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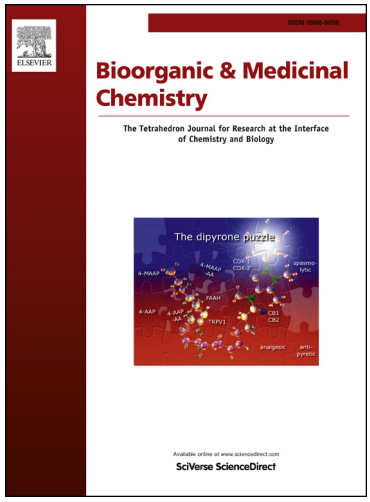

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Synthesis and biological evaluation of enantiomerically pure glyceric acid derivatives as LpxC inhibitors

Giovanni Tangherlini[a,b], Tullio Torregrossa[a], Oriana Agoglitta[a,c], Jens Köhler[a], Jelena Melesina[d], Wolfgang Sippl[d], Ralph Holl[a,b,*]

[a]Institut für Pharmazeutische und Medizinische Chemie der Westfälischen Wilhelms-Universität Münster, Corrensstr. 48, D-48149 Münster, Germany

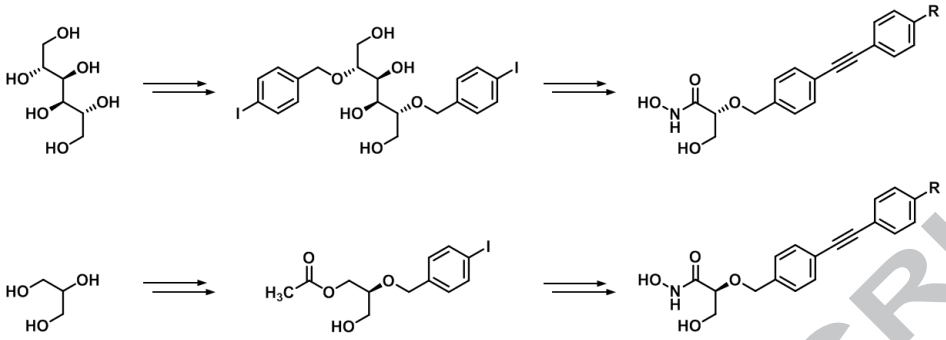
[b]Cells-in-Motion Cluster of Excellence (EXC 1003 - CiM), University of Münster, Germany

[c]NRW Graduate School of Chemistry, University of Münster, Germany

[d]Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Wolfgang-Langenbeck Str. 4, 06120 Halle (Saale), Germany

*To whom correspondence should be addressed. Tel.: +49-251-8333372; Fax: +49-251-8332144; E-mail: hollr@uni-muenster.de.

Graphical Abstract



Key words

LpxC inhibitors, structure–activity relationships, glyceric acid derivatives, chiral pool synthesis, enantioselective desymmetrization, molecular docking studies

Abstract

Inhibitors of the UDP-3-O-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine deacetylase (LpxC) represent a promising class of novel antibiotics, selectively combating Gram-negative bacteria. In order to elucidate the impact of the hydroxymethyl groups of diol (*S*,*S*)-**4** on the inhibitory activity against LpxC, glyceric acid ethers (*R*)-**7a**, (*S*)-**7a**, (*R*)-**7b**, and (*S*)-**7b**, lacking the hydroxymethyl group in benzylic position, were synthesized. The compounds were obtained in enantiomerically pure form by a chiral pool synthesis and a lipase-catalyzed enantioselective desymmetrization, respectively. The enantiomeric hydroxamic acids (*R*)-**7b** (K_i = 230 nM) and (*S*)-**7b** (K_i = 390 nM) show promising enzyme inhibition. However, their inhibitory activities do not substantially differ from each other leading to a low eudismic ratio. Generally, the synthesized glyceric acid derivatives **7** show antibacterial activities against two *E. coli* strains exceeding the ones of their respective regioisomes **6**.

1. Introduction

Antimicrobial resistance poses a severe problem for modern medicine.[1,2] Besides causing high economic cost, antimicrobial-resistant infections claim numerous human lives, with at least 48,000 people dying from these infections every year in Europe and the US alone.[3] Furthermore, the drastic decrease in the number of novel antibiotics released onto the market over the last decades has exacerbated the situation.[4,5] Therefore, in order to prevent an accentuation of this scenario, it is urgently necessary to find new antibacterial targets and to consequently develop antibiotics possessing novel mechanisms of action, which are able to circumvent established mechanisms of resistance.[6]

Especially among Gram-negative bacteria, resistance is a matter of great concern as the majority of antimicrobial-resistant infections are caused by these bacteria.[7-9] Gram-negative bacterial infections are difficult to treat as these bacteria possess an additional outer membrane, which acts as a permeability barrier shielding the bacteria from external agents like antibiotics.[10,11] The outer monolayer of the outer membrane of Gram-negative bacteria mainly consists of lipid A, the hydrophobic membrane anchor of lipopolysaccharides (LPS).[12] On the one hand, lipid A is required for growth and viability of most Gram-negative bacteria.[13] On the other, it is primarily responsible for the biological effects of LPS in the human host, triggering an innate immune response, which can lead to a life-threatening septic shock.[14,15] For these reasons, the inhibition of the biosynthesis of lipid A represents a promising strategy for antibacterial drug development.

One of the best characterized but so far clinically unexploited bacterial targets within the biosynthetic pathway of lipid A is the Zn^{2+}-dependent deacetylase LpxC.[16] This enzyme is present in virtually all Gram-negative bacteria, exhibiting a high sequence similarity among the orthologues but showing no sequence homology to any mammalian protein.[17] LpxC catalyses the irreversible deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**), representing the first committed step of lipid A biosynthesis (Figure 1).[18] Structural studies revealed that LpxC displays a "β-α-α-β sandwich fold", being made up by two domains with similar topologies.[19] At the interface of these two domains, the conical active site cleft is located.[20] The catalytic Zn^{2+}-ion resides at the bottom of the active site cleft, being coordinated by one aspartate and two histidine residues.[21] Another characteristic structural element of LpxC is a ~15 Å long, hydrophobic tunnel, which leads out of the active site cleft,

binding the 3-*O*-[(*R*)-3-hydroxymyristoyl] substituent of the natural substrate **1** during catalysis.[22]

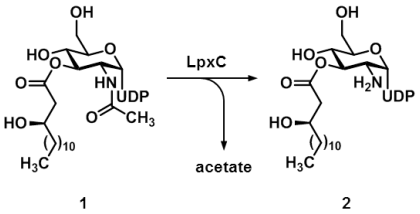


Figure 1: LpxC catalyzed deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**1**).

The deacetylase has been validated as an antibacterial target and several classes of small-molecule LpxC inhibitors have been described so far.[23-31] Most of them possess a Zn^{2+}-binding hydroxamate moiety as well as a structural element addressing the hydrophobic tunnel of the enzyme. E.g. the *N*-aroyl-L-threonine hydroxamic acid derivatives CHIR-090 (**3a**, Figure 2) and LPC-009 (**3b**) are able to potently inhibit diverse LpxC orthologues and exhibit antibacterial activity against various Gram-negative bacteria.[30,21] The compounds could be crystallized with *Yersinia enterocolitica* LpxC and *Escherichia coli* LpxC, respectively, displaying similar binding modes.[32,21] Whereas the lipophilic side chain of the inhibitors penetrates through the hydrophobic tunnel of LpxC, their hydroxamate moiety chelates the catalytic Zn^{2+}-ion in the active site and was found to be involved in polar interactions with the catalytically important residues E78, T191 and H264.[22,32,21]

CHIR-090 (**3a**, Figure 2) served as lead compound for the development of benzyloxyacetohydroxamic acid derivatives, in which the amide group of CHIR-090 is replaced by an ether moiety.[33] In our previous studies, dealing with the synthesis and biological evaluation of these benzyloxyacetohydroxamic acid derivatives, diol (*S*,*S*)-**4**, which exhibits a K_I-value of 358 nM against *E. coli* LpxC, was identified as promising lead structure for the development of potent LpxC inhibitors.[34] Therefore, its structure was further varied by removing its hydroxymethyl groups. Whereas the removal of both hydroxymethyl groups of (*S*,*S*)-**4** was detrimental for the inhibitory activity of benzyloxyacetohydroxamic acid derivatives **5a** and **5b** (Table 1), the truncation of the hydroxymethyl group in α position of the hydroxamate moiety led to

an increase in the biological activity.[35] In fact, the phenylethylene glycol derivatives (*S*)-**6a** and (*S*)-**6b** showed inhibition of LpxC with K_I-values of 66 nM and 95 nM, respectively. This latter finding was somewhat surprising, as in case of threonine derivative LPC-009 (**3b**) and its analogs, the hydroxy group in Cβ position of the hydroxamate moiety was shown to undergo favorable hydrogen bonding interactions with a highly conserved lysine residue (K239) in the active site of the enzyme, indicating that this functionality is important for inhibitor binding.[21]

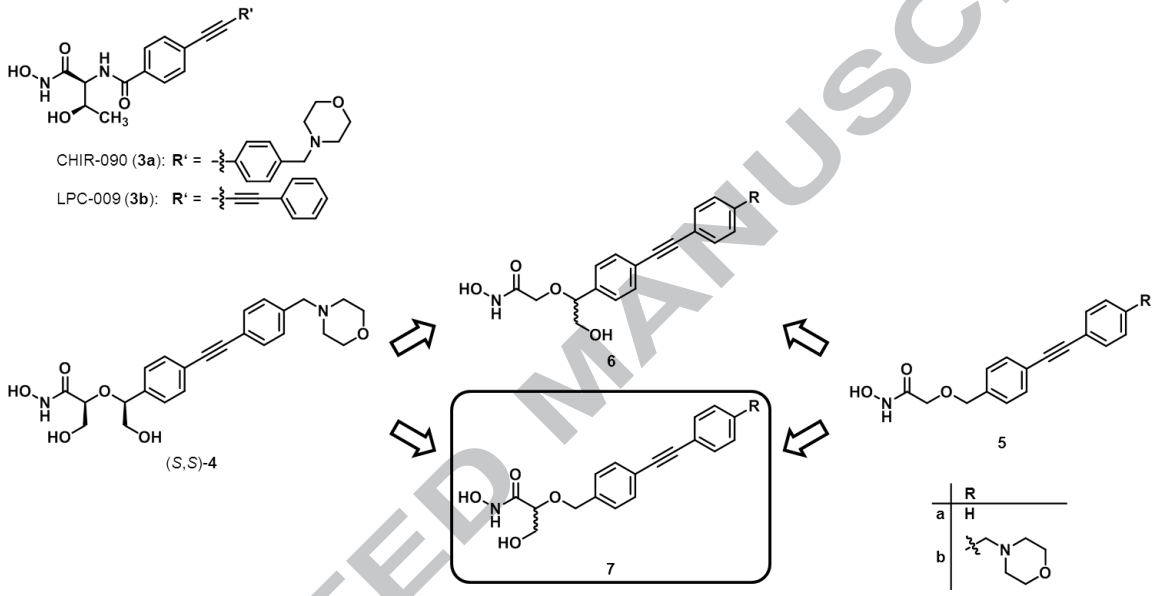
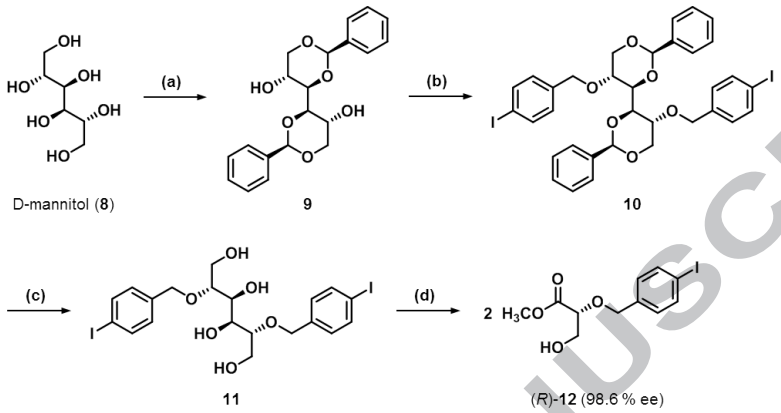


Figure 2: Chemical structures of reported and envisaged LpxC inhibitors.

To learn more about the effect of the hydroxymethyl group in α-position of the hydroxamate moiety, glyceric acid ethers **7a** and **7b**, bearing no hydroxymethyl group in benzylic position, should be synthesized. Moreover, to determine the eutomer, both stereoisomers of these glyceric acid derivatives should be prepared in enantiomerically pure form.

2. Results and Discussion

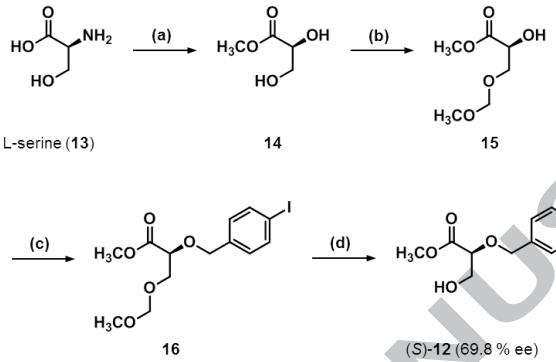
2.1. Chemistry



Scheme 1: Reagents and conditions: (a) benzaldehyde, H_2SO_4, DMF, rt, 3 d, 26 %; (b) 4-iodobenzyl bromide, LiHMDS, TBAI, THF, Δ, 16 h, 69 %; (c) *p*-TsOH, propane-1,3-diol, MeOH, Δ, 4 h, 88 %; (d) 1. $NaIO_4$, MeOH, rt, 2 h, 2. Br_2, $NaHCO_3$, MeOH, H_2O, 61 %.

The envisaged enantiomerically pure glyceric acid ethers should be accessed by performing chiral pool syntheses. For the preparation of the (*R*)-configured hydroxamic acids (*R*)-**5a** and (*R*)-**5b** D-mannitol (**8**) was used as chiral starting material (Scheme 1). In the first reaction step, an acid-catalyzed acetalisation of D-mannitol with benzaldehyde was performed, yielding 1,3:4,6-di-*O*-benzylidene-D-mannitol (**9**).[36] Subsequently, the unprotected hydroxy groups of 1,3-dioxane derivative **9** were benzylated with 4-iodobenzyl bromide in the presence of catalytic amounts of tetrabutylammonium iodide using lithium bis(trimethylsilyl)amide as base. The two benzylidene acetals of the resulting bis(4-iodobenzyl) ether **10** were then cleaved by heating the compound in methanol in the presence of 1,3-propanediol and *p*-toluenesulfonic acid to access tetrol **11**. In the next reaction step, the C-C bond between C-3 and C-4 of glycol derivative **11** should be cleaved. Therefore, a glycol cleavage with sodium periodate was performed yielding two identical (*R*)-configured aldehydes, which were directly subjected to a subsequent oxidation. Using bromine in the presence of sodium bicarbonate dissolved in a 9:1 mixture of methanol and water, the oxidation directly yielded glyceric acid ester (*R*)-**12**.[37] As this ester

represents a central intermediate of the envisaged synthesis, its optical purity was determined with a chiral HPLC method. The analysis revealed an enatiomeric excess (*ee*) of 98.6 % proofing the effectiveness of the performed chiral pool synthesis for the preparation of the enantiomerically pure glyceric acid ether (*R*)-**12**.

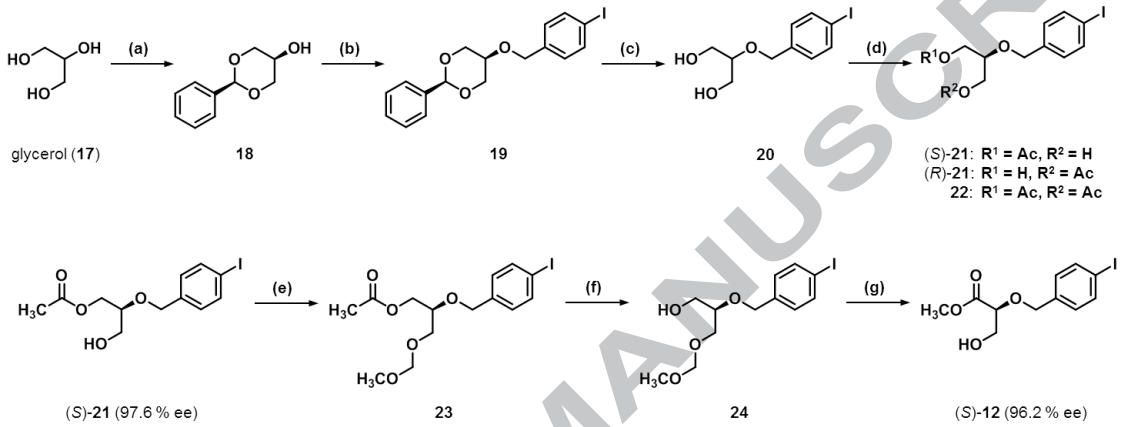


Scheme 2: Reagents and conditions: (a) 1. $NaNO_2$, H_2SO_4, H_2O, 0 °C, 48 h, 2. trimethyl orthoformate, *p*-TsOH, MeOH, Δ, 16 h, 23 %; (b) chloromethyl methyl ether, DIPEA, H_3CCN, rt, 16 h, 35 %; (c) 4-iodobenzyl bromide, NaH, TBAI, H_3CCN, 80 °C, 16 h, 22 %; (d) HCl (g), MeOH, rt, 16 h, 31 %.

In contrast, the enantiomeric glyceric acid ester (*S*)-**12** should be synthesized in a chiral pool synthesis using L-serine (**13**) as starting material (Scheme 2). (*S*)-Configured methyl glycerate (**14**) was prepared according to a literature procedure, which reported its synthesis in high optical purity.[38] Therefore, L-serine was diazotized in an aqueous medium leading to (*S*)-configured glyceric acid. The net retention of configuration in this reaction is due to two subsequent inversions.[39,40] Presumably, at first an intermediate α-lactone is formed with an inversion of configuration. Then, accompanied by a second inversion, the α-lactone reacts with water, yielding (*S*)-glyceric acid. Without intermediate purification, this compound was directly transformed into its methyl ester **14**.

In the next reaction step, the primary alcohol of ester **14** was MOM-protected by reacting glycol **14** with chloromethyl methyl ether. Then, the resulting secondary alcohol **15** was benzylated with 4-iodobenzyl bromide in acetonitrile at 80 °C using sodium hydride as base. The reaction yielded benzyl ether **16**, whose MOM

protective group was subsequently cleaved under acidic conditions to give glyceric acid ether (*S*)-**12**. However, the determination of the optical purity of glyceric acid derivative (*S*)-**12** revealed that this synthetic route had given the desired compound with an *ee* of only 69.8 %. This finding indicated that most probably a partial racemization had occurred during the diazotisation step.



Scheme 3: Reagents and conditions: (a) benzaldehyde, H_2SO_4, toluene, Δ, 50 %; (b) 4-iodobenzyl bromide, NaH, DMF, rt, 24 h, 42 %; (c) *p*-TsOH, THF, MeOH, rt, 16 h, 86 %; (d) IPA, Amano Lipase AK (*Pseudomonas fluorescens*), TBME, -10 °C, 24 h, (*S*)-**21** 62 %; (e) chloromethyl methyl ether, DIPEA, H_3CCN, rt, 21 h, 90 %; (f) K_2CO_3, MeOH, rt, 2 h, 87 %; (g) 1. CrO_3, H_5IO_6, H_2O, H_3CCN, 0 °C, 24 h, 2. HCl (g), MeOH, Δ, 16 h, 49 %.

Therefore, in order to obtain enantiomer (*S*)-**12** in high enantiomeric excess, a pathway involving a lipase catalyzed enantioselective desymmetrization was envisaged. At first, 1,3-dioxane derivative **18** was synthesized in an acid-catalyzed acetalisation of glycerol (**17**) and benzaldehyde.[41] The remaining hydroxy group of benzylidene acetal **18** was then benzylated with 4-iodobenzyl bromide to give ether **19**. The acetal **19** was subsequently cleaved under acidic conditions yielding diol **20**. In the key step for the introduction of chirality, an enantioselective acetylation of prochiral diol **20** should be performed.[42] In a lipase screening Amano Lipase AK from *Pseudomonas fluorescens* was found to be the best catalyst for the enantioselective desymmetrization of diol **20**. Using isopropenyl acetate (IPA) as acylating agent in *tert*-butyl methyl ether, the enzyme gave the desired (*S*)-configured monoacetate (*S*)-

21 and diacetate **22** occurred as a side product. In order to optimize the reaction conditions, the course of the reaction was investigated by performing analytical scale conversions (5 mg of diol **20**). As shown in Figure 3, at a temperature of +25 °C the monoacetate **21** was produced very fast at the beginning of the reaction (Figure 3, sample A: 76.1 % (*S*)-**21**, 90.5 % *ee*). However, the enantiomeric excess of (*S*)-**21** increased significantly by formation of diacetate **22**, since the undesired monoacetate (*R*)-**21** was converted preferentially (Figure 3, sample B: 56.6 % (*S*)-**21**, 97.5 % *ee*). Lowering the temperature to -10 °C led to an additionally increased enantioselectivity of the lipase (Figure 3, sample C: 82.6 % (*S*)-**21**, 94.8 % *ee*, sample D: 69.4 % (*S*)-**21**, 97.8 % *ee*). Therefore, large scale conversions of diol **20** (1.0 g, 3.3 mmol) were performed at -10 °C. Upon isolation the desired (*S*)-configured monoacetate (*S*)-**21** was obtained in 62 % yield with an *ee* of 97.6 %.

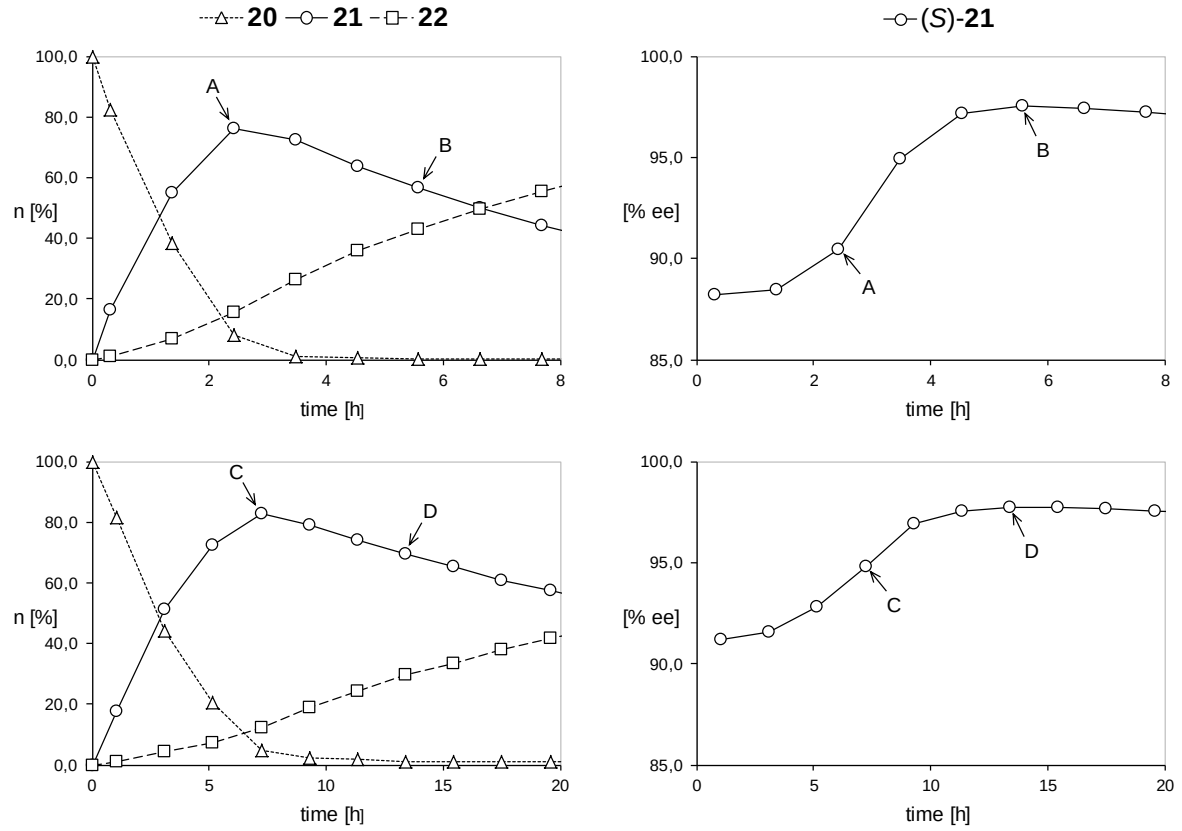
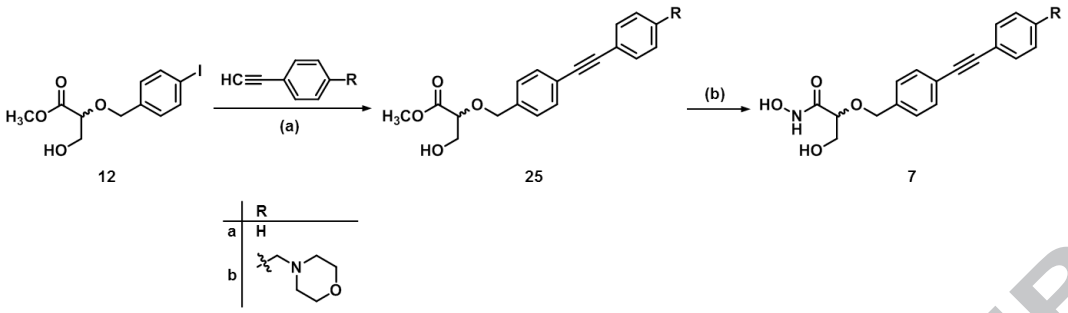


Figure 3: Lipase catalyzed conversion of diol **20** using Amano lipase AK from *Pseudomonas fluorescens* in a mixture of *tert*-butyl methyl ether : isopropenyl acetate (50 : 1, 1.5 mL), left: amount of compounds **20**, **21** and **22** (n [%]), right: enantiomeric

excess of (*S*)-**21** (% *ee*), top: carried out at +25 °C (sample A: 76.1 % (*S*)-**21**, 90.5 % *ee*, sample B: 56.6 % (*S*)-**21**, 97.5 % *ee*), bottom: carried out at -10 °C (sample C: 82.6 % (*S*)-**21**, 94.8 % *ee*, sample D: 69.4 % (*S*)-**21**, 97.8 % *ee*).

The absolute configuration of monoacetate (*S*)-**21** was unequivocally proven after its transformation into ester (*S*)-**12**. At first, MOM-protection of the hydroxy group of monoacetate (*S*)-**21** gave ester **23**, which was then saponified to yield alcohol **24**. In order to obtain ester (*S*)-**12**, alcohol **24** was transformed into the corresponding carboxylic acid using an oxidant solution which contained periodic acid and catalytic amounts of CrO_3 in wet acetonitrile. Heating the intermediately formed carboxylic acid in methanol under acidic conditions did not only lead to the esterification of the carboxylate moiety but also caused the cleavage of the MOM protective group yielding ester (*S*)-**12**. In contrast to its dextrorotatory enantiomer (*R*)-**12**, which was obtained from D-mannitol, ester (*S*)-**12** is levorotatory, confirming the (*S*)-configuration of the compound and consequently of monoacetate (*S*)-**21**. At this stage of the synthesis the *ee* was determined again. The *ee* of (*S*)-**12** was found to be 96.2 %, indicating that the optical purity did not markedly decrease during the synthetic transformations of monoacetate (*S*)-**21**.

With the two optically pure enantiomes (*R*)-**12** and (*S*)-**12** in hand, the synthesis of the envisaged hydroxamic acids was pursued. Sonogashira couplings of 4-iodobenzyl ethers (*R*)-**12** and (*S*)-**12** with phenylacetylene and morpholinomethyl-substituted phenylacetylene[33] gave diphenylacetylene derivatives (*R*)-**25a**, (*S*)-**25a**, (*R*)-**25b**, and (*S*)-**25b**, respectively. The desired hydroxamic acids (*R*)-**7a**, (*S*)-**7a**, (*R*)-**7b**, and (*S*)-**7b** were obtained in a final aminolysis of methyl esters (*R*)-**25a**, (*S*)-**25a**, (*R*)-**25b**, and (*S*)-**25b** with hydroxylamine. Although TLC control of the reactions indicated nearly complete conversion of the esters, the yields of the obtained hydroxamic acids were rather poor, as the purification of the compounds was difficult due to their high polarity.



Scheme 4: Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, NEt_3, H_3CCN, rt, (*R*)-**25a** 83 %, (*S*)-**25a** 81 %, (*R*)-**25b** 88 %, (*S*)-**25b** 69 %; (b) $H_2NOH{\cdot}HCl$, NaOMe, MeOH, rt, (*R*)-**7a** 24 %, (*S*)-**7a** 43 %, (*R*)-**7b** 20 %, (*S*)-**7b** 33 %.

2.2. Biological evaluation

compound	zone of inhibition [mm]		MIC [µM]		enzyme assay	
	E. coli BL21	*E. coli* D22	*E. coli* BL21	*E. coli* D22	IC_{50} [µM]	K_i [µM]
(*R*)-**7a**	10.3 ± 2.5	17.0 ± 1.0	256	8	1.87 ± 0.85	0.26 ± 0.12
(*S*)-**7a**	11.7 ± 0.6	20.7 ± 1.7	128	4	1.96 ± 0.36	0.27 ± 0.05
(*R*)-**7b**	12.3 ± 0.6	22.0 ± 1.3	128	4	1.66 ± 0.31	0.23 ± 0.04
(*S*)-**7b**	15.7 ± 0.6	25.8 ± 1.9	64	2	2.82 ± 0.5	0.39 ± 0.07
(*R*)-**6a**	9.1 ± 0.4	13.0 ± 1.7	256	32	31.6 ± 6.0	4.4 ± 0.8
(*S*)-**6a**	9.5 ± 0.4	20.5 ± 0.2	256	4	0.48 ± 0.23	0.066 ± 0.032
(*R*)-**6b**	8.7 ± 0.7	12.3 ± 1.6	>256	4	198 ± 12	27.3 ± 1.7
(*S*)-**6b**	13.4 ± 0.5	21.2 ± 0.6	64	0.5	0.69 ± 0.30	0.095 ± 0.042
(*S,S*)-**4**	9.0 ± 0.5	20.8 ± 0.6	>256	4	2.6 ± 0.3	0.36 ± 0.04
5a	10.6 ± 0.4	13.2 ± 1.6	128	4	> 200	-
5b	16.5 ± 0.4	20.3 ± 1.0	64	1	10.5 ± 2.5	1.45 ± 0.35
CHIR-090	24.6 ± 1.9	30.3 ± 2.5	1	0.032	0.058 ± 0.002	0.008 ± 0.0003

Table 1: MIC values as well as the results of the disc diffusion assays and the *E. coli* LpxC enzyme assay for the synthesized glyceric acid derivatives **7** and already known benzyloxyacetohydroxamic acid derivatives **4 – 6**.[35,34]

In order to determine the antibacterial activities of the synthesized hydroxamic acids, disc diffusion tests were performed and the MIC (minimal inhibitory concentration) values were determined. Additionally, a LpxC enzyme assay was performed (Table 1). In this fluorescence-based enzyme assay, the extent of the LpxC-catalyzed deacetylation of the enzyme's natural substrate **1** in the presence of varying concentrations of the putative inhibitors is determined by transforming the resulting primary amine **2** into a fluorescent isoindole.[24]

In contrast to the ethylene glycol derivatives **6a** and **6b**, for which a pronounced difference between the inhibitory activities of the enantiomers had been observed, in case of the glyceric acid derivatives (*R*)-**7a**, (*S*)-**7a**, (*R*)-**7b**, and (*S*)-**7b**, low eudismic ratios can be observed, indicating that stereochemistry apparently does not play a key role for the inhibitory activity of these compounds. The finding that the glyceric acid derivatives show low eudismic ratios was astonishing as in case of the *N*-aroyl-L-threonine hydroxamic acid derivatives (*S*)-configuration in α-position of the hydroxamate moiety was found to be essential for high antibacterial activity.[43] In fact, with K_i-values of 0.26 μM and 0.23 μM the (*R*)-configured glyceric acid derivatives (*R*)-**7a** and (*R*)-**7b** even exhibit slightly lower K_i-values than their (*S*)-configured enantiomes.

Whereas the (*S*)-configured glyceric acid derivatives (*S*)-**7a** and (*S*)-**7b** show an about 4-fold reduced activity compared to the respective ethylene glycol derivatives (*S*)-**6a** and (*S*)-**6b**, the inhibitory activities of (*R*)-**7a** and (*R*)-**7b** are considerably increased relative to the ones of their (*R*)-configured regioisomers (*R*)-**6a** and (*R*)-**6b**. When comparing the inhibitory activity of diol (*S*,*S*)-**4** with the ones of hydroxamic acids (*S*)-**7b** and (*S*)-**6b**, lacking one of the two hydroxymethyl groups of (*S*,*S*)-**4**, it can be observed, that the removal of the hydroxymethyl group in α-position of the hydroxamate moiety yielding ethylene glycol derivative (*S*)-**6b** caused a pronounced increase in inhibitory activity. In contrast, the removal of the hydroxymethyl group in benzylic position of diol (*S*,*S*)-**4**, leading to glyceric acid derivative (*S*)-**7b**, did not cause a considerable change of the inhibitory activity towards LpxC. These results indicate, that the presence of both of the two hydroxymethyl groups of diol (*S*,*S*)-**4** is not required for LpxC inhibition, with the removal of the group in α-position of the hydroxamate moiety being rather beneficial as it leads to an increased inhibitory activity.

In the disc diffusion assays, the synthesized glyceric acid derivatives were tested against *E. coli* BL21 (DE3) and the *E. coli* D22 strain, which is more sensitive towards LpxC inhibition. Against both *E. coli* strains, all of the newly synthesized glyceric acid derivatives **7** showed antibacterial properties, which were superior to the ones of their respective ethylene glycol derivatives **6**. Especially the (*S*)-configured glyceric acid derivatives (*S*)-**7a** and (*S*)-**7b** were more active than their (*R*)-configured enantiomers (*R*)-**7a** and (*R*)-**7b** as well as of their respective ethylene glycol derivatives (*S*)-**6a** and (*S*)-**6b**, which is in contrast to the results of the LpxC enzyme assay.

Generally, the same trends, which were observed in the disc diffusion assays, were also found when determining the MIC values. Only the (*S*)-configured glyceric acid derivative (*S*)-**7b**, which caused the largest halo of inhibition in the disc diffusion assay against *E. coli* D22, showed a 4-fold increased MIC value against this *E. coli* strain compared to its regioisomer (*S*)-**6b**.

As it had already been observed in the series of the (*S*)-configured ethylene glycol derivatives, also in case of the glyceric acid derivatives the presence of a morpholinomethyl substituent generally resulted in a higher antibacterial activity against both *E. coli* strains in comparison to the compounds bearing no substituent at their distal phenyl ring. Although the presence of the morpholinomethyl substituent causes increased antibacterial activities, the opposite effect can be observed for the inhibitory activity, with the unsubsituted compound (*S*)-**7a** inhibiting LpxC with a 1.4-fold lower K_i value than (*S*)-**7b**. The same trend had also been observed for the ethylene glycol derivatives (*S*)-**6a** and (*S*)-**6b**.

2.3. Molecular docking studies

To rationalize the structure-activity relationships of the synthesized LpxC inhibitors, molecular docking studies were carried out. All compounds show a similar binding pose in the substrate pocket of *E. coli* LpxC (Figure 4). The hydroxamic acid chelates the Zn^{2+}-ion and is involved in hydrogen-bonding to E78 and T191, while the lipophilic distal part is placed in the hydrophobic tunnel formed by I198, M195, F212 and V217, respectively. The morpholine group (if present) is sticking out of the tunnel into the solvent. The variability of interactions of distinct compounds with the protein is observed in the linker region. Only the hydrogen bond between the ether group of the linker and the hydroxy group of T191 is observed in all cases. Therefore, according to this binding hypothesis, the relatively high activity of inhibitors CHIR-090 (**3a**), (*S*,*S*)-**4**, (*S*)-**6a**, (*S*)-**6b** and **7a,b** in comparison to other reported compounds is due to favorable interactions of their linker groups with surrounding amino acid residues, especially hydrogen bonding interactions with polar residues of LpxC (Figure 4).

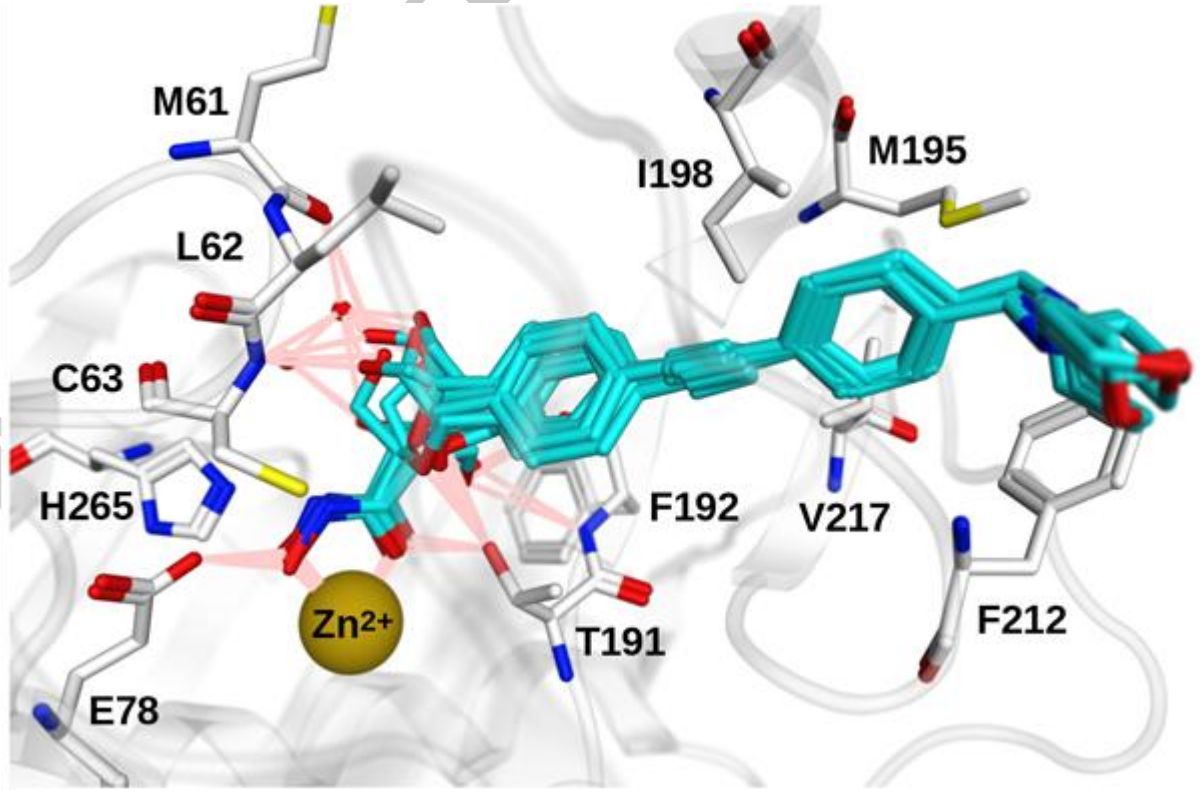


Figure 4: Putative binding mode of reported LpxC inhibitors CHIR-090 and **4**-**7b** (cyan carbons) docked to the *E. coli* LpxC X-ray structure (white carbons, PDB ID

3P3G).[21] In this and following figures heteroatoms are colored as following: nitrogen atoms – blue, oxygen atoms – red, sulfur atoms – yellow. The protein backbone is depicted as white ribbon, the Zn^{2+}-ion as golden ball, conserved water molecules as red balls and interactions of the ligands with the protein atoms are shown as salmon lines.

The dramatic difference between the K_i-values of the enantiomers of ethylene glycol derivatives **6a** and **6b** (around 70- and 300-fold, respectively) can be explained partially by their different interactions of the linker region, as suggested previously.[35] Another reason might be the displacement of a conserved water molecule by the *S*-stereoisomer. As shown by the docking results, the hydroxy group of the *R*-enantiomers (*R*)-**6a** and (*R*)-**6b** makes water-mediated hydrogen bonds with M61 and C63 (Figure 5a). However, the hydroxy group of the *S*-enantiomers (*S*)-**6a** and (*S*)-**6b** instead of interacting with a conserved water molecule, displaces it and reproduces its hydrogen-bond interactions with M61 and C63 backbone atoms (Figure 5b). The release of the water molecule may cause a large entropic gain, which would explain the observed activity cliff. Surprisingly, in case of the glyceric acid derivatives (*R*)-**7a**, (*S*)-**7a**, (*R*)-**7b**, and (*S*)-**7b** no significant difference in the K_i-values was observed between the enantiomers. Analysis of the docking poses suggests that this is because, in contrast to compounds **6a** and **6b**, there is no water displacement effect. The *R*-enantiomers (*R*)-**7a** and (*R*)-**7b** make water-mediated hydrogen bond interactions with M61 and C63 (Figure 5c) similar to (*R*)-**6a** and (*R*)-**6b** (Figure 5a). The difference in the biological activity probably comes from more favorable linker geometry of (*R*)-**7a** and (*R*)-**7b** compared to (*R*)-**6a** and (*R*)-**6b**. The hydroxy group of the *S*-enantiomers (*S*)-**7a** and (*S*)-**7b** is involved in two hydrogen bonds with the backbone NH and CO of F192 located on the opposite side of the pocket (Figure 5d). Since *R*- and *S*-stereoisomers of **7a** and **7b** are able to adopt an equally favorable binding mode, their biological activity is similar. The influence of the morpholine group is difficult to explain, because it is placed at the entrance of the pocket and is solvent-exposed.

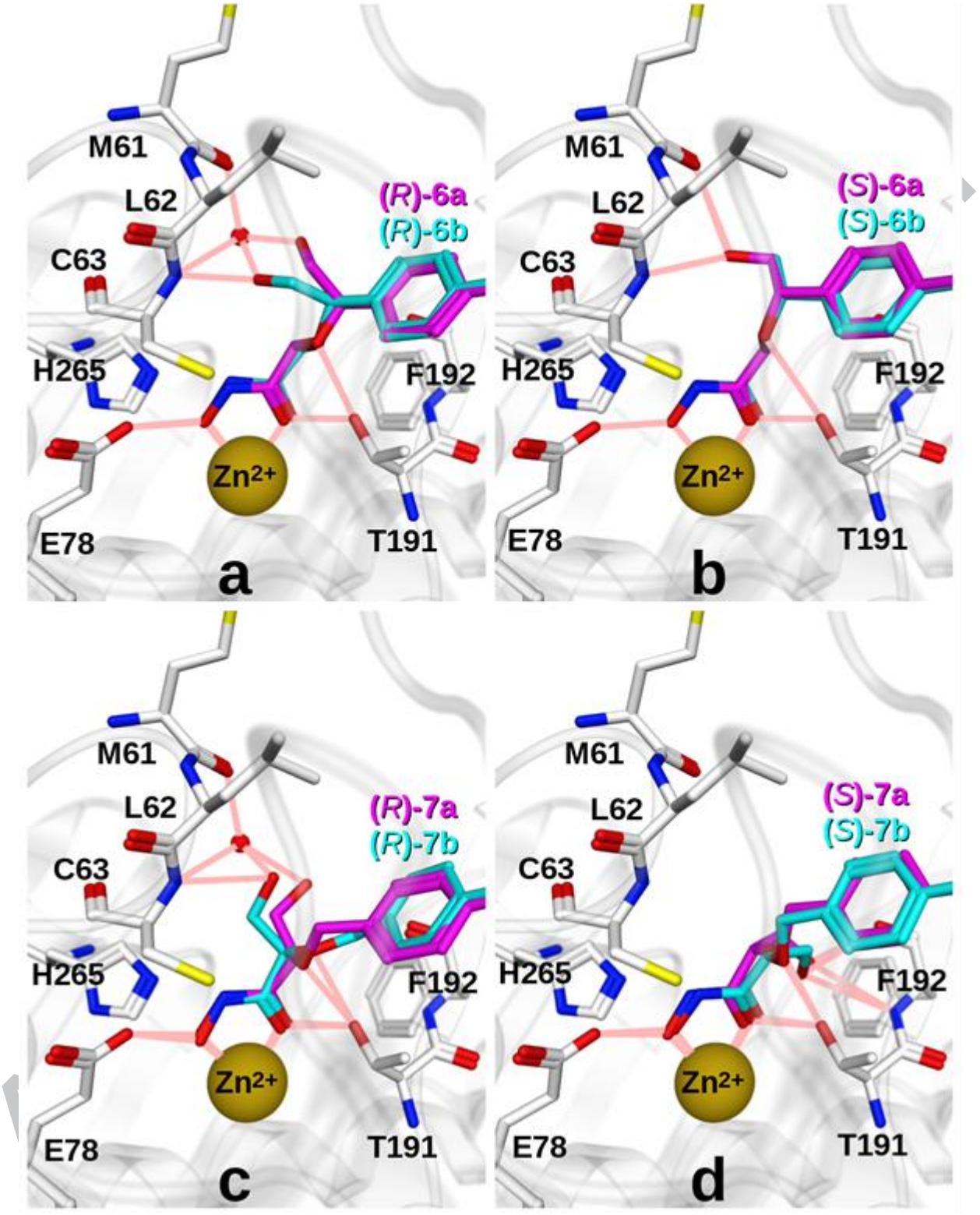


Figure 5: Docking poses of compounds: a) (*R*)-**6a** (magenta) and (*R*)-**6b** (cyan), b) (*S*)-**6a** (magenta) and (*S*)-**6b** (cyan), c) (*R*)-**7a** (magenta) and (*R*)-**7b** (cyan), d) (*S*)-**7a** (magenta) and (*S*)-**7b** (cyan) in the X-ray structure of *E. coli* LpxC (white carbons, PDB ID 3P3G).[21] Only the region nearby the Zn^{2+}-ion is shown for clarity.

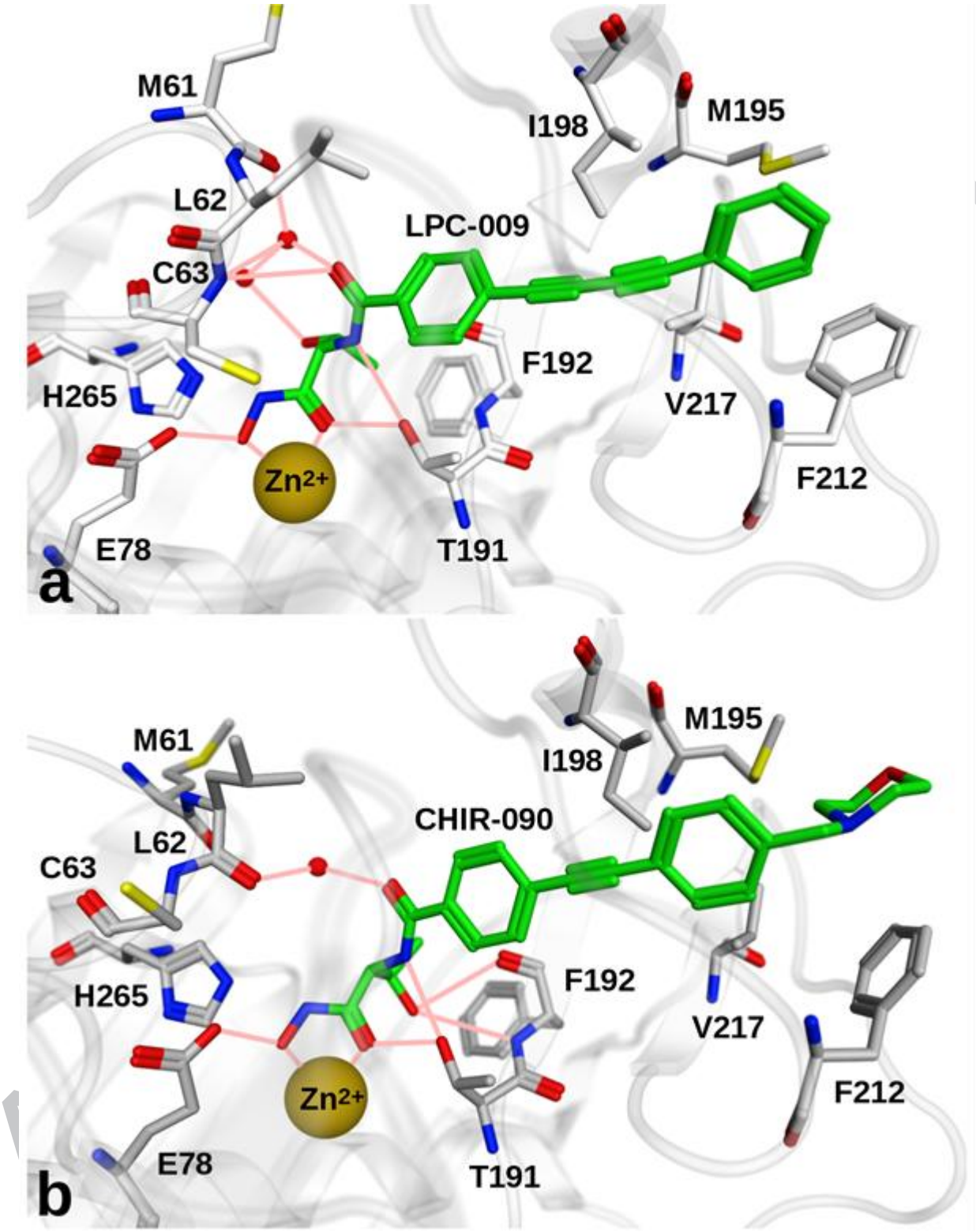


Figure 6: Binding mode of (a) LPC-009 (green carbons) in its X-ray structure with *E. coli* LpxC (white carbons, PDB ID 3P3G)[21] and (b) CHIR-090 (green carbons) in its X-ray structure with *Yersinia enterocolitica* LpxC (light gray carbons, PDB ID 3NZK).[32]

Interestingly, the suggested docking poses of compounds **7a** and **7b** are similar to the binding modes of known potent LpxC inhibitors. The position of the hydroxy group of (*R*)-**7a** and (*R*)-**7b** is the same as the carbonyl group of LPC-009 (**3b**) in its crystal structure with *E. coli* LpxC (Figure 6a).[21] In case of (*S*)-**7a** and (*S*)-**7b** the hydroxy group is located at the same position as observed for the hydroxy group of CHIR-090 (**3a**) in its crystal structure with *Yersinia enterocolitica* LpxC (Figure 6b).[32] This observation suggests that the presented docking poses are realistic.

3. Conclusions

In a chiral pool synthesis starting from D-mannitol, glyceric acid ester (*R*)-**12** was accessed in enantiomerically pure form. Its (*S*)-configured enantiomer (*S*)-**12** was obtained in high enantiomeric excess via an enantioselective desymmetrization of diol **20** using Amano Lipase AK from *Pseudomonas fluorescens*. From these intermediates, enantiomerically pure glyceric acid derivatives (*R*)-**7a**, (*S*)-**7a**, (*R*)-**7b**, and (*S*)-**7b** were synthesized and tested for their inhibitory activities against LpxC as well as their antibacterial activities against two *E. coli* strains.

The biological evaluation of the synthesized glyceric acid derivatives showed that the configuration in α-position of the hydroxamate moiety has only a minor effect on the compounds' inhibitory activity against LpxC. With K_i-values of 0.27 µM and 0.39 µM, the (*S*)-configured hydroxamic acids (*S*)-**7a** and (*S*)-**7b** show approximately the same inhibitory activities as their enantiomers (*R*)-**7a** and (*R*)-**7b**, exhibiting K_i-values of 0.26 µM and 0.23 µM, respectively. These observations are in agreement with favorable docking poses derived for both classes of isomers.

The comparison of the inhibitory activities of glyceric acid derivatives (*S*)-**7a** and (*S*)-**7b** with the ones of their regioisomers (*S*)-**6a** and (*S*)-**6b** showed, that the shift of the hydroxymethyl group from the benzylic position (compounds (*S*)-**6a** and (*S*)-**6b**) to the α-position of the hydroxamate moiety (compounds (*S*)-**7a** and (*S*)-**7b**) led to slightly reduced inhibitory activities of the benzyloxyacetohydroxamic acid derivatives.

However, the antibacterial properties of the glyceric acid derivatives **7** were generally higher or at least equal to the ones of the respective ethylene glycol derivatives **6**, thus making these newly synthesized compounds leads for further optimization steps.

Acknowledgement

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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 F_{254} 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 (m.p.): 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): Agilent DD2 400 MHz spectrometer; δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. 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 x 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 %. Method 3: Merck Hitachi Equipment; diode array detector: L-7455; pump: L-6200A; column: DiacelChiralpak IA; flow rate: 1.00 mL/min; injection: manual, Rheodyne 7725i; injection volume: 5.0 µL; detection at λ = 235 nm for 40 min; solvent: isohexane : ethanol = 9 : 1. Method 4: Merck Hitachi Equipment; diode array detector: L-7455;

pump: L-6200A; column: DiacelChiralpak IA; flow rate: 1.00 mL/min; injection: manual, Rheodyne 7725i; injection volume: 5.0 µL; detection at λ = 235 nm for 60 min; solvent: isohexane : isopropanol = 95 : 5.

4.2. Synthetic procedures

4.2.1. (2*R*,2'*R*,4*R*,4'*R*,5*R*,5'*R*)-2,2'-Diphenyl-[4,4'-bi(1,3-dioxane)]-5,5'-diol (9)

Benzaldehyde (1.2 mL, 1.3 g, 12 mmol) was added to a stirred solution of D-mannitol (1.0 g, 5.5 mmol) in DMF (30 mL). After cooling to 0 °C, concentrated sulfuric acid (0.21 mL, 380 mg, 3.9 mmol) was added dropwise. The reaction was stirred at ambient temperature for 3 d. Then the solution was poured into a mixture of ice water (120 mL), potassium carbonate (1.2 g) and petroleum ether (10 mL). The white precipitate being formed was filtered off and washed with fresh petroleum ether. The solid residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.11) to give **9** as colourless solid (500 mg, 1.4 mmol, 26% yield). m.p. = 180 °C; $[\alpha]_D^{20}$ = -3.3 (2.9, methanol); ^{1}H NMR (DMSO-d_6): δ [ppm] = 3.55 (t, *J* = 10.4 Hz, 2H, OC*H*$_2$CHOHCHO), 3.75 – 3.85 (m, 2H, OCH$_2$C*H*OHCHO), 3.91 (d, *J* = 9.2 Hz, 2H, OCH$_2$CHOHC*H*O), 4.16 (dd, *J* = 10.4/5.3 Hz, 2H, OC*H*$_2$CHOHCHO), 5.35 (d, *J* = 5.9 Hz, 2H, OCH$_2$CHO*H*CHO), 5.51 (s, 2H, PhC*H*), 7.31 – 7.46 (m, 10H, $H_{arom.}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 58.8 (2C, OCH$_2$*C*HOHCHO), 71.0 (2C, O*C*H$_2$CHOHCHO), 78.1 (2C, OCH$_2$CHOH*C*HO), 100.1 (2C, Ph*C*H), 126.1 (4C, $C_{arom.}$), 128.0 (4C, $C_{arom.}$), 128.5 (2C, $C_{arom.}$), 138.3 (2C, $C_{arom.}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3476, 2978, 2859, 1447, 1412, 1362, 1223, 1099, 1026, 968, 926, 775, 737, 698, 629; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{20}H_{23}O_6$, 359.1489; found, 359.1499; HPLC (method 1): t_R = 17.3 min, purity 99.2 %.

4.2.2. (2*R*,2'*R*,4*R*,4'*R*,5*R*,5'*R*)-5,5'-Bis[(4-iodobenzyl)oxy]-2,2'-diphenyl-4,4'-bi(1,3-dioxane) (10)

Under nitrogen atmosphere, a 1 M solution of lithium hexamethyldisilazane in THF (3.7 mL, 3.7 mmol) was added to a solution of **9** (550 mg, 1.5 mmol) in anhydrous THF (50 mL). Tetrabutylammonium iodide (56 mg, 0.15 mmol) and 4-iodobenzyl bromide (1.0 g, 3.4 mmol) were added and the mixture was heated to reflux overnight. 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 (Ø = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 9/1, R_f = 0.19) to afford **10** as colorless solid (840 mg, 1.1 mmol, 69 % yield). m.p. = 118 °C; $[\alpha]_D^{20}$ = -60.6 (1.5,

CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.65 (t, *J* = 10.2 Hz, 2H, OC*H*$_2$CHCHO), 3.92 – 4.03 (m, 4H, OCH$_2$C*H*C*H*O), 4.33 (dd, *J* = 10.6/4.7 Hz, 2H, OC*H*$_2$CHCHO), 4.48 (d, *J* = 12.0 Hz, 2H, OC*H*$_2$Ar), 4.53 (d, *J* = 12.0 Hz, 2H, OC*H*$_2$Ar), 5.37 (s, 2H, PhC*H*), 6.97 – 7.02 (m, 4H, 2'-H$_{4\text{-iodophenyl}}$, 6'-H$_{4\text{-iodophenyl}}$), 7.34 – 7.45 (m, 10H, H$_{phenyl}$), 7.55 – 7.59 (m, 4H, 3'-H$_{4\text{-iodophenyl}}$, 5'-H$_{4\text{-iodophenyl}}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 66.9 (2C, OCH$_2$*C*HCHO), 69.6 (2C, O*C*H$_2$CHCHO), 72.0 (2C, O*C*H$_2$Ar), 77.4 (2C, OCH$_2$CH*C*HO), 93.8 (2C, C-4'$_{4\text{-iodophenyl}}$), 101.2 (2C, Ph*C*H), 126.3 (4C, C$_{phenyl}$), 128.4 (4C, C$_{phenyl}$), 129.2 (2C, C$_{phenyl}$), 129.9 (4C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 137.6 (4C, C-1'$_{4\text{-iodophenyl}}$ (2C), C$_{phenyl}$ (2C)), 137.7 (4C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2866, 1485, 1450, 1400, 1369, 1219, 1092, 1030, 1007, 972, 926, 795, 748, 737, 694; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{34}H_{33}I_2O_6$, 791.0361; found, 791.0320; HPLC (method 1): t_R = 26.1 min, purity 99.0 %.

4.2.3. (2*R*,3*S*,4*S*,5*R*)-2,5-Bis[(4-iodobenzyl)oxy]hexane-1,3,4,6-tetraol (11)

p-Toluenesulfonic acid (120 mg, 0.64 mmol) and 1,3-propanediol (2.3 mL, 2.4 g, 32 mmol) were added to a solution of **10** (2.5 g, 3.2 mmol) in methanol (200 mL). The reaction was heated to reflux for 4 h. Then the solvent was evaporated. The residue was dissolved in a saturated aqueous solution of $NaHCO_3$ 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 (Ø = 3 cm, h = 15 cm, V = 20 mL, CH_2Cl_2/methanol = 9.5/0.5, R_f = 0.27) to give **11** as colourless solid (1.7 g, 2.8 mmol, 88% yield). m.p. = 151 °C; $[\alpha]_D^{20}$ = +17.5 (1.7, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 3.56 – 3.60 (m, 2H, HOCH$_2$C*H*CHOH), 3.75 (dd, *J* = 11.9/4.6 Hz, 2H, HOC*H*$_2$CHCHOH), 3.92 – 3.95 (m, 4H, HOC*H*$_2$CHCHOH (2H), HOCH$_2$CHC*H*OH), 4.55 (d, *J* = 11.7 Hz, 2H, OC*H*$_2$Ar), 4.70 (d, *J* = 11.7 Hz, 2H, OC*H*$_2$Ar), 7.15 – 7.18 (m, 4H, 2'-H$_{4\text{-iodophenyl}}$, 6'-H$_{4\text{-iodophenyl}}$), 7.64 – 7.67 (m, 4H, 3'-H$_{4\text{-iodophenyl}}$, 5'-H$_{4\text{-iodophenyl}}$); ^{13}C NMR (CD_3OD): δ [ppm] = 62.1 (2C, HO*C*H$_2$CHCHOH), 70.1 (2C, HOCH$_2$CH*C*HOH), 72.7 (2C, O*C*H$_2$Ar), 81.5 (2C, HOCH$_2$*C*HCHOH), 93.4 (2C, C-4'$_{4\text{-iodophenyl}}$), 130.9 (4C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 138.5 (4C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 140.0 (2C, C-1'$_{4\text{-iodophenyl}}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3426, 3306, 2886, 1589, 1481, 1404, 1304, 1234, 1084,

1038, 1003, 829, 795, 621; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{20}H_{25}I_2O_6$, 614.9735; found, 614.9725; HPLC (method 1): t_R = 18.5 min, purity 95.6 %.

4.2.4. Methyl (*S*)-2,3-dihydroxypropanoate (14)

Concentrated sulphuric acid (4.1 mL, 7.5 g) was added to a solution of L-serine (12 g, 110 mmol) in water (150 mL) at 0 °C. Afterwards, a solution of $NaNO_2$ (6.8 g, 99 mmol) in water (75 mL) was added over a period of 24 h. Then a cooled solution of concentrated sulphuric acid (3.3 mL, 6.0 g) in water (20 mL) was added and again a solution of $NaNO_2$ (6.8 g, 99 mmol) in water (75 mL) was added over a period of 24 h. Afterwards, the solution was stirred at ambient temperature for additional 24 h. After the volume of the solution had been reduced *in vacuo* to about 1/3, an aqueous solution of NaOH (3.8 g, 95 mmol) was added. Then methanol (100 mL) and acetone (30 mL) were added and the mixture was filtered. After evaporation of the filtrate, the residue was dissolved in methanol (100 mL). Trimethyl orthoformate (75 mL) and *p*-toluenesulfonic acid (1.9 g, 10 mmol) were added and the mixture was heated to reflux 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 (Ø = 8 cm, h = 15 cm, V = 65 mL, cyclohexane/ethyl acetate = 1/2, R_f = 0.17) to give **14** as colourless oil (3.0 g, 25 mmol, 23%). $[\alpha]_D^{20}$ = -2.5 (1.1, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.84 (s, 3H, CO_2CH_3), 3.85 (dd, *J* = 11.7/3.9 Hz, 1H, $HOCHCH_2OH$), 3.91 (dd, *J* = 11.7/3.3 Hz, 1H, $HOCHCH_2OH$), 4.28 (t, *J* = 3.6 Hz, 1H, $HOCHCH_2OH$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 53.1 (1C, CO_2CH_3), 64.1 (1C, $HOCHCH_2OH$), 71.6 (1C, $HOCHCH_2OH$), 173.6 (1C, CO_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3402, 2955, 1732, 1439, 1215, 1115, 1061, 1007, 972, 648; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_4H_9O_4$, 121.0495; found, 121.0517.

4.2.5. Methyl (*S*)-2-hydroxy-3-(methoxymethoxy)propanoate (15)

Under N_2 atmosphere *N*,*N*-diisopropylethylamine (2.5 mL, 15 mmol) and chloromethyl methyl ether (1.1 mL, 15 mmol) were added to a solution of **14** (1.5 g, 12 mmol) in acetonitrile (50 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 (Ø = 4 cm, h = 15 cm, V = 30 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.18) to give **15** as colorless oil (710 mg, 4.3 mmol, 35%). $[\alpha]_D^{20}$ = -14.0 (0.7, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.36 (s, 3H, OCH_2OCH_3), 3.80 (dd, *J* = 10.8/2.8 Hz, 1H, $OCHCH_2O$), 3.82 (s, 3H, CO_2CH_3), 3.94 (dd, *J* = 10.8/3.7 Hz, 1H, $OCHCH_2O$), 4.32 – 4.34 (m, 1H, $OCHCH_2O$), 4.63 (d, *J* = 6.7 Hz, 1H, OCH_2OCH_3), 4.66 (d, *J* = 6.7 Hz, 1H, OCH_2OCH_3); ^{13}C NMR ($CDCl_3$): δ [ppm] = 52.8 (1C, CO_2CH_3), 55.7 (1C, OCH_2OCH_3), 70.3 (1C, $OCHCH_2O$), 70.9 (1C, $OCHCH_2O$), 97.2 (1C, OCH_2OCH_3), 173.1 (1C, CO_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3441, 2951, 2889, 1740, 1439, 1211, 1111, 1026, 918; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_6H_{13}O_5$, 165.0757; found, 165.0733.

5.2.6. Methyl (*S*)-2-[(4-iodobenzyl)oxy]-3-(methoxymethoxy)propanoate (16)

Under N_2 atmosphere sodium hydride (55% suspension in paraffin oil, 28 mg, 0.64 mmol) was added to a solution of **15** (70 mg, 0.43 mmol) in acetonitrile (20 mL). Then 4-iodobenzyl bromide (380 mg, 1.3 mmol) and tetrabutylammonium iodide (32 mg, 0.09 mmol) were added and the mixture was stirred at 80 °C 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 (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 8/2, R_f = 0.34) to give **16** as colourless oil (35 mg, 0.09 mmol, 22%). $[\alpha]_D^{20}$ = -34.6 (0.7, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.34 (s, 3H, OCH_2OCH_3), 3.78 (s, 3H, CO_2CH_3), 3.82 – 3.87 (m, 2H, $OCHCH_2O$), 4.15 (dd, *J* = 5.1/3.9 Hz, 1H, $OCHCH_2O$), 4.48 (d, *J* = 12.0 Hz, 1H, OCH_2Ar), 4.63 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.65 (d, *J* = 6.6 Hz, 1H, OCH_2OCH_3), 4.73 (d, *J* = 12.0 Hz, 1H, OCH_2Ar), 7.11 – 7.14 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.66 – 7.68 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 52.3 (1C, OCH_3), 55.5 (1C, OCH_2OCH_3), 68.0 (1C, $OCHCH_2O$), 72.1 (1C, OCH_2Ar), 77.9 (1C, $OCHCH_2O$), 93.6 (1C, C-4'$_{4\text{-iodophenyl}}$), 96.8 (1C, OCH_2OCH_3), 129.9 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 137.1 (1C, C-1'$_{4\text{-iodophenyl}}$), 137.7

(2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 170.9 (1C, *C*O_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2947, 2886, 1748, 1485, 1439, 1265, 1207, 1115, 1038, 1007, 918, 799; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{13}H_{18}IO_5$, 381.0193; found, 381.0187; HPLC (method 1): t_R = 19.3 min, purity 97.1 %.

4.2.7. *cis*-2-Phenyl-1,3-dioxan-5-ol (18)

Concentrated sulfuric acid (3 drops) was added to a mixture of glycerol (44 mL, 55 g, 0.60 mol) and freshly distilled benzaldehyde (48 mL, 50 g, 0.47 mol) in toluene (69 mL). The resulting mixture was heated to reflux in a Dean-Stark water separator. When the separation of water was complete (8.5 mL), the reaction mixture was allowed to cool to room temperature and the solvent removed *in vacuo*. The resulting white solid was recrystallized from isopropyl ether/petroleum ether. The precipitate was filtered off and purified by flash column chromatography (Ø = 6 cm, h = 15 cm, V = 50 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.16) to give **18** as colourless solid (42.6 g, 0.24 mol, 50% yield). m.p. = 81 °C; ^{1}H NMR (DMSO-d_6): δ [ppm] = 3.48 – 3.52 (m, 1H, OCH_2CHOH), 3.91 – 3.97 (m, 2H, OCH_2CHOH), 4.01 – 4.07 (m, 2H, OCH_2CHOH), 4.99 (d, *J* = 4.8 Hz, 1H, O*H*), 5.53 (s, 1H, C*H*Ph), 7.33 – 7.40 (m, 3H, 3'-H$_{phenyl}$, 4'-H$_{phenyl}$, 5'-H$_{phenyl}$), 7.42 – 7.47 (m, 2H, 2'-H$_{phenyl}$, 6'-H$_{phenyl}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 62.4 (1C, OCH_2CHOH), 71.4 (2C, OCH_2CHOH), 100.2 (1C, *C*HPh), 126.2 (2C, C-2'$_{phenyl}$, C-6'$_{phenyl}$), 127.9 (2C, C-3'$_{phenyl}$, C-5'$_{phenyl}$), 128.5 (1C, C-4'$_{phenyl}$), 138.9 (1C, C-1'$_{phenyl}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3271, 2916, 2851, 1450, 1385, 1339, 1277, 1153, 1084, 995, 976, 806, 741, 694; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{10}H_{13}O_3$, 181.0859; found, 181.0878; HPLC (method 1): t_R = 11.7 min, purity 95.4 %.

4.2.8. *cis*-5-[(4-Iodobenzyl)oxy]-2-phenyl-1,3-dioxane (19)

4-Iodobenzyl bromide (5.0 g, 17 mmol) and sodium hydride (55 % suspension in paraffin oil, 0.81 g, 19 mmol) were added to a solution of **18** (2.6 g, 14 mmol) in DMF (27 mL). After stirring the mixture at ambient temperature for 24 h, a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with ethyl acetate (4×). 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 (Ø = 8 cm, h = 15 cm, V = 60 mL, cyclohexane/ethyl acetate = 9/1, R_f = 0.18) to give **19** as colourless solid (2.4 g, 6.1 mmol, 42% yield). m.p. = 125 °C; ^{1}H NMR (DMSO-d_6): δ [ppm] = 3.38 – 3.41 (m, 1H, OCH_2CHO), 4.02 – 4.08 (m, 2H, OCH_2CHO), 4.19 – 4.25 (m, 2H, OCH_2CHO), 4.56 (s, 2H, OCH_2Ar), 5.58 (s, 1H, CHPh), 7.19 – 7.23 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.33 – 7.43 (m, 5H, H_{phenyl}), 7.70 – 7.74 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 68.2 (2C, OCH_2CHO), 68.7 (1C, OCH_2Ar), 69.9 (1C, OCH_2CHO), 93.2 (1C, C-4'$_{4\text{-iodophenyl}}$), 100.1 (1C, $CHPh$), 126.0 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$), 127.9 (2C, C-3$_{phenyl}$, C-5$_{phenyl}$), 128.6 (1C, C-4$_{phenyl}$), 129.7 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 137.0 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 138.6 (1C, C-1'$_{4\text{-iodophenyl}}$), 138.7 (1C, C-1$_{phenyl}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2970, 2859, 1481, 1454, 1377, 1335, 1277, 1238, 1153, 1096, 1007, 980, 799, 745, 698; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{17}H_{18}IO_3$, 397.0295; found, 397.0309; HPLC (method 1): t_R = 23.1 min, purity 99.7 %.

4.2.9. 2-[(4-Iodobenzyl)oxy]propane-1,3-diol (20)

p-Toluenesulfonic acid monohydrate (230 mg, 1.2 mmol) was added to a solution of **19** (2.4g, 6.1 mmol) in a mixture of methanol (80 mL) and tetrahydrofuran (1 mL). After stirring the mixture at ambient temperature for 16 h, a saturated aqueous solution of $NaHCO_3$ and brine were added and the mixture was extracted with ethyl acetate (4×). 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 (Ø = 5 cm, h = 15 cm, V = 30 mL, cyclohexane/ethyl acetate = 1/2, R_f = 0.15) to give **20** as colourless solid (1.6 g, 5.3 mmol, 86% yield). m.p. = 95 °C; ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.56 – 3.61 (m, 1H, $HOCH_2CHO$), 3.73 (dd, *J* = 11.7/4.9 Hz, 2H, $HOCH_2CHO$), 3.80 (dd, *J* = 11.7/4.4 Hz, 2H, $HOCH_2CHO$), 4.61 (s, 2H, OCH_2Ar), 7.09 – 7.12 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.67 – 7.71 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 62.6 (2C, $HOCH_2CHO$), 71.4 (1C, OCH_2Ar), 79.5 (1C, $HOCH_2CHO$), 93.6 (1C, C-4'$_{4\text{-iodophenyl}}$), 129.8 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 137.8 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 137.9 (1C, C-1'$_{4\text{-iodophenyl}}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3167, 2913, 1481, 1462, 1339, 1119, 1069, 1038, 1003, 887, 795, 679; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{10}H_{14}IO_3$, 308.9982; found, 308.9989; HPLC (method 1): t_R = 15.4 min, purity 98.5 %.

4.2.10. (*S*)-3-Hydroxy-2-[(4-iodobenzyl)oxy]propyl acetate ((*S*)-21)

Amano lipase AK from *Pseudomonas fluorescens* (1.1 g) was added to a solution of **20** (1.0 g, 3.3 mmol) in a mixture of *tert*-butyl methyl ether (150 mL) and isopropenyl acetate (3 mL, 28 mmol) at -10 °C. The reaction mixture was stirred at -10 °C for 24 h. Then the mixture was filtered and the solvent was evaporated *in vacuo*. The residue was purified by flash column chromatography (Ø = 4 cm, h = 15 cm, V = 30 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.20) to give (*S*)-**21** as colorless oil (0.72 g, 2.1 mmol, 62% yield). $[\alpha]_D^{20}$ = +5.1 (2.7, methanol); ^{1}H NMR (DMSO-d_6): δ [ppm] = 2.00 (s, 3H, H_3CCO_2), 3.43 – 3.52 (m, 2H, HOCH_2CHO), 3.53 – 3.59 (m, 1H, $HOCH_2CHO$), 4.02 (dd, *J* = 11.7/6.1 Hz, 1H, $H_3CCO_2CH_2$), 4.19 (dd, *J* = 11.7/3.7 Hz, 1H, $H_3CCO_2CH_2$), 4.53 (d, *J* = 12.6 Hz, 1H, OCH_2Ar), 4.57 (d, *J* = 12.6 Hz, 1H, OCH_2Ar), 4.78 (t, *J* = 5.6 Hz, 1H, O*H*), 7.13 – 7.18 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.68 – 7.72 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 20.7 (1C, H_3CCO_2), 60.2 (1C, $HOCH_2CHO$), 63.4 (1C, $H_3CCO_2CH_2$), 70.1 (1C, OCH_2Ar), 77.6 (1C, $HOCH_2CHO$), 93.2 (1C, C-4'$_{4\text{-iodophenyl}}$), 129.7 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 136.9 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 138.6 (1C, C-1'$_{4\text{-iodophenyl}}$), 170.3 (1C, H_3CCO_2); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3441, 2924, 2870, 1732, 1485, 1366, 1234, 1107, 1045, 1007, 799; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{12}H_{16}IO_4$, 351.0088; found, 351.0076; HPLC (method 1): t_R = 18.8 min, purity 99.5 %; enantiomeric ratio (HPLC method 4): t_R = 25.5 min, (*S*):(*R*) = 98.8:1.2.

4.2.11. (*S*)-2-[(4-Iodobenzyl)oxy]-3-(methoxymethoxy)propyl acetate (23)

Under N_2 atmosphere *N*,*N*-diisopropylethylamine (1.1 mL, 6.4 mmol) and chloromethyl methyl ether (0.46 mL, 6.1 mmol) were added to a solution of (*S*)-**21** (710 mg, 2.0 mmol) in acetonitrile (25 mL) at 0 °C. After stirring the mixture at ambient temperature for 21 h, water and brine were 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 (Ø = 2.5 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 9/1, R_f = 0.13) to give **23** as colorless oil (720 mg, 1.8 mmol, 90%). $[\alpha]_D^{20}$ =

+6.7 (3.2, methanol); ^{1}H NMR (DMSO-d_6): δ [ppm] = 2.01 (s, 3H, H_3CCO_2), 3.24 (s, 3H, OCH_2OCH_3), 3.52 – 3.60 (m, 2H, $H_3COCH_2OCH_2CHO$), 3.72 – 3.78 (m, 1H, OCH_2CHO), 4.07 (dd, *J* = 11.7/5.9 Hz, 1H, $H_3CCO_2CH_2$), 4.18 (dd, *J* = 11.7/4.0 Hz, 1H, $H_3CCO_2CH_2$), 4.53 – 4.60 (m, 4H, OCH_2Ar, OCH_2OCH_3), 7.13 – 7.17 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.69 – 7.73 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 20.6 (1C, H_3CCO_2), 54.6 (1C, OCH_2OCH_3), 63.2 (1C, $H_3CCO_2CH_2$), 66.3 (1C, $H_3COCH_2OCH_2CHO$), 70.2 (1C, OCH_2Ar), 75.6 (1C, OCH_2CHO), 93.3 (1C, C-4'$_{4\text{-iodophenyl}}$), 95.9 (1C, OCH_2OCH_3), 129.7 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 136.9 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 138.4 (1C, C-1'$_{4\text{-iodophenyl}}$), 170.2 (1C, H_3CCO_2); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2936, 2882, 1736, 1485, 1366, 1231, 1107, 1038, 1007, 918, 799; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{14}H_{20}IO_5$, 395.0350; found, 395.0339; HPLC (method 1): t_R = 22.0 min, purity 99.7 %.

4.2.12. (*R*)-2-[(4-Iodobenzyl)oxy]-3-(methoxymethoxy)propan-1-ol (24)

Potassium carbonate (0.79 g, 5.7 mmol) was added to a solution of **23** (1.0 g, 2.6 mmol) in methanol (40 mL) and the mixture was stirred at ambient temperature for 2 h. Then water and brine were added and the mixture was extracted with ethyl acetate (4×). 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 (Ø = 3 cm, h = 15 cm, V = 20 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.16) to afford **24** as colorless oil (780 mg, 2.2 mmol, 87% yield). $[\alpha]_D^{20}$ = +2.7 (8.4, methanol); ^{1}H NMR (DMSO-d_6): δ [ppm] = 3.24 (s, 3H, OCH_2OCH_3), 3.45 – 3.54 (m, 4H, $HOCH_2CHO$, $H_3COCH_2OCH_2$ (1H)), 3.56 – 3.63 (m, 1H, $H_3COCH_2OCH_2$), 4.53 – 4.60 (m, 4H, OCH_2Ar, OCH_2OCH_3), 4.68 (t, *J* = 5.5 Hz, 1H, OH), 7.14 – 7.19 (m, 2H, 2'-$H_{4\text{-iodophenyl}}$, 6'-$H_{4\text{-iodophenyl}}$), 7.67 – 7.72 (m, 2H, 3'-$H_{4\text{-iodophenyl}}$, 5'-$H_{4\text{-iodophenyl}}$); ^{13}C NMR (DMSO-d_6): δ [ppm] = 54.6 (1C, OCH_2OCH_3), 60.7 (1C, $HOCH_2CHO$), 67.1 (1C, $H_3COCH_2OCH_2$), 70.1 (1C, OCH_2Ar), 79.1 (1C, $HOCH_2CHO$), 93.0 (1C, C-4'$_{4\text{-iodophenyl}}$), 95.9 (1C, OCH_2OCH_3), 129.6 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 136.9 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 138.9 (1C, C-1'$_{4\text{-iodophenyl}}$). IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3433, 2928, 2882, 1481, 1400, 1211, 1107, 1034, 1007, 918, 799; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{12}H_{18}IO_4$, 353.0244; found, 353.0249; HPLC (method 1): t_R = 18.9 min, purity 99.5 %.

4.2.13. Methyl (*R*)-3-hydroxy-2-[(4-iodobenzyl)oxy]propanoate ((*R*)-12)

$NaIO_4$ (130 mg, 0.6 mmol) was added to a solution of **11** (250 mg, 0.4 mmol) in methanol (40 mL) and the mixture was stirred at ambient temperature for 2 h. Then the solution was concentrated *in vacuo*, brine 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 a mixture of methanol and water (9/1, 30 mL) and $NaHCO_3$ (1.0 g, 12 mmol) was added. Then a 1 M solution of Br_2 in the same mixture of methanol and water (1.2 mL, 1.2 mmol) was added. The flask was covered with aluminum foil and stirred at room temperature overnight. Afterwards, sodium thiosulfate and water were added and the mixture was extracted with EtOAc (3×). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.27) to give (*R*)-**12** as colourless oil (160 mg, 0.48 mmol, 61%). $[\alpha]_D^{20}$ = +49.2 (21, methanol); HPLC (method 1): t_R = 16.8 min, purity 95.6 %; enantiomeric ratio (HPLC method 3): t_R = 31.2 min, (*R*):(*S*) = 99.3:0.7.

4.2.14. Methyl (*S*)-3-hydroxy-2-[(4-iodobenzyl)oxy]propanoate ((*S*)-12)

method 1:

HCl-saturated methanol (0.5 mL) was added to a solution of **16** (120 mg, 0.31 mmol) in methanol (20 mL). The reaction mixture was stirred at room 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 (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.27) to give (*S*)-**12** as colourless oil (33 mg, 0.10 mmol, 31%). HPLC (method 1): t_R = 16.7 min, purity 97.1 %; enantiomeric ratio (HPLC method 3): t_R = 14.2 min, (*R*):(*S*) = 15.1:84.9.

method 2:

An oxidant solution was prepared by dissolving H_5IO_6 (2.3 g, 10 mmol) and CrO_3 (10 mg, 0.10 mmol) in acetonitrile (23 mL) and water (2 drops). The complete dissolution of the solids typically required 2 h. The oxidant solution (3.2 mL) was added to a solution of **24** (210 mg, 0.59 mmol) in acetonitrile (2.8 mL) and water (2 drops). The mixture was stirred at 0 °C for 24 h. Then water was added and the mixture extracted with ethyl acetate (4×). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The crude product was dissolved in methanol (5 mL) and a saturated solution of hydrochloric acid in methanol (1 mL) was added. The mixture was heated to reflux for 16 h. Then the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Ø = 1.5 cm, h = 15 cm, V = 5 mL, cyclohexane/ethyl acetate = 8/2, R_f = 0.13) to give (*S*)-**12** as colourless oil (98 mg, 0.29 mmol, 49% yield). $[\alpha]_D^{20}$ = -32.1 (3.4, methanol); HPLC (method 1): t_R = 18.4 min, purity 95.6 %; enantiomeric ratio (HPLC method 3): t_R = 15.0 min, (*S*):(*R*) = 98.1:1.9.

4.2.15. Spectroscopic data of (*R*)-12 and (*S*)-12:

^{1}H NMR ($CDCl_3$): δ [ppm] = 3.78 (s, 3H, OC*H*$_3$), 3.80 – 3.94 (m, 2H, OCHC*H*$_2$OH), 4.08 (dd, *J* = 5.7/3.7 Hz, 1H, OC*H*CH$_2$OH), 4.45 (d, *J* = 11.6 Hz, 1H, OC*H*$_2$Ar), 4.76 (d, *J* = 11.6 Hz, 1H, OC*H*$_2$Ar), 7.10 – 7.15 (m, 2H, 2'-H$_{4\text{-iodophenyl}}$, 6'-H$_{4\text{-iodophenyl}}$), 7.66 – 7.72 (m, 2H, 3'-H$_{4\text{-iodophenyl}}$, 5'-H$_{4\text{-iodophenyl}}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 52.4 (1C, O*C*H$_3$), 63.6 (1C, OCH*C*H$_2$OH), 72.3 (1C, O*C*H$_2$Ar), 78.8 (1C, O*C*HCH$_2$OH), 93.9 (1C, C-4'$_{4\text{-iodophenyl}}$), 130.1 (2C, C-2'$_{4\text{-iodophenyl}}$, C-6'$_{4\text{-iodophenyl}}$), 136.8 (1C, C-1'$_{4\text{-iodophenyl}}$), 137.8 (2C, C-3'$_{4\text{-iodophenyl}}$, C-5'$_{4\text{-iodophenyl}}$), 171.0 (1C, *C*O$_2$CH$_3$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3449, 2947, 2874, 1740, 1481, 1385, 1273, 1204, 1123, 1057, 1007, 795; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{11}H_{14}IO_4$, 336.9931; found, 336.9935.

4.2.16. Methyl (*R*)-3-hydroxy-2-{[4-(phenylethynyl)benzyl]oxy}propanoate ((*R*)-25a)

Under N_2 atmosphere triethylamine (0.39 mL, 280 mg, 2.8 mmol), copper(I) iodide (15 mg, 0.08 mmol) and tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.04 mmol) were added to a solution of (*R*)-**12** (140 mg, 0.40 mmol) in acetonitrile (30 mL). Then

phenylacetylene (0.4 mL, 370 mg, 3.62 mmol) was added and the mixture was stirred at ambient temperature for 3 h. After evaporation of the solvent the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.27) to give (*R*)-**25a** as colorless solid (100 mg, 0.34 mmol, 83% yield). m.p. = 97 °C; $[\alpha]_D^{20}$ = +37.9 (1.9, methanol); HPLC (method 1): t_R = 19.8 min, purity 97.3 %.

4.2.17. Methyl (*R*)-3-hydroxy-2-{[4-(phenylethynyl)benzyl]oxy}propanoate ((*S*)-25a)

Under N_2 atmosphere triethylamine (0.17 mL, 120 mg, 1.2 mmol), copper(I) iodide (6 mg, 0.03 mmol) and tetrakis(triphenylphosphine)palladium(0) (18 mg, 0.015 mmol) were added to a solution of (*S*)-**12** (51 mg, 0.15 mmol) in acetonitrile (15 mL). Then phenylacetylene (0.15 mL, 140 mg, 1.40 mmol) was added and the mixture was stirred at ambient temperature for 3 h. After evaporation of the solvent the residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.27) to give (*R*)-**25a** as colorless solid (38 mg, 0.12 mmol, 81% yield). m.p. = 93 °C; $[\alpha]_D^{20}$ = -34.9 (1.3, methanol); HPLC (method 1): t_R = 19.5 min, purity 99.7 %.

4.2.18. Spectroscopic data of (*S*)-25a and (*R*)-25a:

^{1}H NMR ($CDCl_3$): δ [ppm] = 3.79 (s, 3H, OCH_3), 3.85 (dd, *J* = 11.8/5.7 Hz, 1H, OCHCH_2OH), 3.92 (dd, *J* = 11.8/3.7 Hz, 1H, OCHCH_2OH), 4.11 (dd, *J* = 5.7/3.7 Hz, 1H, OC*H*CH_2OH), 4.53 (d, *J* = 11.7 Hz, 1H, OCH_2Ar), 4.84 (d, *J* = 11.7 Hz, 1H, OCH_2Ar), 7.32 – 7.38 (m, 5H, $H_{arom.}$), 7.50 – 7.56 (m, 4H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 52.4 (1C, O*C*H_3), 63.7 (1C, OCH*C*H_2OH), 72.6 (1C, O*C*H_2Ar), 78.8 (1C, O*C*HCH_2OH), 89.2 (1C, *C*≡C), 89.9 (1C, *C*≡C), 123.2 (1C, $C_{arom.}$), 123.3 (1C, $C_{arom.}$), 128.2 (2C, $C_{arom.}$), 128.4 (1C, $C_{arom.}$), 128.5 (2C, $C_{arom.}$), 131.8 (2C, $C_{arom.}$), 131.9 (2C, $C_{arom.}$), 137.3 (1C, $C_{arom.}$), 171.1 (1C, *C*O_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3518, 2924, 1748, 1508, 1443, 1335, 1207, 1115, 1096, 1057, 1011, 964, 891, 826, 760, 691; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{19}H_{19}O_4$, 311.1278; found, 311.1277.

4.2.19. Methyl (*R*)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]propanoate ((*R*)-25b)

Under N_2 atmosphere triethylamine (1.2 mL, 880 mg, 8.7 mmol), copper(I) iodide (48 mg, 0.25 mmol) and tetrakis(triphenylphosphine)palladium(0) (140 mg, 0.12 mmol) were added to a solution of (*R*)-**12** (420 mg, 1.3 mmol) in acetonitrile (70 mL). Then 4-(4-ethynylbenzyl)morpholine (2.0 g, 9.9 mmol) was added and the mixture was stirred at ambient temperature for 3 h. After evaporation of the solvent the residue was purified by flash column chromatography (Ø = 3 cm, h = 15 cm, V = 15 mL, cyclohexane/ethyl acetate = 2/1, R_f = 0.12) to give (*R*)-**25b** as colorless oil (450 mg, 1.1 mmol, 88% yield). $[\alpha]_D^{20}$ = +36.1 (4.0, methanol); HPLC (method 1): t_R = 16.4 min, purity 99.8 %.

4.2.20. Methyl (*S*)-3-hydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]propanoate ((*S*)-25b)

Under N_2 atmosphere, copper(I) iodide (17 mg, 0.09 mmol), tetrakis(triphenylphosphine)palladium(0) (70 mg, 0.06 mmol) and triethylamine (0.33 mL, 240 mg, 2.4 mmol) were added to a solution of (*S*)-**12** (99 mg, 0.29 mmol) in dry acetonitrile (10 mL) at ambient temperature. Then a solution of 4-(4-ethynylbenzyl)morpholine (65 mg, 0.32 mmol) in dry acetonitrile (4 mL) was added dropwise over a period of 3 h. Afterwards, the solvent was removed *in vacuo* and the residue was purified twice by flash column chromatography (Ø = 1.5 cm, h = 15 cm, V = 5 mL, cyclohexane/ethyl acetate = 1/2, R_f = 0.14) to give (*S*)-**25b** as colorless oil (83 mg, 0.20 mmol, 69% yield). $[\alpha]_D^{20}$ = -53.1 (1.8, methanol); HPLC (method 1): t_R = 15.7 min, purity 95.0 %.

4.2.21. Spectroscopic data of (*R*)-25b and (*S*)-25b:

^{1}H NMR (CD_3OD): δ [ppm] = 2.44 – 2.49 (m, 4H, NC*H*$_2$CH_2O), 3.54 (s, 2H, NC*H*$_2$Ar), 3.68 – 3.71 (m, 4H, NCH_2C*H*$_2$O), 3.76 (s, 3H, OC*H*$_3$), 3.80 (dd, *J* = 11.8/5.5 Hz, 1H, OCHC*H*$_2$OH), 3.84 (dd, *J* = 11.8/3.8 Hz, 1H, OCHC*H*$_2$OH), 4.11 (dd, *J* = 5.5/3.8 Hz,

1H, OC*H*CH_2OH), 4.55 (d, *J* = 11.9 Hz, 1H, OCH_2Ar), 4.74 (d, *J* = 11.9 Hz, 1H, OCH_2Ar), 7.35 – 7.38 (m, 2H, $H_{arom.}$), 7.41 – 7.44 (m, 2H, $H_{arom.}$), 7.46 – 7.51 (m, 4H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ [ppm] = 52.4 (1C, O*C*H_3), 54.7 (2C, NCH_2CH_2O), 63.9 (1C, OCH*C*H_2OH), 64.0 (1C, N*C*H_2Ar), 67.8 (2C, NCH_2*C*H_2O), 73.1 (1C, O*C*H_2Ar), 81.0 (1C, O*C*HCH_2OH), 90.0 (1C, *C*≡C), 90.1 (1C, *C*≡C), 123.6 (1C, $C_{arom.}$), 124.0 (1C, $C_{arom.}$), 129.2 (2C, $C_{arom.}$), 130.7 (2C, $C_{arom.}$), 132.4 (2C, $C_{arom.}$), 132.5 (2C, $C_{arom.}$), 139.1 (1C, $C_{arom.}$), 139.6 (1C, $C_{arom.}$), 172.9 (1C, *C*O_2CH_3); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3426, 2951, 2855, 2808, 1748, 1516, 1454, 1350, 1288, 1204, 1115, 1069, 1007, 864, 822; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{24}H_{28}NO_5$, 410.1962; found, 410.1960.

4.2.22. (*R*)-*N*,3-Dihydroxy-2-{[4-(phenylethynyl)benzyl]oxy}propanamide ((*R*)-7a)

Hydroxylamine hydrochloride (70 mg, 1.0 mmol) and a 2 M solution of sodium methoxide in methanol (0.34 mL, 0.68 mmol) were added to a solution of (*R*)-**25a** (104 mg, 0.34 mmol) in dry methanol (20 mL) and the mixture was stirred at ambient temperature for 16 h. Then 1 M HCl 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 (Ø = 1 cm, h = 15 cm, V = 5 mL, CH_2Cl_2/methanol = 9.5/0.5, R_f = 0.21) to give (*R*)-**7a** as colorless solid (25 mg, 0.08 mmol, 24% yield). m.p. = 128 °C; $[\alpha]_D^{20}$ = +44.9 (0.5, methanol); HPLC (method 2): t_R = 16.3 min, purity 97.6 %.

4.2.23. (*S*)-*N*,3-Dihydroxy-2-{[4-(phenylethynyl)benzyl]oxy}propanamide ((*S*)-7a)

Hydroxylamine hydrochloride (81 mg, 1.2 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.2 mL, 1.1 mmol) were added to a solution of (*S*)-**25a** (55 mg, 0.18 mmol) in dry methanol (8 mL) and the mixture was stirred at ambient temperature for 14 h. Then the mixture was acidified with 1 M HCl, water was added and the mixture extracted with ethyl acetate (4×). 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 (Ø = 1.5 cm, h = 15 cm, V = 5 mL, CH_2Cl_2/methanol = 9.5/0.5, R_f = 0.14) to give (*S*)-**7a** as colorless solid (24 mg, 0.08

mmol, 43% yield). m.p. = 144 °C; $[\alpha]_D^{20}$ = -35.9 (2.5, methanol); HPLC (method 2): t_R = 15.7 min, purity 98.5 %.

4.2.24. Spectroscopic data of (*R*)-7a and (*S*)-7a:

^{1}H NMR (CD_3OD): δ [ppm] = 3.76 (dd, *J* = 11.7/5.7 Hz, 1H, OCHC*H*$_2$OH), 3.81 (dd, *J* = 11.7/3.9 Hz, 1H, OCHC*H*$_2$OH), 3.93 (dd, *J* = 5.7/3.9 Hz, 1H, OC*H*CH$_2$OH), 4.58 (d, *J* = 12.3 Hz, 1H, OC*H*$_2$Ar), 4.70 (d, *J* = 12.3 Hz, 1H, OC*H*$_2$Ar), 7.34 – 7.39 (m, 3H, $H_{arom.}$), 7.42 – 7.45 (m, 2H, $H_{arom.}$), 7.48 – 7.53 (m, 4H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ [ppm] = 63.7 (1C, OCH*C*H$_2$OH), 72.9 (1C, O*C*H$_2$Ar), 81.3 (1C, O*C*HCH$_2$OH), 89.9 (1C, *C*≡C), 90.3 (1C, *C*≡C), 124.1 (1C, $C_{arom.}$), 124.5 (1C, $C_{arom.}$), 129.2 (2C, $C_{arom.}$), 129.5 (1C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 132.5 (2C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 139.3 (1C, $C_{arom.}$), 169.5 (1C, *C*ONHOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3503, 3194, 2924, 2862, 1663, 1508, 1443, 1346, 1258, 1103, 1065, 1042, 1003, 837, 756, 687; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{18}NO_4$, 312.1230; found, 312.1242.

4.2.25. (*R*)-*N*,3-Dihydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]propanamide ((*R*)-7b)

Hydroxylamine hydrochloride (610 mg, 8.8 mmol) and a 1 M solution of sodium methoxide in methanol (8.8 mL, 8.8 mmol) were added to a solution of (*R*)-**25b** (600 mg, 1.5 mmol) in dry methanol (30 mL) and the mixture was stirred at ambient temperature for 4 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 (Ø = 3 cm, h = 15 cm, V = 20 mL, CH_2Cl_2/methanol = 9.5/0.5, R_f = 0.05) to give (*R*)-**7b** as colorless oil (120 mg, 0.29 mmol, 20% yield). $[\alpha]_D^{20}$ = +31.9 (1.4, methanol); HPLC (method 2): t_R = 12.6 min, purity 99.9 %.

4.2.26. (*S*)-*N*,3-Dihydroxy-2-[(4-{[4-(morpholinomethyl)phenyl]ethynyl}benzyl)oxy]propanamide ((*S*)-7b)

Hydroxylamine hydrochloride (27 mg, 0.39 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.07 mL, 0.38 mmol) were added to a solution of (*S*)-**25b** (24

mg, 0.06 mmol) in dry methanol (4 mL) and the mixture was stirred at ambient temperature for 15 h. Then water was added and the mixture extracted with ethyl acetate (4×). 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 (Ø = 1.5 cm, h = 15 cm, V = 5 mL, CH_2Cl_2/methanol = 9.5/0.5, R_f = 0.05) to give (*S*)-**7b** as colorless oil (8 mg, 0.02 mmol, 33% yield). $[\alpha]_D^{20}$ = -26.0 (2.3, methanol); HPLC (method 2): t_R = 12.1 min, purity 95.3 %.

4.2.27. Spectroscopic data of (*R*)-7b and (*S*)-7b:

^{1}H NMR (CD_3OD): δ [ppm] = 2.44 – 2.50 (m, 4H, NC*H*$_2$CH$_2$O), 3.54 (s, 2H, NC*H*$_2$Ar), 3.67 – 3.71 (m, 4H, NCH$_2$C*H*$_2$O), 3.76 (dd, *J* = 11.7/5.7 Hz, 1H, OCHC*H*$_2$OH), 3.81 (dd, *J* = 11.7/3.9 Hz, 1H, OCHC*H*$_2$OH), 3.93 (dd, *J* = 5.7/3.9 Hz, 1H, OC*H*CH$_2$OH), 4.57 (d, *J* = 12.2 Hz, 1H, OC*H*$_2$Ar), 4.70 (d, *J* = 12.2 Hz, 1H, OC*H*$_2$Ar), 7.35 – 7.39 (m, 2H, $H_{arom.}$), 7.41 – 7.45 (m, 2H, $H_{arom.}$), 7.46 – 7.52 (m, 4H, $H_{arom.}$); ^{13}C NMR (CD_3OD): δ [ppm] = 54.7 (2C, NCH_2CH_2O), 63.7 (1C, OCHCH_2OH), 64.0 (1C, NCH_2Ar), 67.8 (2C, NCH$_2$CH_2O), 72.9 (1C, OCH_2Ar), 81.3 (1C, OCHCH$_2$OH), 90.0 (1C, *C*≡C), 90.2 (1C, *C*≡C), 123.6 (1C, $C_{arom.}$), 124.1 (1C, $C_{arom.}$), 129.2 (2C, $C_{arom.}$), 130.7 (2C, $C_{arom.}$), 132.5 (2C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 139.1 (1C, $C_{arom.}$), 139.3 (1C, $C_{arom.}$), 169.5 (1C, *C*ONHOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3368, 3287, 2928, 2870, 2828, 1647, 1512, 1454, 1412, 1350, 1300, 1261, 1107, 1065, 1026, 999, 918, 864, 841, 791; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{23}H_{27}N_2O_5$, 411.1914; found, 411.1944.

4.2.28. Reaction courses of lipase catalyzed reactions

The courses of the lipase catalyzed reactions shown in Figure 3 were performed in a 1.5 mL vial with a magnetic stirring bar. 5.0 mg of the prochiral diol **20** were dissolved in a mixture of *tert*-butyl methyl ether : isopropenyl acetate (50 : 1, 1.5 mL) and the solution was added to 5.0 mg of Amano lipase AK from *Pseudomonas fluorescens*. The vial was placed in a HPLC system (Merck Hitachi Equipment; UV detector: L-7400; pump: L-7150) equipped with an autosampler (L-7200) that was modified by replacing the regular sample rack with a magnet stirrer (IKA-mini-MR). The temperature of the reaction vessel was adjusted with an attached cryogenic system

(Julabo FP40). Samples (10 µL) were taken automatically and analyzed using the conditions of HPLC method 4 (column: DiacelChiralpak IA with guard column; flow rate: 1.00 mL/min; detection at λ = 235 nm for 60 min; solvent: isohexane : isopropanol = 95 : 5). The lipase was removed from the samples by use of a 0.2 µm inline filter (Agilent 5067-1555).

4.3. Biological evaluation

4.3.1. Agar diffusion clearance assay

The antibiotic activity of the synthesized inhibitors was determined by agar disc diffusion clearance assays. Liquid cultures of *E. coli* BL21 (DE3) and the defective strain *E. coli* D22[44] were grown overnight in LB broth[45] at 37 °C, 200 rpm. 150 µL of an overnight cell suspension were spread evenly onto LB agar petri dishes. 15 µL of each compound (10 mM in DMSO) were applied onto circular filter paper (Ø = 6 mm, thickness 0.75 mm, Carl Roth). Pure DMSO, serving as a negative and CHIR-090,[30] 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 diameters are given as mean value ± SD from three independent experiments.

4.3.2. Minimum Inhibitory Concentration (MIC)

The MIC values of the compounds were determined by means of the microdilution method[46] using a 96-well plate and LB medium in the presence of 5% DMSO.

E. coli BL21 (DE3) and *E. coli* D22 were grown overnight in LB medium at 37 °C and 200 rpm. The overnight suspension was diluted 1:100 in fresh LB broth and 190 µL of the inoculated medium were dispensed to each well of a 96-well plate. 10 µL of a twofold dilution series of the compounds in DMSO (ranging from 5.12 mM to 20 nM) was added to the inoculated medium resulting in a final concentration range between 256 µM to 1 nM. Then the plates were incubated for 20 h at 37 °C, 200 rpm. The lowest concentrations at which no visible growth of bacteria could be observed were taken as the MIC values.

4.3.3. Protein purification

The plasmid for the expression of LpxCC63A (pETEcLpxCC63A) was kindly provided by Carol Fierke.[47] The C63A mutation lowers the undesired influence of Zn^{2+}-concentration on enzymatic activity. The purification of LpxC was performed essentially as previously described.[48] 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 20 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 120 mL of Superdex 200 (HiLoad 16/600) (GE Healthcare). LpxCC63A emerged in a peak after 80 mL of elution buffer. The purified LpxC was concentrated with MWCO columns and stored in 50 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 was quantified by use of an Implen NanoPhotometer showing a concentration of 500 µg*mL^{-1}.

4.3.4. LpxC assay

A fluorescence-based microplate assay for LpxC activity was performed as described by Clements *et al*.[24] The wells in a black, non-binding, 96 wells fluorescence microplate (Greiner Bio One, Frickenhausen) were filled with 93 µL of a 40 mM sodium morpholinoethanesulfonic acid buffer (pH 6.0) containing 26.9 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. After addition of 250 ng purified LpxC, the microplate was incubated for 30 min at 37 °C in a plate shaker. Then the biochemical reaction was stopped by adding 40 µL of 0.625 M sodium hydroxide. The reaction mixture was further incubated for 10 min and neutralized 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[49] 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 calculated from the Lineweaver-Burk plot. 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

To perform molecular docking studies we followed a slightly modified protocol validated previously.[35] The crystal structure of *Escherichia coli* LpxC in complex with the inhibitor LPC-009,[21] which has a similar structure as the compounds under study, was downloaded from the Protein Data Bank (PDB ID 3P3G)[50] and used as a protein model. Protein preparation was done using Schrödinger's Protein Preparation Wizard[51] in the following way. The complex was cleaned of the solvent particles, except of two conserved water molecules situated near the zinc binding group of the ligand. Hydrogen atoms were added automatically and then the tautomeric forms and protonation states of the amino acid residues were assigned using PROPKA application[51] at pH 7.0. For H265 residue both the neutral and charged states were considered. A total of eight protein models were used for docking, which had a different distribution of conserved water molecules and a different His265 protonation state. On the final protein preparation step, each model was energy minimized using OPLS-2005 force field with restrains (RMSD of the atom displacement for terminating the minimization 0.3 Å). The ligand preparation was carried out in MOE.[52] A conformational search was performed for all inhibitors using the Low Mode MD sampling in MOE (minimum RMSD between conformations 1 Å) to produce multiple low energy starting conformations. Docking studies were done using the Glide program[51] and applying metal constraint to the Zn^{2+}-ion, two hydrogen-bond constraints to E78 and T191 and a positional constraint placed on the carbon atom of the benzamide of LPC-009 connected to the diacetylene linker. The Glide Score calculated in standard precision mode (SP) was used to rank the docking poses.

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