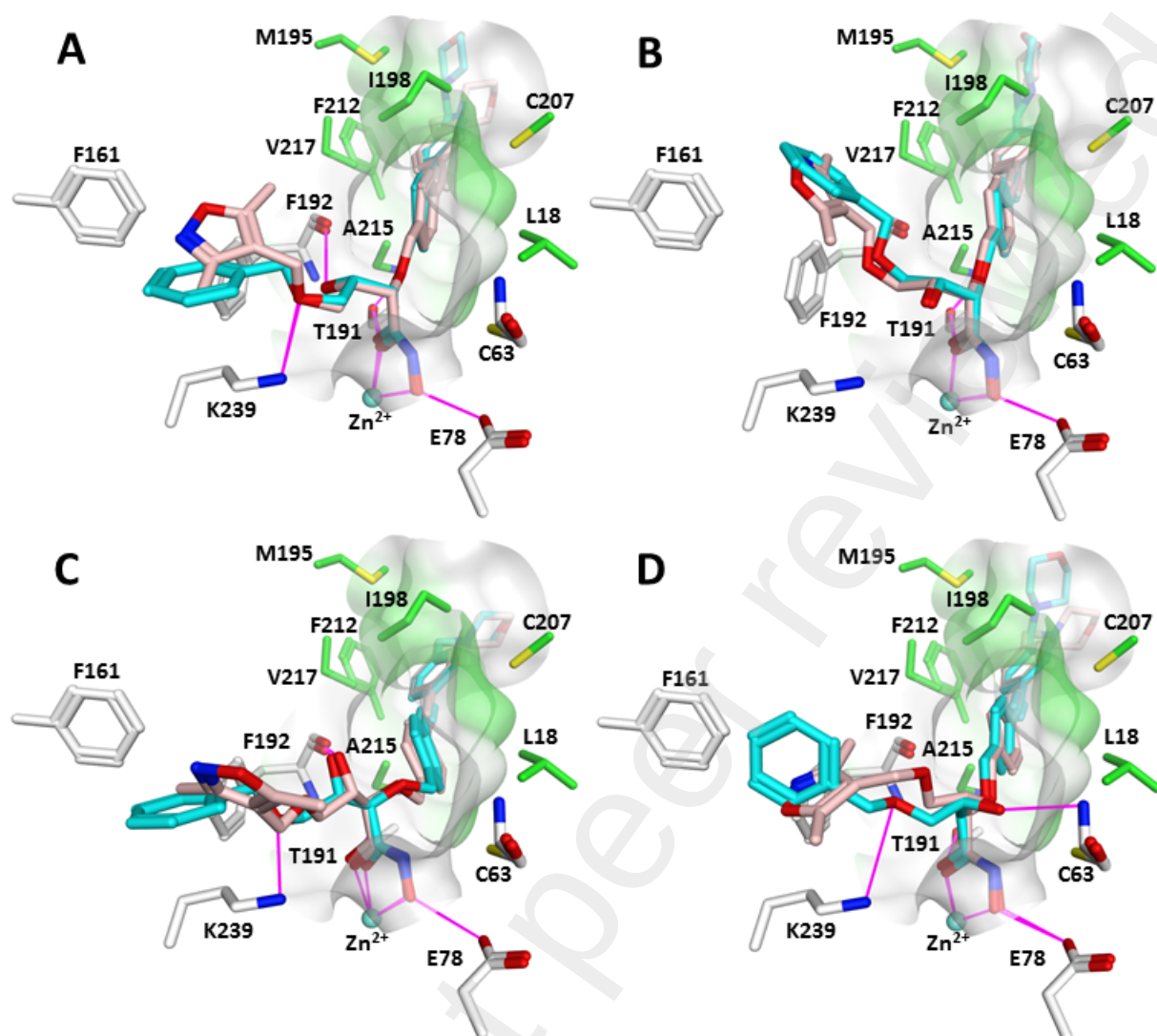


Figure 4: Docking poses in *E. coli* LpxC (PDB ID: 3P3G): A) **17a** (cyan) – **17b** (light pink), B) **21a** (cyan) – **21b** (light pink), C) *ent*-**21a** (cyan) – *ent*-**21b** (light pink), D) *ent*-**17a** (cyan) – *ent*-**17b** (light pink). The side chains of amino acid residues are shown in stick representation with green carbon atoms for the hydrophobic tunnel and white carbon atoms for the hydrophilic pocket. The surface of the binding pocket is shown in green for hydrophobic parts and white for other parts. The Zn²⁺-ion is shown as a cyan sphere. Hydrogen bonds and metal ion coordination are shown as magenta colored lines.

The (2*S*,3*S*)-configured derivatives having the most favorable LpxC inhibitory activities, **17a** and **17b**, showed similar docking poses in LpxC (Figure 4A). The hydroxy group in β -position approaches F192 and is engaged in a hydrogen-bond interaction with the backbone of this residue. The benzyl and isoxazolylmethyl residues are able to make π - π interactions with F161 (like the uridine moiety of **2**) and F192 of the UDP pocket. Additionally, the oxygen of the ether group in γ -position makes a hydrogen bond interaction with K239 in both cases. Due to the observed high flexibility of K239, for instance, in the crystal structure of LpxC with the catalytic reaction product **2** (Figure 1, PDB ID 4MDT), it is also possible that this residue is involved in cation- π interactions with the distal aromatic rings of **17a** and **17b**.

In case of the (2*S*,3*R*)-configured derivatives **21a** and **21b** (Figure 4B), the inversion of the configuration at the β -carbon atom of **17a** and **17b** resulted in a significant loss of LpxC activity. The hydroxy group in β -position is turned out of the pocket towards the solvent. Moreover, the benzyl and isoxazolylmethyl groups are shifted away from the UDP pocket into the solvent and the hydrogen-bond interaction between the ether oxygen atom and the K239 side chain is lost, leading to a significant decrease in LpxC activity in case of **21a** and **21b**.

The docking poses of the (2*R*,3*S*)-configured hydroxamic acids *ent*-**21a** and *ent*-**21b** (Figure 4C) were compared with the ones of their (2*S*,3*S*)-configured diastereomers **17a** and **17b**. In case of *ent*-**21a** and *ent*-**21b**, the hydroxy group approaches the F192 backbone and exhibits a hydrogen-bond interaction with this residue, resembling the binding mode of **17a** and **17b**. The benzyl ether part of *ent*-**21a** exhibits a hydrogen bond with K239 as well as π - π interactions with F161 and F192 like **17a**. Meanwhile, these interactions are lost in *ent*-**21b** due to the less favorable orientation of the

isoxazolylmethyl ether group. This explains the better inhibitory activity of *ent*-**21a** in comparison to *ent*-**21b**.

The obtained docking poses of the (2*R*,3*R*)-configured derivatives *ent*-**17a** and *ent*-**17b** (Figure 4D) revealed that the hydroxy group makes a direct hydrogen-bond interaction with the backbone nitrogen atom of C63. This residue, which is opposite to F192 in the active site pocket of LpxC, is interacting with some of the active compounds including **17a**, **17b**, *ent*-**21a** and *ent*-**21b**. The interactions of the benzyl ether and isoxazolylmethyl ether groups of *ent*-**17a** and *ent*-**17b** follow the same trend as observed in case of *ent*-**21a** and *ent*-**21b**. Namely, *ent*-**17a** preserves interactions with F161, F192, and K239, while *ent*-**17b** does not make these interactions (Figure 4D).

Further biological evaluation

- *Antibacterial activities against clinically relevant Gram-negative bacteria*

Being the most potent LpxC inhibitor of the investigated aldotetronic acid derivatives and having shown promising antibacterial activity when tested against the common laboratory *E. coli* strains *E. coli* BL21(DE3) and *E. coli* D22, the antibacterial activity of benzyl ether **17a** was further characterized by testing the compound against a series of Gram-negative wild type strains representing clinically relevant species, including *Enterobacterales* and *P. aeruginosa* (Table 2). C-Furanoside **6**, the most potent stereoisomer of a previously synthesized series of C-glycosidic hydroxamic acids, was tested against the same strains for comparison.

17a exhibited activity against a panel of *E. coli* reference strains, substantiating the finding obtained with *E. coli* laboratory strains. Findings for other *Enterobacterales* were variable, with consistent activity only against *C. freundii*. **17a** was also able to inhibit *P. aeruginosa* growth, at least to some extent. In comparison, C-furanoside **6** was also found to inhibit growth of *E. coli* and *C. freundii*, but in addition, inhibition zones were also recorded for *P. mirabilis* and *K. aerogenes*. *P. aeruginosa* showed weak susceptibility against C-furanoside **6**. Of notice, both compounds had no activity against *K. pneumoniae* ATCC 700603.

Table 2: Antibacterial activity of hydroxamic acids **6** and **17a** against clinically relevant species.

strain	compound	6	17a
		inhibition zone [mm]	
<i>E. coli</i> ATCC 25922		18.0	15.0
<i>E. coli</i> ATCC 35218		18.0	15.0
<i>E. coli</i> ATCC 8739		19.0	18.0
<i>E. coli</i> NCTC 13476		16.0	14.0
<i>K. pneumoniae</i> NCTC 23442		9.0	7.0
<i>K. pneumoniae</i> ATCC 700603		<6	<6
<i>K. aerogenes</i> ATCC 13048		10.0	<6
<i>P. mirabilis</i> ATCC 12453		8.0	<6
<i>C. freundii</i> ATCC 43864		19.0	18.0
<i>P. aeruginosa</i> ATCC 9027		7.0	12.0
<i>P. aeruginosa</i> ATCC 27853		7.0	7.0

- Cellular uptake and efflux

To investigate whether the improved antibacterial potency of **17a** was due to an increased uptake into the cell and particular into the cytoplasm, the compartment where LpxC is located, its quantity in the cytoplasm, periplasm, and membrane fractions of *E. coli* (BW25113) and the corresponding $\Delta toI/C$ strain (JW5503-1) was determined by a combination of cellular fractionation with liquid chromatography-coupled to mass spectrometry.⁵¹

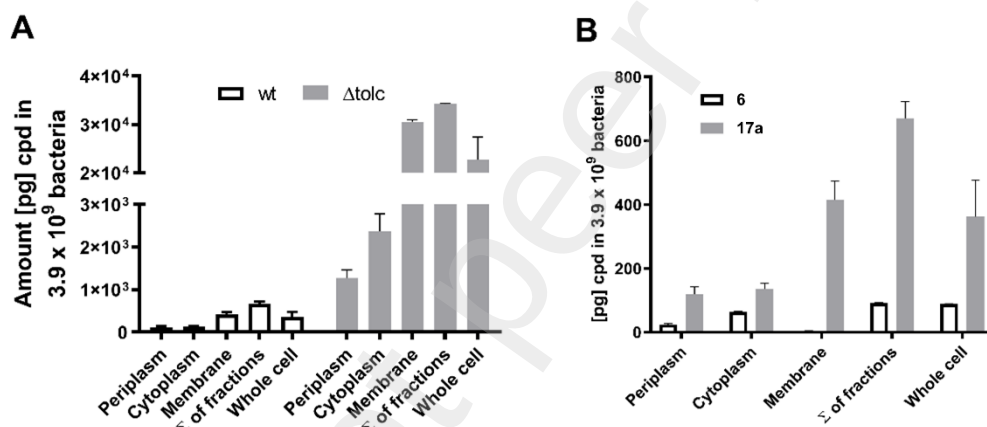


Figure 5: Accumulation of **17a** in *E. coli*. A: The quantities of **17a**, expressed in pg per 3.9×10^9 bacteria, in the periplasm, cytoplasm, and the membranes of two *E. coli* strains (BW25113 and its isogenic $\Delta toI/C$ mutant), following a 10 min exposure of 100 ng/mL, are depicted. B: Uptake comparison of C-furanoside **6** and **17a** in *E. coli* BW25113 strains.

The majority of **17a** was found in membrane fractions (61% for the wild-type strain and 89% for the $\Delta toI/C$ strain). Notably, a 15-fold higher quantity was found in the efflux-deficient strain, pointing out that the compound is prone to efflux (Figure 5 A). However,

compared to C-furanoside **6**, a much higher amount of **17a** accumulated in *E. coli* (BW25113) (Figure 5B). The cytosolic amount of **17a** was slightly higher compared to **6** after a short incubation of 10 min; this finding is in line with the improved antimicrobial activity.

In contrast to C-furanoside **6**, which was primarily located intracellularly, the more hydrophobic compound **17a** showed a very high membrane association. Moreover, it was found to be effluxed more efficiently *via* TolC in comparison to **6**, which showed a 6-fold reduction in the cytoplasmic values for the wild-type *E. coli* strain compared to the Δ *tolC* strain in a previous study.³⁴

As efflux pump systems are substantially contributing to the intrinsic resistance of Gram-negative bacteria to various antibacterial compounds, it was further investigated, whether **17a** is a substrate of multidrug resistance (MDR) efflux pump systems (Table 3). Therefore, the MIC values of **17a** against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were determined in the absence and the presence of phenylalanine-arginine- β -naphthylamide (PA β N), an inhibitor of efflux pumps belonging to the resistance-nodulation-division (RND) type (such as AcrAB-TolC in *E. coli* and MexCD-OprJ in *P. aeruginosa*). In both cases, in the presence of PA β N a pronounced decrease in the MIC of **17a** (more than four serial dilution steps) was observed, additionally confirming that **17a** is prone to efflux in *E. coli* as well as *P. aeruginosa*. This is in contrast to C-furanoside **6**, for which in the presence of PA β N no change in the MIC value against *E. coli* ATCC 25922 and only a reduction by three serial dilution steps against *P. aeruginosa* ATCC 27853 had been observed in a previous study.³⁴

Table 3: MIC values of **17a** in $\mu\text{g} \cdot \text{mL}^{-1}$ against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 in the absence (-) and presence (+) of the efflux pump inhibitor PA β N (64 $\mu\text{g} \cdot \text{mL}^{-1}$). * Δ MIC indicates the decrease in the MIC value in serial dilutions steps.

strain	17a MIC [$\mu\text{g} \cdot \text{mL}^{-1}$] (Δ MIC)*	6 ³⁴
<i>E. coli</i> ATCC 25922 (-PA β N)	32	4
<i>E. coli</i> ATCC 25922 (+PA β N)	1 (5)	4 (0)
<i>P. aeruginosa</i> ATCC 27853 (-PA β N)	>64	16
<i>P. aeruginosa</i> ATCC 27853 (+PA β N)	4 (>4)	2 (3)

- *Inhibition of the virulence factor LasB from Pseudomonas aeruginosa*

As shown above, hydroxamic acid **17a** exhibits antibacterial activity against *P. aeruginosa*, which can be attributed to its inhibitory activity toward *P. aeruginosa* LpxC. Other strategies aiming to combat *P. aeruginosa*, which is among the three most critical pathogens on the “WHO priority list for research and development of new antibiotics for antibiotic-resistant bacteria”,¹ target bacterial virulence factors like the extracellular elastase B (LasB).⁵²⁻⁵⁵ LasB is a secreted zinc metalloprotease, which plays a crucial role in host invasion, by degrading components of the connective tissue like elastin or collagen,⁵⁶⁻⁵⁷ biofilm formation,⁵⁸⁻⁵⁹ and immune evasion, by cleaving IgG, cytokines, and complement factor C3,⁶⁰⁻⁶² amongst others. Due to the important role of LasB in the infection process, pathoblockers inhibiting this major virulence factor of *P. aeruginosa* have been developed,^{52-53, 55, 63} among which hydroxamic acids can be found.⁵⁴ Therefore, the inhibitory activity of the newly synthesized hydroxamic acids against the zinc-containing enzyme LasB was evaluated (Table 4).

Table 4: *In vitro* activities of the newly synthesized hydroxamic acids against LasB.

compound	Inhibition at 20 μ M [%]	IC ₅₀ [μ M]
17a	61.0 $\pm$ 0.6	17.7 $\pm$ 0.8
17b	8.6 $\pm$ 3.3	-
21a	64.9 $\pm$ 3.2	14.8 $\pm$ 0.4
21b	13.6 $\pm$ 3.9	-
<i>ent</i> - 21a	-0.6 $\pm$ 1.3	-
<i>ent</i> - 21b	-1.8 $\pm$ 4.6	-
<i>ent</i> - 17a	4.3 $\pm$ 3.3	-
<i>ent</i> - 17b	0.6 $\pm$ 4.5	-

Among the investigated hydroxamic acids, the (2*S*)-configured benzyl ethers **17a** and **21a** were found to be the most potent LasB inhibitors. The replacement of the benzyl group by a (3,5-dimethylisoxazol-4-yl)methyl substituent as well as the inversion of configuration in position 2 were found to be detrimental for LasB inhibition. With IC₅₀ values of 17.7 μ M and 14.8 μ M, respectively, aldotetronic acid derivatives **17a** and **21a** represent promising starting points for further optimization.

Summary and conclusions

A series of aldotetronic acid-based LpxC inhibitors exhibiting a substituent to address the UDP binding site of the enzyme were obtained in chiral pool syntheses. Whereas the (2*S*)-configured hydroxamic acids were synthesized from 4,6-*O*-benzylidene-D-glucose, their (2*R*)-configured enantiomers were accessed from L-arabinitol. To vary the configuration in position 3, Mitsunobu reactions were employed.

The evaluation of the LpxC inhibitory activity of the newly synthesized aldotetronic acid derivatives revealed the benzyl ethers to inhibit the enzymatic activity LpxC more potently than the respective isoxazolylmethyl ethers. Additionally, a pronounced influence of the configuration of both stereocenters on the compounds' LpxC inhibitory activity was observed, with the (2*S*,3*S*)-configured stereoisomers **17a** and **17b** being the most potent LpxC inhibitors of the respective series of ethers. Particularly, (3*R*)-configuration was detrimental to the inhibitory activity of the hydroxamic acids. These findings are in contrast to the stereochemical requirements previously observed for threonine-based LpxC inhibitors like LPC-011, for which the absolute configuration in β -position was found to be less critical than the configuration in α -position.²⁶ The higher sensitivity to the configuration of the α -carbon atom in the LPC compounds published by Liang *et al.* probably occurs due to more rigid structure of these inhibitors. The presence of the amide linker increases the planarity of the molecule and forces it in a certain orientation by the change of the chirality at the α -carbon atom. As shown by the structural models generated by the authors, the change of configuration at the α -carbon atom from (2*S*) to (2*R*) induces clashes with protein residues. Meanwhile, the ether linker of our compounds is more flexible and leaves opportunities to find a suitable orientation of the ligand and interaction partners. On the other hand, rigidity of the molecule is beneficial due to entropic win if the configuration is suitable.

Even though the LpxC inhibitory activity of benzyl ether **17a** only slightly exceeded the one of lead compound **8**, the observation that the introduction of a substituent at the β -carbon atom of glyceric acid **8** did not diminish inhibitory activity shows the feasibility of our approach to grow this inhibitor into the UDP binding site of LpxC.

Molecular docking studies were performed to rationalize the observed LpxC inhibitory activities of the newly synthesized aldotetronic acid derivatives. The docking pose of **17a** revealed its hydroxy group in β -position to be engaged in a hydrogen-bond interaction with the backbone of F192, its benzyl residues in π - π interactions with F161 and F192 of the UDP pocket, and the oxygen of the ether group in γ -position in a hydrogen bond interaction with K239. As a result of the molecular-docking study, we observed that the aromatic interactions with F161 and F192 as well as the hydrogen-bond interactions with F192 and K239 in the UDP pocket seem favorable for LpxC inhibitory activity. Particularly, the loss of the K239 interaction in the docking experiments corresponded to a significant decrease in the inhibitory activity.

When determining the antibacterial activities of the newly synthesized hydroxamic acids against *E. coli* BL21(DE3) and the defective *E. coli* D22 strain, the most potent LpxC inhibitor **17a** exhibited the lowest MIC values of all aldotetronic acid derivatives and also showed higher antibacterial activity than lead compounds **6**, **7**, and **8**. In disc diffusion assays, **17a** also exhibited promising antibacterial activity against a panel of *E. coli* reference strains, *C. freundii* ATCC 43864, and *P. aeruginosa* ATCC 9027.

To test whether hydroxamic acid **17a** is able to cross the bacterial cell wall, thus reaching its cytoplasmic target, its concentration in subcellular compartments of *E. coli* (BW25113) was determined. Even though the majority of **17a** was found in membrane fractions, the amount of **17a** found in the cytoplasm was higher compared to the more polar C-furanoside **6**, which is in agreement with the higher antimicrobial activity of the

aldotetronic acid derivative. When investigating the accumulation of **17a** in the corresponding $\Delta toI/C$ strain (JW5503-1), a 15-fold higher quantity of the compound was found, indicating that benzyl ether **17a** is prone to efflux. This observation was confirmed by antimicrobial susceptibility tests against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, in which the presence of the efflux pump inhibitor Pa β N caused a pronounced decrease in the MIC of **17a**. Taken together these data suggest that a reduction of the propensity of **17a** to efflux by structural optimization might lead to compounds with even higher antibacterial potency.

Finally, the newly synthesized aldotetronic acid derivatives were tested against another Zn²⁺-dependent enzyme, the virulence factor LasB from *P. aeruginosa*, revealing the (2*S*)-configured benzyl ethers **17a** and **21a** as the most potent LasB inhibitors of this series of compounds. With an IC₅₀ value of 17.7 μ M, aldotetronic acid derivative **17a** represents a promising starting point for further optimization steps, which might possibly lead to dual LpxC/LasB inhibitors, being able to simultaneously attack *P. aeruginosa* from the inside (LpxC) and the outside (LasB).

Experimental Section

Chemistry, general

All experiments involving water- or air-sensitive compounds were carried out under anhydrous conditions (N_2 atmosphere). Reagents were purchased from various suppliers and were used without further purification, unless otherwise noted. Anhydrous solvents were purchased from Acros Organics (extra dry over molecular sieves). Solvents for flash column chromatography were purchased in technical grade and distilled prior to use. Ultrapure water for reversed-phase chromatography was purified using a Sartorius arium[®] pro system (Sartopore 0.2 μm , UV). ACN for reversed-phase chromatography was purchased from VWR (HPLC grade). Flash column chromatography on silica gel was performed using Macherey Nagel silica gel 60 M (0.040–0.063 mm). Parentheses include diameter of the column, fraction size, eluent and R_f value. Thin-layer chromatography was performed on Macherey Nagel precoated TLC sheets (ALUGRAM[®] Xtra SIL G/UV₂₅₄). Visualization was achieved by heat-staining using a cerium molybdate dipping bath [$Ce(SO_4)_2$ (1.8 g), $(NH_4)_6Mo_7O_{24} \times 4 H_2O$ (45 g), conc. H_2SO_4 (45 g), H_2O (900 mL)]. Automatic reversed-phase flash column chromatography was performed using Biotage[®] SNAP KP-C18-HS 12 g columns on an Isolera[™] One (Biotage[®]). Product-containing fractions were lyophilized using a Christ Alpha 2-4 LDplus freeze-dryer. Melting points were measured with a Büchi Melting Point M-565 and are uncorrected. Optical rotation α [deg] was determined with a P8000 polarimeter (A. Krüss Optronic GmbH); path length 1 dm, wavelength 589 nm (sodium D line); the unit of the specific rotation $[\alpha]_D^{20}$ [deg $\cdot$ mL $\cdot$ dm⁻¹ $\cdot$ g⁻¹] is omitted; the concentration of the sample c [mg $\cdot$ mL⁻¹] and the solvent used are given in brackets. IR spectra were recorded on a Bruker Alpha FT-IR Platinum ATR spectro-photometer. NMR solvents were purchased from Eurisotop (CD_3OD) and

Deutero (DMSO- d_6). NMR-spectra were recorded at room temperature on Bruker Avance I 400, DRX 500 and Avance III 600 instruments. High-resolution mass spectrometry was performed using an Agilent 6224 ESI-TOF instrument. HPLC methods for the determination of product purity: Method 1: VWR Hitachi equipment; UV/VIS detector: 5420; autosampler: 5260; pump: 5160; column: LiChrospher® 60 RP-select B (5 μ m); LiChroCART® 250-4 mm cartridge. flow rate: 1.00 mL/min; injection volume: 5.0 μ L; detection at λ = 210 nm for 30 min; solvents: A: water with 0.05% (V/V) trifluoroacetic acid; B: acetonitrile with 0.05% (V/V) trifluoroacetic acid: gradient elution: (A%): 0 – 4 min: 90% , 4 – 29 min: gradient from 90% to 0%, 29 – 31 min: 0%, 31 – 31.5 min: gradient from 0% to 90%, 31.5 – 40 min: 90%. Method 2: VWR Hitachi equipment; UV/VIS detector: 5420; autosampler: 5260; pump: 5160; column: phenomenex Gemini® 5 μ m C6-Phenyl 110 Å; LC Column 250 $\times$ 4.6 mm; flow rate: 1.00 mL/min; injection volume: 5.0 μ L; detection at λ = 254 nm for 20 min; solvents: A: acetonitrile : 10 mM ammonium formate = 10 : 90 with 0.1% formic acid; B: acetonitrile : 10 mM ammonium formate = 90 : 10 with 0.1% formic acid; gradient elution: (A%): 0 – 5 min: 100% , 5 – 12 min: gradient from 100% to 0%, 12 – 20 min: 0%, 20 – 22 min: gradient from 0% to 100%, 22 – 30 min: 100%. For all methods, data were collected and evaluated by Chromaster software.

(2*R*,4*S*,5*R*)-4-(Hydroxymethyl)-2-phenyl-1,3-dioxan-5-ol (11)

Sodium bicarbonate (3.8 g, 46 mmol) and sodium metaperiodate (7.0 g, 33 mmol) were added to a solution of 4,6-*O*-benzylidene-D-glucose (5.2 g, 19 mmol) in water (140 mL) and THF (60 mL) and the resulting solution was stirred for 2 h at ambient temperature, whereby a colourless precipitate formed. After cooling to 0 °C, sodium borohydride (1.2 g, 33 mmol) was added and the mixture was stirred for additional 45 min at 0 °C and

for 45 min at ambient temperature. The precipitate was filtered off and the filtrate was extracted with ethyl acetate (3× 150 mL). The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate (150 mL) and a saturated aqueous solution of sodium thiosulfate (100 mL), dried over sodium sulfate, and filtered. Evaporation of the solvent *in vacuo* gave **11** (3.0 g, 14 mmol, 75%) as colourless solid. TLC (ethyl acetate): R_f = 0.45; m.p. = 134 °C; $[\alpha]_D^{20}$ = -95.6 (2.9, methanol); ^1H NMR (400 MHz, DMSO- d_6): δ [ppm] = 3.41 – 3.59 (m, 4H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$ (1H), $\text{CH}_2\text{CHCHCH}_2\text{OH}$, $\text{CH}_2\text{CHCHCH}_2\text{OH}$, $\text{CH}_2\text{CHCHCH}_2\text{OH}$ (1H)), 3.69 – 3.79 (m, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 4.04 – 4.14 (m, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 4.64 (t, J = 5.5 Hz, 1H, CH_2OH), 5.16 (d, J = 4.6 Hz, 1H, $\text{CH}_2\text{CHOHCHCH}_2\text{OH}$), 5.50 (s, 1H, PhCH), 7.31 – 7.39 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.41 – 7.48 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = 60.8 (2C, $\text{CH}_2\text{CHCHCH}_2\text{OH}$, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 70.8 (1C, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 83.1 (1C, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 100.1 (1C, PhCH), 126.3 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.9 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 128.6 (1C, C-4'_{phenyl}), 138.3 (1C, C-1'_{phenyl}); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3247, 2971, 2943, 2930, 2868, 2752, 1452, 1426, 1389, 1379, 1335, 1312, 1305, 1270, 1245, 1220, 1188, 1140, 1081, 1065, 1018, 988, 976, 941, 901, 854, 752, 695, 640, 531, 462, 446, 405; HRMS (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{11}\text{H}_{14}\text{NaO}_4$: 233.0784, found: 233.0780; HPLC (method 1): t_R = 13.2 min, purity 99.5%.

(2R,4S,5R)-4-([(tert-Butyldimethylsilyl)oxy]methyl)12-2-phenyl-1,3-dioxan-5-ol
(12)

Under nitrogen atmosphere, triethylamine (0.73 mL, 0.53 g, 5.2 mmol) was added to an ice-cooled solution of **11** (3.0 g, 14 mmol) in dry THF (40 mL). After 5 min, *tert*-butyldimethylsilyl chloride (2.7 g, 18 mmol) and DMAP (88 mg, 0.72 mmol) were added

and the solution was stirred at 0 °C for 1 h and at ambient temperature for 6 h. After adding additional triethylamine (1.7 mL, 1.3 g, 13 mmol) the mixture was stirred for additional 12 h at ambient temperature. Then ice-cold water (40 mL) was added and the mixture was extracted with ethyl acetate (3× 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 20 cm, V = 20 mL, petroleum ether/ethyl acetate = 4/1, R_f = 0.61) to give **12** (4.3 g, 13 mmol, 95%) as colourless oil. $[\alpha]_D^{20}$ = -42.3 (3.1, methanol); ^1H NMR (500 MHz, DMSO- d_6): δ [ppm] = 0.02 (s, 3H, Si(CH₃)₂), 0.03 (s, 3H, Si(CH₃)₂), 0.86 (s, 9H, C(CH₃)₃), 3.45 – 3.53 (m, 2H, CH₂CHCHCH₂OSi, CH₂CHCHCH₂OSi (1H)), 3.54 – 3.59 (m, 1H, CH₂CHCHCH₂OSi), 3.77 (dd, J = 11.6/5.6 Hz, 1H, CH₂CHCHCH₂OSi), 3.93 (dd, J = 11.6/1.7 Hz, 1H, CH₂CHCHCH₂OSi), 4.05 – 4.13 (m, 1H, CH₂CHCHCH₂OSi), 5.18 (d, J = 5.3 Hz, 1H, OH), 5.50 (s, 1H, PhCH), 7.31 – 7.38 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.38 – 7.44 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = -5.2 (1C, Si(CH₃)₂), -5.1 (1C, Si(CH₃)₂), 18.1 (1C, C(CH₃)₃), 25.8 (3C, C(CH₃)₃), 60.4 (1C, CHOH), 62.7 (1C, CH₂OSi), 70.7 (1C, CH₂CHOH), 82.7 (1C, CHCH₂OSi), 100.0 (1C, PhCH), 126.1 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.9 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 128.6 (1C, C-4'_{phenyl}), 138.2 (1C, C-1'_{phenyl}); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3444, 3037, 2953, 2928, 2856, 1596, 1462, 1391, 1360, 1309, 1291, 1253, 1215, 1173, 1154, 1085, 1027, 974, 943, 912, 877, 832, 814, 778, 752, 697, 674, 652, 612, 550, 505, 474; HRMS (m/z): [M+Na]⁺ calcd for C₁₇H₂₈NaO₄Si: 347.1649, found: 347.1647; HPLC (method 1): t_R = 25.7 min, purity 98.5%.

{(2*R*,4*S*,5*R*)-5-[(4-Iodobenzyl)oxy]-2-phenyl-1,3-dioxan-4-yl}methanol (13)

Sodium hydride (60% in mineral oil, 0.35 g, 8.8 mmol) was added to a solution of **12** (1.8 g, 5.4 mmol) in DMF (30 mL) at 0 °C and the mixture was stirred for 40 min at 0 °C. Then a solution of 4-iodobenzyl bromide (2.6 g, 8.7 mmol) in DMF (3 mL) was added dropwise to the reaction mixture over a period of 5 min at 0 °C. After stirring at 0 °C for 30 min, the mixture was stirred for 1.5 h at ambient temperature. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography ($\varnothing$ = 4 cm, h = 15 cm, V = 15 mL, cyclohexane/ethyl acetate = 10/1). The fractions containing the intermediate benzyl ether (R_f = 0.80) were evaporated and the residue was dissolved in THF (30 mL). Tetrabutylammonium fluoride trihydrate (3.8 g, 12 mmol) was dissolved in THF (30 mL) and the reaction mixture was stirred for 1.5 h at ambient temperature. After the addition of water (30 mL), the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified twice by flash column chromatography ($\varnothing$ = 4 cm, h = 15 cm, V = 15 mL, cyclohexane/ethyl acetate = 8/2, R_f = 0.21) to give **13** (1.8 g, 4.3 mmol, 80%) as colourless solid. m.p. = 128 °C; $[\alpha]_D^{20}$ = -25.0 (3.0, CH₂Cl₂); ¹H NMR (400 MHz, DMSO-d₆): δ [ppm] = 3.50 – 3.62 (m, 3H, CH₂CHCHCH₂OH (1H), CH₂CHCHCH₂OH (1H), CH₂CHCHCH₂OH), 3.61 – 3.75 (m, 2H, CH₂CHCHCH₂OH (1H), CH₂CHCHCH₂OH), 4.28 – 4.39 (m, 1H, CH₂CHCHCH₂OH), 4.52 (d, J = 12.1 Hz, 1H, OCH₂Ar), 4.58 (d, J = 12.1 Hz, 1H, OCH₂Ar), 4.76 (t, J = 5.8 Hz, 1H, OH), 5.54 (s, 1H, PhCH), 7.13 – 7.20 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.32 – 7.40 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.40 – 7.47 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}), 7.70 – 7.76 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ¹³C NMR (101 MHz, DMSO-d₆): δ [ppm] = 60.6 (1C, CH₂CHCHCH₂OH), 68.3 (1C, CH₂CHCHCH₂OH), 68.4 (1C, CH₂CHCHCH₂OH), 70.5

(1C, OCH₂Ar), 81.2 (1C, CH₂CHCHCH₂OH), 93.6 (1C, C-4''₄-iodophenyl), 100.1 (1C, PhCH), 126.3 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.9 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 128.7 (1C, C-4'_{phenyl}), 129.9 (2C, C-2''₄-iodophenyl, C-6''₄-iodophenyl), 137.0 (2C, C-3''₄-iodophenyl, C-5''₄-iodophenyl), 138.0 (1 C, C-1'_{phenyl}), 138.1 (1C, C-1''₄-iodophenyl); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3314, 2955, 2870, 1377, 1099, 1026, 1007, 980, 799, 745, 691; HRMS (*m/z*): [M+H]⁺ calcd for C₁₈H₂₀IO₄: 427.0401, found: 427.0379; HPLC (method 1): *t*_R = 23.2 min, purity 99.3%.

(2*R*,3*S*)-4-(Benzyloxy)-2-[(4-iodobenzyl)oxy]butane-1,3-diol (14a)

Sodium hydride (60% in mineral oil, 130 mg, 3.3 mmol) was added to a solution of **13** (890 mg, 2.1 mmol) in DMF (10 mL) at 0 °C and the mixture was stirred for 40 min. Then a solution of benzyl bromide (0.40 mL, 570 mg, 3.3 mmol) in DMF (1 mL) was added dropwise over 5 min at 0 °C. After the addition of tetrabutylammonium iodide (9 mg, 0.02 mmol) the mixture was stirred for 2 h at ambient temperature. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The obtained crude product was dissolved in a mixture of methanol (10 mL) and THF (0.1 mL). *p*-Toluenesulfonic acid (13 mg, 0.07 mmol) was added and the mixture was stirred for 5.5 h at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. Purification of the residue by flash column chromatography (Ø = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 1/1, *R*_f = 0.26) gave **14a** (670 mg, 1.6

mmol, 76%) as colorless oil. $[\alpha]_D^{20} = -23.1$ (3.5, methanol); HPLC (method 2): $t_R = 17.1$ min, purity 97.7%.

(2S,3R)-4-(Benzyloxy)-2-[(4-iodobenzyl)oxy]butane-1,3-diol (*ent*-14a)

Under nitrogen atmosphere, triphenylphosphine (1.5 g, 5.7 mmol), *p*-nitrobenzoic acid (0.96 g, 5.7 mmol), and *ent*-18a (410 mg, 0.96 mmol) were dissolved in dry THF (20 mL). Diisopropyl azodicarboxylate (1.1 mL, 1.2 g, 5.7 mmol) was added dropwise at 0 °C over a period of 20 min. After stirring the mixture at ambient temperature for 3 d, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography ($\varnothing = 4$ cm, $h = 15$ cm, $V = 20$ mL, petroleum ether/ethyl acetate = 2/1). The fractions containing the intermediately formed diester were combined and the solvent was removed *in vacuo*. The residue was dissolved in methanol (7 mL), potassium carbonate (0.53 g, 3.8 mmol) was added, and the mixture was stirred at ambient temperature for 1 d. Then water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography ($\varnothing = 4$ cm, $h = 15$ cm, $V = 20$ mL, petroleum ether/ethyl acetate = 1/1, $R_f = 0.29$) to give *ent*-14a (0.23 g, 0.53 mmol, 55%) as yellow oil. $[\alpha]_D^{20} = +15.2$ (2.3, methanol); HPLC (method 1): $t_R = 21.7$ min, purity 99.7%.

Spectroscopic data of 14a and *ent*-14a:

^1H NMR (400 MHz, CDCl_3): δ [ppm] = 3.52 (dt, $J = 6.8/4.3$ Hz, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.58 (dd, $J = 9.6/5.6$ Hz, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.64 (dd, $J = 9.6/3.7$ Hz, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.79 (dd, $J = 11.9/4.1$ Hz, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.84 (dd, $J =$

11.9/4.5 Hz, 1H, HOCH₂CHCHCH₂), 3.92 – 3.97 (m, 1H, HOCH₂CHCHCH₂), 4.47 (d, *J* = 11.8 Hz, 1H, OCH₂Ph), 4.51 (d, *J* = 11.8 Hz, 1H, OCH₂Ph), 4.57 (d, *J* = 11.8 Hz, 1H, OCH₂Ph), 4.58 (d, *J* = 11.8 Hz, 1H, OCH₂Ph), 6.99 – 7.05 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.28 – 7.40 (m, 5H, H_{phenyl}), 7.63 – 7.69 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ¹³C NMR (101 MHz, CDCl₃): δ [ppm] = 61.8 (1C, HOCH₂CHCHCH₂), 70.9 (1C, HOCH₂CHCHCH₂), 71.0 (1C, HOCH₂CHCHCH₂), 71.7 (1C, OCH₂Ph), 73.7 (1C, OCH₂Ph), 79.2 (1C, HOCH₂CHCHCH₂), 93.5 (1C, C-4''_{4-iodophenyl}), 128.0 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 128.1 (1C, C-4'_{phenyl}), 128.7 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 129.8 (2C, C-2''_{4-iodophenyl}, C-6''_{4-iodophenyl}), 137.72 (2C, C-3''_{4-iodophenyl}, C-5''_{4-iodophenyl}), 137.74 (1C, C_{arom.}), 137.75 (1C, C_{arom.}); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3406, 2866, 1589, 1481, 1454, 1076, 1007, 799, 737, 698; HRMS (*m/z*): [M+H]⁺ calcd for C₁₈H₂₂IO₄: 429.0557, found: 429.0538.

(2*R*,3*S*)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-2-[(4-iodobenzyl)oxy]butane-1,3-diol (14b)

Sodium hydride (60% in mineral oil, 130 mg, 3.3 mmol) was added to a solution of **13** (880 mg, 2.1 mmol) in DMF (10 mL) at 0 °C and the mixture was stirred for 40 min at 0 °C. Then a solution of 4-chloromethyl-3,5-dimethylisoxazole (480 mg, 3.3 mmol) in DMF (1 mL) was added dropwise over a period of 5 min at 0 °C. Tetrabutylammonium iodide (62 mg, 0.17 mmol) was added and the mixture was stirred for 2 h at ambient temperature. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The obtained crude product was dissolved in methanol (10 mL) and THF (0.1 mL). *p*-Toluenesulfonic acid (62 mg, 0.36 mmol) and the mixture was stirred for 5 h at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic

layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 1/1, R_f = 0.34) to give **14b** (560 mg, 1.2 mmol, 61%) as yellow oil. $[\alpha]_D^{20}$ = -19.4 (2.7, methanol); HPLC (method 2): t_R = 15.9 min, purity 95.3%.

(2S,3R)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-2-[(4-iodobenzyl)oxy]butane-1,3-diol (*ent*-14b)

Under nitrogen atmosphere, triphenylphosphine (1.7 g, 6.6 mmol) and *p*-nitrobenzoic acid (1.1 g, 6.6 mmol) were added to an ice-cooled solution of *ent*-**18b** (0.50 g, 1.1 mmol) in dry THF (20 mL). After adding diisopropyl azodicarboxylate (1.3 mL, 1.3 g, 6.6 mmol) dropwise over a period of 30 min, the mixture was stirred at ambient temperature for 3 d. Then the solvent was removed *in vacuo* and the residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 15 cm, V = 30 mL, petroleum ether/ethyl acetate = 2/1). The fractions containing the intermediate diester were concentrated *in vacuo* and the residue was dissolved in methanol (10 mL). Potassium carbonate (0.61 g, 4.4 mmol) was added and the solution was stirred at ambient temperature for 24 h. Then water (20 mL) and brine (20 mL) were added and the mixture was extracted with ethyl acetate (3× 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 20 cm, V = 30 mL, CH₂Cl₂/methanol = 19/1, R_f = 0.23) to give *ent*-**14b** (0.27 g, 0.61 mmol, 55% over two steps) as yellowish oil. $[\alpha]_D^{20}$ = +28.6 (2.2, methanol); HPLC (method 1): t_R = 19.2 min, purity 98.1%.

Spectroscopic data of **14b and ent-**14b**:**

¹H NMR (400 MHz, CD₃OD): δ [ppm] = 2.23 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.47 (ddd, *J* = 6.8/5.0/3.6 Hz, 1H, HOCH₂CHCHCH₂), 3.51 (dd, *J* = 9.9/5.8 Hz, 1H, HOCH₂CHCHCH₂), 3.59 (dd, *J* = 9.9/3.4 Hz, 1H, HOCH₂CHCHCH₂), 3.70 (dd, *J* = 11.9/5.0 Hz, 1H, HOCH₂CHCHCH₂), 3.81 – 3.87 (m, 2H, HOCH₂CHCHCH₂ (1H), HOCH₂CHCHCH₂), 4.33 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.37 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.49 (d, *J* = 11.9 Hz, 1H, CHOCH₂PhI), 4.67 (d, *J* = 11.9 Hz, 1H, CHOCH₂PhI), 7.10 – 7.15 (m, 2H, 2'-H₄-iodophenyl, 6'-H₄-iodophenyl), 7.65 – 7.70 (m, 2H, 3'-H₄-iodophenyl, 5'-H₄-iodophenyl); ¹³C NMR (101 MHz, CD₃OD): δ [ppm] = 10.0 (1C, CH₃), 10.8 (1C, CH₃), 61.9 (1C, HOCH₂CHCHCH₂), 62.6 (1C, CH₂OCH₂Ar), 71.1 (1C, HOCH₂CHCHCH₂), 72.4 (1C, HOCH₂CHCHCH₂), 72.7 (1C, CHOCH₂PhI), 81.8 (1C, HOCH₂CHCHCH₂), 93.5 (1C, C-4'-iodophenyl), 112.8 (1C, C-4''-isoxazole), 130.9 (2C, C-2'-iodophenyl, C-6'-iodophenyl), 138.5 (2C, C-3'-iodophenyl, C-5'-iodophenyl), 139.9 (1C, C-1'-iodophenyl), 161.5 (1C, C-5''-isoxazole), 169.0 (1C, C-3''-isoxazole); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3291, 2897, 1636, 1462, 1416, 1385, 1099, 1030, 1007, 941, 876, 795; HRMS (*m/z*): [M+H]⁺ calcd for C₁₇H₂₃INO₅: 448.0615, found: 448.0580.

Methyl (2S,3S)-4-(benzyloxy)-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (15a)

(Diacetoxiodo)benzene (1.6 g, 4.9 mmol) and TEMPO (80 mg, 0.51 mmol) were added to a solution of **14a** (710 mg, 1.7 mmol) in acetonitrile (1.4 mL) and water (1.4 mL). After stirring the reaction mixture at ambient temperature for 4 h, a saturated aqueous solution of sodium thiosulfate was added. The mixture was acidified with hydrochloric acid (0.5 N) until pH 1 was reached and extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and evaporated

under reduced pressure. The obtained crude product was dissolved in methanol (16 mL) and *p*-toluenesulfonic acid (30 mg, 0.16 mmol) was added. The mixture was stirred for 14 h at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the organic solvent was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate (3×) and the combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 8/2, R_f = 0.16) to give **15a** (410 mg, 0.90 mmol, 54%) as pale yellow oil. $[\alpha]_D^{20}$ = -33.6 (2.3, CH₂Cl₂); HPLC (method 2): t_R = 18.3 min, purity 91.8%.

Methyl (2*R*,3*R*)-4-(benzyloxy)-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (ent-15a)

N-Chlorosuccinimide (0.25 g, 1.9 mmol) and TEMPO (88 mg, 0.567 mmol) were added to a solution of *ent*-**14a** (0.20 g, 0.47 mmol) in a mixture of acetonitrile (4 mL) and a saturated aqueous solution of NaHCO₃ (4 mL). After stirring the reaction mixture for 3 h at 90 °C, hydrochloric acid (0.5 N) was added until pH 1 was reached. The mixture was extracted with ethyl acetate (3×), the combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The obtained crude product was dissolved in methanol (7 mL) and *p*-toluenesulfonic acid monohydrate (0.13 g, 0.70 mmol) was added. After stirring the mixture at 75 °C overnight, a saturated aqueous solution of NaHCO₃ was added, the organic solvent was removed under reduced pressure, and the aqueous phase was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15

cm, V = 15 mL, petroleum ether/ethyl acetate = 4/1, R_f = 0.17) to give **ent-15a** (75 mg, 0.16 mmol, 35%) as colorless oil. $[\alpha]_D^{20}$ = +21.5 (2.4, CH_2Cl_2); HPLC (method 1): t_R = 24.0 min, purity 98.2%.

Spectroscopic data of 15a and ent-15a:

^1H NMR (400 MHz, CDCl_3): δ [ppm] = 2.51 – 2.57 (m, 1H, OH), 3.58 – 3.65 (m, 2H, CHCHCH_2), 3.73 (s, 3H, CO_2CH_3), 4.04 – 4.14 (m, 2H, CHCHCH_2), 4.37 (d, J = 11.7 Hz, 1H, CHOCH_2PhI), 4.48 (d, J = 11.8 Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.54 (d, J = 11.8 Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.64 (d, J = 11.7 Hz, 1H, CHOCH_2PhI), 7.02 – 7.08 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.25 – 7.39 (m, 5H, 2'-H_{phenyl}, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}, 6'-H_{phenyl}), 7.63 – 7.69 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ^{13}C NMR (101 MHz, CDCl_3): δ [ppm] = 52.3 (1C, CO_2CH_3), 69.9 (1C, CHCHCH_2), 71.2 (1C, CHCHCH_2), 72.4 (1C, CHOCH_2PhI), 73.6 (1C, $\text{CH}_2\text{OCH}_2\text{Ph}$), 78.9 (1C, CHCHCH_2), 93.8 (1C, C-4''_{4-iodophenyl}), 127.99 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 128.04 (1C, C-4'_{phenyl}), 128.6 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 130.1 (2C, C-2''_{4-iodophenyl}, C-6''_{4-iodophenyl}), 136.8 (1C, C-1''_{4-iodophenyl}), 137.7 (2C, C-3''_{4-iodophenyl}, C-5''_{4-iodophenyl}), 137.8 (1C, C-1'_{phenyl}), 171.3 (1C, CO_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3494, 3191, 2943, 2875, 1736, 1463, 1234, 1204, 1121, 1063, 1007, 798, 751, 701; HRMS (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{21}\text{INO}_5$: 479.0326, found: 479.0313.

Methyl (2S,3S)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-iodobenzyl) oxy]butanoate (15b)

(Diacetoxiodo)benzene (1.2 g, 3.7 mmol) and TEMPO (60 mg, 0.38 mmol) were added to a solution of **14b** (550 mg, 1.2 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) and stirred for 3 h at ambient temperature. Then a saturated aqueous solution of

sodium thiosulfate was added and the mixture was acidified with hydrochloric acid (0.5 N) until pH 1 was reached. The mixture was extracted with ethyl acetate (3×), dried over sodium sulfate, filtered, and evaporated under reduced pressure. The obtained crude product was dissolved in methanol (12 mL) and *p*-toluenesulfonic acid (16 mg, 0.08 mmol) was added. The mixture was stirred for 17 h at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the organic solvent was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate (3×) and the combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 3/1, R_f = 0.17) to give **15b** (280 mg, 0.58 mmol, 47%) as pale yellow oil. $[\alpha]_D^{20}$ = -12.7 (3.0, CH₂Cl₂); HPLC (method 2): t_R = 17.1 min, purity 93.4%.

Methyl (2*R*,3*R*)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (*ent*-15b)

A saturated aqueous solution of sodium bicarbonate (6 mL) was added to a solution of *ent*-**14b** (0.22 g, 0.49 mmol) in acetonitrile (6 mL), whereby a colourless precipitate formed. After adding TEMPO (93 mg, 0.060 mmol) and *N*-chlorosuccinimide (0.23 g, 1.7 mmol), the mixture was stirred at 90 °C for 9 h. Afterwards, a 0.5 M aqueous solution of hydrogen chloride (15 mL) was added until pH = 1 was reached. The mixture was extracted with ethyl acetate (3× 30 mL), the combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in methanol (20 mL) and *p*-toluenesulfonic acid monohydrate (0.14 g, 0.75 mmol) was added. After stirring for 39 h at 75 °C, a saturated aqueous solution of sodium bicarbonate (20 mL) and brine (30 mL) were added and the mixture was

extracted with ethyl acetate (3× 30 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 15 cm, V = 20 mL, petroleum ether/ethyl acetate = 1/1, R_f = 0.29) to give **ent-15b** (0.14 g, 0.29 mmol, 60% over two steps) as yellow oil. $[\alpha]_D^{20}$ = +17.6 (19.3, methanol); HPLC (method 1): t_R = 21.7 min, purity 94.7%.

Spectroscopic data of 15b and ent-15b:

^1H NMR (600 MHz, CD_3OD): δ [ppm] = 2.22 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.51 (dd, J = 9.8/5.5 Hz, 1H, CHCHCH_2), 3.57 (dd, J = 9.8/5.1 Hz, 1H, CHCHCH_2), 3.67 (s, 3H, CO_2CH_3), 4.00 (q, J = 5.3 Hz, 1H, CHCHCH_2), 4.05 (d, J = 5.4 Hz, 1H, CHCHCH_2), 4.32 (s, 2H, $\text{CH}_2\text{OCH}_2\text{Ar}$), 4.41 (d, J = 11.8 Hz, 1H, CHOCH_2PhI), 4.60 (d, J = 11.8 Hz, 1H, CHOCH_2PhI), 7.12 – 7.15 (m, 2H, 2'- $\text{H}_{4\text{-iodophenyl}}$, 6'- $\text{H}_{4\text{-iodophenyl}}$), 7.67 – 7.70 (m, 2H, 3'- $\text{H}_{4\text{-iodophenyl}}$, 5'- $\text{H}_{4\text{-iodophenyl}}$); ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 10.0 (1C, CH_3), 10.8 (1C, CH_3), 52.3 (1C, CO_2CH_3), 62.6 (1C, $\text{CH}_2\text{OCH}_2\text{Ar}$), 71.2 (1C, CHCHCH_2), 72.3 (1C, CHCHCH_2), 73.1 (1C, CHOCH_2PhI), 80.8 (1C, CHCHCH_2), 93.9 (1C, C-4' $_{4\text{-iodophenyl}}$), 112.7 (1C, C-4'' $_{\text{isoxazole}}$), 131.1 (2C, C-2' $_{4\text{-iodophenyl}}$, C-6' $_{4\text{-iodophenyl}}$), 138.6 (2C, C-3' $_{4\text{-iodophenyl}}$, C-5' $_{4\text{-iodophenyl}}$), 138.8 (1C, C-1' $_{4\text{-iodophenyl}}$), 161.5 (1C, C-5'' $_{\text{isoxazole}}$), 169.1 (1C, C-3'' $_{\text{isoxazole}}$), 172.8 (1C, CO_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3379, 2924, 1736, 1682, 1639, 1435, 1204, 1138, 1099, 1007, 799, 721; HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{23}\text{INO}_6$: 476.0565, found: 476.0528.

Methyl

(2S,3S)-4-(benzyloxy)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl} benzyl)oxy]butanoate (16a)

Under nitrogen atmosphere, copper(I) iodide (30 mg, 0.16 mmol), triethylamine (0.76 mL, 560 mg, 5.5 mmol) and tetrakis(triphenylphosphine)palladium(0) (90 mg, 0.08 mmol) were added to a solution of **15a** (370 mg, 0.81 mmol) in dry acetonitrile (10 mL). A solution of 4-(4-ethynylbenzyl)morpholine (1.2 g, 6.1 mmol) in dry acetonitrile (4.5 mL) was added dropwise over a period of 3 h at ambient temperature. Then the solvent was evaporated under reduced pressure and the residue was purified twice by flash column chromatography ($\varnothing = 2$ cm, $h = 15$ cm, $V = 10$ mL, cyclohexane/ethyl acetate = 1/1, $R_f = 0.32$) to give **16a** (360 mg, 0.68 mmol, 84%) as yellow oil. $[\alpha]_D^{20} = -32.8$ (1.8, CH_2Cl_2); HPLC (method 2): $t_R = 14.2$ min, purity 85.6%.

Methyl (2R,3R)-4-(benzyloxy)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanoate (*ent*-16a)

Under nitrogen atmosphere, copper(I) iodide (4.2 mg, 0.022 mmol), triethylamine (0.03 mL, 22 mg, 0.22 mmol) and tetrakis(triphenylphosphine)palladium(0) (13 mg, 0.011 mmol) were added to a solution of *ent*-**15a** (51 mg, 0.11 mmol) in dry acetonitrile. A solution of 4-(4-ethynylbenzyl)morpholine (34 mg, 0.17 mmol) in dry acetonitrile was added dropwise over a period of 15 min at ambient temperature. The solvent was evaporated under reduce pressure and the residue was purified once by flash column chromatography ($\varnothing = 2$ cm, $h = 15$ cm, $V = 10$ mL, dichloromethane/methanol = 19/1, $R_f = 0.37$) and twice by automatic flash column chromatography to give *ent*-**16a** (39 mg, 0.073 mmol, 66%) as yellow oil. $[\alpha]_D^{20} = +37.6$ (2.1, CH_2Cl_2); HPLC (method 1): $t_R = 19.8$ min, purity 99.7%.

Spectroscopic data of 16a and ent-16a:

^1H NMR (400 MHz, DMSO- d_6): δ [ppm] = 2.31 – 2.40 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.44 – 3.51 (m, 3H, CHCHCH_2 (1H), ArCH_2N), 3.53 – 3.60 (m, 5H, CHCHCH_2 (1H), $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.62 (s, 3H, CO_2CH_3), 3.90 – 3.97 (m, 1H, CHCHCH_2), 4.04 (d, J = 5.5 Hz, 1H, CHCHCH_2), 4.43 – 4.51 (m, 3H, CHOCH_2Ar (1H), $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.61 (d, J = 12.4 Hz, 1H, CHOCH_2Ar), 5.35 (d, J = 6.1 Hz, 1H, OH), 7.24 – 7.40 (m, 9H, 2'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 6'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 3''- $\text{H}_{4-(\text{morpholinomethyl})\text{phenyl}}$, 5''- $\text{H}_{4-(\text{morpholinomethyl})\text{phenyl}}$, 2'''- H_{phenyl} , 3'''- H_{phenyl} , 4'''- H_{phenyl} , 5'''- H_{phenyl} , 6'''- H_{phenyl}), 7.48 – 7.54 (m, 4H, 3'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 5'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 2''- $\text{H}_{4-(\text{morpholinomethyl})\text{phenyl}}$, 6''- $\text{H}_{4-(\text{morpholinomethyl})\text{phenyl}}$); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = 51.6 (1C, CO_2CH_3), 53.2 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 62.0 (1C, ArCH_2N), 66.2 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 70.5 (1C, CHCHCH_2), 70.7 (1C, CHCHCH_2), 71.3 (1C, CHOCH_2Ar), 72.3 (1C, $\text{CH}_2\text{OCH}_2\text{Ph}$), 79.8 (1C, CHCHCH_2), 89.1 (1C, $\text{C}\equiv\text{C}$), 89.4 (1C, $\text{C}\equiv\text{C}$), 120.9 (1C, $\text{C}_{\text{arom.}}$), 121.5 (1C, $\text{C}_{\text{arom.}}$), 127.4 (1C, C-4''' $_{\text{phenyl}}$), 127.5 (2C, C-2''' $_{\text{phenyl}}$, C-6''' $_{\text{phenyl}}$), 127.9 (2C, C-2'- $_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, C-6'- $_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$), 128.2 (2C, C-3''' $_{\text{phenyl}}$, C-5''' $_{\text{phenyl}}$), 129.2 (2C, C-3''- $_{4-(\text{morpholinomethyl})\text{phenyl}}$, C-5''- $_{4-(\text{morpholinomethyl})\text{phenyl}}$), 131.2 (2C, $\text{C}_{\text{arom.}}$), 131.3 (2C, $\text{C}_{\text{arom.}}$), 138.4 (1C, C-1''' $_{\text{phenyl}}$), 138.5 (1C, C-1'- $_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$), 138.9 (1C, C-4''- $_{4-(\text{morpholinomethyl})\text{phenyl}}$), 170.9 (1C, CO_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3402, 2859, 1744, 1454, 1204, 1115, 1007, 914, 864, 737, 698; HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{32}\text{H}_{36}\text{NO}_6$: 530.2537, found: 530.2517.

Methyl (2S,3S)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-[[4-(morpholino-methyl)phenyl]ethynyl]benzyl)oxy]butanoate (16b)

Under nitrogen atmosphere, copper(I) iodide (20 mg, 0.10 mmol), triethylamine (0.50 mL, 360 mg, 3.6 mmol) and tetrakis(triphenylphosphine)palladium(0) (58 mg, 0.05

mmol) were added to a solution of **15b** (250 mg, 0.52 mmol) in dry acetonitrile (6 mL). A solution of 4-(4-ethynylbenzyl)morpholine (800 mg, 4.0 mmol) in dry acetonitrile (3 mL) was added dropwise over a period of 3 h at ambient temperature and the mixture was stirred for additional 16 h. Then the solvent was evaporated under reduced pressure and purified twice by flash column chromatography ($\varnothing$ = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 1/1, R_f = 0.17) and automatic flash column chromatography (100% H₂O $\rightarrow$ 100% acetonitrile, Biotage® SNAP C18 30 g) to give **16b** (250 mg, 0.46 mmol, 87%) as yellow oil. $[\alpha]_D^{20}$ = -30.9 (4.0, methanol); HPLC (method 2): t_R = 13.8 min, purity 95.4%.

Methyl (2*R*,3*R*)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[[4-[(4-morpholinomethyl)phenyl]ethynyl]benzyl]oxy]butanoate (*ent*-16b)

Under nitrogen atmosphere, copper(I) iodide (10 mg, 0.053 mmol), tetrakis(triphenylphosphine)palladium(0) (15 mg, 0.013 mmol), and triethylamine (60 μ L, 43 mg, 0.42 mmol) were added to a solution of *ent*-**15b** (100 mg, 0.21 mmol) in dry acetonitrile (5 mL). After adding a solution of 4-(4-ethynylbenzyl)morpholine (51 mg, 0.25 mmol) in dry acetonitrile (6 mL) dropwise over a period of 30 min, the mixture was stirred at ambient temperature for 22 h. Then the solution was concentrated *in vacuo* and the residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 25 cm, V = 20 mL, CH₂Cl₂/methanol = 9/1, R_f = 0.55) and by automated flash column chromatography (10% $\rightarrow$ 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give *ent*-**16b** (85 mg, 0.15 mmol, 74%) as yellow oil. $[\alpha]_D^{20}$ = + 5.9 (11.1, methanol); HPLC (method 1): t_R = 18.0 min, purity 92.2%.

Spectroscopic data of 16b and ent-16b:

¹H NMR (600 MHz, CD₃OD): δ [ppm] = 2.23 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 2.43 – 2.51 (m, 4H, N(CH₂CH₂)₂O), 3.52 (dd, *J* = 9.8/5.5 Hz, 1H, CHCHCH₂), 3.54 (s, 2H, ArCH₂N), 3.58 (dd, *J* = 9.8/5.1 Hz, 1H, CHCHCH₂), 3.68 (s, 3H, CO₂CH₃), 3.68 – 3.71 (m, 4H, N(CH₂CH₂)₂O), 4.02 (q, *J* = 5.3 Hz, 1H, CHCHCH₂), 4.07 (d, *J* = 5.4 Hz, 1H, CHCHCH₂), 4.33 (s, 2H, CH₂OCH₂Ar), 4.48 (d, *J* = 11.9 Hz, 1H, CHOCH₂Ar), 4.67 (d, *J* = 11.9 Hz, 1H, CHOCH₂Ar), 7.35 – 7.39 (m, 4H, 2'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 6'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 3''-H₄-(morpholinomethyl)phenyl, 5''-H₄-(morpholinomethyl)phenyl), 7.47 – 7.50 (m, 4H, 3'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 5'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 2''-H₄-(morpholinomethyl)phenyl, 6''-H₄-(morpholinomethyl)phenyl); ¹³C NMR (151 MHz, CD₃OD): δ [ppm] = 10.0 (1C, CH₃), 10.8 (1C, CH₃), 52.3 (1C, CO₂CH₃), 54.7 (2C, N(CH₂CH₂)₂O), 62.6 (1C, CH₂OCH₂Ar), 64.0 (1C, ArCH₂N), 67.8 (2C, N(CH₂CH₂)₂O), 71.2 (1C, CHCHCH₂), 72.3 (1C, CHCHCH₂), 73.4 (1C, CHOCH₂Ar), 80.8 (1C, CHCHCH₂), 90.0 (1C, C≡C), 90.2 (1C, C≡C), 112.7 (1C, C-4'''_{isoxazole}), 123.6 (1C, C_{arom.}), 124.1 (1C, C_{arom.}), 129.2 (2C, C-2'4'-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, C-6'H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl), 130.7 (2C, C-3'4'-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, C-5''4-(morpholinomethyl)phenyl), 132.46 (2C, C_{arom.}), 132.48 (2C, C_{arom.}), 139.1 (1C, C-4''4-(morpholinomethyl)phenyl), 139.3 (1C, C-1'4'-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl), 161.5 (1C, C-5'''_{isoxazole}), 169.1 (1C, C-3'''_{isoxazole}), 172.9 (1C, CO₂CH₃); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3391, 2859, 2808, 1740, 1636, 1516, 1435, 1350, 1261, 1204, 1115, 1069, 1007, 864, 822; HRMS (*m/z*): [M+H]⁺ calcd for C₃₁H₃₇N₂O₇: 549.2595, found: 549.2545.

(2S,3S)-4-(Benzyloxy)-N,3-dihydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanamide (17a)

Hydroxylamine hydrochloride (40 mg, 0.57 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.15 mL, 0.81 mmol) were added to a solution of **16a** (100 mg, 0.19 mmol) in methanol (5 mL) and the mixture was stirred for 2 d at ambient temperature. Then the solvent was evaporated under reduced pressure and the residue was purified by automatic flash column chromatography (100% H₂O → 100% acetonitrile, Biotage® SNAP C18 30g) and flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, dichloromethane/methanol = 96/4, R_f = 0.30) to give **17a** (22 mg, 0.04 mmol, 22%) as pale yellow solid. m.p. = 144 °C; $[\alpha]_D^{20} = -39.0$ (3.0, methanol); HPLC (method 2): t_R = 13.1 min, purity 99.6%.

(2R,3R)-4-(Benzyloxy)-N,3-dihydroxy-2-[(4-{4-(morpholinomethyl)phenyl}ethynyl)benzyl]oxy]butanamide (ent-17a)

Under nitrogen atmosphere, a 5.4 M solution of sodium methoxide in methanol (0.87 mL, 4.7 mmol) and hydroxylamine hydrochloride (0.33 g, 4.7 mmol) were added to a solution of *ent*-**16a** (25 mg, 0.047 mmol) in methanol (5 mL) and the mixture was stirred at ambient temperature overnight. Then the solvent was removed under reduced pressure and the residue was purified by automatic flash column chromatography to give *ent*-**17a** (10 mg, 0.019 mmol, 40%) as colorless solid. $[\alpha]_D^{20} = +52.0$ (1.0, methanol); HPLC (method 2): t_R = 13.3 min, purity 99.5%.

Spectroscopic data of 17a and ent-17a:

¹H NMR (400 MHz, CD₃OD): δ [ppm] = 2.41 – 2.52 (m, 4H, N(CH₂CH₂)₂O), 3.54 (s, 2H, ArCH₂N), 3.59 – 3.68 (m, 2H, CHCHCH₂), 3.68 – 3.73 (m, 4H, N(CH₂CH₂)₂O), 3.90 (d, J = 6.6 Hz, 1H, CHCHCH₂), 3.99 – 4.06 (m, 1H, CHCHCH₂), 4.45 (d, J = 12.1 Hz,

1H, CHOCH₂Ar), 4.48 (d, *J* = 11.9 Hz, 1H, CH₂OCH₂Ph), 4.59 (d, *J* = 11.9 Hz, 1H, CH₂OCH₂Ph), 4.64 (d, *J* = 12.1 Hz, 1H, CHOCH₂Ar), 7.21 – 7.41 (m, 9H, 2'-H_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}, 6'-H_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}, 3''_{4-(morpholinomethyl)phenyl}, 5''_{4-(morpholinomethyl)phenyl}, 2'''-H_{phenyl}, 3'''-H_{phenyl}, 4'''-H_{phenyl}, 5'''-H_{phenyl}, 6'''-H_{phenyl}), 7.41 – 7.53 (m, 4H, 3'-H_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}, 5'-H_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}, 2''_{4-(morpholinomethyl)phenyl}, 6''_{4-(morpholinomethyl)phenyl}); ¹³C NMR (101 MHz, CD₃OD): δ [ppm] = 54.6 (2C, N(CH₂CH₂)₂O), 64.0 (1C, ArCH₂N), 67.8 (2C, N(CH₂CH₂)₂O), 71.9 (1C, CHCHCH₂), 72.0 (1C, CHCHCH₂), 73.0 (1C, CHOCH₂Ar), 74.4 (1C, CH₂OCH₂Ph), 80.0 (1C, CHCHCH₂), 90.0 (1C, C≡C), 90.2 (1C, C≡C), 123.6 (1C, C-1''_{4-(morpholinomethyl)phenyl}), 124.1 (1C, C-4'_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}), 128.6 (1C, C-4'''_{phenyl}), 128.9 (2C, C-2'''_{phenyl}, C-6'''_{phenyl}), 129.2 (2C, C-2'_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}, C-6'_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}), 129.3 (2C, C-3'''_{phenyl}, C-5'''_{phenyl}), 130.7 (2C, C-3''_{4-(morpholinomethyl)phenyl}, C-5''_{4-(morpholinomethyl)phenyl}), 132.48 (2C, C_{arom.}), 132.52 (2C, C_{arom.}), 139.0 (1C, C-4''_{4-(morpholinomethyl)phenyl}), 139.2 (1C, C-1'_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}), 139.6 (1C, C-1'''_{phenyl}), 169.6 (1C, CONHOH); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3375, 3175, 3032, 2913, 2862, 1659, 1516, 1454, 1277, 1111, 1007, 914, 868, 818, 741, 698; HRMS (*m/z*): [M+H]⁺ calcd for C₃₁H₃₅N₂O₆: 531.2490, found: 531.2527.

(2S,3S)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-N,3-dihydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanamide (17b)

Hydroxylamine hydrochloride (33 mg, 0.48 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.13 mL, 0.69 mmol) were added to a solution of **16b** (88 mg, 0.16 mmol) in methanol (5 mL) and the mixture was stirred for 2 d at ambient temperature. Then the solvent was evaporated under reduced pressure and the

residue was purified by automatic flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP C18 30 g) to give **17b** (34 mg, 0.06 mmol, 39%) as colorless solid. TLC (dichloromethane/methanol = 20/1): R_f = 0.30; m.p. = 122 °C; $[\alpha]_D^{20}$ = -86.7 (2.0, methanol); HPLC (method 2): t_R = 12.7 min, purity 97.2%.

(2*R*,3*R*)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-*N*,3-dihydroxy-2-[[4-[(4-(morpholinomethyl)phenyl)ethynyl]benzyl]oxy]butanamide (*ent*-17b**)**

Under nitrogen atmosphere, a 50% aqueous solution of hydroxylamine (0.24 mL, 129 mg, 3.9 mmol) was added to a solution of *ent*-**16b** (70 mg, 0.13 mmol) in methanol (1.5 mL) and THF (1.5 mL). After adding potassium cyanide (8 mg, 0.12 mmol), the mixture was stirred at ambient temperature for 24 h. The resulting colourless solution was purified by automated flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give *ent*-**17b** (70 mg, 0.13 mmol, quant.) as colourless solid. TLC: R_f = 0.38 (CH₂Cl₂/methanol = 9/1); m.p. = 60 °C; $[\alpha]_D^{20}$ = + 23.7 (4.2, methanol); HPLC (method 2): t_R = 12.8 min, purity 99.9%.

Spectroscopic data of **17b and *ent*-**17b**:**

¹H NMR (500 MHz, CD₃OD): δ [ppm] = 2.23 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 2.44 – 2.49 (m, 4H, N(CH₂)₂), 3.54 (s, 2H, ArCH₂N), 3.56 (dd, J = 10.1/5.8 Hz, 1H, CHCHCH₂), 3.60 (dd, J = 10.1/3.5 Hz, 1H, CHCHCH₂), 3.67 – 3.72 (m, 4H, N(CH₂CH₂)₂O), 3.86 (d, J = 6.3 Hz, 1H, CHCHCH₂), 4.01 (td, J = 5.9/3.5 Hz, 1H, CHCHCH₂), 4.33 (d, J = 12.2 Hz, 1H, CH₂OCH₂Ar), 4.38 (d, J = 12.2 Hz, 1H, CH₂OCH₂Ar), 4.46 (d, J = 12.0 Hz, 1H, CHOCH₂Ar), 4.65 (d, J = 12.0 Hz, 1H, CHOCH₂Ar), 7.34 – 7.39 (m, 4H, 2'-H₄-[4-

(morpholinomethyl)phenyl]ethynyl]phenyl),

6'-H₄-[4-(morpholinomethyl)phenyl]ethynyl]phenyl),

3''H₄-

(morpholinomethyl)phenyl, $^5\text{H}_{4-(\text{morpholinomethyl)phenyl}}$, 7.46 – 7.50 (m, 4H, $2'\text{-H}_{4-[[4-(\text{morpholinomethyl)phenyl]ethynyl}]phenyl}$, $6'\text{-H}_{4-[[4-(\text{morpholinomethyl)phenyl]ethynyl}]phenyl}$, $3'\text{H}_{4-(\text{morpholinomethyl)phenyl}}$, $^5\text{H}_{4-(\text{morpholinomethyl)phenyl}}$); ^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 10.0 (1C, CH_3), 10.8 (1C, CH_3), 54.7 (2C $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 62.6 (1C, $\text{CH}_2\text{OCH}_2\text{Ar}$), 64.0 (1C, ArCH_2N), 67.8 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 71.7 (1C, CHCHCH_2), 71.9 (1C, CHCHCH_2), 73.0 (1C, CHOCH_2Ar), 80.3 (1C, CHCHCH_2), 89.9 (1C, $\text{C}\equiv\text{C}$), 90.3 (1C, $\text{C}\equiv\text{C}$), 112.7 (1C, $\text{C-4}''''_{\text{isoxazole}}$), 123.5 (1C, $\text{C-1}''_{4-(\text{morpholinomethyl)phenyl}}$), 124.2 (1C, $\text{C-4}'_{4-[[4-(\text{morpholinomethyl)phenyl]ethynyl}]phenyl}$), 129.2 (2C, $\text{C-2}'_{4-[[4-(\text{morpholinomethyl)phenyl]ethynyl}]phenyl}$, $\text{C-6'H}_{4-[[4-(\text{morpholinomethyl)phenyl]ethynyl}]phenyl}$), 130.7 (2C, $\text{C-3}''_{4-(\text{morpholinomethyl)phenyl}}$, $\text{C-5}''_{4-(\text{morpholinomethyl)phenyl}}$), 132.49 (2C, $\text{C}_{\text{arom.}}$), 132.54 (2C, $\text{C}_{\text{arom.}}$), 139.1 (1C, $\text{C}_{\text{arom.}}$), 139.2 (1C, $\text{C}_{\text{arom.}}$), 160.2 (1C, $\text{C-5}''''_{\text{isoxazole}}$), 167.6 (1C, $\text{C-3}''''_{\text{isoxazole}}$), 169.4 (1C, CONHOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3225, 2920, 2862, 1732, 1663, 1516, 1454, 1242, 1111, 1072, 1007, 864, 822; HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{30}\text{H}_{36}\text{N}_3\text{O}_7$: 550.2548, found: 550.2562.

(2R,3R)-4-(Benzyloxy)-2-[(4-iodobenzyl)oxy]butane-1,3-diol (18a)

Under nitrogen atmosphere, triphenylphosphine (2.5 g, 9.5 mmol) and *p*-nitrobenzoic acid (1.6 g, 9.5 mmol) were added to an ice-cooled solution of **14a** (680 mg, 1.6 mmol) in dry THF (20 mL). After adding diisopropyl azodicarboxylate (1.9 mL, 1.9 g, 9.5 mmol) dropwise over a period of 30 min, the mixture was stirred at ambient temperature for 3 d. Then the solvent was removed *in vacuo* and the residue was purified by flash column chromatography ($\varnothing$ = 6 cm, *h* = 20 cm, *V* = 30 mL, petroleum ether/ethyl acetate = 2/1). The fractions containing the intermediate diester were concentrated *in vacuo* and the residue was dissolved in methanol (20 mL). Potassium carbonate (880 mg, 6.4 mmol) was added and the solution was stirred at ambient temperature for 21 h. Then

water (30 mL) and brine (30 mL) were added and the mixture was extracted with ethyl acetate (3× 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 6 cm, h = 30 cm, V = 30 mL, CH₂Cl₂/methanol = 19/1, R_f = 0.24) and by automated flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give **18a** (330 mg, 0.77 mmol, 48% over two steps) as colourless oil. $[\alpha]_D^{20}$ = -23.8 (3.4, methanol); HPLC (method 1): t_R = 21.5 min, purity 99.7%.

(2S,3S)-4-(Benzyloxy)-2-[(4-iodobenzyl)oxy]butane-1,3-diol (*ent*-18a)

Under nitrogen atmosphere, sodium hydride (60% mineral oil, 0.13 g, 3.4 mmol) was added to a solution of **26** (0.48 g, 1.1 mmol) in dry THF (10 mL) at 0 °C and the mixture was stirred at 0 °C for 20 min. Then a solution of benzyl bromide (0.17 mL, 0.25 g, 1.5 mmol) in THF (2 mL) was added dropwise over 5 min at 0 °C. After the addition of a spatula tip of tetrabutylammonium iodide, the mixture was stirred at ambient temperature overnight. The reaction was stopped by adding a saturated aqueous solution of sodium hydrogen carbonate and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The obtained crude product was dissolved in a mixture of methanol (10 mL) and THF (0.1 mL). *p*-Toluenesulfonic acid monohydrate (0.32 g, 1.7 mmol) was added and the mixture was stirred for 3.3 h at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 15 mL, petroleum ether/ethyl acetate = 1/1,

$R_f = 0.22$) to give **ent-18a** (0.33 g, 0.76 mmol, 68%) as colorless solid. m.p. = 61 °C; $[\alpha]_D^{20} = +22.5$ (2.9, methanol); HPLC (method 1): $t_R = 21.5$ min, purity 96.9%.

Spectroscopic data of 18a and ent-18a:

^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ [ppm] = 3.37 (dd, $J = 9.5/6.4$ Hz, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.41 – 3.44 (m, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.46 – 3.53 (m, 2H, $\text{HOCH}_2\text{CHCHCH}_2$ (1H), $\text{HOCH}_2\text{CHCHCH}_2$ (1H)), 3.57 – 3.62 (m, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 3.76 – 3.80 (m, 1H, $\text{HOCH}_2\text{CHCHCH}_2$), 4.42 (d, $J = 12.1$ Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.44 – 4.48 (m, 2H, CHOCH_2Ar (1H), $\text{CH}_2\text{OCH}_2\text{Ph}$ (1H)), 4.58 (t, $J = 5.5$ Hz, 1H, $\text{HOCH}_2\text{CHCHOHCH}_2$), 4.61 (d, $J = 12.2$ Hz, 1H, CHOCH_2Ar), 4.65 (d, $J = 6.2$ Hz, 1H, $\text{HOCH}_2\text{CHCHOHCH}_2$), 7.12 – 7.16 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.25 – 7.36 (m, 5H, H_{phenyl}), 7.65 – 7.70 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): δ [ppm] = 60.3 (1C, $\text{HOCH}_2\text{CHCHCH}_2$), 68.9 (1C, $\text{HOCH}_2\text{CHCHCH}_2$), 71.2 (1C, $\text{HOCH}_2\text{CHCHCH}_2$), 71.2 (1C, CHOCH_2Ar), 72.2 (1C, $\text{CH}_2\text{OCH}_2\text{Ph}$), 80.4 (1C, $\text{HOCH}_2\text{CHCHCH}_2$), 93.1 (1C, C-4''_{4-iodophenyl}), 127.4 (1C, C-4'_{phenyl}), 127.5 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 128.2 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 129.8 (2C, C-2''_{4-iodophenyl}, C-6''_{4-iodophenyl}), 136.8 (2C, C-3''_{4-iodophenyl}, C-5''_{4-iodophenyl}), 138.5 (1C, C-1'_{phenyl}), 139.0 (1C, C-1''_{4-iodophenyl}); IR (neat): ν [cm^{-1}] = 3224, 3029, 2900, 2868, 1728, 1590, 1484, 1464, 1452, 1402, 1386, 1362, 1339, 1305, 1291, 1264, 1250, 1207, 1178, 1135, 1106, 1073, 1061, 1019, 1007, 979, 944, 919, 886, 821, 797, 755, 732, 703, 695, 628, 611, 542, 514, 484, 470, 437; HRMS (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{21}\text{INO}_4$: 451.0377, found: 451.0370.

(2R,3R)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-2-[(4-iodobenzyl)oxy]butane-1,3-diol (18b)

Under nitrogen atmosphere, **14b** (740 mg, 1.7 mmol), triphenylphosphine (2.6 g, 9.9 mmol), and *p*-nitrobenzoic acid (1.7 g, 9.9 mmol) were dissolved in dry THF (40 mL). Diisopropyl azodicarboxylate (1.9 mL, 2.0 g, 9.9 mmol) was added dropwise over a period of 1 h at 0 °C. Then the mixture was stirred for 3 d at ambient temperature. The crude product was purified by flash column chromatography ($\varnothing$ = 8 cm, h = 10 cm, V = 60 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.25). The fractions containing the desired diester were concentrated *in vacuo*, the residue was dissolved in methanol (40 mL), and an aqueous solution of potassium hydroxide (2 M, 10 mL) was added. The mixture was stirred at 60 °C for 2 d. Then hydrochloric acid (0.5 N) was added until pH 1 was reached. The organic solvent was removed *in vacuo* and the aqueous phase was extracted with ethyl acetate (5×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified twice by flash column chromatography (1. $\varnothing$ = 8 cm, h = 15 cm, V = 60 mL, cyclohexane/ethyl acetate = 1/2 $\rightarrow$ 0/1; 2. $\varnothing$ = 4 cm, h = 15 cm, V = 30 mL, ethyl acetate) to give **18b** (520 mg, 1.2 mmol, 70%) as colorless solid. TLC (cyclohexane/ethyl acetate = 1/3): R_f = 0.13; m.p. = 73 °C; $[\alpha]_D^{20}$ = -49.0 (2.7, methanol); HPLC (method 1): t_R = 17.8 min, purity 88.5%.

(2S,3S)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-2-[(4-iodobenzyl)oxy]butane-1,3-diol (*ent*-18b)

Under nitrogen atmosphere, sodium hydride (60% in mineral oil, 0.31 g, 7.8 mmol) was added to an ice-cooled solution of **26** (2.1 g, 4.9 mmol) in dry DMF (30 mL). After stirring the mixture for 1 h at 0 °C, a solution of 4-chloromethyl-3,5-dimethylisoxazole (0.96 mL, 1.1 g, 7.8 mmol) and tetra-*n*-butylammonium iodide (140 mg, 0.39 mmol) in dry DMF (2 mL) was added dropwise over a period of 5 min and the solution was stirred

at ambient temperature for 17 h. Then a saturated aqueous solution of sodium bicarbonate (30 mL) and brine (10 mL) were added and the mixture was extracted with ethyl acetate (4× 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in methanol (20 mL) and THF (0.2 mL) and *p*-toluenesulfonic acid monohydrate (1.4 g, 7.4 mmol) was added. After stirring for 24 h at 85 °C, a saturated aqueous solution of sodium hydrogen carbonate (30 mL) was added and the mixture was extracted with ethyl acetate (3× 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, V = 30 mL, CH₂Cl₂/methanol = 19/1, R_f = 0.23) to give **ent-18b** (1.9 g, 4.4 mmol, 90% over two steps) as yellowish oil. $[\alpha]_D^{20} = +10.3$ (4.6, methanol); HPLC (method 1): t_R = 19.0 min, purity 96.4%.

Spectroscopic data of 18b and ent-18b:

¹H NMR (600 MHz, CD₃OD): δ [ppm] = 2.23 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.47 (dd, *J* = 9.7/6.3 Hz, 1H, HOCH₂CHCHCH₂), 3.49 – 3.56 (m, 2H, HOCH₂CHCHCH₂, HOCH₂CHCHCH₂ (1H)), 3.67 (dd, *J* = 11.5/5.6 Hz, 1H, HOCH₂CHCHCH₂), 3.77 (dd, *J* = 11.5/5.1 Hz, 1H, HOCH₂CHCHCH₂), 3.85 – 3.90 (m, 1H, HOCH₂CHCHCH₂), 4.29 – 4.36 (m, 2H, CH₂OCH₂Ar), 4.51 (d, *J* = 12.0 Hz, 1H, CHOCH₂PhI), 4.69 (d, *J* = 12.0 Hz, 1H, CHOCH₂PhI), 7.13 – 7.18 (m, 2H, 2'-H₄-iodophenyl, 6'-H₄-iodophenyl), 7.65 – 7.70 (m, 2H, 3'-H₄-iodophenyl, 5'-H₄-iodophenyl); ¹³C NMR (151 MHz, CD₃OD): δ [ppm] = 10.0 (1C, CH₃), 10.8 (1C, CH₃), 62.1 (1C, HOCH₂CHCHCH₂), 62.5 (1C, CH₂OCH₂Ar), 71.2 (1C, HOCH₂CHCHCH₂), 72.1 (1C, HOCH₂CHCHCH₂), 73.3 (1C, CHOCH₂PhI), 81.5 (1C, HOCH₂CHCHCH₂), 93.5 (1C, C-4'-iodophenyl), 112.7 (1C, C-4''-isoxazole), 131.0 (2C, C-2'-iodophenyl, C-6'-iodophenyl), 138.5 (2C, C-3'-iodophenyl, C-5'-iodophenyl), 140.0 (1C, C-1'-iodophenyl).

iodophenyl), 161.4 (1C, C-5" isoxazole), 169.0 (1C, C-3" isoxazole); IR (neat): ν [cm⁻¹] = 3256, 2940, 2882, 1651, 1458, 1423, 1396, 1323, 1207, 1084, 1053, 1007, 988, 876, 849, 806, 768, 721, 679; HRMS (m/z): [M+H]⁺ calcd for C₁₇H₂₃INO₅: 448.0615, found: 448.0646.

Methyl (2S,3R)-4-(benzyloxy)-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (19a)

A saturated aqueous solution of sodium bicarbonate (10 mL) was added to a solution of **18a** (0.29 g, 0.68 mmol) in acetonitrile (10 mL). After adding TEMPO (0.13 g, 0.81 mmol) and *N*-chlorosuccinimide (0.37 g, 2.7 mmol), the mixture was stirred at 90 °C for 24 h. Afterwards, a 0.5 M aqueous solution of hydrogen chloride (20 mL) was added until pH = 1 was reached. The mixture was extracted with ethyl acetate (3× 40 mL), the combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in methanol (10 mL) and *p*-toluenesulfonic acid monohydrate (0.19 g, 1.0 mmol) was added. After stirring for 21 h at 75 °C, a saturated aqueous solution of sodium bicarbonate (20 mL) and brine (20 mL) were added and the mixture was extracted with ethyl acetate (3× 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 6 cm, h = 20 cm, V = 20 mL, petroleum ether/ethyl acetate = 2/1 → 1/1) to give **19a** (0.12 g, 0.26 mmol, 39% over two steps) as yellow oil. TLC: R_f = 0.39 (petroleum ether/ethyl acetate = 2/1); $[\alpha]_D^{20}$ = -77.4 (1.4, methanol); HPLC (method 1): t_R = 24.0 min, purity 94.0%.

Methyl (2R,3S)-4-(benzyloxy)-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (ent-19a)

N-Chlorosuccinimide (0.30 g, 2.3 mmol) and TEMPO (0.11 g, 0.67 mmol) were added to a solution of **ent-18a** (0.24 g, 0.56 mmol) in a mixture of acetonitrile (4 mL) and a saturated aqueous solution of NaHCO₃ (4 mL). After stirring the reaction mixture for 5 h at 90 °C, hydrochloric acid (0.5 N) was added until pH 1 was reached. The mixture was extracted with ethyl acetate (3×), the combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The obtained crude product was dissolved in methanol (15 mL) and *p*-toluenesulfonic acid monohydrate (0.16 g, 0.84 mmol) was added. After stirring the mixture at 75 °C overnight, a saturated aqueous solution of NaHCO₃ was added, the organic solvent was removed under reduced pressure, and the aqueous phase was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, petroleum ether/ethyl acetate = 4/1, R_f = 0.24) to give **ent-19a** (0.14 g, 0.31 mmol, 55%) as pale yellow oil. $[\alpha]_D^{20} = +51.3$ (2.9, methanol); HPLC (method 1): t_R = 24.0 min, purity 98.8%.

Spectroscopic data of 19a and ent-19a:

¹H NMR (500 MHz, CDCl₃): δ [ppm] = 3.51 – 3.60 (m, 2H, CHCHCH₂), 3.73 (s, 3H, CO₂CH₃), 4.10 – 4.15 (m, 2H, CHCHCH₂), 4.37 (d, *J* = 11.7 Hz, 1H, CHOCH₂Ph), 4.47 (d, *J* = 11.8 Hz, 1H, CH₂OCH₂Ph), 4.52 (d, *J* = 11.8 Hz, 1H, CH₂OCH₂Ph), 4.73 (d, *J* = 11.7 Hz, 1H, CHOCH₂Ph), 7.04 – 7.08 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.27 – 7.32 (m, 3H, 2'-H_{phenyl}, 4'-H_{phenyl}, 6'-H_{phenyl}), 7.32 – 7.37 (m, 2H, 3'-H_{phenyl}, 5'-H_{phenyl}), 7.64 – 7.68 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ¹³C NMR (126 MHz, CDCl₃): δ [ppm] = 52.3 (1C, CO₂CH₃), 70.1 (1C, CHCHCH₂), 71.3 (1C, CHCHCH₂), 72.5 (1C, CHOCH₂Ph), 73.6 (1C, CH₂OCH₂Ph), 77.9 (1C, CHCHCH₂), 93.8 (1C, C-4''_{4-iodophenyl}),

127.95 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.97 (1C, C-4'_{phenyl}), 128.6 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 130.2 (2C, C-2''_{4-iodophenyl}, C-6''_{4-iodophenyl}), 136.9 (1C, C-1''_{4-iodophenyl}), 137.7 (2C, C-3''_{4-iodophenyl}, C-5''_{4-iodophenyl}), 137.9 (1C, C-1'_{phenyl}), 171.3 (1C, CO₂CH₃); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3451, 2950, 2901, 2867, 1744, 1484, 1453, 1273, 1205, 1075, 1005, 794, 736, 697; HRMS (*m/z*): [M+Na]⁺ calcd for C₁₉H₂₁INO₅: 479.0326, found: 479.0349.

Methyl (2S,3R)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (19b)

(Diacetoxiodo)benzene (1.0 g, 3.2 mmol) and TEMPO (47 mg, 0.30 mmol) were added to a solution of **18b** (490 mg, 1.1 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) and the mixture was stirred for 4 h at ambient temperature. Then a saturated aqueous solution of sodium thiosulfate was added. The mixture was acidified with hydrochloric acid (0.5 N) until pH 1 was reached and extracted with ethyl acetate (4×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The obtained crude product was dissolved in dry methanol (20 mL) and *p*-toluenesulfonic acid (19 mg, 0.11 mmol) was added. The mixture was stirred for 2 d at 75 °C. Then a saturated aqueous solution of sodium hydrogen carbonate was added, the organic solvent was removed *in vacuo*, and the aqueous phase was extracted with ethyl acetate (7×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified twice by flash column chromatography (1. Ø = 3 cm, h = 15 cm, V = 10 mL, ethyl acetate, 2. Ø = 1 cm, h = 15 cm, V = 5 mL, ethyl acetate) to give **19b** (190 mg, 0.41 mmol, 37% yield) as colorless oil. TLC (cyclohexane/ethyl acetate = 3/1): R_f = 0.15; $[\alpha]_D^{20}$ = -79.6 (0.5, CH₂Cl₂); HPLC (method 1): t_R = 20.1 min, purity 95.6%.

Methyl (2R,3S)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-iodobenzyl)oxy]butanoate (*ent*-19b)

A saturated aqueous solution of sodium bicarbonate (10 mL) was added to a solution of *ent*-18b (0.69 g, 1.5 mmol) in acetonitrile (10 mL), whereby a colourless precipitate formed. After adding TEMPO (0.29 g, 1.9 mmol) and *N*-chlorosuccinimide (0.83 g, 6.2 mmol), the mixture was stirred at 90 °C for 4 h. Afterwards a 0.5 M aqueous solution of hydrogen chloride (10 mL) was added until pH = 1 was reached and the mixture was extracted with ethyl acetate (3× 30 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in methanol (15 mL) and *p*-toluenesulfonic acid monohydrate (0.44 g, 2.3 mmol) was added. After stirring the solution for 18 h at 75 °C, a saturated aqueous solution of sodium bicarbonate (15 mL) was added and the mixture was extracted with ethyl acetate (3× 30 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 30 mL, petroleum ether/ethyl acetate = 1/1, R_f = 0.30) to give *ent*-19b (0.67 g, 1.4 mmol, 91% over two steps) as yellow oil. $[\alpha]_D^{20}$ = + 36.3 (5.6, methanol); HPLC (method 1): t_R = 21.5 min, purity 94.7%.

Spectroscopic data of 19b and *ent*-19b:

^1H NMR (600 MHz, CD_3OD): δ [ppm] = 2.21 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.45 (dd, J = 9.4/5.9 Hz, 1H, CHCHCH_2), 3.54 (dd, J = 9.4/6.7 Hz, 1H, CHCHCH_2), 3.74 (s, 3H, CO_2CH_3), 4.02 – 4.09 (m, 1H, CHCHCH_2), 4.10 (d, J = 3.3 Hz, 1H, CHCHCH_2), 4.31 (s, 2H, $\text{CH}_2\text{OCH}_2\text{Ar}$), 4.35 (d, J = 11.8 Hz, 1H, CHOCH_2PhI), 4.71 (d, J = 11.8 Hz, 1H, CHOCH_2PhI), 7.12 – 7.17 (m, 2H, 2'- $\text{H}_{4\text{-iodophenyl}}$, 6'- $\text{H}_{4\text{-iodophenyl}}$), 7.66 – 7.71 (m, 2H, 3'- $\text{H}_{4\text{-iodophenyl}}$, 5'- $\text{H}_{4\text{-iodophenyl}}$); ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 10.0 (1C, CH_3), 10.8

(1C, CH₃), 52.4 (1C, CO₂CH₃), 62.6 (1C, CH₂OCH₂Ar), 71.2 (1C, CHCHCH₂), 72.1 (1C, CHCHCH₂), 73.3 (1C, CHOCH₂PhI), 80.2 (1C, CHCHCH₂), 93.9 (1C, C-4'-iodophenyl), 112.6 (1C, C-4'' isoxazole), 131.2 (2C, C-2'-iodophenyl, C-6'-iodophenyl), 138.6 (2C, C-3'-iodophenyl, C-5'-iodophenyl), 138.9 (1C, C-1'-iodophenyl), 161.4 (1C, C-5'' isoxazole), 169.0 (1C, C-3'' isoxazole), 173.0 (1C, C=O); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3410, 2947, 2866, 1748, 1636, 1427, 1269, 1204, 1138, 1080, 1007, 883, 837, 799, 768, 667; HRMS (*m/z*): [M+H]⁺ calcd for C₁₈H₂₃INO₆: 476.0565, found: 476.0554.

Methyl (2S,3R)-4-(benzyloxy)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanoate (20a)

Under nitrogen atmosphere, copper(I) iodide (8 mg, 0.04 mmol), tetrakis(triphenylphosphine)palladium(0) (12 mg, 0.010 mmol), and triethylamine (60 μ L, 41 mg, 0.40 mmol) were added to a solution of **19a** (92 mg, 0.20 mmol) in dry acetonitrile (5 mL). After adding a solution of 4-(4-ethynylbenzyl)morpholine (49 mg, 0.24 mmol) in dry acetonitrile (3 mL) dropwise over a period of 30 min, the mixture was stirred at ambient temperature for 18 h. Then the solution was concentrated *in vacuo* and the residue was purified by flash column chromatography ($\varnothing$ = 3 cm, h = 20 cm, V = 20 mL, CH₂Cl₂/methanol = 19/1, R_f = 0.38) and by automated flash column chromatography (10% $\rightarrow$ 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give **20a** (73 mg, 0.14 mmol, 68%) as colourless syrup. $[\alpha]_D^{20}$ = -32.7 (1.1, methanol); HPLC (method 1): t_R = 19.9 min, purity 99.6%.

Methyl (2R,3S)-4-(benzyloxy)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanoate (ent-20a)

Under nitrogen atmosphere, copper(I) iodide (13 mg, 0.066 mmol), triethylamine (0.09 mL, 67 mg, 0.66 mmol), and tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.033 mmol) were added to a solution of *ent*-**19a** (150 mg, 0.33 mmol) in dry acetonitrile. A solution of 4-(4-ethynylbenzyl)morpholine (99 mg, 0.49 mmol) in dry acetonitrile was added dropwise over a period of 15 min at ambient temperature. Then the solvent was evaporated under reduced pressure and the residue was purified twice by flash column chromatography ($\varnothing$ = 3 cm, h = 15 cm, V = 15 mL, dichloromethane/methanol = 19/1) and once by automatic flash column chromatography to give *ent*-**20a** (110 mg, 0.21 mmol, 64%) as a yellow oil. $[\alpha]_D^{20}$ = +63.2 (3.0, methanol); HPLC (method 1): t_R = 19.8 min, purity 99.7%.

Spectroscopic data of *20a* and *ent*-20a**:**

^1H NMR (DMSO- d_6): δ [ppm] = 2.32 – 2.38 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.40 (dd, J = 9.3/5.8 Hz, 1H, CHCHCH_2), 3.49 (s, 2H, ArCH_2N), 3.52 (dd, J = 9.3/7.4 Hz, 1H, CHCHCH_2), 3.56 – 3.60 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.66 (s, 3H, CO_2CH_3), 3.96 – 4.02 (m, 1H, CHCHCH_2), 4.12 (d, J = 3.2 Hz, 1H, CHCHCH_2), 4.41 (d, J = 12.2 Hz, 1H, CHOCH_2Ar), 4.42 (d, J = 12.0 Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.48 (d, J = 12.0 Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.69 (d, J = 12.2 Hz, 1H, CHOCH_2Ar), 5.15 (d, J = 7.2 Hz, 1H, OH), 7.25–7.31 (m, 3H, 2''- H_{phenyl} , 4'''- H_{phenyl} , 6'''- H_{phenyl}), 7.31 – 7.35 (m, 2H, 3'''- H_{phenyl} , 5'''- H_{phenyl}), 7.35 – 7.39 (m, 4H, 2'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 6'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 3''-4-(morpholinomethyl)phenyl, 5''-4-(morpholinomethyl)phenyl), 7.49 – 7.53 (m, 4H, 3'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 5'- $\text{H}_{4-[[4-(\text{morpholinomethyl})\text{phenyl}]\text{ethynyl}]\text{phenyl}}$, 2''-4-(morpholinomethyl)phenyl, 6''-4-(morpholinomethyl)phenyl); ^{13}C NMR (DMSO- d_6): δ [ppm] = 51.6 (1C, CO_2CH_3), 53.1 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 62.0 (1C, ArCH_2N), 66.2 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 70.18 (1C, CHCHCH_2), 70.21 (1C, CHCHCH_2), 71.5 (1C, CHOCH_2Ar), 72.2 (1C,

CH₂OCH₂Ph), 79.0 (1C, CHCHCH₂), 89.0 (1C, C≡C), 89.3 (1C, C≡C), 120.8 (1C, C-1''⁴-(morpholinomethyl)phenyl), 121.4 (1C, C-4'⁴-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl), 127.4 (1C, C-4'''^{phenyl}), 127.5 (2C, C-2'''^{phenyl}, C-6'''^{phenyl}), 127.9 (2C, C-2'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, C-6'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl), 128.2 (2C, C-3'''^{phenyl}, C-5'''^{phenyl}), 129.2 (2C, C-3''⁴-(morpholinomethyl)phenyl, C-5''⁴-(morpholinomethyl)phenyl), 131.15 (2C, C_{arom.}), 131.21 (2C, C_{arom.}), 138.2 (1C, C-1'''^{phenyl}), 138.6 (1C, C-1'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl), 138.8 (1C, C-4''⁴-(morpholinomethyl)phenyl), 171.1 (1C, CO₂CH₃); .IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3409, 2952, 2861, 1749, 1518, 1454, 1395, 1349, 1277, 1205, 1113, 1071, 1006, 913, 865, 840, 819, 792, 737, 698; HRMS (*m/z*): [M+H]⁺ calcd for C₃₂H₃₆NO₆: 530.2537; found: 530.2539.

Methyl (2S,3R)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanoate (20b)

Under nitrogen atmosphere, copper(I) iodide (13 mg, 0.07 mmol), triethylamine (0.26 mL, 0.19 g, 1.9 mmol) and tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.04 mmol) were added to a solution of **19b** (170 mg, 0.36 mmol) in dry acetonitrile (4.0 mL). A solution of 4-(4-ethynylbenzyl)morpholine (570 mg, 2.8 mmol) in dry acetonitrile (2.0 mL) was added dropwise over a period of 2 h at ambient temperature. The reaction was stirred for 1 d. Then the solvent was removed *in vacuo* and the residue was purified by automatic flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP C18 30 g) to give **20b** (170 mg, 0.31 mmol, 85%) as brown oil. TLC (CH₂Cl₂/methanol = 10/1): R_f = 0.37; [α]_D²⁰ = -79.6 (0.5, methanol); HPLC (method 1): t_R = 17.1 min, purity 86.9%.

Methyl (2R,3S)-4-[(3,5-dimethylisoxazol-4-yl)methoxy]-3-hydroxy-2-[[4-[[4-(morpholinomethyl)phenyl]ethynyl]benzyl]oxy]butanoate (*ent*-20b)

Under nitrogen atmosphere, copper(I) iodide (28 mg, 0.15 mmol), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.033 mmol), and triethylamine (0.19 mL, 0.14 g, 1.4 mmol) were added to a solution of *ent*-19b (0.33 g, 0.69 mmol) in dry acetonitrile (10 mL). After adding a solution of 4-(4-ethynylbenzyl)morpholine (0.17 g, 0.84 mmol) in dry acetonitrile (5 mL) dropwise over a period of 20 min, the mixture was stirred at ambient temperature for 18 h. The resulting solution was concentrated *in vacuo* and the residue was purified twice by flash column chromatography ($\varnothing$ = 6 cm, h = 15/25 cm, V = 20 mL, CH₂Cl₂/methanol = 9:1, R_f = 0.11) and by automated flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give *ent*-20b (0.19 g, 0.34 mmol, 49%) as yellow oil. $[\alpha]_D^{20}$ = +47.5 (3.5, methanol); HPLC (method 1): t_R = 17.9 min, purity 94.5%.

Spectroscopic data of 20b and ent-20b:

¹H NMR (600 MHz, CD₃OD): δ [ppm] = 2.21 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.42 – 2.49 (m, 4H, N(CH₂CH₂)₂O), 3.46 (dd, *J* = 9.4/5.9 Hz, 1H, CHCHCH₂), 3.54 (s, 2H, ArCH₂N), 3.56 (dd, *J* = 9.4/6.7 Hz, 1H, CHCHCH₂), 3.67 – 3.71 (m, 4H, N(CH₂CH₂)₂O), 3.75 (s, 3H, CO₂CH₃), 4.05–4.10 (m, 1H, CHCHCH₂), 4.12 (d, *J* = 3.2 Hz, 1H, CHCHCH₂), 4.30 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.33 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.42 (d, *J* = 11.9 Hz, 1H, CHOCH₂Ar), 4.78 (d, *J* = 11.9 Hz, 1H, CHOCH₂Ar), 7.30 – 7.44 (m, 4H, 2'-H₄-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, 6'-H₄-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, 3''-H₄-(morpholinomethyl)phenyl, 5''-H₄-(morpholinomethyl)phenyl), 7.46 – 7.51 (m, 4H, 3'-H₄-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, 5'-H₄-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, 2''-H₄-(morpholinomethyl)phenyl, 6''-H₄-(morpholinomethyl)phenyl); ¹³C NMR (151 MHz, CD₃OD): δ

[ppm] = 10.0 (1C, CH₃), 10.8 (1C, CH₃), 52.4 (1C, CO₂CH₃), 54.7 (2C, N(CH₂CH₂)₂O), 62.6 (1C, CH₂OCH₂Ar), 64.0 (1C, ArCH₂N), 67.8 (2C, N(CH₂CH₂)₂O), 71.3 (1C, CHCHCH₂), 72.1 (1C, CHCHCH₂), 73.6 (1C, CHOCH₂Ar), 80.2 (1C, CHCHCH₂), 90.0 (1C, C≡C), 90.2 (1C, C≡C), 112.6 (1C, C-4'''_{isoxazole}), 123.5 (1C, C_{arom.}), 124.1 (1C, C_{arom.}), 129.3 (2C, C-2'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl, C-6'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl), 130.7 (2C, C-3''4-(morpholinomethyl)phenyl, C-5''4-(morpholinomethyl)phenyl), 132.47 (2C, C_{arom.}), 132.48 (2C, C_{arom.}), 139.1 (1C, C-4''4-(morpholinomethyl)phenyl), 139.4 (1C, C-1'4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl), 161.4 (1C, C-5'''_{isoxazole}), 169.0 (1C, C-3'''_{isoxazole}), 173.0 (1C, C=O); IR (neat): ν [cm⁻¹] = 2951, 2859, 2808, 1736, 1639, 1516, 1454, 1435, 1350, 1204, 1115, 1072, 1007, 914, 864, 841, 822, 795, 667; HRMS (m/z): [M+H]⁺ calcd for C₃₁H₃₇N₂O₇: 549.2595, found: 549.2575.

(2S,3R)-4-(Benzyloxy)-N,3-dihydroxy-2-[[4-[[4-(morpholinomethyl)phenyl]ethynyl]benzyl]oxy]butanamide (21a)

Under nitrogen atmosphere, a 50% aqueous solution of hydroxylamine (0.19 mL, 0.10 g, 3.1 mmol) was added to a solution of **20a** (55 mg, 0.10 mmol) in methanol (1 mL) and THF (1 mL). After adding potassium cyanide (7 mg, 0.1 mmol), the mixture was stirred at ambient temperature for 17 h. The resulting colourless solution was purified by automated flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give **21a** (37 mg, 0.070 mmol, 67%) as colourless solid. TLC: R_f = 0.53 (CH₂Cl₂/methanol = 9:1); m.p. = 106 °C; $[\alpha]_D^{20}$ = -36.5 (1.6, methanol); HPLC (method 2): t_R = 12.8 min, purity 100%.

(2R,3S)-4-(Benzyloxy)-N,3-dihydroxy-2-[[4-[[4-(morpholinomethyl)phenyl]ethynyl]benzyl]oxy]butanamide (ent-21a)

Under nitrogen atmosphere, a 5.4 M solution of sodium methoxide in methanol (3.0 mL, 16 mmol) and hydroxylamine hydrochloride (1.1 g, 16 mmol) were added to a solution of *ent*-**20a** (87 mg, 0.16 mmol) in methanol (10 mL) and the mixture was stirred at ambient temperature overnight. Then the solvent was removed under reduced pressure and the residue was purified by automatic flash column chromatography to give *ent*-**21a** (50 mg, 0.094 mmol, 58%) as colorless solid. $[\alpha]_D^{20} = +45.5$ (2.8, methanol); HPLC (method 2): $t_R = 13.3$ min, purity 99.2%.

Spectroscopic data of 21a and ent-21a:

^1H NMR (400 MHz, DMSO- d_6): δ [ppm] = 2.31 – 2.40 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.30 – 3.40 (m, 1H, CHCHCH_2), 3.44 – 3.51 (m, 3H, ArCH_2N , CHCHCH_2 (1H)), 3.54 – 3.61 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 3.78 (d, $J = 4.0$ Hz, 1H, CHCHCH_2), 3.87 – 3.94 (m, 1H, CHCHCH_2), 4.36 (d, $J = 12.4$ Hz, 1H, CHOCH_2Ar), 4.41 (d, $J = 12.1$ Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.45 (d, $J = 12.1$ Hz, 1H, $\text{CH}_2\text{OCH}_2\text{Ph}$), 4.62 (d, $J = 12.4$ Hz, 1H, CHOCH_2Ar), 5.14 (s br, 1H, CHOH), 7.24 – 7.42 (m, 9H, $2'''$ - H_{phenyl} , $3'''$ - H_{phenyl} , $4'''$ - H_{phenyl} , $5'''$ - H_{phenyl} , $6'''$ - H_{phenyl} , $2'$ - $\text{H}_{4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}}$, $6'$ - $\text{H}_{4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}}$, $3''$ - $4-(\text{morpholinomethyl})\text{phenyl}$, $5''$ - $4-(\text{morpholinomethyl})\text{phenyl}$), 7.47 – 7.54 (m, 4H, $3'$ - $\text{H}_{4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}}$, $5'$ - $\text{H}_{4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}}$, $2''$ - $4-(\text{morpholinomethyl})\text{phenyl}$, $6''$ - $4-(\text{morpholinomethyl})\text{phenyl}$), 8.82 (s br, 1H, CONHOH), 10.61 (s br, 1H, CONHOH); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = 53.2 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 62.0 (1C, ArCH_2N), 66.2 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$), 70.2 (1C, CHCHCH_2), 70.7 (1C, CHCHCH_2), 71.3 (1C, CHOCH_2Ar), 72.2 (1C, $\text{CH}_2\text{OCH}_2\text{Ph}$), 79.2 (1C, CHCHCH_2), 89.1 (1C, $\text{C}\equiv\text{C}$), 89.3 (1C, $\text{C}\equiv\text{C}$), 120.9 (1C, $\text{C}-1''$ - $4-(\text{morpholinomethyl})\text{phenyl}$), 121.4 (1C, $\text{C}-4'$ - $4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}$), 127.4 (1C, $\text{C}-4'''$ - phenyl), 127.5 (2C, $\text{C}-2'''$ - phenyl , $\text{C}-6'''$ - phenyl), 128.0 (2C, $\text{C}-2'$ - $4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}$, $\text{C}-6'$ - $4-\{[4-(\text{morpholinomethyl})\text{phenyl}] \text{ethynyl}\} \text{phenyl}$),

128.2 (2C, C-3'''_{phenyl}, C-5'''_{phenyl}), 129.2 (2C, C-3''_{4-(morpholinomethyl)phenyl}, C-5''_{4-(morpholinomethyl)phenyl}), 131.1 (2C, C_{arom.}) 131.2 (2C, C_{arom.}), 138.4 (1C, C-1'''_{phenyl}), 138.77 (1C, C-1'_{4-[[4-(morpholinomethyl)phenyl]ethynyl]phenyl}), 138.83 (1C, C-4''_{4-(morpholinomethyl)phenyl}), 166.3 (1C, CONHOH); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3238, 2857, 1660, 1517, 1454, 1349, 1113, 1069, 1005, 864, 840, 820, 791, 736, 697; HRMS (*m/z*): [M+H]⁺ calcd for C₃₁H₃₅N₂O₆: 531.2490, found: 531.2556.

(2*S*,3*R*)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-*N*,3-dihydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]butanamide (21*b*)

Hydroxylamine hydrochloride (58 mg, 0.84 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.16 mL, 0.84 mmol) were added to a solution of **20b** (150 mg, 0.28 mmol) in methanol (7 mL) and the mixture was stirred for 12 h at 30 °C. Subsequently the solvent was removed *in vacuo*. The residue was purified by automatic flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP C18 30 g) and flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, CH₂Cl₂/methanol = 10/1, R_f = 0.42) to give **21b** (140 mg, 0.25 mmol, 91%) as a pale yellow solid. m.p. = 70 °C; $[\alpha]_D^{20}$ = -62.4 (2.3, methanol); HPLC (method 2): t_R = 12.7 min, purity 98.3%.

(2*R*,3*S*)-4-[(3,5-Dimethylisoxazol-4-yl)methoxy]-*N*,3-dihydroxy-2-{[4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl]oxy}butanamide (ent-21*b*)

Under nitrogen atmosphere, a 50 % aqueous solution of hydroxylamine (0.16 mL, 86 mg, 2.6 mmol) was added to a solution of ent-**20b** (46 mg, 0.084 mmol) in methanol (2 mL) and THF (2 mL). After adding potassium cyanide (6 mg, 0.1 mmol), the mixture

was stirred at ambient temperature for 19 h. The resulting colourless solution was purified by automated flash column chromatography (10% → 100% acetonitrile in H₂O, Biotage® SNAP KP-C18-HS 30 g) to give *ent*-**21b** (33 mg, 0.060 mmol, 72%) as colourless solid. TLC: R_f = 0.37 (CH₂Cl₂/methanol = 9/1); m.p. = 69 °C; $[\alpha]_D^{20} = +52.4$ (1.5, methanol); HPLC (method 2): t_R = 12.8 min, purity 99.6%.

Spectroscopic data of 21b and ent-21b:

¹H NMR (600 MHz, CD₃OD): δ [ppm] = 2.22 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.45 – 2.52 (m, 4H, N(CH₂CH₂)₂O), 3.43 (dd, *J* = 9.7/6.1 Hz, 1H, CHCHCH₂), 3.49 (dd, *J* = 9.7/5.9 Hz, 1H, CHCHCH₂), 3.55 (s, 2H, ArCH₂N), 3.67 – 3.73 (m, 4H, N(CH₂CH₂)₂O), 3.93 (d, *J* = 3.8 Hz, 1H, CHCHCH₂), 4.00 (td, *J* = 6.0/3.8 Hz, 1H, CHCHCH₂), 4.28 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.31 (d, *J* = 12.3 Hz, 1H, CH₂OCH₂Ar), 4.43 (d, *J* = 12.1 Hz, 1H, CHOCH₂Ar), 4.72 (d, *J* = 12.0 Hz, 1H, CHOCH₂Ar), 7.34 – 7.42 (m, 4H, 2'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 6'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 3''-H₄-(morpholinomethyl)phenyl, 5''-H₄-(morpholinomethyl)phenyl), 7.46 – 7.53 (m, 4H, 3'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 5'-H₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, 2''-H₄-(morpholinomethyl)phenyl, 6''-H₄-(morpholinomethyl)phenyl); ¹³C NMR (151 MHz, CD₃OD): δ [ppm] = 10.0 (1C, CH₃), 10.8 (1C, CH₃), 54.6 (2C N(CH₂CH₂)₂O), 62.6 (1C, CH₂OCH₂Ar), 63.9 (1C, ArCH₂N), 67.7 (2C, N(CH₂CH₂)₂O), 71.4 (1C, CHCHCH₂), 71.8 (1C, CHCHCH₂), 73.7 (1C, CHOCH₂Ar), 80.7 (1C, CHCHCH₂), 89.9 (1C, C≡C), 90.3 (1C, C≡C), 112.6 (1C, C-4'''-isoxazole), 123.6 (1C, C-1''₄-(morpholinomethyl)phenyl), 124.2 (1C, C-4'₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl), 129.5 (2C, C-2'₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl, C-6'₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl), 130.8 (2C, C-3''₄-(morpholinomethyl)phenyl, C-5''₄-(morpholinomethyl)phenyl), 132.5 (2C, C_{arom.}), 132.6 (2C, C_{arom.}), 139.0 (1C, C-4''₄-(morpholinomethyl)phenyl), 139.1 (1C, C-1'₄-{[4-(morpholinomethyl)phenyl]ethynyl}phenyl), 161.4 (1C, C-

5'''_{isoxazole}), 169.0 (1C, C-3'''_{isoxazole}), 169.7 (1C, C=O); IR (neat): ν [cm⁻¹] = 3186, 2928, 2866, 1732, 1667, 1516, 1454, 1373, 1242, 1111, 1072, 1045, 1007, 914, 841, 822, 791, 667; HRMS (m/z): [M+H]⁺ calcd. for C₃₀H₃₆N₃O₇: 550.2548, found: 550.2560.

(S)-1-[(2R,4S,5S)-5-Hydroxy-2-phenyl-1,3-dioxan-4-yl]ethane-1,2-diol (23)

Hydrogen chloride gas, generated by the dropwise addition of concentrated sulfuric acid to sodium chloride, was bubbled through a mixture of L-arabinitol (5.0 g, 33 mmol) and freshly distilled benzaldehyde (3.4 mL, 3.5 g, 33 mmol) for 30 min. The mixture was stirred at room temperature overnight. The solidified mass was broken up with mortar and pestle. The solid was triturated with 20% aqueous NaHCO₃ (20 mL) and diethyl ether (10 mL). The solid was filtered off and rinsed with water (10 mL) and diethyl ether (10 mL). The resulting solid was recrystallized from propan-2-ol to afford **23** as colorless solid (5.7 g, 24 mmol, 71%). TLC (ethyl acetate): R_f = 0.3; m.p. = 149 °C; $[\alpha]_D^{20}$ = -9.0 (2.9, methanol); ¹H NMR (500 MHz, CD₃OD): δ [ppm] = 3.64 – 3.68 (m, 1H, CH₂CHCHCHCH₂OH), 3.75 – 3.79 (m, 1H, CH₂CHCHCHCH₂OH), 3.79 – 3.81 (m, 1H, CH₂CHCHCHCH₂OH), 3.87 – 3.91 (m, 2H, CH₂CHCHCHCH₂OH), 4.12 (dd, J = 12.1/1.5 Hz, 1H, CH₂CHCHCHCH₂OH), 4.20 (dd, J = 12.1/1.7 Hz, 1H, CH₂CHCHCHCH₂OH), 5.60 (s, 1H, PhCH), 7.30 – 7.37 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.49-7.54 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}); ¹³C NMR (126 MHz, CD₃OD): δ [ppm] = 64.0 (1C, CH₂CHCHCHCH₂OH), 64.1 (1C, CH₂CHCHCHCH₂OH), 71.0 (1C, CH₂CHCHCHCH₂OH), 73.8 (1C, CH₂CHCHCHCH₂OH), 80.1 (1C, CH₂CHCHCHCH₂OH), 102.6 (1C, PhCH), 127.4 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 129.0 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 129.7 (1C, 4'-H_{phenyl}), 139.9 (1C, C-1'_{phenyl}); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3286, 2961, 2930, 2855, 1449, 1409, 1390, 1361, 1311, 1218, 1157, 1086, 1012, 990,

957, 886, 814, 744, 694, 656, 565, 451; HRMS (m/z): $[M+Na]^+$ calcd for $C_{12}H_{16}NaO_5$: 263.0890, found: 263.0887; HPLC (method 1): t_R = 11.5 min, purity 99.7%.

(2*R*,4*S*,5*S*)-4-(Hydroxymethyl)-2-phenyl-1,3-dioxan-5-ol (24)

A solution of **23** (2.7 g, 11 mmol), sodium metaperiodate (4.2 g, 20 mmol) and sodium hydrogen carbonate (2.4 g, 28 mmol) in water (72 mL) and THF (32 mL) was stirred for 1 h at ambient temperature. Then, the mixture was cooled to 0 °C for 10 min, sodium borohydride (0.75 g, 20 mmol) was added, and the mixture was stirred for 1 h at ambient temperature. The resulting precipitate was filtered off and the filtrate was extracted with ethyl acetate (3×). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (1×) and a saturated aqueous solution of sodium thiosulfate (1×), dried over sodium sulfate, and filtered. Evaporation of the solvent under reduced pressure gave **24** (1.4 g, 6.5 mmol, 59%) as colorless solid. TLC (petroleum ether/ethyl acetate = 1/2): R_f = 0.18; m.p. = 132 °C; $[\alpha]_D^{20}$ = +5.6 (2.6, methanol); 1H NMR (400 MHz, DMSO- d_6): δ [ppm] = 3.48 – 3.59 (m, 3H, $CH_2CHCHCH_2OH$), 3.85 – 3.89 (m, 1H, $CH_2CHCHCH_2OH$), 3.99 – 4.01 (m, 2H, $CH_2CHCHCH_2OH$), 4.64 (t, J = 5.7 Hz, 1H, CH_2OH), 4.75 (d, J = 6.6 Hz, 1H, $CHOH$), 5.55 (s, 1H, $PhCH$), 7.32 – 7.38 (m, 3H, 3'- H_{phenyl} , 4'- H_{phenyl} , 5'- H_{phenyl}), 7.46 – 7.50 (m, 2H, 2'- H_{phenyl} , 6'- H_{phenyl}); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = 61.0 (1C, $CH_2CHCHCH_2OH$), 62.4 (1C, $CH_2CHCHCH_2OH$), 72.2 (1C, $CH_2CHCHCH_2OH$), 80.0 (1C, $CH_2CHCHCH_2OH$), 100.3 (1C, $PhCH$), 126.4 (2C, C-2' $_{phenyl}$, C-6' $_{phenyl}$), 127.8 (2C, C-3' $_{phenyl}$, C-5' $_{phenyl}$), 128.5 (1C, 4'- H_{phenyl}), 138.8 (1C, C-1' $_{phenyl}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3228, 2935, 2855, 1460, 1442, 1407, 1314, 1219, 1146, 1058, 1001, 984, 957, 921, 900, 884, 821, 758, 698, 658, 633, 545; HRMS (m/z): $[M+Na]^+$ calcd for $C_{11}H_{14}NaO_4$: 233.0784, found: 233.0796; HPLC (method 1): t_R = 11.7 min, purity 96.6%.

(2R,4S,5S)-4-[[*tert*-Butyldimethylsilyl]oxy]methyl]-2-phenyl-1,3-dioxan-5-ol (25**)**

Under nitrogen atmosphere, triethylamine (0.84 mL, 0.61 g, 6.1 mmol) was added to an ice-cooled solution of **24** (1.0 g, 4.8 mmol) in dry THF (20 mL). After 5 min, *tert*-butyldimethylsilyl chloride (0.91 g, 6.1 mmol) and DMAP (30 mg, 0.24 mmol) were added. The solution was stirred at 0 °C for 1 h and at ambient temperature for 24 h. Then ice-cold water (20 mL) was added and the mixture was extracted with ethyl acetate (3× 40 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 5 cm, h = 15 cm, V = 30 mL, petroleum ether/ethyl acetate = 4/1, R_f = 0.67) to give **25** (1.5 g, 4.5 mmol, 94%) as colourless solid. m.p. = 41 °C; $[\alpha]_D^{20}$ = + 3.4 (11.4, methanol); ^1H NMR (400 MHz, DMSO- d_6): δ [ppm] = 0.03 (s, 3H, Si(CH₃)₂), 0.04 (s, 3H, Si(CH₃)₂), 0.86 (s, 9H, C(CH₃)₃), 3.47 – 3.51 (m, 1H, CHOH), 3.67 (dd, J = 10.5/6.6 Hz, 1H, CH₂OSi), 3.77 (dd, J = 10.5/5.6 Hz, 1H, CH₂OSi), 3.90 – 3.95 (m, 1H, CHCH₂OSi), 3.97 – 4.05 (m, 2H, CH₂CHOH), 4.80 (d, J = 6.6 Hz, 1H, OH), 5.57 (s, 1H, PhCH), 7.31 – 7.39 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.43 – 7.49 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}); ^{13}C NMR (101 MHz, DMSO- d_6): δ [ppm] = -5.3 (1C, Si(CH₃)₂), -5.2 (1C, Si(CH₃)₂), 18.1 (1C, C(CH₃)₃), 25.8 (3C, C(CH₃)₃), 62.2 (1C, CHOH), 62.8 (1C, CH₂OSi), 72.2 (1C, CH₂CHOH), 79.7 (1C, CHCH₂OSi), 100.2 (1C, PhCH), 126.3 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.8 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 128.6 (1C, C-4'_{phenyl}), 138.7 (1C, C-1'_{phenyl}); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3309, 2929, 2856, 1452, 1388, 1361, 1250, 1155, 1084, 1004, 833, 775, 742, 696; HRMS (m/z): [M+Na]⁺ calcd for C₁₇H₂₈NaO₄Si: 347.1649, found: 347.1648; HPLC (method 1): t_R = 25.6 min, purity 97.4%.

{(2R,4S,5S)-5-[(4-iodobenzyl)oxy]-2-phenyl-1,3-dioxan-4-yl}methanol (26**)**

Under nitrogen atmosphere, sodium hydride (60% in mineral oil, 0.49 g, 12 mmol) was added to a solution of **25** (2.7 g, 8.2 mmol) in DMF (65 mL) at 0 °C and the mixture was stirred at 0 °C for 20 min. Then a solution of 4-iodobenzyl bromide (3.6 g, 12 mmol) in DMF (7 mL) was added dropwise and the reaction mixture was stirred at 0 °C for 40 min. Thereafter, the mixture was stirred at ambient temperature overnight. Then a saturated aqueous solution of sodium hydrogen carbonate was added and the resulting mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 4 cm, h = 15 cm, V = 35 mL, petroleum ether/ethyl acetate = 8/1). The fractions containing the intermediate benzyl ether were evaporated and the residue was dissolved in THF. After the addition of tetrabutylammonium fluoride trihydrate (3.9 g, 12 mmol), the reaction was stirred at ambient temperature overnight. Then water (55 mL) was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over sodium sulfate, filtered, and and concentrated *in vacuo*. The residue was purified by flash column chromatography ($\varnothing$ = 4 cm, h = 15 cm, V = 35 mL, petroleum ether/ethyl acetate = 4/1, R_f = 0.10) to give **26** (2.1 g, 5.0 mmol, 61%) as colorless solid. m.p. = 140 °C; $[\alpha]_D^{20}$ = +29.6 (2.4, methanol); ^1H NMR (500 MHz, DMSO- d_6): δ [ppm] = 3.44 – 3.47 (m, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 3.58 (t, J = 5.7 Hz, 2H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 3.91 – 3.96 (m, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 3.99 (td, J = 6.2/1.7 Hz, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 4.32 – 4.36 (m, 1H, $\text{CH}_2\text{CHCHCH}_2\text{OH}$), 4.45 (d, J = 12.2 Hz, 1H, OCH_2Ar), 4.65 (d, J = 12.2 Hz, 1H, OCH_2Ar), 4.73 (t, J = 5.5 Hz, 1H, OH), 5.60 (s, 1H, PhCH), 7.18 – 7.22 (m, 2H, 2''-H_{4-iodophenyl}, 6''-H_{4-iodophenyl}), 7.33 – 7.38 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.40 – 7.44 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}), 7.70 – 7.74 (m, 2H, 3''-H_{4-iodophenyl}, 5''-H_{4-iodophenyl}); ^{13}C

NMR (126 MHz, DMSO- d_6): δ [ppm] = 60.6 (1C, CH₂CHCHCH₂OH), 67.4 (1C, CH₂CHCHCH₂OH), 69.5 (1C, OCH₂Ar), 70.1 (1C, CH₂CHCHCH₂OH), 79.5 (1C, CH₂CHCHCH₂OH), 93.2 (1C, C-4''_{4-iodophenyl}), 100.0 (1C, PhCH), 126.2 (2C, C-2'_{phenyl}, C-6'_{phenyl}), 127.9 (2C, C-3'_{phenyl}, C-5'_{phenyl}), 128.5 (1C, C-4'_{phenyl}), 129.8 (2C, C-2''_{4-iodophenyl}, C-6''_{4-iodophenyl}), 136.9 (2C, C-3''_{4-iodophenyl}, C-5''_{4-iodophenyl}), 138.60 (1C, C_{arom.}), 138.63 (1C, C_{arom.}); IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3332, 2966, 2864, 1484, 1449, 1368, 1337, 1246, 1219, 1158, 1129, 1086, 1029, 1006, 967, 892, 855, 818, 801, 744, 695, 582, 511, 479; HRMS (m/z): [M+Na]⁺ calcd for C₁₈H₁₉INaO₄: 449.0220, found: 449.0189; HPLC (method 1): t_R = 22.8 min, purity 99.5%.

Biological evaluation

Disk diffusion assay

The antibiotic activity of the synthesized inhibitors was determined by disk diffusion assays. Liquid cultures of *E. coli* BL21(DE3) and the defective strain *E. coli* D22⁵⁰ were grown overnight in lysogeny broth (LB)⁶⁴ at 37 °C, 200 rpm. 150 μ L of an overnight cell suspension were spread evenly onto LB agar plates. 0.15 μ mol of each compound (dissolved in 10 μ L or 15 μ L DMSO) were applied onto circular filter paper ($\varnothing$ = 6 mm, GE Healthcare). Pure DMSO, serving as a negative and CHIR-090, serving as a positive control were also spotted. The agar plates were incubated overnight at 37 °C and the diameter of the zone of growth inhibition was measured for each compound. Each assay was performed at least three times on separate days.

The disc diffusion assays against Gram-negative wild type strains were performed as follows: The bacteria were grown overnight on a blood agar plate. The next day, one single colony was diluted with sterile saline to yield a suspension visually equivalent to a 0.5 McFarland standard. The suspension was spread evenly onto Mueller-Hinton

agar plates. 0.10 μmol of each compound (dissolved in 10 μL DMSO) were applied onto circular filter paper. After incubating the agar plates for 20 h at 37 °C, the diameter of the zone of growth inhibition was measured for each compound.

Minimum Inhibitory Concentration (MIC)

The MIC values of the compounds were determined by means of the microdilution method using 96-well plates.

To determine the MIC values against *E. coli* BL21(DE3) and *E. coli* D22, the bacteria were grown overnight in LB at 37 °C and 200 rpm. The overnight suspension was diluted 1:1000 in fresh LB. 10 μL of a twofold dilution series of the compounds in DMSO and 90 μL of LB were dispensed to each well of a 96-well plate. Then, 100 μL of the inoculated medium were added, resulting in $5 \cdot 10^5 \text{ cfu} \cdot \text{mL}^{-1}$, 5% DMSO, and a final concentration range of the test compounds between $64 \mu\text{g} \cdot \text{mL}^{-1}$ and $0.031 \mu\text{g} \cdot \text{mL}^{-1}$. The plates were incubated for 20 h at 37 °C. The MIC was defined as the lowest concentration of the compounds that prevented visible growth after incubation. Each assay was performed at least three times on separate days.

To determine the MIC against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 in the presence/absence of the efflux pump inhibitor PA β N, the bacteria were grown overnight on a LB-agar plate. The next day, one single colony was diluted with sterile saline to yield a suspension visually equivalent to a 0.5 McFarland standard. This initial inoculum was consecutively diluted 1:10 with MHB twice. 25 μL of a two-fold dilution series of the compounds in MHB (ranging from $512 \mu\text{g} \cdot \text{mL}^{-1}$ to $1 \mu\text{g} \cdot \text{mL}^{-1}$) were dispensed to each well of a 96-well-plate. Then, 25 μL MHB or 25 μL PA β N ($256 \mu\text{g} \cdot \text{mL}^{-1}$ in MHB) were added, respectively. Finally, 50 μL of the prepared inoculum were added, resulting in $5 \cdot 10^5 \text{ cfu} \cdot \text{mL}^{-1}$, a final concentration range of the test

compounds between $128 \mu\text{g} \cdot \text{mL}^{-1}$ and $0.25 \mu\text{g} \cdot \text{mL}^{-1}$, and, in case the efflux pump inhibitor was present, a PA β N concentration of $64 \mu\text{g} \cdot \text{mL}^{-1}$. The plates were incubated for 18 h at 37 °C. The MIC was defined as the lowest concentration of the compounds that prevented visible growth after incubation.

Protein expression

The expression of LpxCC63A was performed essentially as previously described.⁶⁵ The C63A mutation lowers the undesired influence of the Zn^{2+} -concentration on the enzymatic activity.

The plasmid pETecLpxCC63A, which was kindly provided by Carol Fierke,⁴⁸ was transformed into *E. coli* BL21(DE3) cells. The overnight culture was prepared by growing a single colony in 50 mL LB supplemented with carbenicillin (0.1 mM) and glucose (0.5%) at 37 °C and 200 rpm. The next day, 2 mL of this culture were used to inoculate 400 mL of fresh LB, containing carbenicillin (0.1 mM) and glucose (0.5%). After reaching an OD₆₀₀ of 0.6 – 0.8, the culture was cooled to 30 °C and induced with IPTG (1 mM) and ZnCl_2 (100 μM). After being grown for additional 4 h at 30 °C, the cells were cooled on ice for 20 min and then harvested by centrifugation (4 °C, 5000 g, 15 min) and stored at -20 °C.

Protein purification

Unless otherwise specified, all steps were carried out at 4 °C.

The harvested cells were thawed on ice and resuspended in 50 mL anion exchange (AEX)-buffer (25 mM Hepes, 2 mM DTT, pH = 7.0), containing benzamidine (15 $\mu\text{g} \cdot \text{mL}^{-1}$) and PMSF (1 mM) as protease inhibitors. Afterwards, the cells were

disrupted by sonication (5×40 s). Then, the cellular debris were removed by centrifugation ($4\text{ }^{\circ}\text{C}$, 5000 g , 90 min) and the supernatant was filtered ($0.2\text{ }\mu\text{m}$).

The cleared lysate was loaded onto a 20 mL -AEX-column (HiPrep Q HP $16/10$, GE Healthcare) and eluted at a flow rate of $0.5\text{ mL} \cdot \text{min}^{-1}$ using a linear potassium chloride gradient ($0\text{ M} \rightarrow 0.5\text{ M}$) in AEX-buffer. The fractions containing LpxC were concentrated using molecular weight cut off (MWCO) spin columns (10 kDa), loaded onto a 120 mL -size exclusion (SEC)-column (HiLoad $16/600$ Superdex 200 , GE Healthcare) and eluted at a flowrate of $0.5\text{ mL} \cdot \text{min}^{-1}$ in SEC-buffer (50 mM Bis/Tris, 150 mM NaCl, $\text{pH} = 6.0$). The presence of the enzyme during the purification progress was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie brilliant blue staining. The purified enzyme was quantified by use of a Nanodrop 2000C, diluted with SEC-buffer to $0.5\text{ mg} \cdot \text{mL}^{-1}$ and stored at $-80\text{ }^{\circ}\text{C}$.

The purification of LasB from *P. aeruginosa* P14 supernatant was performed as described previously.⁵⁵

Enzyme inhibition assays

A fluorescence-based microplate assay for LpxC activity was performed as described by Clements *et al.*⁴⁷ The wells in a black, non-binding, 96-well fluorescence microplate (Greiner Bio One, Frickenhausen) were filled with $93\text{ }\mu\text{L}$ of $26.9\text{ }\mu\text{M}$ UDP-3-O-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine in assay buffer (40 mM sodium morpholinoethanesulfonic acid, $80\text{ }\mu\text{M}$ dithiothreitol, 0.02% Brij 35 ($\text{pH} 6.0$)). In order to assay the inhibitors at final concentrations from 200 nM up to $200\text{ }\mu\text{M}$, $2\text{ }\mu\text{L}$ of a respective dilution of the compounds in DMSO were added. The addition of $5\text{ }\mu\text{L}$ of a solution of purified LpxC ($10\text{ }\mu\text{g} \cdot \text{mL}^{-1}$) in assay buffer led to final concentrations of

25 μ M UDP-3-O-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine, 14.7 nM LpxC, 2% DMSO, and from 200 nM up to 200 μ M inhibitor. The microplate was incubated for 30 min at 37 °C in a plate shaker. Then, the biochemical reaction was stopped by adding 40 μ L of 0.625 M sodium hydroxide. The reaction mixture was further incubated for 10 min and neutralized by adding 40 μ L of 0.625 M acetic acid. The deacetylated product UDP-3-O-[(*R*)-3-hydroxymyristoyl]glucosamine was converted into a fluorescing isoindole by adding 120 μ L of a *o*-phthaldialdehyde-2-mercaptoethanol solution, which was prepared by dissolving 10 mg *o*-phthaldialdehyde in 1 mL methanol, diluting the mixture with 24 mL sodium borate buffer (0.1 M), and finally adding 2.5 μ L 2-mercaptoethanol.⁶⁶ Fluorescence was measured with a Tristar² plate reader (Berthold, Bad Wildbad) at 340 nm excitation and 460 nm emission wavelengths. Each assay was performed at least three times on separate days. The IC₅₀ values were calculated *via* Probit-log concentration graphs (Figures S2-S9) with the aid of the software Origin.

The performance of the FRET-based LasB inhibition assay was performed as described previously.⁵⁵ All samples were run in duplicate for each condition, and experiments were conducted independently at least twice.

Quantification of 17a in subcellular compartments of *E. coli*

Compound **17a** was quantified using our previously described protocol for strain cultivation, incubation, and cellular fractionation.⁵¹ For LC/MS/MS analysis, a sample volume of 1 μ L was injected. LC separations were achieved with a YMC - Pack TMS 100 mm $\times$ 2.1 mm /S3- μ m /12 nm column (YMC Europe, Dinslaken, Germany). A linear gradient using solvent A (H₂O + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid) was applied from 1% B to 99% B within 8.5 min at a constant flow of

0.7 mL/min. The compound eluted at a retention time of 1.60 min. A MRM (multiple reaction monitoring) method was applied, which allowed for a sensitive detection of the compound of interest. The Q1/Q3 transition masses used for compound identification are given in Table S1. The calibration curve (Figure S1) was linear over a range of 0.4 – 100 ng/mL, with an $r^2 = 0.9930$. The LOD in *E. coli* whole-cell matrix was found to be 22 pg/mL.

The reported data represent the average of two biological replicates performed on different days.

Computational methods

Molecular docking studies were performed using similar protocols as reported in our previous studies.^{37, 67} The crystal structure of *E. coli* LpxC (PDB ID: 3P3G) in complex with LPC-009 was retrieved from the Protein Data Bank (www.rcsb.org)⁶⁸ to conduct molecular docking. This protein structure was chosen due to the similarity of LPC-009 to the synthesized compounds. The LpxC protein was prepared using Protein Preparation Wizard by adding the hydrogen atoms and missing side chains in Schrödinger suite.⁶⁹ Solvent molecules were removed. Tautomeric states and protonation states of the amino acids were adjusted with PROPKA tool at pH 7.0. OPLS3e force field was applied to minimize the complex to remove the steric clashes, bad contacts and unsuitable torsional angles.⁷⁰ Ligands were prepared using the Ligprep tool by applying the OPLS3e force field. Subsequently, 64 conformers per ligand were generated using the Confgen tool with force field minimization on output conformers.

Molecular docking studies were performed in Glide from Schrödinger suite.⁶⁹ Grid files were prepared with default settings by applying box-size as 15 Å * 15 Å * 15 Å.

Standard Precision (SP) mode with flexible ligand sampling and enhanced planarity of conjugated π groups were used for docking studies. Moreover, metal constraint to the Zn^{2+} -ion, two hydrogen-bond constraints to E78 and T191 and a positional constraint placed on the diphenylacetylene linker group were set. The validation of the docking protocol was done by re-docking. The root mean square deviation (RMSD) value of the re-docked ligand compared to its observed binding mode in the crystal structure was 0.68 Å. The docking results were visually analyzed in MOE.⁷¹

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Conflict of interest

The authors declare no conflict of interest.

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