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Synthesis, biological evaluation and molecular docking studies of benzyloxyacetohydroxamic acids as LpxC inhibitors

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ABSTRACT

The inhibition of the UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine deacetylase (LpxC) represents a promising strategy to combat infections caused by multidrug-resistant Gram-negative bacteria. In order to elucidate the functional groups being important for the inhibition of LpxC, the structure of our previously reported hydroxamic acid **4** should be systematically varied. Therefore, a series of benzyloxyacetohydroxamic acids was prepared, of which the diphenylacetylene derivatives **28** (K_i = 95 nM) and **21** (K_i = 66 nM) were the most potent inhibitors of *Escherichia coli* LpxC. These compounds could be synthesized in a stereoselective manner employing a Sharpless asymmetric dihydroxylation and a Sonogashira coupling in the key steps. The obtained structure–activity relationships could be rationalized by molecular docking studies.

1. Introduction

Gram-negative bacteria cause several serious diseases, such as pneumonia or sepsis, which can be fatal without treatment.[1,2] Although a number of antibiotics, addressing different bacterial targets, are currently available to treat these infections, more and more bacterial strains evolve which are resistant to most of the available antibiotics, and the number of infections caused by multidrug-resistant Gram-negative bacteria is steadily increasing.[3,4] At the same time, only few novel antibacterial agents are in the research pipeline.[5] These alarming data emphasize the necessity to develop new antibacterials, which act upon unexploited bacterial targets, thereby circumventing established mechanisms of resistance.

The inhibition of the biosynthesis of lipid A represents a promising strategy to combat infections caused by Gram-negative germs.[6] Lipid A is a phosphorylated, (1→6)-linked glucosamine disaccharide, which acts as the hydrophobic membrane anchor of lipopolysaccheride (LPS) in the outer leaflet of the outer membrane of Gram-negative bacteria, and is essential for virulence and viability of the cells.[7] Bacteria with a defective lipid A synthesis show a higher membrane permeability leading to an increased sensitivity to a range of antibiotics and a decreased viability.[8,9] The inhibition of lipid A biosynthesis results in cell death.[10]

The first irreversible step of lipid A biosynthesis is the deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**), which is catalyzed by the Zn^{2+}-dependent enzyme LpxC (Fig. 1). This enzyme, which shows no homology to any mammalian protein, could be validated as antibacterial drug target and different structural classes of LpxC inhibitors have been described in the literature.[6,10–17] Amongst them, the *N*-aroyl-L-threonine hydroxamic acid derivatives CHIR-090 and LPC-009 represent potent LpxC inhibitors (Fig. 2), which show excellent antibacterial activities against clinically important Gram-negative pathogens including *Escherichia coli* and *Pseudomonas aeruginosa*.[18]

Solution as well as crystal structures of various LpxC orthologs complexed with different LpxC inhibitors have been reported.[14,18–23] They show that the enzyme consists of two domains. Each of them contains two α-helices, which are packed against a β-sheet. As in the overall structure the α-helices are sandwiched between the β-sheets, the enzyme displays a 'β–α–α–β sandwich' fold. In addition, each domain contains a unique insert. Whilst Insert I of Domain I partially defines the boundary of the active site where the catalytic Zn^{2+}-ion is located, Insert II of Domain II largely forms a hydrophobic passage, which during normal function of the enzyme hosts the fatty acid chain of its natural substrate **1**.

The diphenyldiacetylene derivative LPC-009 could be crystallized with LpxC orthologs from different Gram-negative species thus giving insight into the structural requirements for ligands binding to these enzymes as well as into the inherent conformational variations of the individual LpxC orthologs.[18]

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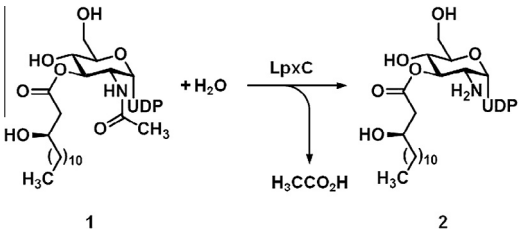


Figure 1. Deacetylation of uridine diphosphate-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**) catalyzed by LpxC.

The crystal structure of LPC-009 in complex with *E. coli* LpxC shows important interactions between the inhibitor and the enzyme (Fig. 3). In the active site, the catalytic Zn^{2+}-ion is complexed by the hydroxamate moiety of LPC-009. Additionally, its threonyl group interacts with conserved residues in the active site. Whilst the methyl group forms van der Waals contacts with the phenyl ring of Phe192, a hydrogen bond is formed between the hydroxyl group and Lys239. Thr191 and the backbone amide of Cys63 form additional hydrogen bonds with the NH and the carbonyl of the amide moiety of LPC-009, respectively. The diacetylene group of the inhibitor penetrates through the hydrophobic tunnel. The terminal phenyl ring of LPC-009 forms van der Waals contacts with the hydrophobic residues of Ile198, Met195, Phe212 and Val217 outside the substrate-binding passage.

CHIR-090, a slow, tight-binding inhibitor of LpxC, showed a similar binding mode in a solution structure of the *Aquifex aeolicus* LpxC–CHIR-090 complex, with its lipophilic side chain occupying the hydrophobic channel and the threonine hydroxamic acid moiety binding to the active site of the enzyme.[16,21] This diphenylacetylene derivative kills *E. coli* and *P. aeruginosa* with an efficiency comparable to that of ciprofloxacin and was chosen as lead compound for the development of benzyloxyacetohydroxamic acid derivatives **3** and **4** (Fig. 2).[24,25] In our previous studies we showed that the open chain derivative **4** is more active than the conformationally constrained *C*-glycoside **3** in the LpxC assay as well as in disc diffusion assays against *E. coli*.[25] Therefore, the structure of diol **4** was further investigated. In a deconstruction reconstruction approach, the structural features of **4** which are important for binding to LpxC were elucidated. In particular, attention was paid to the length of the lipophilic side chain, the hydroxymethyl groups as well as the stereochemistry (Fig. 2). In order to gain a deeper understanding of the deduced structure activity relationships of the synthesized compounds, docking studies were performed.

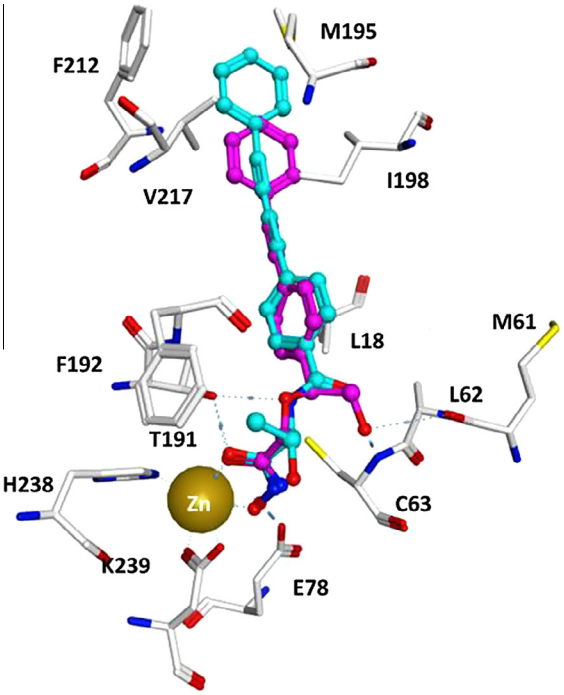


Figure 3. Comparison between docking results of compound **21** (magenta) and the cocrystallized inhibitor LPC-009 (3P3G, inhibitor colored cyan). The zinc ion is shown as brown ball. Hydrogen bonds are shown as dashed line.

2. Results and discussion

2.1. Chemistry

First, benzyloxyacetohydroxamic acid derivatives bearing no hydroxymethyl side chain were synthesized (Scheme 1). For this purpose, ethyl bromoacetate was reacted with 4-iodobenzyl alcohol to give benzyl ether **5**. Then a Sonogashira reaction was performed to establish the lipophilic side chain. The C–C coupling with phenylacetylene and 4-(4-ethynylbenzyl)morpholine[24] gave diphenylacetylene derivatives **6** and **7**, respectively. The reaction of esters **6** and **7** with hydroxylamine led to hydroxamates **8** and **9**.

For the synthesis of the phenyl ethylene glycol derivatives, styrene and 4-bromostyrene were used as starting materials (Scheme 2). According to a literature procedure, these compounds were subjected to a Sharpless asymmetric dihydroxylation using AD-mix-α to obtain the enantiomerically pure (*S*)-configured

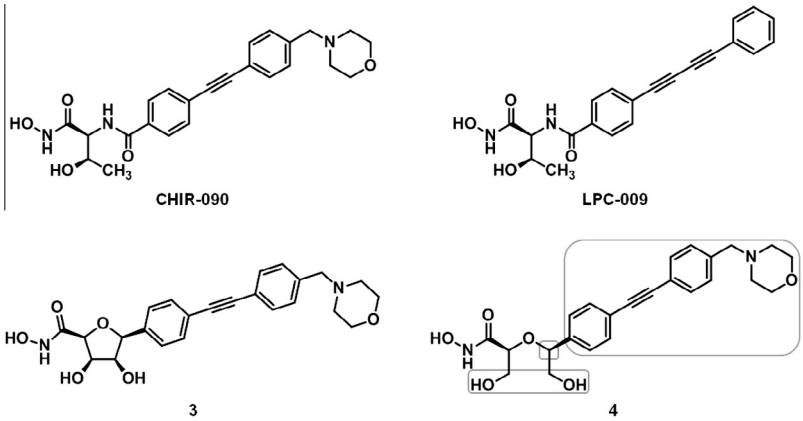
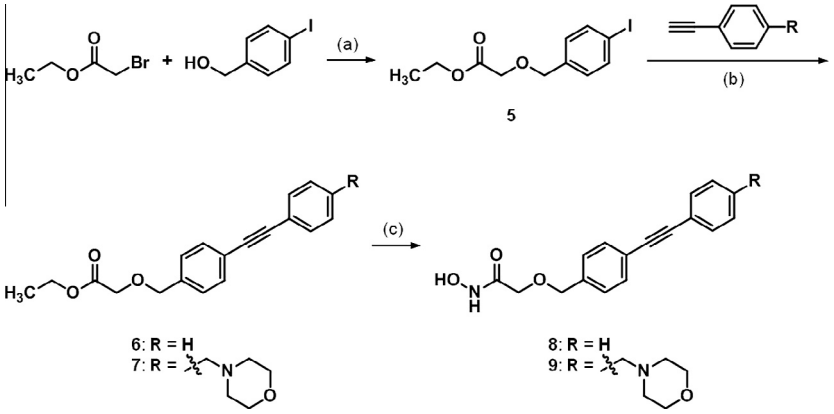
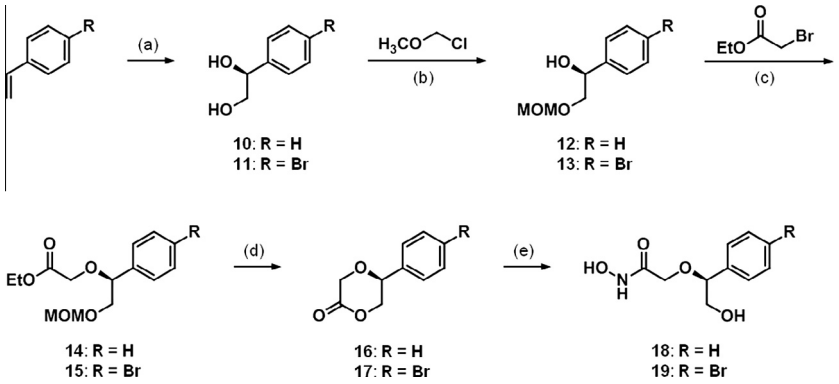


Figure 2. Chemical structures of *N*-aroyl-L-threonine hydroxamic acids CHIR-090 and LPC-009 and lead compounds **3** and **4**.



Scheme 1. Reagents and conditions: (a) NaHMDS, THF, Δ, 8 h, 62%; (b) $Pd(PPh_3)_4$, CuI, NEt_3, H_3CCN, rt, 2 h, **6** 92%, **7** 84%; (c) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, **8** 27%, **9** 16%.



Scheme 2. Reagents and conditions: (a) AD-mix-α, *t*BuOH/H_20 (1:1), 0 °C, 16 h, **10** 94%, **11** 82%; (b) DIPEA, H_3CCN, rt, 16 h, **12** 30%, **13** 36%; (c) LiHMDS, THF, Δ, 16 h, **14** 62%, **15** 73%; (d) (1) *p*TsOH, MeOH, rt, 16 h, (2) *p*TsOH, H_3CCN, rt, 16 h, **16** 63%, **17** 50%; (e) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, **18** 36%, **19** 64%.

glycols **10** and **11**, respectively.[26,27] Then, the primary alcohol of the diols was protected. Several protective groups were tested (MOM, BOM, TBDMS). With respect to protection, stability and deprotection, the MOM protective group proved to be superior. Therefore, diols **10** and **11** were reacted with MOM-Cl to obtain the methoxymethyl ethers **12** and **13**.[28] However, only moderate regioselectivity was observed. Whilst in case of the protection of diol **11** the desired compound **13** was obtained in 36% yield, its regioisomer as well as the bis-protected compound were isolated in 11% and 10% yield, respectively. Etherification of the secondary alcohols **12** and **13** with ethyl bromoacetate yielded the benzyloxyacetic acid derivatives **14** and **15**. Then, under acidic conditions the MOM protective group was cleaved and the resulting primary alcohols were subsequently transformed into lactones **16** and **17**.[29] In order to introduce various side chains to the lactones, Sonogashira couplings were performed with the 4-bromobenzene derivative **17** (Scheme 3). However, in contrast to the reaction of the iodine-substituted compound **5**, the transformations of the bromine-substituted compounds required somewhat harsher reaction conditions and were performed in triethylamine as solvent under refluxing conditions. Whilst the coupling with phenylacetylene gave access to diphenylacetylene **20**, the coupling with trimethylsilylacetylene yielded the acetylene derivative **22**. The terminal alkyne **24** was obtained by cleaving the TMS-protective group of **22** with TBAF. The morpholin-4-ylmethyl substituted diphenylacetylene derivative **27** was synthesized via a Sonogashira coupling of alkyne **24** with 4-(4-iodobenzyl)morpholine (**26**). Finally, the lactones **16**, **17**, **20**, **22**, **24** and **27** were reacted with hydroxylamine to yield the desired hydroxamic acids **18**, **19**, **21**, **23**, **25** and **28**, respectively. The corresponding (*R*)-configured hydroxamic acids were obtained in the same way by using AD-mix-β in the dihydroxylation step of styrene and 4-bromostyrene.

2.2. Biological evaluation

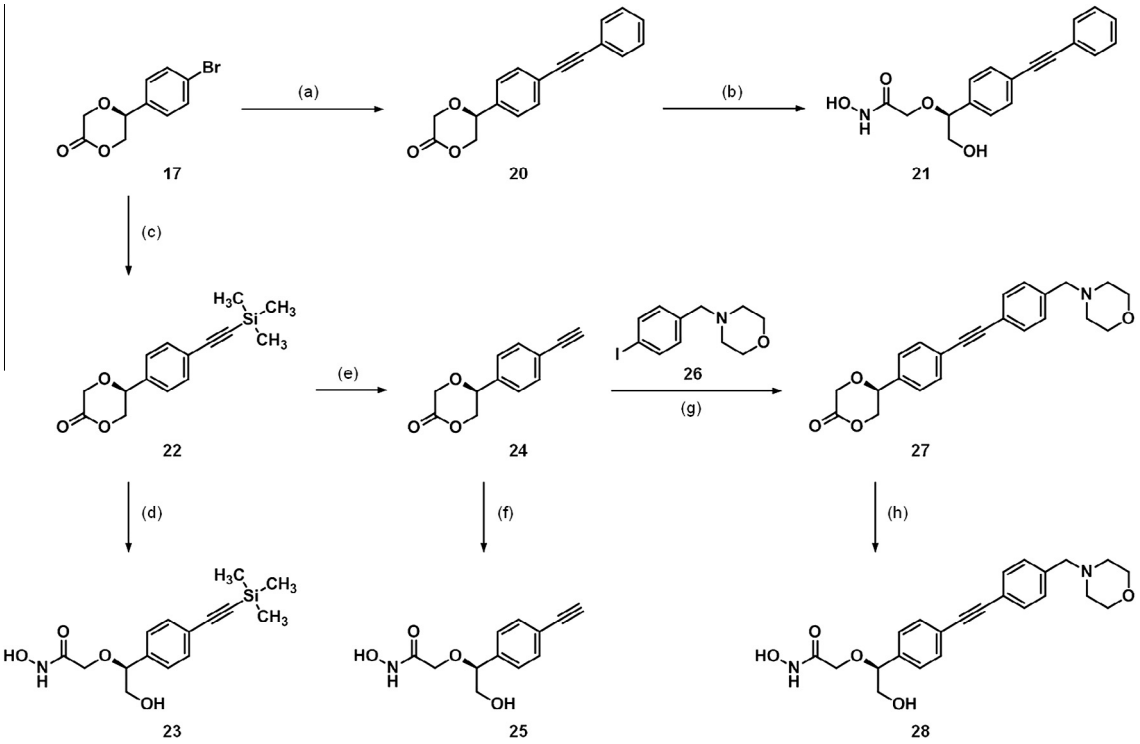
The antibacterial activity of the synthesized benzyloxyacetohydroxamic acids was determined in an LpxC enzyme assay as well as in disc diffusion tests (Table 1).

In the enzyme assay, purified *E. coli* LpxC and the enzyme's natural substrate were employed. The inhibition of the enzyme could be determined by measuring the amount of the deacetylated product. For this purpose, the resulting primary amine **2** was transformed into a fluorescent isoindole with phthalaldehyde and 2-mercaptoethanol.

When comparing the IC_{50}- as well as the resultant K_i-values, several structure–activity relationships can be deduced.

In case of the hydroxamates bearing a morpholin-4-ylmethyl-substituted diphenylacetylene moiety, the removal of both hydroxymethyl groups of hydroxamic acid **4**, leading to **9**, causes a 4-fold decrease in inhibitory activity. However, **28**, which retains the hydroxymethyl group in benzylic position, shows a 15-fold increase in inhibitory activity compared to **9** and also outperforms the activity of lead compound **4**.

The deconstruction of the lipophilic side chain of ethylene glycol ether **28**, leading to the unsubstituted phenyl derivative **18**, was detrimental to the inhibitory activity. In order to observe inhibition of *E. coli* LpxC, a certain minimal length of the hydrophobic side chain is required for the ethylene glycol ethers. Like the unsubstituted hydroxamate **18**, the bromo-, the acetylene- and the TMS-acetylene-substituted compounds **19**, **25** and **23** were



Scheme 3. Reagents and conditions: (a) phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 16 h, 65%; (b) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, 33%; (c) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 16 h, 64%; (d) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, 16%; (e) TBAF, THF, rt, 2 h, 31%; (f) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, 26%; (g) $Pd(PPh_3)_4$, CuI, NEt_3, H_3CCN, rt, 2 h, 91%; (h) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, 16 h, 25%.

Table 1
Results of the agar diffusion clearance assays, the LpxC assay and molecular docking studies (higher ChemPLP Score values indicate more favourable interactions)

Compound	Substitution pattern	Stereochemistry	Zone of inhibition [mm]		IC_{50} (μM)	K_i (μM)	ChemPLP Score
			E. coli BL21 (DE3)	*E. coli* D22			
CHIR-090			24.6 ± 1.9	30.3 ± 2.5	0.058 ± 0.002	0.008 ± 0.0003	73.75
3	Morpholinomethylphenylethynyl	(2*S*,3*R*,4*S*,5*S*)	<6	13.1 ± 0.8	>200	–	71.32
4	Morpholinomethylphenylethynyl	(*S*,*S*)	9.0 ± 0.5	20.8 ± 0.6	2.6 ± 0.3	0.358 ± 0.038	70.76
8	Phenylethynyl		10.6 ± 0.4	13.2 ± 1.6	>200	–	53.94
9	Morpholinomethylphenylethynyl		16.5 ± 0.4	20.3 ± 1.0	10.5 ± 2.5	1.45 ± 0.35	69.47
18	H	(*S*)	<6	<6	>200	–	57.42
ent-18	H	(*R*)	<6	<6	>200	–	53.05
19	Br	(*S*)	<6	<6	>200	–	60.67
ent-19	Br	(R)	<6	<6	>200	–	51.06
25	Ethynyl	(*S*)	<6	<6	>200	–	63.37
ent-25	Ethynyl	(*R*)	<6	<6	>200	–	37.56
23	Trimethylsilylethynyl	(*S*)	<6	7.5 ± 0.3	>200	–	59.57
ent-23	Trimethylsilylethynyl	(*R*)	7.1 ± 0.1	7.2 ± 0.2	>200	–	59.87
21	Phenylethynyl	(*S*)	9.5 ± 0.4	20.5 ± 0.2	0.48 ± 0.23	0.066 ± 0.032	76.24
ent-21	Phenylethynyl	(*R*)	9.1 ± 0.4	13.0 ± 1.7	31.6 ± 6.0	4.4 ± 0.8	69.03
28	Morpholinomethylphenylethynyl	(*S*)	13.4 ± 0.5	21.2 ± 0.6	0.69 ± 0.30	0.095 ± 0.042	73.06
ent-28	Morpholinomethylphenylethynyl	(*R*)	8.7 ± 0.7	12.3 ± 1.6	198 ± 12	27.3 ± 1.7	66.29

unable to inhibit the LpxC-catalyzed deacetylation of **1** at concentrations up to 200 μM. In contrast, compounds **21** and **28**, bearing a diphenylacetylene moiety in their side chains, inhibit LpxC at nanomolar concentrations. With K_i-values of 66 nM and 95 nM, respectively, these compounds are only about 10-fold less potent than the lead compound CHIR-090.

A comparison of the activities of the enantiomeric pairs shows, that generally the (*S*)-configured phenyl ethylene glycol derivatives possess higher inhibitory activities than their (*R*)-configured counterparts.

In the disc diffusion assays, the synthesized compounds were tested against *E. coli* BL21 (DE3) and the *E. coli* D22 strain, which is more sensitive towards LpxC inhibition. CHIR-090 and DMSO were used as positive and negative control, respectively.

When tested against *E. coli* D22, for the phenyl ethylene glycol derivatives similar trends could be observed as in the enzyme assay. For a relatively high antibacterial activity these compounds require a lipophilic side chain of a certain length. In case of the diphenylacetylene derivatives, representing the most active antibacterials in this series of compounds, the (*S*)-configured hydroxamates possess a higher antibacterial activity than their (*R*)-configured enantiomers.

The comparison of the morpholin-4-ylmethyl-substituted hydroxamic acids **28**, **4** and **9** reveals that, in spite of their differing K_i values, the compounds are almost equally active against *E. coli* D22. When tested against the wild type strain *E. coli* BL21 (DE3), compound **9**, which is the least active inhibitor of *E. coli* LpxC, showed the biggest halo of inhibition on the agar plate. Most

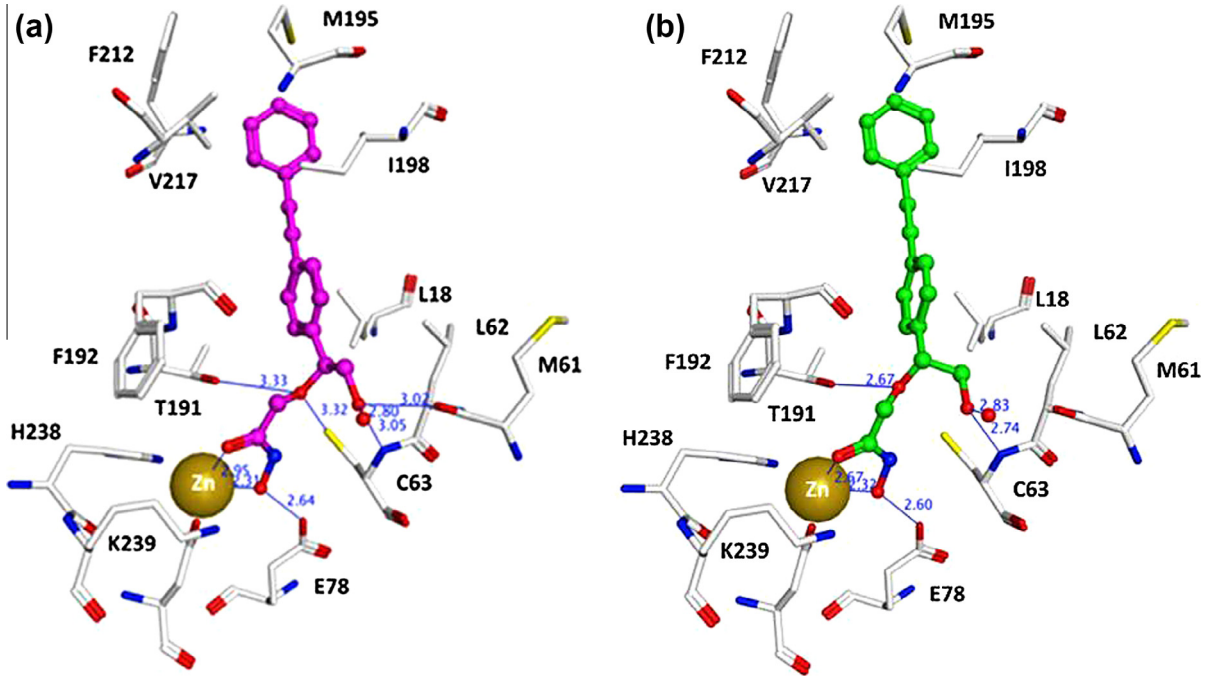


Figure 4. Docking results obtained for a) compound **21** (magenta) in comparison with b) the stereoisomer ent-**21** (green). Hydrogen bonds between LpxC and the inhibitors are shown as blue lines and distances are given in Å.

probably, these findings are due to kinetic reasons like a higher stability towards metabolic inactivation by the bacteria or a higher membrane permeability of compound **9** lacking the hydroxymethyl groups.

Also in case of the diphenylacetylene derivatives **28** and **21**, the antibacterial activities against *E. coli* BL21 (DE3) do not fully match the inhibitory activities against the enzyme. The morpholin-4-ylmethyl-substituted hydroxamic acid **28**, which is slightly less active against purified *E. coli* LpxC, was shown to be significantly more active in the disc diffusion assay. This might result from the fact that **21** does not contain a polar functional group in its lipophilic side chain and is therefore more likely to be trapped within the cell membrane.

2.3. Ligand docking studies

In order to rationalize the obtained biological data (K_i on LpxC from *E. coli*) all compounds were docked into the crystal structure of LpxC. The docking studies show that all compounds are able to coordinate the zinc ion of LpxC with the hydroxamic acid group and are able to mimic some of the interactions observed for LPC-009 (Fig. 3). In general, a good agreement between the ChemPLP docking scores and binding affinities is obtained for this homogenous data set (Table 1). A correlation coefficient (r^2) between pK_i and ChemPLP Score values of 0.81 (RMSE 0.49) is observed and the model is able to discriminate between active and inactive compounds with only one exception, namely compound **3** (Fig. S1, Supporting Information).

Besides the docking scores, also the coordination to the zinc ion, the hydrogen-bonds as well as the interaction with the cluster of hydrophobic residues have to be considered to explain the biological data. In case of **18**, ent-**18**, **19**, ent-**19**, **25**, and ent-**25** (Figs. S2 and S3, Supporting Information) the important interaction with the cluster of hydrophobic residues (Ile198, Met195, Phe212 and Val217) is missing, resulting in a decrease of the docking score and loss of inhibitory activity. In case of **23** and ent-**23**, the bulky trimethylsilyl group is too large for the narrow channel and resulting in an altered orientation of the hydroxymethyl group (Fig. S3, Supporting Information). As a consequence the hydrogen bond to Cys63 is lost resulting in lower scores. For compounds **8** and **9** no hydrogen bond to Met61 or Cys63 is observed resulting in diminished activities. Docking of compound **3** shows a favourable score as observed for the active inhibitors. However, analyzing the docking pose of compound **3** it is recognized that only one hydroxyl group of the tetrahydrofuran ring is engaged in a hydrogen bond with the backbone of Phe192 whereas the second OH-group is located nearby the aromatic ring of Phe212 at the same place as the methyl group of the threonyl-fragment of LPC-009 (Fig. S4, Supporting Information). So it seems to be unfavourable to place a polar substituent at this region which could explain the loss of activity of **3**. However, this observation is not reflected by the docking score.

Docking of the most-active inhibitors **21** and **28**, having the hydroxymethyl group in (*S*)-configuration, results in favourable docking scores (76.24 and 73.06, respectively). The hydroxyl group is hydrogen-bonded to the backbone of Met61 (CO) and Cys63 (NH) and is mimicking the interaction of the amide group of LPC-009 (Fig. 4a). Another hydrogen bond is observed between the Thr191 hydroxyl group and the ether-oxygen of **21**/**28**. The reduced affinity compared to LPC-009 can be explained by the missing hydrophobic interaction with Phe192. The morphonyl-substituent of **28** is located nearby the solvated entrance of the tunnel and is not making significant contribution to the interaction (similar scores for **21** and **28** are obtained) (Fig. S5, Supporting Information). For the two (*R*)-configured stereoisomers (ent-**21** and ent-**28**) the hydroxymethyl group makes only a hydrogen bond to Cys63 whereas the hydrogen bond to Met61 is lost (Fig. 4b). The docking scores obtained for the two enantiomers ent-**21** and ent-**28** are significantly lower, explaining the reduced activities (Fig. S2, Supporting Information). For **4**, a similar orientation and docking score is obtained as observed for **21** and **28**. One hydroxyl group is involved in hydrogen bonds to Met61/Cys63

whereas the second hydroxyl group is not directly hydrogen-bonded, but nearby Lys239 (4.5 Å) enabling a water-bridged interaction (Fig. S4, Supporting Information).

3. Conclusions

With the phenyl ethylene glycol ethers this study identified a new class of LpxC inhibitors, which serves to broaden the current scope for compounds acting at this enzyme. A convenient synthesis to access these compounds in enantiomerically pure form was presented, allowing especially variations of the lipophilic side chain. The biological evaluation identified the diphenylacetylene derivatives **28** (K_i = 95 nM) and **21** (K_i = 66 nM) as the most potent inhibitors of the presented series of compounds. A molecular docking study was performed to help elucidating the molecular interactions, which underlie these activities. The derived model emphasized the importance of the long lipophilic side chain, the hydroxymethyl group in benzylic position and the (*S*)-configuration of the stereocenter. With their promising biological activities hydroxamic acids **28** and **21** may serve as leads for the further development of antibacterials targeting LpxC.

4. Experimental section

4.1. Chemistry, general

Unless otherwise mentioned, THF was dried with sodium/benzophenone and was freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Macherey-Nagel); parentheses include: diameter of the column, fraction size, eluent, R_f value. Melting point (mp): Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. Optical rotation α [deg] was determined with a Polarimeter 341 (Perkin Elmer); path length 1 dm, wavelength 589 nm (sodium D line); the unit of the specific rotation $[\alpha]_D^{20}$ [deg mL dm^{-1} g^{-1}] is omitted; the concentration of the sample c [mg mL^{-1}] and the solvent used are given in brackets. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. Where necessary, the assignment of the signals in the ^{1}H NMR and ^{13}C NMR spectra was performed using ^{1}H–^{1}H and ^{1}H–^{13}C COSEY NMR spectra as well as NOE (nuclear Overhauser effect) difference spectroscopy. IR: IR Prestige-21(Shimadzu). HRMS: MicrOTOF-QII (Bruker). HPLC methods for the determination of product purity: Method 1: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 μm); LiCroCART® 250-4 mm cartridge; flow rate: 1.00 mL/min; injection volume: 5.0 μL; 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: Merck Hitachi Equipment; UV detector: L-7400; pump: L-6200A; column: phenomenex Gemini® 5 μm C6-Phenyl 110 Å; LC Column 250 × 4.6 mm; flow rate: 1.00 mL/min; injection volume: 5.0 μL; 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%, 5–15 min: gradient from 100% to 0%, 15–20 min: 0%, 20–22 min: gradient from 0% to 100%, 22–30 min: 100%. Chiral HPLC for the determination of enantiomeric ratios: Method 3: equipment: pump: L-6200A; Injection: manual, Rheodyne 7725i; Diode Array Detector: L-7455; data transfer: D-line; data acquisition: HSM-Software (all except Rheodyne 7725i: LaChrom, Merck Hitachi); column: Daicel Chiralpak IB, 5 μm, 250 mm/4.6 mm; solvent/isohexane/ethanol = 30:1; flow rate: 1.0 mL/min; injection volume: 15 μL; detection detection at λ = 220 nm for 30 min. Synthetic starting materials were purchased from Sigma-Aldrich and ACROS ORGANICS.

4.2. Synthetic procedures

4.2.1. General synthetic procedures

4.2.1.1. Synthesis of hydroxamic acids. Hydroxylamine hydrochloride and a 2 M solution of sodium methoxide in methanol were added to a solution of the lactone in methanol and the mixture was stirred at ambient temperature for 16 h. Then water was added and the mixture extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography.

4.2.2. Ethyl 2-[(4-iodobenzyl)oxy]acetate (5)

Under N_2 atmosphere a 2 M solution of sodium hexamethyldisilazane in THF (2.67 mL, 5.34 mmol) and ethyl bromoacetate (1.42 mL, 2.14 g, 12.8 mmol) were added to a solution of 4-iodobenzyl alcohol (1.0 g, 4.27 mmol) in THF (50 mL) and the mixture was heated to reflux for 8 h. Then water was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (4 cm, 30 mL, cyclohexane/ethyl acetate = 9:1, R_f = 0.38) to give **5** as colorless oil (850 mg, 2.65 mmol, 62%). ^{1}H NMR ($CDCl_3$): δ 1.29 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 4.08 (s, 2H, OCH_2CO_2Et), 4.23 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 4.57 (s, 2H, OCH_2Ar), 7.11–7.14 (m, 2H, 2′-$H_{4\text{-iodophenyl}}$, 6′-$H_{4\text{-iodophenyl}}$), 7.67–7.70 (m, 2H, 3′-$H_{4\text{-iodophenyl}}$, 5′-$H_{4\text{-iodophenyl}}$); ^{13}C NMR ($CDCl_3$): δ 14.4 (1C, OCH_2CH_3), 61.1 (1C, OCH_2CH_3), 67.5 (1C, OCH_2CO_2Et), 72.8 (1C, OCH_2Ar), 93.7 (1C, C-4′$_{4\text{-iodophenyl}}$), 130.0 (2C, C-2′$_{4\text{-iodophenyl}}$, C-6′$_{4\text{-iodophenyl}}$), 137.0 (1C, C-1′$_{4\text{-iodophenyl}}$), 137.7 (2C, C-3′$_{4\text{-iodophenyl}}$, C-5′$_{4\text{-iodophenyl}}$), 170.3 (1C, CO_2Et); IR (neat): ν [cm^{-1}] = 2978, 1747, 1485, 1200, 1269, 1007, 795; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{11}H_{14}IO_3$, 320.9982; found, 320.9964; HPLC (method 1): t_R = 23.5 min, purity 97.0%.

4.2.3. Ethyl 2-{[4-(phenylethynyl)benzyl]oxy}acetate (6)

Under N_2 atmosphere triethylamine (1.0 mL, 7.0 mmol), copper(I) iodide (38 mg, 0.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.1 mmol) were added to a solution of **5** (320 mg, 1.0 mmol) in acetonitrile (25 mL). Then a solution of phenylacetylene (0.9 mL, 8.3 mmol) in acetonitrile (10 mL) was added dropwise over a period of 2 h. After evaporation of the solvent the residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 9:1, R_f = 0.36) to give **6** as colorless solid (270 mg, 0.92 mmol, 92%). Mp: 124 °C; ^{1}H NMR ($CDCl_3$): δ 1.30 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 4.11 (s, 2H, OCH_2CO_2Et), 4.24 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 4.65 (s, 2H, OCH_2Ar), 7.32–7.38 (m, 5H, $H_{arom.}$), 7.51–7.55 (m, 4H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 14.4 (1C, OCH_2CH_3), 61.1 (1C, OCH_2CH_3), 67.5 (1C, OCH_2CO_2Et), 73.1 (1C, OCH_2Ar), 89.3 (1C, C≡C), 89.7 (1C, C≡C), 123.0 (1C, $C_{arom.}$), 123.3 (1C, $C_{arom.}$), 128.0 (2C, $C_{arom.}$), 128.4 (1C, $C_{arom.}$), 128.5 (2C, $C_{arom.}$), 131.8 (2C, $C_{arom.}$), 131.9 (2C, $C_{arom.}$), 137.5 (1C, $C_{arom.}$), 170.4 (1C, CO_2Et); IR (neat): ν [cm^{-1}] = 3048, 2889, 1740, 1512, 1435, 1393, 1215, 1146, 937, 833, 752, 691; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{19}H_{19}O_3$, 295.1329; found, 295.1353; HPLC (method 1): t_R = 25.9 min, purity 99.0%.

4.2.4. Ethyl 2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl} benzyl)oxy]acetate (7)

Under N_2 atmosphere triethylamine (0.61 mL, 4.4 mmol), copper(I) iodide (24 mg, 0.13 mmol) and tetrakis(triphenylphosphine)palladium(0) (72 mg, 0.063 mmol) were added to a solution of **5** (200 mg, 0.63 mmol) in acetonitrile (25 mL). Then a solution of

4-(4-ethynylbenzyl)morpholine (253 mg, 1.26 mmol) in acetonitrile (10 mL) was added dropwise over a period of 2 h. After evaporation of the solvent the residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 2:1, R_f = 0.20) to give **7** as colorless solid (207 mg, 0.53 mmol, 84%). Mp: 69 °C; ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.42–2.46 (m, 4H, NCH_2CH_2O), 3.50 (s, 2H, NCH_2Ar), 3.70–3.73 (m, 4H, NCH_2CH_2O), 4.11 (s, 2H, OCH_2CO_2Et), 4.24 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.64 (s, 2H, OCH_2Ar), 7.30–7.33 (m, 2H, $H_{arom.}$), 7.34–7.37 (m, 2H, $H_{arom.}$), 7.46–7.50 (m, 2H, $H_{arom.}$), 7.50–7.53 (m, 2H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 14.4 (1C, OCH_2CH_3), 53.8 (2C, NCH_2CH_2O), 61.1 (1C, OCH_2CH_3), 63.3 (1C, NCH_2Ar), 67.1 (2C, NCH_2CH_2O), 67.5 (1C, OCH_2CO_2Et), 73.1 (1C, OCH_2Ar), 89.2 (1C, $C{\equiv}C$), 89.7 (1C, $C{\equiv}C$), 122.1 (1C, $C_{arom.}$), 123.1 (1C, $C_{arom.}$), 128.0 (2C, $C_{arom.}$), 129.3 (2C, $C_{arom.}$), 131.7 (2C, $C_{arom.}$), 131.8 (2C, $C_{arom.}$), 137.4 (1C, $C_{arom.}$), 138.4 (1C, $C_{arom.}$), 170.4 (1C, CO_2Et); IR (neat): ν [cm^{-1}] = 2808, 1744, 1516, 1454, 1292, 1204, 1138, 1111, 1022, 864; HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{28}NO_4$, 394.2013; found, 394.1991; HPLC (method 1): t_R = 19.2 min, purity 97.7%.

4.2.5. *N*-Hydroxy-2-{[4-(phenylethynyl)benzyl]oxy}acetamide (8)

Hydroxylamine hydrochloride (76 mg, 1.1 mmol) and a 2 M solution of sodium methoxide in methanol (0.55 mL, 1.1 mmol) were added to a solution of **6** (130 mg, 0.44 mmol) in methanol (30 mL) and the mixture was stirred at ambient temperature for 16 h. Then the solvent was evaporated and the residue was purified by flash column chromatography (1 cm, 5 mL, CH_2Cl_2/methanol = 9.5:0.5, R_f = 0.37) to give **8** as colorless solid (33 mg, 0.12 mmol, 27%). Mp: 136 °C; ^{1}H NMR (CD_3OD): δ 4.01 (s, 2H, $OCH_2CONHOH$), 4.61 (s, 2H, OCH_2Ar), 7.36–7.42 (m, 5H, $H_{arom.}$), 7.50–7.53 (m, 4H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ 69.3 (1C, $OCH_2CONHOH$), 73.9 (1C, OCH_2Ar), 89.9 (1C, $C{\equiv}C$), 90.3 (1C, $C{\equiv}C$), 124.2 (1C, $C_{arom.}$), 124.5 (1C, $C_{arom.}$), 129.1 (2C, $C_{arom.}$), 129.5 (1C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 132.5 (2C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 139.1 (1C, $C_{arom.}$), 168.9 (1C, $CONHOH$); IR (neat): ν [cm^{-1}] = 3248, 2909, 1636, 1512, 1485, 1439, 1358, 1281, 1099, 976, 837, 752, 687; HRMS (m/z): $[M+H]^+$ calcd for $C_{17}H_{16}NO_3$, 282.1125; found, 282.1125; HPLC (method 2): t_R = 16.8 min, purity 99.3%.

4.2.6. *N*-Hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl] ethynyl}benzyl)oxy]acetamide (9)

Hydroxylamine hydrochloride (59 mg, 0.85 mmol) and a 2 M solution of sodium methoxide in methanol (0.43 mL, 0.85 mmol) were added to a solution of **7** (140 mg, 0.36 mmol) in methanol (30 mL) and the mixture was stirred at ambient temperature for 16 h. Then the solvent was evaporated and the residue was purified by flash column chromatography (1 cm, 5 mL, CH_2Cl_2/methanol = 9.5:0.5, R_f = 0.34) to give **9** as colorless solid (22 mg, 0.06 mmol, 16%). Mp: 113 °C; ^{1}H NMR (CD_3OD): δ 2.45–2.49 (m, 4H, NCH_2CH_2O), 3.54 (s, 2H, NCH_2Ar), 3.68–3.71 (m, 4H, NCH_2CH_2O), 4.01 (s, 2H, $OCH_2CONHOH$), 4.61 (s, 2H, OCH_2Ar), 7.35–7.42 (m, 4H, $H_{arom.}$), 7.47–7.53 (m, 4H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ 54.6 (2C, NCH_2CH_2O), 63.9 (1C, NCH_2Ar), 67.7 (2C, NCH_2CH_2O), 69.3 (1C, $OCH_2CONHOH$), 73.9 (1C, OCH_2Ar), 89.9 (1C, $C{\equiv}C$), 90.2 (1C, $C{\equiv}C$), 123.6 (1C, $C_{arom.}$), 124.2 (1C, $C_{arom.}$), 129.1 (2C, $C_{arom.}$), 130.7 (2C, $C_{arom.}$), 132.5 (2C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 139.0 (1C, $C_{arom.}$), 139.1 (1C, $C_{arom.}$), 168.9 (1C, $CONHOH$); IR (neat): ν [cm^{-1}] = 3213, 2932, 2812, 1647, 1516, 1454, 1408, 1350, 1288, 1103, 1072, 999, 868, 845, 818, 787; HRMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_4$, 381.1809; found, 381.1824; HPLC (method 2): t_R = 13.1 min, purity 99.7%.

4.2.7. (*S*)-1-(4-Bromophenyl)ethane-1,2-diol (11)

AD-mix-α (77 g) was added to a mixture of *tert*-butyl alcohol (275 mL) and water (275 mL). The mixture was cooled to 0 °C, 4-bromostyrene (7.2 mL, 55 mmol) was added and the reaction mixture was stirred at 0 °C for 16 h. Then sodium sulfite (82.5 g) was added and the mixture was allowed to warm to room temperature and stirred for 1 h. Ethyl acetate was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (8 cm, 60 mL, cyclohexane/ethyl acetate = 2:1, R_f = 0.14) to give **11** as colorless solid (9.8 g, 45 mmol, 82%). Mp: 108 °C; $[\alpha]_D^{20}$ 41.9 (3.1; CH_2Cl_2); HPLC (method 1): t_R = 12.5 min, purity 99.6%; enantiomeric ratio (HPLC method 3): t_R = 25.0 min, (*R*):(*S*) = 1.4:98.6.

4.2.8. (*R*)-1-(4-Bromophenyl)ethane-1,2-diol (ent-11)

As described for the preparation of **11**, 4-bromostyrene (1.3 mL, 10 mmol) was reacted with AD-mix-β (14 g) in a mixture of *tert*-butyl alcohol (50 mL) and water (50 mL) to give ent-**11** as colorless solid (1.9 g, 8.8 mmol, 88%). Mp: 108 °C; $[\alpha]_D^{20}$ −45.3 (2.7; CH_2Cl_2); HPLC (method 1): t_R = 12.7 min, purity 99.5%; enantiomeric ratio (HPLC method 3): t_R = 23.6 min, (*R*):(*S*) = 98.9:1.1.

4.2.9. Spectroscopic data for 11 and ent-11

^{1}H NMR (D_3COD): δ 3.58 (dd, J = 11.3/6.8 Hz, 1H, $HOCHCH_2OH$), 3.61 (dd, J = 11.3/5.2 Hz, 1H, $HOCHCH_2OH$), 4.65 (dd, J = 6.8/5.2 Hz, 1H, $HOCHCH_2OH$), 7.28–7.32 (m, 2H, 2′-$H_{4\text{-bromophenyl}}$, 6′-$H_{4\text{-bromophenyl}}$), 7.46–7.50 (m, 2H, 3′-$H_{4\text{-bromophenyl}}$, 5′-$H_{4\text{-bromophenyl}}$); ^{13}C NMR (D_3COD): δ 68.5 (1C, $HOCHCH_2OH$), 75.2 (1C, $HOCHCH_2OH$), 122.1 (1C, C-4′$_{4\text{-bromophenyl}}$), 129.4 (2C, C-2′$_{4\text{-bromophenyl}}$, C-6′$_{4\text{-bromophenyl}}$), 132.3 (2C, C-3′$_{4\text{-bromophenyl}}$, C-5′$_{4\text{-bromophenyl}}$), 142.8 (1C, C-1′$_{4\text{-bromophenyl}}$); IR (neat): ν [cm^{-1}] = 3368, 2924, 1481, 1392, 1346, 1227, 1061, 1011, 895, 826; HRMS (m/z): $[M+Na]^+$ calcd for $C_8H_9{}^{79}BrNaO_2$, 238.9678; found, 238.9684.

4.2.10. (*S*)-2-(Methoxymethoxy)-1-phenylethanol (12)[28]

Under N_2 atmosphere *N*,*N*-diisopropylethylamine (6.4 mL, 5.0 g, 39 mmol) and chloromethyl methyl ether (2.1 mL, 2.25 g, 27.9 mmol) were added to a solution of (*S*)-1-phenylethane-1,2-diol (**10**) (1.54 g, 11.1 mmol) in acetonitrile (100 mL) at 0 °C. After stirring the mixture at ambient temperature for 16 h, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (5 cm, 50 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.15) to give **12** as colorless oil (600 mg, 3.3 mmol, 30%). $[\alpha]_D^{20}$ 43.1 (0.5; CH_2Cl_2); HPLC (method 1): t_R = 12.2 min, purity 99.6%.

4.2.11. (*R*)-2-(Methoxymethoxy)-1-phenylethanol (ent-12)

As described for the preparation of **12**, the enantiomer (*R*)-1-phenylethane-1,2-diol ent-**10** (792 mg, 5.73 mmol) was reacted with *N*,*N*-diisopropylethylamine (3.32 mL, 2.59 g, 20.1 mmol) and chloromethyl methyl ether (1.09 mL, 1.15 g, 14.3 mmol) in acetonitrile (50 mL) to give ent-**12** as colorless oil (450 mg, 2.47 mmol, 43%). $[\alpha]_D^{20}$ −45.8 (4.8; CH_2Cl_2); HPLC (method 1): t_R = 12.6 min, purity 97.8%.

4.2.12. Spectroscopic data for 12 and ent-12

^{1}H NMR ($CDCl_3$): δ 3.06 (d, J = 2.7 Hz, 1H, OH), 3.39 (s, 3H, OCH_2OCH_3), 3.59 (dd, J = 10.6/8.6 Hz, 1H, $HOCHCH_2O$), 3.79 (dd, J = 10.6/3.1 Hz, 1H, $HOCHCH_2O$), 4.69 (d, J = 6.6 Hz, 1H, OCH_2OCH_3), 4.72 (d, J = 6.6 Hz, 1H, OCH_2OCH_3), 4.90 (dt, J = 8.6/2.8 Hz, 1H, $HOCHCH_2O$), 7.27–7.41 (m, 5H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 55.7 (1C, OCH_2OCH_3), 73.2 (1C, $HOCHCH_2O$), 74.6 (1C, $HOCHCH_2O$), 97.2 (1C, OCH_2OCH_3), 126.3 (2C, C-2′$_{phenyl}$, C-6′$_{phenyl}$), 128.0 (1C, C-4′$_{phenyl}$), 128.6 (2C, C-3′$_{phenyl}$, C-5′$_{phenyl}$), 140.3 (1C, C-1′$_{phenyl}$); IR (neat): ν [cm^{-1}] = 3441,

2932, 2886, 1454, 1211, 1150, 1111, 1026, 914, 756, 698; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{10}H_{15}O_3$, 183.1016; found, 183.1023.

4.2.13. (*S*)-1-(4-Bromophenyl)-2-(methoxymethoxy)ethanol (13)

Under N_2 atmosphere *N*,*N*-diisopropylethylamine (18.7 mL, 110 mmol) and chloromethyl methyl ether (5.6 mL, 73.2 mmol) were added to a solution of **11** (7.9 g, 36.6 mmol) in acetonitrile (70 mL) at 0 °C. After stirring the mixture at ambient temperature for 16 h, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (8 cm, 60 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.14) to give **13** as colorless oil (3.4 g, 13.0 mmol, 36%). $[\alpha]_D^{20}$ 40.9 (3.9; CH_2Cl_2); HPLC (method 1): t_R = 16.0 min, purity 98.6%.

4.2.14. (*R*)-1-(4-Bromophenyl)-2-(methoxymethoxy)ethanol (ent-13)

As described for the preparation of **13**, the enantiomer ent-**11** (1.7 g, 7.8 mmol) was reacted with *N*,*N*-diisopropylethylamine (4.33 mL, 3.39 g, 26.2 mmol) and chloromethyl methyl ether (1.42 mL, 1.51 g, 18.7 mmol) in acetonitrile (50 mL) to give ent-**13** as colorless oil (940 mg, 3.6 mmol, 46%). $[\alpha]_D^{20}$ −34.3 (3.2; CH_2Cl_2); HPLC (method 1): t_R = 16.3 min, purity 99.2%.

4.2.15. Spectroscopic data for 13 and ent-13

^{1}H NMR ($CDCl_3$): δ 3.18 (s br, 1H, O*H*), 3.39 (s, 3H, OCH_2OCH_3), 3.55 (dd, *J* = 10.7/8.5 Hz, 1H, $HOCHCH_2O$), 3.77 (dd, *J* = 10.7/3.1 Hz, 1H, $HOCHCH_2O$), 4.68 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.71 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.85 (dd, *J* = 8.5/3.1 Hz, 1H, $HOCHCH_2O$), 7.26–7.30 (m, 2H, 2′-$H_{4\text{-bromophenyl}}$, 6′-$H_{4\text{-bromophenyl}}$), 7.47–7.50 (m, 2H, 3′-$H_{4\text{-bromophenyl}}$, 5′-$H_{4\text{-bromophenyl}}$); ^{13}C NMR ($CDCl_3$): δ 55.7 (1C, OCH_2OCH_3), 72.6 (1C, $HOCHCH_2O$), 74.4 (1C, $HOCHCH_2O$), 97.3 (1C, OCH_2OCH_3), 121.8 (1C, C-4′$_{4\text{-bromophenyl}}$), 128.0 (2C, C-2′$_{4\text{-bromophenyl}}$, C-6′$_{4\text{-bromophenyl}}$), 131.6 (2C, C-3′$_{4\text{-bromophenyl}}$, C-5′$_{4\text{-bromophenyl}}$), 139.4 (1C, C-1′$_{4\text{-bromophenyl}}$); IR (neat): ν [cm^{-1}] = 3426, 2932, 2886, 1485, 1400, 1211, 1150, 1111, 1069, 1030, 1011, 822; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{10}H_{14}{}^{79}BrO_3$, 261.0121; found, 261.0118.

4.2.16. (*S*)-Ethyl 2-[2-(methoxymethoxy)-1-phenylethoxy] acetate (14)

Under N_2 atmosphere a 1 M solution of LiHMDS in THF (7.5 mL, 7.5 mmol) was added to a solution of **12** (910 mg, 5.0 mmol) in THF (50 mL). Then ethyl bromoacetate (1.1 mL, 10 mmol) was added and the mixture was heated to refulx for 16 h. After cooling the mixture to ambient temperature, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (4 cm, 30 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.28) to give **14** as colorless oil (830 mg, 3.1 mmol, 62%). $[\alpha]_D^{20}$ +63.4 (4.0; CH_2Cl_2); HPLC (method 1): t_R = 17.9 min, purity 95.6%.

4.2.17. (*R*)-Ethyl 2-[2-(methoxymethoxy)-1-phenylethoxy] acetate (ent-14)

As described for the preparation of **14**, the enantiomer ent-**12** (1.37 g, 7.52 mmol) was reacted with a 2 M solution of NaHMDS in THF (5.6 mL, 11.3 mmol) and ethyl bromoacetate (2.5 mL, 22.6 mmol) in THF (50 mL) to give ent-**14** as colorless oil (1.31 g, 4.88 mmol, 65%). $[\alpha]_D^{20}$ −74.4 (2.8; CH_2Cl_2); HPLC (method 1): t_R = 18.3 min, purity 93.4%.

4.2.18. Spectroscopic data for 14 and ent-14

^{1}H NMR ($CDCl_3$): δ 1.24 (t, *J* = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.30 (s, 3H, OCH_2OCH_3), 3.70 (dd, *J* = 10.9/4.1 Hz, 1H, $OCHCH_2O$), 3.84 (dd, *J* = 10.9/7.5 Hz, 1H, $OCHCH_2O$), 3.97 (d, *J* = 16.2 Hz, 1H, OCH_2CO_2Et), 4.10 (d, *J* = 16.2 Hz, 1H, OCH_2CO_2Et), 4.13–4.20 (m, 2H, $CO_2CH_2CH_3$), 4.65 (d, *J* = 6.5 Hz, 1H, OCH_2OCH_3), 4.67 (d, *J* = 6.5 Hz, 1H, OCH_2OCH_3), 4.68 (dd, *J* = 7.5/4.1 Hz, 1H, $OCHCH_2O$), 7.29–7.37 (m, 5H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 14.3 (1C, $CO_2CH_2CH_3$), 55.4 (1C, OCH_2OCH_3), 60.9 (1C, $CO_2CH_2CH_3$), 66.4 (1C, OCH_2CO_2Et), 71.6 (1C, $OCHCH_2O$), 81.7 (1C, $OCHCH_2O$), 96.7 (1C, OCH_2OCH_3), 127.4 (2C, C-2′$_{phenyl}$, C-6′$_{phenyl}$), 128.5 (1C, C-4′$_{phenyl}$), 128.7 (2C, C-3′$_{phenyl}$, C-5′$_{phenyl}$), 138.1 (1C, C-1′$_{phenyl}$), 170.3 (1C, CO_2Et); IR (neat): ν [cm^{-1}] = 2932, 1751, 1450, 1200, 1111, 1030, 918, 760, 702; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{14}H_{21}O_5$, 269.1384; found, 269.1390.

4.2.19. (*S*)-Ethyl 2-[1-(4-bromophenyl)-2-(methoxymethoxy) ethoxy]acetate (15)

Under N_2 atmosphere a 1 M solution of LiHMDS in THF (32.1 mL, 32.1 mmol) was added to a solution of **13** (5.6 g, 21.4 mmol) in THF (70 mL). Then ethyl bromoacetate (7.1 mL, 10.7 g, 64.2 mmol) was added and the mixture was heated to reflux for 16 h. After cooling the mixture to ambient temperature, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (8 cm, 60 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.28) to give **15** as colorless oil (5.4 mg, 15.6 mmol, 73%).$[\alpha]_D^{20}$ 77.2 (3.0; CH_2Cl_2); HPLC (method 1): t_R = 19.9 min, purity 97.8%.

4.2.20. (*R*)-Ethyl 2-[1-(4-bromophenyl)-2-(methoxymethoxy) ethoxy]acetate (ent-15)

As described for the preparation of **15**, the enantiomer ent-**13** (996 mg, 3.81 mmol) was reacted with a 1 M solution of LiHMDS in THF (5.72 mL, 5.72 mmol) and ethyl bromoacetate (1.27 mL, 1.91 g, 11.4 mmol) to give ent-**15** as colorless oil (857 mg, 2.47 mmol, 65%).$[\alpha]_D^{20}$ −64.7 (3.4; CH_2Cl_2); HPLC (method 1): t_R = 20.1 min, purity 95.9%.

4.2.21. Spectroscopic data for 15 and ent-15

^{1}H NMR (D_3COD): δ 1.24 (t, *J* = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.25 (s, 3H, OCH_2OCH_3), 3.64 (dd, *J* = 10.7/4.6 Hz, 1H, $OCHCH_2O$), 3.78 (dd, *J* = 10.7/6.9 Hz, 1H, $OCHCH_2O$), 4.01 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.09 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.16 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.59 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.62 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.65 (dd, *J* = 6.9/4.6 Hz, 1H, $OCHCH_2O$), 7.28–7.34 (m, 2H, 2′-$H_{4\text{-bromophenyl}}$, 6′-$H_{4\text{-bromophenyl}}$), 7.50–7.55 (m, 2H, 3′-$H_{4\text{-bromophenyl}}$, 5′-$H_{4\text{-bromophenyl}}$); ^{13}C NMR (D_3COD): δ 14.4 (1C, $CO_2CH_2CH_3$), 55.5 (1C, OCH_2OCH_3), 61.9 (1C, $CO_2CH_2CH_3$), 67.4 (1C, OCH_2CO_2Et), 72.2 (1C, $OCHCH_2O$), 82.1 (1C, $OCHCH_2O$), 97.6 (1C, OCH_2OCH_3), 123.0 (1C, C-4′$_{4\text{-bromophenyl}}$), 130.3 (2C, C-2′$_{4\text{-bromophenyl}}$, C-6′$_{4\text{-bromophenyl}}$), 132.6 (2C, C-3′$_{4\text{-bromophenyl}}$, C-5′$_{4\text{-bromophenyl}}$), 139.2 (1C, C-1′$_{4\text{-bromophenyl}}$), 171.9 (1C, CO_2Et); IR (neat): ν [cm^{-1}] = 2932, 1751, 1485, 1377, 1281, 1200, 1111, 1034, 918, 822; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{14}H_{20}{}^{79}BrO_5$, 347.0489; found, 347.0496.

4.2.22. (*S*)-5-Phenyl-1,4-dioxan-2-one (16)[29]

p-Toluenesulfonic acid monohydrate (255 mg, 1.34 mmol) was added to a solution of **14** (720 mg, 2.68 mmol) in methanol (50 mL) and the mixture was stirred at ambient temperature for 16 h. After TLC had indicated that all of staring material was consumed, the solvent was removed in vacuo. The residue was dissolved in acetonitrile (50 mL), additional *p*-toluenesulfonic acid monohydrate (255 mg, 1.34 mmol) was added and the mixture was stirred at ambient temperature for 16 h. Then a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The

residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.28) to give **16** as colorless oil (300 mg, 1.68 mmol, 63%). $[\alpha]_D^{20}$ +104.3 (0.3; CH_2Cl_2); HPLC (method 1): t_R = 14.3 min, purity 97.4%.

4.2.23. (*R*)-5-Phenyl-1,4-dioxan-2-one (ent-16)

As described for the preparation of **16**, the enantiomer ent-**14** (1.23 g, 4.58 mmol) was reacted with *p*-toluenesulfonic acid monohydrate (436 mg, 2.29 mmol) in methanol (50 mL) and the crude product of the transformation was reacted with *p*-toluenesulfonic acid monohydrate (436 mg, 2.29 mmol) acetonitrile (50 mL) to give ent-**16** as colorless oil (433 mg, 2.43 mmol, 53%).$[\alpha]_D^{20}$ −117.4 (2.6; CH_2Cl_2); HPLC (method 1): t_R = 14.2 min, purity 87.3%.

4.2.24. Spectroscopic data for 16 and ent-16

^{1}H NMR ($CDCl_3$): δ 4.44 (dd, J = 11.5/10.1 Hz, 1H, OCHC*H*$_2$O), 4.50 (d, J = 17.8 Hz, 1H, OC*H*$_2$CO$_2$), 4.51 (dd, J = 11.5/3.2 Hz, 1H, OCHC*H*$_2$O), 4.67 (d, J = 17.8 Hz, 1H, OC*H*$_2$CO$_2$), 4.86 (dd, J = 10.1/3.2 Hz, 1H, OC*H*CH$_2$O), 7.35–7.44 (m, 5H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 66.2 (1C, OCH$_2$CO$_2$), 73.2 (1C, OCHCH$_2$O), 74.2 (1C, OCHCH$_2$O), 126.3 (2C, C-2′$_{phenyl}$, C-6′$_{phenyl}$), 129.0 (2C, C-3′$_{phenyl}$, C-5′$_{phenyl}$), 129.2 (1C, C-4′$_{phenyl}$), 135.1 (1C, C-1′$_{phenyl}$), 166.6 (1C, OCH$_2$*C*O$_2$); IR (neat): ν [cm^{-1}] = 2951, 2882, 1744, 1454, 1408, 1323, 1223, 1115, 1026, 895, 752, 698; HRMS (m/z): [M+H]$^+$ calcd for $C_{10}H_{11}O_3$, 179.0703; found, 179.0699.

4.2.25. (*S*)-5-(4-Bromophenyl)-1,4-dioxan-2-one (17)

p-Toluenesulfonic acid monohydrate (1.5 g, 7.8 mmol) was added to a solution of **15** (5.4 g, 15.6 mmol) in methanol (70 mL) and the mixture was stirred at ambient temperature for 16 h. After TLC had indicated that all of staring material was consumed, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was dissolved in acetonitrile (70 mL), *p*-toluenesulfonic acid monohydrate (1.5 mg, 7.8 mmol) was added and the mixture was stirred at ambient temperature for 16 h. Then water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (8 cm, 60 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.10) to give **17** as colorless solid (2.0 g, 7.8 mmol, 50%). Mp: 84 °C;$[\alpha]_D^{20}$ 81.2 (3.2; CH_2Cl_2); HPLC (method 1): t_R = 16.6 min, purity 98.5%.

4.2.26. (*R*)-5-(4-Bromophenyl)-1,4-dioxan-2-one (ent-17)

As described for the preparation of **17**, the enantiomer ent-**15** (401 mg, 1.16 mmol) was reacted with *p*-toluenesulfonic acid monohydrate (110 mg, 0.58 mmol) in methanol (50 mL) and the crude product of the transformation was reacted with *p*-toluenesulfonic acid monohydrate (110 mg, 0.58 mmol) acetonitrile (50 mL) to give ent-**17** as colorless solid (175 mg, 0.68 mmol, 59%).$[\alpha]_D^{20}$ −72.7 (2.6; CH_2Cl_2); HPLC (method 1): t_R = 16.8 min, purity 98.6%.

4.2.27. Spectroscopic data for 17 and ent-17

^{1}H NMR ($CDCl_3$): δ 4.38 (dd, J = 11.5/10.3 Hz, 1H, OCHC*H*$_2$O), 4.48 (dd, J = 11.5/3.0 Hz, 1H, OCHC*H*$_2$O), 4.49 (d, J = 17.8 Hz, 1H, OC*H*$_2$CO$_2$), 4.66 (d, J = 17.8 Hz, 1H, OC*H*$_2$CO$_2$), 4.82 (dd, J = 10.3/3.0 Hz, 1H, OC*H*CH$_2$O),), 7.24–7.27 (m, 2H, 2′-H$_{4\text{-bromophenyl}}$, 6′-H$_{4\text{-bromophenyl}}$), 7.52–7.56 (m, 2H, 3′-H$_{4\text{-bromophenyl}}$, 5′-H$_{4\text{-bromophenyl}}$); ^{13}C NMR ($CDCl_3$): δ 66.1 (1C, OCH$_2$CO$_2$), 72.8 (1C, OCHCH$_2$O), 73.6 (1C, OCHCH$_2$O), 123.2 (1C, C-4′$_{4\text{-bromophenyl}}$), 127.9 (2C, C-2′$_{4\text{-bromophenyl}}$, C-6′$_{4\text{-bromophenyl}}$), 132.2 (2C, C-3′$_{4\text{-bromophenyl}}$, C-5′$_{4\text{-bromophenyl}}$), 134.1 (1C, C-1′$_{4\text{-bromophenyl}}$), 166.2 (1C, OCH$_2$*C*O$_2$); IR (neat): ν [cm^{-1}] = 2951, 2889, 1744, 1485, 1454, 1400, 1327, 1223, 1119, 1011, 895, 826, 779; HRMS (m/z): [M+H]$^+$ calcd for $C_{10}H_{10}{}^{79}BrO_3$, 256.9808; found, 256.9806.

4.2.28. (*S*)-*N*-Hydroxy-2-(2-hydroxy-1-phenylethoxy)acetamide (18)

As described in the general procedure for the synthesis of hydroxamic acids, **16** (100 mg, 0.56 mmol) was reacted with hydroxylamine hydrochloride (117 mg, 1.68 mmol) and a 2 m solution of sodium methoxide in methanol (0.56 mL, 1.12 mmol) in methanol (10 mL) to give **18** as colorless solid (43 mg, 0.20 mmol, 36%). R_f = 0.42 (CH_2Cl_2/methanol = 9:1); mp: 114 °C; $[\alpha]_D^{20}$ +117.4 (1.3; methanol); HPLC (method 2): t_R = 12.5 min, purity 95.3%.

4.2.29. (*R*)-*N*-Hydroxy-2-(2-hydroxy-1-phenylethoxy)acetamide (ent-18)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**16** (100 mg, 0.56 mmol) was reacted with hydroxylamine hydrochloride (78 mg, 1.12 mmol) and a 2.5 M solution of sodium methoxide in methanol (0.67 mL, 1.68 mmol) in methanol (30 mL) to give ent-**18** as colorless solid (23 mg, 0.11 mmol, 19%). R_f = 0.42 (CH_2Cl_2/methanol = 9:1); mp: 114 °C; $[\alpha]_D^{20}$ −109.7 (1.4; methanol); HPLC (method 2): t_R = 12.4 min, purity 95.2%.

4.2.30. Spectroscopic data for 18 and ent-18

^{1}H NMR (CD_3OD): δ 3.61 (dd, J = 11.9/3.4 Hz, 1H, OCHC*H*$_2$O), 3.70 (dd, J = 11.9/8.3 Hz, 1H, OCHC*H*$_2$O), 3.86 (d, J = 14.9 Hz, 1H, OC*H*$_2$CONHOH), 3.94 (d, J = 14.9 Hz, 1H, OC*H*$_2$CONHOH), 4.46 (dd, J = 8.3/3.4 Hz, 1H, OC*H*CH$_2$O), 7.30–7.41 (m, 5H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ 67.6 (1C, OCHCH$_2$O), 68.3 (1C, OCH$_2$CONHOH), 85.7 (1C, OCHCH$_2$O), 128.0 (2C, C-2′$_{phenyl}$, C-6′$_{phenyl}$), 129.5 (1C, C-4′$_{phenyl}$), 129.7 (2C, C-3′$_{phenyl}$, C-5′$_{phenyl}$), 139.0 (1C, C-1′$_{phenyl}$), 169.1 (1C, OCH$_2$CONHOH); IR (neat): ν [cm^{-1}] = 3248, 2862, 1639, 1524, 1489, 1454, 1339, 1196, 1111, 1049, 895, 756, 698; HRMS (m/z): [M+H]$^+$ calcd for $C_{10}H_{14}NO_4$, 212.0917; found, 212.0927.

4.2.31. (*S*)-2-[1-(4-Bromophenyl)-2-hydroxyethoxy]-*N*-hydroxyacetamide (19)

As described in the general procedure for the synthesis of hydroxamic acids, **17** (100 mg, 0.39 mmol) was reacted with hydroxylamine hydrochloride (54 mg, 0.78 mmol) and a 2 M solution of sodium methoxide in methanol (0.58 mL, 1.17 mmol) in methanol (30 mL) to give **19** as colorless solid (72 mg, 0.25 mmol, 64%). R_f = 0.39 (CH_2Cl_2/methanol = 9:1); mp: 119 °C; $[\alpha]_D^{20}$ 95.8 (1.1; methanol); HPLC (method 2): t_R = 14.3 min, purity 88.9%.

4.2.32. (*R*)-2-[1-(4-Bromophenyl)-2-hydroxyethoxy]-*N*-hydroxyacetamide (ent-19)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**17** (100 mg, 0.39 mmol) was reacted with hydroxylamine hydrochloride (54 mg, 0.78 mmol) and a 2 M solution of sodium methoxide in methanol (0.58 mL, 1.17 mmol) in methanol (30 mL) to give ent-**19** as colorless solid (71 mg, 0.24 mmol, 63%). R_f = 0.39 (CH_2Cl_2/methanol = 9:1); Mp: 119 °C; $[\alpha]_D^{20}$ −95.7 (1.1; methanol); HPLC (method 2): t_R = 14.0 min, purity 98.3%.

4.2.33. Spectroscopic data for 19 and ent-19

^{1}H NMR (CD_3OD): δ 3.61 (dd, J = 11.9/3.6 Hz, 1H, OCHC*H*$_2$O), 3.67 (dd, J = 11.9/7.8 Hz, 1H, OCHC*H*$_2$O), 3.88 (d, J = 14.8 Hz, 1H, OC*H*$_2$CONHOH), 3.95 (d, J = 14.8 Hz, 1H, OC*H*$_2$CONHOH), 4.45 (dd, J = 7.8/3.6 Hz, 1H, OC*H*CH$_2$O), 7.26–7.30 (m, 2H, 2′-H$_{4\text{-bromophenyl}}$, 6′-H$_{4\text{-bromophenyl}}$), 7.52–7.56 (m, 2H, 3′-H$_{4\text{-bromophenyl}}$, 5′-H$_{4\text{-bromophenyl}}$);

$_{13}$C NMR (CD_3OD): δ 67.2 (1C, OCHCH$_2$O), 68.4 (1C, OCH$_2$CONHOH), 84.9 (1C, OCHCH$_2$O), 123.2 (1C, C-4′$_{4\text{-bromophenyl}}$), 130.0 (2C, C-2′$_{4\text{-bromophenyl}}$, C-6′$_{4\text{-bromophenyl}}$), 132.8 (2C, C-3′$_{4\text{-bromophenyl}}$, C-5′$_{4\text{-bromophenyl}}$), 138.5 (1C, C-1′$_{4\text{-bromophenyl}}$), 168.9 (1C, OCH$_2$*C*ONHOH); IR (neat): ν [cm^{-1}] = 3240, 2913, 2866, 1643, 1531, 1485, 1439, 1404, 1339, 1281, 1111, 1042, 1007, 818, 667; HRMS (m/z): [M+H]$^+$ calcd for $C_{10}H_{13}{}^{79}BrNO_4$, 290.0022; found, 290.0012.

4.2.34. (*S*)-5-[4-(Phenylethynyl)phenyl]-1,4-dioxan-2-one (20)

Under N_2 atmosphere copper(I) iodide (11 mg, 0.06 mmol), tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.04 mmol) and phenylacetylene (0.30 mL, 276 mg, 2.7 mmol) were added to a solution of **17** (500 mg, 1.94 mmol) in triethylamine (50 mL). The mixture was heated to reflux and additional phenylacetylene (0.30 mL, 276 mg, 2.7 mmol) was added. After stirring at reflux for 16 h, the solvent was evaporated and the residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.26) to give **20** as colorless solid (350 mg, 1.26 mmol, 65%). Mp: 134 °C; $[\alpha]_D^{20}$ 108.4 (0.8; CH_2Cl_2); HPLC (method 1): t_R = 20.2 min, purity 95.8%.

4.2.35. (*R*)-5-[4-(Phenylethynyl)phenyl]-1,4-dioxan-2-one (ent-20)

As described for the preparation of **20**, the enantiomer ent-**17** (313 mg, 1.22 mmol) was reacted with copper(I) iodide (7 mg, 0.04 mmol), tetrakis(triphenylphosphine)palladium(0) (21 mg, 0.02 mmol) and phenylacetylene (0.20 mL, 184 mg, 1.81 mmol) in triethylamine (50 mL) to give ent-**20** as colorless solid (138 mg, 0.50 mmol, 41%). Mp: 134 °C; $[\alpha]_D^{20}$ −94.1 (1.8; CH_2Cl_2); HPLC (method 1): t_R = 21.1 min, purity 95.1%.

4.2.36. Spectroscopic data for 20 and ent-20

^{1}H NMR ($CDCl_3$): δ 4.42 (dd, J = 11.5/10.3 Hz, 1H, OCHCH_2O), 4.51 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.52 (dd, J = 11.5/3.0 Hz, 1H, OCHCH_2O), 4.68 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.87 (dd, J = 10.3/3.0 Hz, 1H, OC*H*CH$_2$O), 7.34–7.38 (m, 5H, H$_{arom.}$), 7.51–7.58 (m, 4H, H$_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 66.2 (1C, OCH$_2$CO$_2$), 72.9 (1C, OCHCH$_2$O), 73.9 (1C, OCHCH$_2$O), 88.7 (1C, *C*≡C), 90.5 (1C, *C*≡C), 123.0 (1C, C$_{arom.}$), 124.3 (1C, C$_{arom.}$), 126.3 (2C, C$_{arom.}$), 128.5 (2C, C$_{arom.}$), 128.7 (1C, C$_{arom.}$), 131.8 (2C, C$_{arom.}$), 132.2 (2C, C$_{arom.}$), 135.0 (1C, C$_{arom.}$), 166.3 (1C, OCH$_2$*C*O$_2$); IR (neat): ν [cm^{-1}] = 2870, 1740, 1512, 1400, 1223, 1107, 1022, 891, 837, 760, 691; HRMS (m/z): [M+H]$^+$ calcd for $C_{18}H_{15}O_3$, 279.1016; found, 279.0992.

4.2.37. (*S*)-*N*-Hydroxy-2-{2-hydroxy-1-[4-(phenylethynyl) phenyl]ethoxy}acetamide (21)

As described in the general procedure for the synthesis of hydroxamic acids, **20** (57 mg, 0.20 mmol) was reacted with hydroxylamine hydrochloride (28 mg, 0.41 mmol) and a 2 M solution of sodium methoxide in methanol (0.31 mL, 0.61 mmol) in methanol (30 mL) to give **21** as colorless solid (21 mg, 0.07 mmol, 33%). R_f = 0.21 (CH_2Cl_2/methanol = 9.5:0.5); mp: 128 °C; $[\alpha]_D^{20}$ 98.8 (1.1; methanol); HPLC (method 2): t_R = 16.0 min, purity 97.9%.

4.2.38. (*R*)-*N*-Hydroxy-2-{2-hydroxy-1-[4-(phenylethynyl) phenyl]ethoxy}acetamide (ent-21)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**20** (99 mg, 0.36 mmol) was reacted with hydroxylamine hydrochloride (49 mg, 0.71 mmol) and a 2.5 M solution of sodium methoxide in methanol (0.43 mL, 1.1 mmol) methanol (30 mL) to give ent-**21** as colorless solid (62 mg, 0.20 mmol, 56%). R_f = 0.21 (CH_2Cl_2/methanol = 9.5:0.5); mp: 128 °C; $[\alpha]_D^{20}$ −99.5 (1.8; methanol); HPLC (method 2): t_R = 16.1 min, purity 95.2%.

4.2.39. Spectroscopic data for 21 and ent-21

^{1}H NMR (CD_3OD): δ 3.64 (dd, J = 11.9/3.6 Hz, 1H, OCHCH_2O), 3.71 (dd, J = 11.9/7.9 Hz, 1H, OCHCH_2O), 3.90 (d, J = 14.8 Hz, 1H, OCH_2CONHOH), 3.98 (d, J = 14.8 Hz, 1H, OCH_2CONHOH), 4.49 (dd, J = 7.9/3.6 Hz, 1H, O*C*HCH$_2$O), 7.36–7.39 (m, 5H, H$_{arom.}$), 7.49–7.55 (m, 4H, H$_{arom.}$); ^{13}C NMR (CD_3OD): δ 67.3 (1C, OCH*C*H$_2$O), 68.5 (1C, O*C*H$_2$CONHOH), 85.3 (1C, O*C*HCH$_2$O), 89.7 (1C, *C*≡C), 90.5 (1C, *C*≡C), 124.5 (1C, C$_{arom.}$), 124.7 (1C, C$_{arom.}$), 128.3 (2C, C$_{arom.}$), 129.5 (1C, C$_{arom.}$), 129.6 (2C, C$_{arom.}$), 132.5 (2C, C$_{arom.}$), 132.8 (2C, C$_{arom.}$), 139.5 (1C, C$_{arom.}$), 168.9 (1C, OCH$_2$*C*ONHOH); IR (neat): ν [cm^{-1}] = 3240, 2916, 2862, 1639, 1504, 1443, 1412, 1335, 1196, 1130, 1111, 1045, 829, 752, 687; HRMS (m/z): [M+H]$^+$ calcd for $C_{18}H_{18}NO_4$, 312.1230; found, 312.1217.

4.2.40. (*S*)-5-{4-[(Trimethylsilyl)ethynyl]phenyl}-1,4-dioxan-2-one (22)

Under N_2 atmosphere copper(I) iodide (16 mg, 0.08 mmol), tetrakis(triphenylphosphine)palladium(0) (62 mg, 0.05 mmol) and trimethylsilylacetylene (0.6 mL, 398 mg, 4.1 mmol) were added to a solution of **17** (700 mg, 2.7 mmol) in triethylamine (40 mL). The mixture was heated to reflux and additional trimethylsilylacetylene (0.6 mL, 398 mg, 4.1 mmol) was added. After stirring at reflux for 16 h, the solvent was evaporated and the residue was purified by flash column chromatography (4 cm, 30 mL, cyclohexane/ethyl acetate = 9:1, R_f = 0.25) to give **22** as colorless solid (480 mg, 1.75 mmol, 64%). Mp: 113 °C; $[\alpha]_D^{20}$ 84.4 (1.3; CH_2Cl_2); HPLC (method 1): t_R = 20.5 min, purity 98.9%.

4.2.41. (*R*)-5-{4-[(Trimethylsilyl)ethynyl]phenyl}-1,4-dioxan-2-one (ent-22)

As described for the preparation of **22**, the enantiomer ent-**17** (550 mg, 2.1 mmol) was reacted with copper(I) iodide (12 mg, 0.06 mmol), tetrakis(triphenylphosphine)palladium(0) (49 mg, 0.04 mmol) and trimethylsilylacetylene (twice 0.46 mL, 315 mg, 3.2 mmol) in triethylamine (70 mL) to give ent-**22** as colorless solid (420 mg, 1.53 mmol, 72%). Mp: 113 °C; $[\alpha]_D^{20}$ −84.3 (1.5; CH_2Cl_2); HPLC (method 1): t_R = 20.4 min, purity 95.4%.

4.2.42. Spectroscopic data for 22 and ent-22

^{1}H NMR ($CDCl_3$): δ 0.25 (s, 9H, Si(CH_3)$_3$), 4.38 (dd, J = 11.5/10.3 Hz, 1H, OCHCH_2O), 4.49 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.49 (dd, J = 11.5/3.0 Hz, 1H, OCHCH_2O), 4.66 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.84 (dd, J = 10.3/3.0 Hz, 1H, OC*H*CH$_2$O), 7.29–7.32 (m, 2H, H$_{arom.}$), 7.47–7.51 (m, 2H, H$_{arom.}$); ^{13}C NMR ($CDCl_3$): δ 0.05 (3C, Si(*C*H$_3$)$_3$), 66.1 (1C, OCH$_2$CO$_2$), 72.9 (1C, OCHCH$_2$O), 73.8 (1C, OCHCH$_2$O), 95.6 (1C, SiC≡C), 104.3 (1C, SiC≡*C*), 124.1 (1C, C$_{arom.}$), 126.1 (2C, C$_{arom.}$), 132.5 (2C, C$_{arom.}$), 135.2 (1C, C$_{arom.}$), 166.3 (1C, OCH$_2$*C*O$_2$); IR (neat): ν [cm^{-1}] = 2963, 2153, 1748, 1504, 1404, 1323, 1238, 1119, 1026, 833, 760; HRMS (m/z): [M+H]$^+$ calcd for $C_{15}H_{19}O_3Si$, 275.1098; found, 275.1110.

4.2.43. (*S*)-*N*-Hydroxy-2-(2-hydroxy-1-{4-[(trimethylsilyl) ethynyl]phenyl}ethoxy)acetamide (23)

As described in the general procedure for the synthesis of hydroxamic acids, **22** (120 mg, 0.44 mmol) was reacted with hydroxylamine hydrochloride (76 mg, 1.1 mmol) and a 2 M solution of sodium methoxide in methanol (0.55 mL, 1.1 mmol) in methanol (20 mL) to give **23** as colorless solid (22 mg, 0.07 mmol, 16%). R_f = 0.10 (CH_2Cl_2/methanol = 9.5:0.5); Mp: 112 °C; $[\alpha]_D^{20}$ +104.7 (0.7; methanol); HPLC (method 2): t_R = 15.5 min, purity 99.1%.

4.2.44. (*R*)-*N*-Hydroxy-2-(2-hydroxy-1-{4-[(trimethylsilyl) ethynyl]phenyl}ethoxy)acetamide (ent-23)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**22** (100 mg, 0.36 mmol) was reacted with

hydroxylamine hydrochloride (76 mg, 1.1 mmol) and a 2 M solution of sodium methoxide in methanol (0.36 mL, 0.73 mmol) in methanol (10 mL) to give ent-**23** as colorless solid (86 mg, 0.28 mmol, 77%). R_f = 0.10 (CH_2Cl_2/methanol = 9.5:0.5); mp: 112 °C; $[\alpha]_D^{20}$ −99.4 (1.4; methanol); HPLC (method 2): t_R = 16.2 min, purity 96.9%.

4.2.45. Spectroscopic data for 23 and ent-23

^{1}H NMR (CD_3OD): δ 0.23 (s, 9H, Si(CH_3)$_3$), 3.58–3.71 (m, 2H, OCHCH_2OH), 3.87 (d, J = 14.8 Hz, 1H, OCH_2CONHOH), 3.95 (d, J = 14.8 Hz, 1H, OCH_2CONHOH), 4.44–4.49 (m, 1H, OCHCH$_2$OH), 7.30–7.36 (m, 2H, 2′-H$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$, 6′-H$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$), 7.41–7.46 (m, 2H, 3′-H$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$, 5′-H$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$); ^{13}C-NMR ($CD_3OD$): δ -0.1 (3C, Si(CH_3)$_3$), 67.3 (1C, OCHCH$_2$OH), 68.5 (1C, OCH$_2$CONHOH), 85.3 (1C, OCHCH$_2$OH), 94.8 (1C, SiC≡C), 105.9 (1C, SiC≡C), 124.5 (1C, C-4′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$), 128.1 (2C, C-2′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$, C-6′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$), 133.1 (2C, C-3′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$, C-5′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$), 139.8 (1C, C-1′$_{4\text{-((trimethylsilyl)ethynyl)phenyl}}$), 168.9 (1C, OCH$_2$CONHOH); IR (neat): ν [cm^{-1}] = 3210, 2959, 2901, 2156, 1663, 1501, 1408, 1335, 1250, 1119, 1049, 837, 756, 648; HRMS (m/z): [M+H]$^+$ calcd for $C_{15}H_{22}NO_4Si$, 308.1313; found, 308.1316.

4.2.46. (*S*)-5-(4-Ethynylphenyl)-1,4-dioxan-2-one (24)

Tetrabutylammonium fluoride trihydrate (662 mg, 2.1 mmol) was added to a solution of **22** (480 mg, 1.75 mmol) in THF (30 mL). The mixture was stirred at ambient temperature for 2 h. Then water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 8:2, R_f = 0.23) to give **24** as colorless solid (110 mg, 0.54 mmol, 31%). Mp: 120 °C; $[\alpha]_D^{20}$ +97.0 (0.7; CH_2Cl_2); HPLC (method 1): t_R = 15.2 min, purity 96.1%.

4.2.47. (*R*)-5-(4-Ethynylphenyl)-1,4-dioxan-2-one (ent-24)

As described for the preparation of **24**, the enantiomer ent-**22** (420 mg, 1.53 mmol) was reacted with tetrabutylammonium fluoride trihydrate (579 mg, 1.84 mmol) in THF (50 mL) to give ent-**24** as colorless solid (70 mg, 0.35 mmol, 23%). Mp: 120 °C; $[\alpha]_D^{20}$ −90.9 (1.5; CH_2Cl_2); HPLC (method 1): t_R = 15.4 min, purity 98.8%.

4.2.48. Spectroscopic data for 24 and ent-24

^{1}H NMR ($CDCl_3$): δ 3.11 (s, 1H, C≡CH), 4.39 (dd, J = 11.5/10.3 Hz, 1H, OCHCH_2O), 4.49 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.49 (dd, J = 11.5/3.0 Hz, 1H, OCHCH_2O), 4.66 (d, J = 17.8 Hz, 1H, OCH_2CO$_2$), 4.86 (dd, J = 10.3/3.0 Hz, 1H, OCHCH$_2$O), 7.32–7.35 (m, 2H, 2′-H$_{4\text{-ethynylphenyl}}$, 6′-H$_{4\text{-ethynylphenyl}}$), 7.51–7.54 (m, 2H, 3′-H$_{4\text{-ethynylphenyl}}$, 5′-H$_{4\text{-ethynylphenyl}}$); ^{13}C NMR ($CDCl_3$): δ 66.1 (1C, OCH$_2CO_2$), 72.9 (1C, OCHCH$_2$O), 73.8 (1C, OCHCH$_2$O), 78.3 (1C, C≡CH), 83.0 (1C, C≡CH), 123.1 (1C, C-4′$_{4\text{-ethynylphenyl}}$), 126.2 (2C, C-2′$_{4\text{-ethynylphenyl}}$, C-6′$_{4\text{-ethynylphenyl}}$), 132.7 (2C, C-3′$_{4\text{-ethynylphenyl}}$, C-5′$_{4\text{-ethynylphenyl}}$), 135.7 (1C, C-1′$_{4\text{-ethynylphenyl}}$), 166.2 (1C, OCH$_2CO_2$); IR (neat): ν [cm^{-1}] = 3248, 2878, 2357, 1736, 1504, 1458, 1404, 1323, 1231, 1107, 1026, 895, 837; HRMS (m/z): [M+H]$^+$ calcd for $C_{12}H_{11}O_3$, 203.0703; found, 203.0726.

4.2.49. (*S*)-2-[1-(4-Ethynylphenyl)-2-hydroxyethoxy]-*N*-hydroxyacetamide (25)

As described in the general procedure for the synthesis of hydroxamic acids, **24** (40 mg, 0.2 mmol) was reacted with hydroxylamine hydrochloride (42 mg, 0.6 mmol) and a 2 M solution of sodium methoxide in methanol (0.3 mL, 0.6 mmol) in methanol (20 mL) to give **25** as colorless solid (12 mg, 0.05 mmol, 26%). R_f = 0.47 (CH_2Cl_2/methanol = 9:1); mp: 124 °C; $[\alpha]_D^{20}$ 126.4 (2.7; methanol); HPLC (method 2): t_R = 13.5 min, purity 95.0%.

4.2.50. (*R*)-2-[1-(4-Ethynylphenyl)-2-hydroxyethoxy]-*N*-hydroxyacetamide (ent-25)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**24** (43 mg, 0.21 mmol) was reacted with hydroxylamine hydrochloride (44 mg, 0.64 mmol) and a 2 M solution of sodium methoxide in methanol (0.21 mL, 0.43 mmol) in methanol (5 mL) to give ent-**25** as colorless solid (34 mg, 0.14 mmol, 68%). R_f = 0.47 (CH_2Cl_2/methanol = 9:1); mp: 124 °C; $[\alpha]_D^{20}$ −118.2 (2.1; methanol); HPLC (method 2): t_R = 14.6 min, purity 98.3%.

4.2.51. Spectroscopic data for 25 and ent-25

^{1}H NMR (CD_3OD): δ 3.50 (s, 1H, C≡CH), 3.62 (dd, J = 11.8/3.3 Hz, 1H, OCHCH_2OH), 3.68 (dd, J = 11.8/7.8 Hz, 1H, OCHCH_2OH), 3.88 (d, J = 14.7 Hz, 1H, OCH_2CONHOH), 3.95 (d, J = 14.7 Hz, 1H, OCH_2CONHOH), 4.48 (dd, J = 7.8/3.3 Hz, 1H, OCHCH$_2$OH), 7.31–7.37 (m, 2H, 2′-H$_{4\text{-ethynylphenyl}}$, 6′-H$_{4\text{-ethynylphenyl}}$), 7.45–7.50 (m, 2H, 3′-H$_{4\text{-ethynylphenyl}}$, 5′-H$_{4\text{-ethynylphenyl}}$); ^{13}C NMR ($CD_3OD$): δ 67.3 (1C, OCHCH$_2$OH), 68.5 (1C, OCH$_2$CONHOH), 79.1 (1C, C≡CH), 84.0 (1C, C≡CH), 85.2 (1C, OCHCH$_2$O), 123.9 (1C, C-4′$_{4\text{-ethynylphenyl}}$), 128.2 (2C, C-2′$_{4\text{-ethynylphenyl}}$, C-6′$_{4\text{-ethynylphenyl}}$), 133.3 (2C, C-3′$_{4\text{-ethynylphenyl}}$, C-5′$_{4\text{-ethynylphenyl}}$), 139.9 (1C, C-1′$_{4\text{-ethynylphenyl}}$), 168.9 (1C, OCH$_2$CONHOH); IR (neat): ν [cm^{-1}] = 3271, 2909, 2361, 1643, 1497, 1443, 1393, 1339, 1281, 1123, 1034, 895, 837, 683; HRMS (m/z): [M+H]$^+$ calcd for $C_{12}H_{14}NO_4$, 236.0917; found, 236.0907.

4.2.52. (*S*)-5-(4-{[4-(Morpholinomethyl)phenyl]ethynyl}phenyl)-1,4-dioxan-2-one (27)

Under N_2 atmosphere triethylamine (0.5 mL, 3.8 mmol), copper(I) iodide (21 mg, 0.11 mmol) and tetrakis(triphenylphosphine)palladium(0) (62 mg, 0.054 mmol) were added to a solution of 4-(4-iodobenzyl)morpholine (**26**, 330 mg, 1.09 mmol) in acetonitrile (30 mL). Then a solution of **24** (110 mg, 0.54 mmol) in acetonitrile (10 mL) was added dropwise over a period of 2 h. After evaporation of the solvent the residue was purified by flash column chromatography (3 cm, 20 mL, cyclohexane/ethyl acetate = 1:2, R_f = 0.27) to give **27** as colorless solid (186 mg, 0.49 mmol, 91%). Mp: 143 °C; $[\alpha]_D^{20}$ 70.0 (0.9; CH_2Cl_2); HPLC (method 1): t_R = 15.4 min, purity 95.0%.

4.2.53. (*R*)-5-(4-{[4-(Morpholinomethyl)phenyl]ethynyl}phenyl)-1,4-dioxan-2-one (ent-27)

As described for the preparation of **27**, the enantiomer ent-**24** (50 mg, 0.25 mmol) in acetonitrile (10 mL) was reacted with triethylamine (0.36 mL, 2.6 mmol), copper(I) iodide (4.3 mg, 0.02 mmol), tetrakis(triphenylphosphine)palladium(0) (17 mg, 0.01 mmol) and **26** (225 mg, 0.75 mmol) in acetonitrile (30 mL) to give ent-**27** as colorless solid (80 mg, 0.21 mmol, 86%). Mp: 143 °C; $[\alpha]_D^{20}$ −71.3 (1.6; CH_2Cl_2); HPLC (method 1): t_R = 15.5 min, purity 98.9%.

4.2.54. Spectroscopic data for 27 and ent-27

^{1}H NMR (CD_3OD): δ 2.43–2.49 (m, 4H, NCH_2CH$_2$O), 3.53–3.56 (m, 2H, NCH_2Ar), 3.59–3.77 (m, 6H, NCH$_2$CH_2O, OCHCH_2O), 4.00–4.07 (m, 1H, OCH_2CO$_2$), 4.08–4.14 (m, 1H, OCH_2CO$_2$), 4.52–4.57 (m, 1H, OCHCH$_2$O), 7.34–7.40 (m, 4H, H$_{arom.}$), 7.45–7.54 (m, 4H, H$_{arom.}$); ^{13}C NMR (CD_3OD): δ 54.6 (2C, NCH$_2$CH$_2$O), 63.9 (1C, NCH$_2$-Ar), 67.1 (1C, OCH$_2$CO$_2$), 67.4 (1C, OCHCH$_2$O), 67.8 (2C, NCH$_2$CH$_2$O), 84.7 (1C, OCHCH$_2$O), 89.9 (1C, C≡C), 90.3 (1C, C≡C), 123.5 (1C, C$_{arom.}$), 124.5 (1C, C$_{arom.}$), 128.5 (2C, C$_{arom.}$), 130.8 (2C, C$_{arom.}$), 132.5 (2C, C$_{arom.}$), 132.7 (2C, C$_{arom.}$), 139.1 (1C, C$_{arom.}$), 140.0 (1C, C$_{arom.}$), 172.9 (1C, OCH$_2$CO$_2$); IR (neat): ν [cm^{-1}] = 2808, 1728, 1516, 1450, 1404, 1350, 1323, 1227, 1111, 1007, 895, 868, 829; HRMS (m/z): [M+H]$^+$ calcd for $C_{23}H_{24}NO_4$, 378.1700; found, 378.1700.

4.2.55. (*S*)-*N*-Hydroxy-2-[2-hydroxy-1-(4-{[4-(morpholinomethyl) phenyl]ethynyl}phenyl)ethoxy]acetamide (28)

As described in the general procedure for the synthesis of hydroxamic acids, **27** (100 mg, 0.26 mmol) was reacted with hydroxylamine hydrochloride (46 mg, 0.66 mmol) and a 2 M solution of sodium methoxide in methanol (0.33 mL, 0.66 mmol) in methanol (20 mL) to give **28** as colorless solid (27 mg, 0.066 mmol, 25%). R_f = 0.34 (CH_2Cl_2/methanol = 9:1); mp: 129 °C; $[\alpha]_D^{20}$ 86.4 (7.1; methanol); HPLC (method 2): t_R = 12.0 min, purity 97.7%.

4.2.56. (*R*)-*N*-Hydroxy-2-[2-hydroxy-1-(4-{[4-(morpholinomethyl) phenyl]ethynyl}phenyl)ethoxy]acetamide (ent-28)

As described in the general procedure for the synthesis of hydroxamic acids, ent-**27** (74 mg, 0.2 mmol) was reacted with hydroxylamine hydrochloride (35 mg, 0.5 mmol) and a 2 M solution of sodium methoxide in methanol (0.25 mL, 0.5 mmol) in methanol (20 mL) to give ent-**28** as colorless solid (30 mg, 0.073 mmol, 37%). R_f = 0.34 (CH_2Cl_2/methanol = 9:1); mp: 129 °C; $[\alpha]_D^{20}$ −82.4 (1.4; methanol); HPLC (method 2): t_R = 11.3 min, purity 96.3%.

4.2.57. Spectroscopic data for 28 and ent-28

^{1}H NMR (CD_3OD): δ 2.44–2.50 (m, 4H, NC*H*$_2$CH$_2$O), 3.53–3.56 (m, 2H, NC*H*$_2$Ar), 3.61–3.73 (m, 6H, NCH$_2$C*H*$_2$O, OCHC*H*$_2$OH), 3.86–3.92 (m, 1H, OC*H*$_2$CONHOH), 3.94–4.00 (m, 1H, OC*H*$_2$CONHOH), 4.47–4.51 (m, 1H, OC*H*CH$_2$OH), 7.35–7.39 (m, 4H, H$_{arom.}$), 7.46–7.55 (m, 4H, H$_{arom.}$); ^{13}C NMR (CD_3OD): δ 54.6 (2C, NCH$_2$CH$_2$O), 63.9 (1C, NCH$_2$Ar), 67.3 (1C, OCHCH$_2$OH), 67.7 (2C, NCH$_2$CH$_2$O), 68.5 (1C, OCH$_2$CONHOH), 85.3 (1C, OCHCH$_2$OH), 89.8 (1C, C≡C), 90.4 (1C, C≡C), 123.5 (1C, C$_{arom.}$), 124.7 (1C, C$_{arom.}$), 128.3 (2C, C$_{arom.}$), 130.8 (2C, C$_{arom.}$), 132.5 (2C, C$_{arom.}$), 132.8 (2C, C$_{arom.}$), 139.1 (1C, C$_{arom.}$), 139.5 (1C, C$_{arom.}$), 168.9 (1C, OCH$_2$CONHOH); IR (neat): ν [cm^{-1}] = 3225, 2855, 2812, 1662, 1516, 1454, 1412, 1335, 1292, 1111, 1049, 1007, 864, 829; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{27}N_2O_5$, 411.1914; found, 411.1941.

4.3. Biological evaluation

4.3.1. Agar diffusion clearance assay

The antibacterial activity of the synthesized inhibitors was determined by agar disc diffusion clearance assays. Liquid cultures of *E. coli* BL21 (DE3) and the antibiotic resistant strain *E. coli* D22[30] were grown overnight in LB broth[31] at 37 °C, 200 rpm. 150 µL of overnight cell suspension was spread evenly onto LB agar petri dishes. 15 µL of each compound (10 mM in DMSO) was applied onto circular filter paper (Ø 6 mm, thickness 0.75 mm, Carl Roth). Pure DMSO, serving as a negative and CHIR-090,[16] serving as a positive control were also spotted. The petri dishes were incubated overnight at 37 °C and the diameter of the zone of growth inhibition was measured for each compound. The measured diameters are given as mean value ± SD from three independent experiments.

4.3.2. Protein purfication

The plasmid for the expression of LpxCC63A (pETEcLpxCC63A) was kindly provided by Carol Fierke.[32] The C63A mutation lowers the undesired influence of Zn^{2+}-concentration on enzymatic activity. The purification of LpxC was performed essentially as previously described.[33] Weak anion exchange was performed with a column containing 30 mL diethylaminoethylcellulose (DEAE)-Sepharose fast flow media (GE Healthcare). Eluted fractions containing the desired enzyme were concentrated and desalted with molecular weight cut-off (MWCO) spin columns (10 kDa, PALL Corporation). Strong anion exchange was then performed with a column containing 30 mL of quaternary ammonium-sepharose (Q-Sepharose) fast flow media (GE Healthcare). The fractions containing LpxC (peak elution at 18.6 mS × cm^{-1}) were concentrated and desalted as above using MWCO columns. The final step of protein purification was performed with a pre-packed size exclusion chromatography column containing 300 mL of Superdex 200 (GE Healthcare). LpxCC63A emerged in a peak after 200 mL of elution buffer. The purified LpxC was concentrated with MWCO columns and stored in 100 µL aliquots at 80 °C in Bis/Tris buffer 50 mM, pH 6.0, containing 150 mM NaCl. The presence of the enzyme during the purification progress was confirmed by sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) with Coomassie brilliant blue staining. The purified LpxC had a purity above 95% according to SDS–PAGE, and a concentration of 500 µg × mL^{-1} according to the Bradford assay (Bio-Rad protein assay).

4.3.3. LpxC assay

A fluorescence-based microplate assay for LpxC activity was performed as described by Clements et al.[10] The wells in a black non-binding, 96 wells fluorescence microplate (Greiner Bio One, Frickenhausen) were filled with 97 µL of a 40 mM sodium morpholinoethanesulfonic acid buffer (pH 6.0) containing 25 µM UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine, 80 µM dithiothreitol and 0.02% Brij 35. Inhibitors were dissolved in DMSO and assayed over a range starting from 0.2 nM up to 200 µM. The microplate was incubated for 30 min at 37 °C in a plate shaker. After addition of 500 ng purified LpxC, the biochemical reaction was stopped by adding 40 µL of 0.625 m sodium hydroxide, further incubation for 15 min and neutralization by adding 40 µL 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 µL of 250 nM *o*-phthaldialdehyde-2-mercaptoethanol in 0.1 M borax[34] and detected by a Mithras plate reader (Berthold, Bad Wildbad) at 340 nm excitation and 460 nm emission wavelengths. The calculation of the IC_{50} values was performed with the aid of the software GraphPadPrism, which were then converted into K_i values using the Cheng-Prusoff equation. The K_i and IC_{50} values are given as mean value ± SD from three independent experiments. The K_M value was determined using the Lineweaver–Burk plot and was found to be 4 µM being comparable to the data in literature.[35] To validate the test system, the IC_{50} value of CHIR-090 was measured and was found to be comparable to the one in the literature.

4.4. Computational methods

Several crystal structures of LpxC from *Escherichia coli,* containing ligands structurally similar to the ones reported in the current paper, were taken from the Protein Data Bank[36] (PDB ID: 3P3G[18], 3PS1, 3PS2 and 3PS3[19]).[14,18–23] The crystal structures were first taken to evaluate the accuracy of the used docking and scoring methods.

Protein models were prepared with Schrödinger's Protein Preparation Wizard (PPW).[37] All solvent particles were removed except of two conserved water molecules located near the Zn^{2+} ion. Hydrogen atoms were added and subsequently an automated optimization of the hydrogen bond network was performed. In this procedure the hydroxyl and thiol groups, water molecules, amide groups of asparagines and glutamines, and the imidazole ring in histidines are reoriented. Also the protonation state of the structures was predicted with the PROPKA tool at pH 7.0. Both charged and uncharged states of His265 were considered for the docking study. Eight models were generated, which differed in hydration of the binding pocket and His265 protonation state. Finally every protein model was subjected to restrained energy minimization using the force field OPLS2005 (RMSD of the atom displacement for terminating the minimization 0.3 Å).

Ligands were prepared in MOE[38] (version 2012.10, Chemical Computing Group, Montreal, Canada). Conformational search for all structures has been carried out using the Low Mode MD

sampling with a minimum RMSD between the conformations of 1 Å. This procedure was applied to obtain realistic conformations for the flexible ligands including the ring systems.

Molecular docking studies were performed on a Linux cluster using the Glide software (Schrödinger Inc, New York, USA).[37] Constraints to the Zn^{2+} ion were set and the molecular docking was performed in standard precision mode (SP). Glide Score has been used to rank the docking poses. Post-docking filtering was done using the Python script distance_to_smarts (Revision 3.5, Schrödinger). Only those docking poses were selected, where the distance between both oxygen atoms of the hydroxamic acid of the ligand and the Zn^{2+} ion inside the binding pocket of the protein was below 2.7 Å. This value was chosen based on inspection of the available X-ray structures of LpxC. Finally, rescoring of the filtered top-ranked docking poses with GOLD version 5.2 (CCDC)[39] was carried out. The *piecewise linear potential* implemented in the GOLD program (ChemPLP)[40] was used as scoring function to calculate the final scores of the compounds.

As far as all the considered LpxC crystal structures were very similar to each other (RMSD of the backbone atoms between 0.15 and 0.21 Å), only one crystal structure reported by Lee et al. in 2011[18] (PDB ID: 3P3G) was used to dock the inhibitors under study to save computational time.

Re-docking of ligands from 3P3G, 3PS1, 3PS2 and 3PS3 (Fig. S7, Supporting Information) showed, as summarized in Figure S6 and Table S1 (Supporting information), that top-ranked docking poses give RMSD values lower than 1.0 Å indicating that the docking program is able to correctly reproduce the X-ray structure of the LpxC-inhibitor complexes. Visual inspecting of the docking results indicates that the hydroxamic acid is coordinating the zinc ion and making hydrogen bonds with Glu78, Thr191 and His265. The threonine group is engaged in hydrogen bonds with Cys63 (backbone), Thr191, Lys239 and van der Waals interaction with Phe192. The hydrophobic diacetylene and the terminal phenyl are interacting with a cluster of hydrophobic residues, including Leu18, Ile198, Met195, Phe212 and Val217.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.057.

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