Journal Pre-proofs

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Synthesis and biological evaluation of triazolyl-substituted benzyloxyacetohydroxamic acids as LpxC inhibitors

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Dedicated to Prof. Dr. Bernhard Wünsch on the occasion of his 60th birthday

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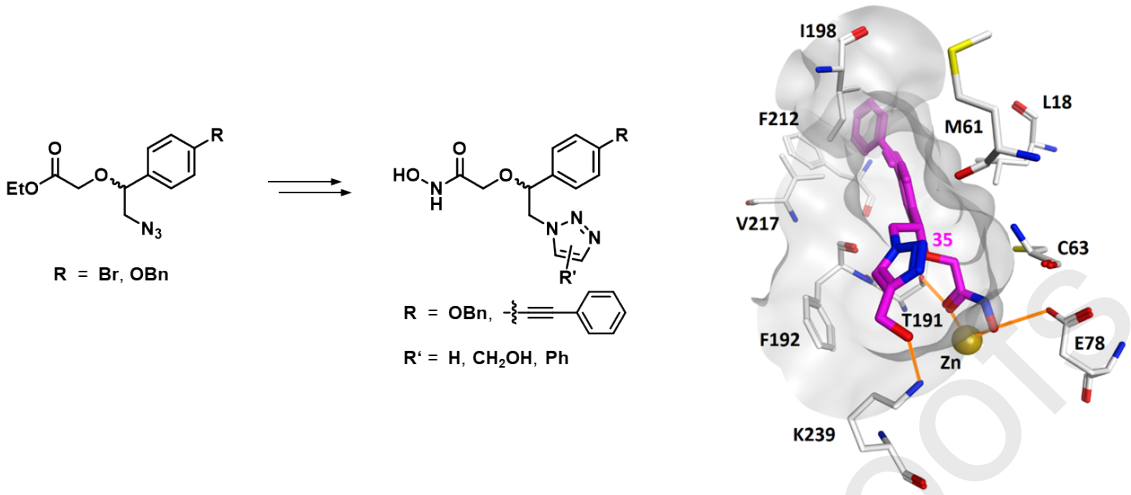
Graphical Abstract



Key words

LpxC inhibitors, structure-activity relationships, azide-alkyne cycloadditions, triazole derivatives, hydroxamic acids, enantioselective synthesis

Abstract

The bacterial deacetylase LpxC is a promising target for the development of antibiotics selectively combating Gram-negative bacteria. To improve the biological activity of the reported benzyloxyacetohydroxamic acid **9** ((*S*)-*N*-hydroxy-2-{2-hydroxy-1-[4-(phenylethynyl)phenyl]ethoxy}acetamide), its hydroxy group was replaced by a triazole ring. Therefore, in divergent syntheses, triazole derivatives exhibiting rigid and flexible lipophilic side chains, different configurations at their stereocenter, and various substitution patterns at the triazole ring were synthesized, tested for antibacterial and LpxC inhibitory activity, and structure-activity relationships were deduced based on docking and binding energy calculations.

1. Introduction

The development of bacterial resistance to commonly used antibiotics is a natural process of adaptation.[1-2] However, the inappropriate and unnecessary use of the available antibiotics enhances the emergence of bacterial resistance, which develops through a multitude of mechanisms.[3-5] Various bacterial species have already developed resistance against most of the available antibiotics.[6-7] These multidrug-resistant bacteria lead to severe difficulties in the treatment of even common infections, cause an increase in the number of fatalities and are associated with higher costs for health care systems.[8-10] Thus, in order to combat the infections caused by multidrug-resistant bacteria, there is an essential need for the development of new antibiotics with novel mechanisms of action.[11-12] However, despite the urgent need for new antibiotics, there is a big lack of new antibacterial drugs in the development pipeline, in particular of those needed to counteract the increasing number of multidrug-resistant Gram-negative bacteria.[13-17]

The bacterial deacetylase LpxC represents a promising target for the development of novel antibiotics selectively combating Gram-negative bacteria.[18] In *Escherichia coli*, the Zn^{2+}-dependent enzyme catalyzes the irreversible deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine (**2**), representing the first irreversible step of lipid A biosynthesis (Figure 1), which is highly conserved in Gram-negative bacteria.[19] Lipid A is the hydrophobic membrane anchor of lipopolysaccharides (LPS), which are the main component of the outer leaflet of the outer membrane of Gram-negative bacteria.[20] Being necessary for the maintenance of an effective outer membrane barrier, lipid A is essential for the growth and viability of Gram-negative bacteria.[19, 21] Thus, bacteria with a defective lipid A biosynthesis grow slowly and are hypersensitive towards several antibiotics, whereas the complete inhibition of lipid A biosynthesis is lethal to Gram-negative bacteria.[20, 22] In consequence, due to the essential role of LpxC in the biosynthesis of lipid A, inhibitors of the deacetylase represent a new class of potential antibiotics.[18] Whereas LpxC orthologues from different Gram-negative species share substantial sequence similarity, the deacetylases exhibit no sequence homology with any mammalian protein.[22] The enzyme displays a novel “β-α-α-β sandwich” fold, which is formed by two domains showing similar topologies.[23] The active site, in which the catalytic Zn^{2+}-ion is located, can be found at the interface of the two domains on one side of the sandwich.[24] Additionally, the enzyme exhibits a

hydrophobic tunnel leading out of the active site, which binds the fatty acyl chain of the enzyme's natural substrate **2** during catalysis.[25]

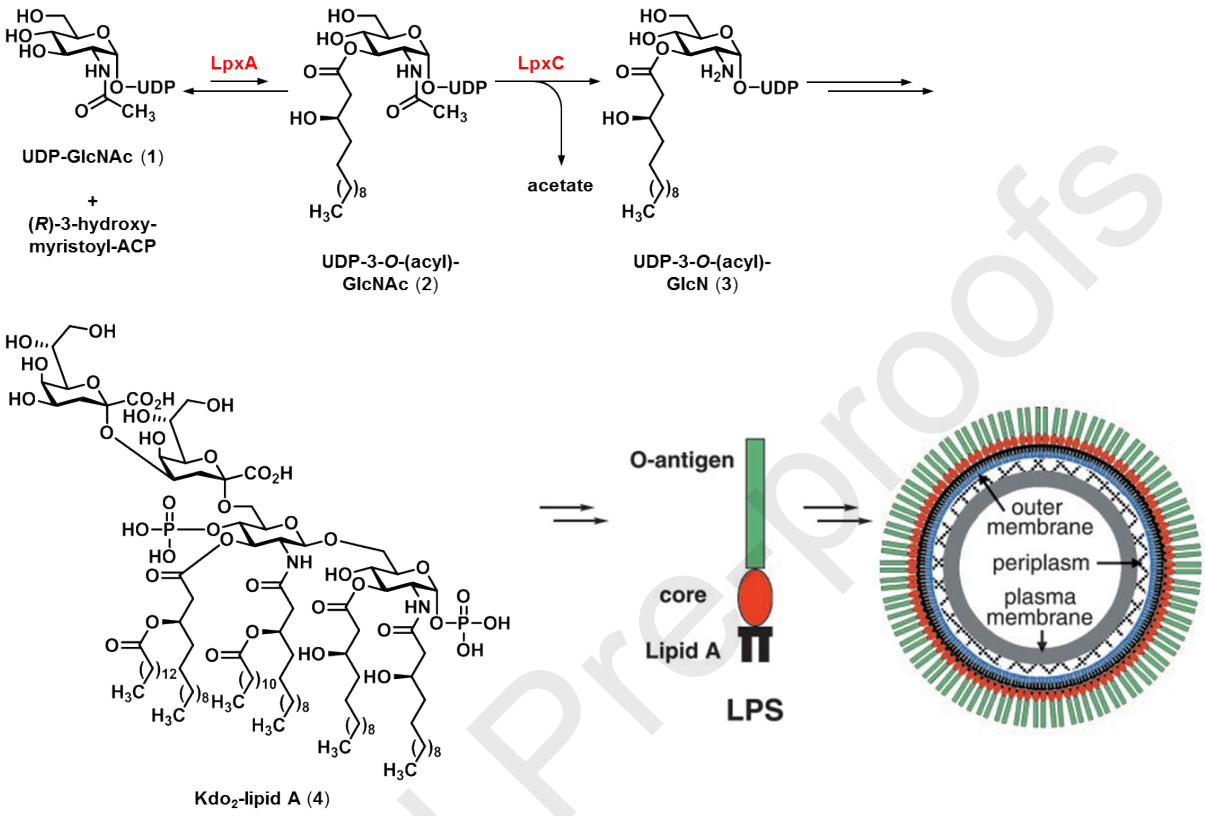


Figure 1: Chemical structure of Kdo_2-lipid A and the first two steps of its biosynthesis in *E. coli*.[18, 23, 26]

Several classes of LpxC inhibitors have already been reported in the literature.[18, 26-35] Most of them share a Zn^{2+}-chelating hydroxamate moiety and a lipophilic side chain binding to the hydrophobic tunnel of LpxC, like e.g. the potent threonine-based LpxC inhibitors CHIR-090 (**5**) and LPC-009 (**6**), tyrosine analogue LPC-051 (**7**), as well as the 3-amino-valine derivative ACHN-975 (**8**), which was the first LpxC inhibitor to enter human clinical trials and was thereby found to exhibit dose-limiting cardiovascular toxicity (Figure 2).[36-39]

Recently, we have described the synthesis and the biological evaluation of benzyloxyacetohydroxamic acid **9** (Figure 2).[40] The compound showed promising inhibitory activity against *E. coli* LpxC and was found to exhibit antibacterial properties against several *E. coli* strains. Therefore, this compound was chosen as lead for further

optimization steps. Docking studies with the benzyloxyacetohydroxamic acid had revealed that the compound's hydroxamate moiety chelates the catalytic Zn^{2+}-ion of LpxC and forms hydrogen bonds with conserved residues of the active site, whereas the diphenylacetylene moiety is placed in the hydrophobic tunnel.[40] The hydroxy group as well as the ether oxygen of the linker region undergo hydrogen-bonding interactions with polar residues of the active site of LpxC. However, the compound leaves the UDP-binding site of the enzyme unoccupied. In order to further optimize hydroxamic acid **9**, its hydroxy group should be replaced by another functional group, which is also able to form hydrogen bonds with the enzyme and additionally offers the possibility of introducing further substituents addressing the so far unoccupied UDP-binding site. As triazoles are able to undergo hydrogen bonds and can be easily accessed via azide-alkyne cycloadditions, thereby enabling the introduction of various substituents, a series of triazole derivatives (**10**) was envisaged to test the feasibility of the triazole moiety as possible linker group. Besides 1-monosubstituted triazole derivatives, 1,4- and 1,5-disubstituted regioisomers should be synthesized to find the optimal position of the substituent at the triazole ring. Additionally, the influence of polar and non-polar substituents at the triazole ring should be investigated. As hydroxamic acid **9** showed considerably higher antibacterial and inhibitory activity than its (*R*)-configured enantiomer *ent*-**9**, mainly (*S*)-configured triazole derivatives should be synthesized. However, to unequivocally confirm that also in case of the triazole derivatives the (*S*)-enantiomers represent the eutomers, for selected triazole derivatives both enantiomers should be synthesized and tested for inhibitory activity against LpxC and antibacterial properties.

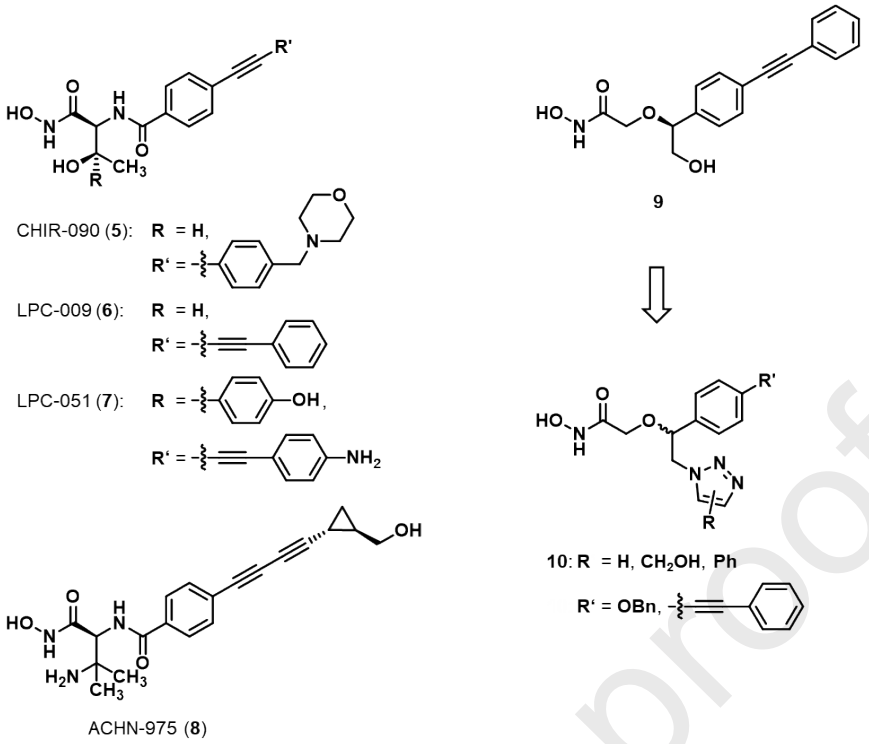
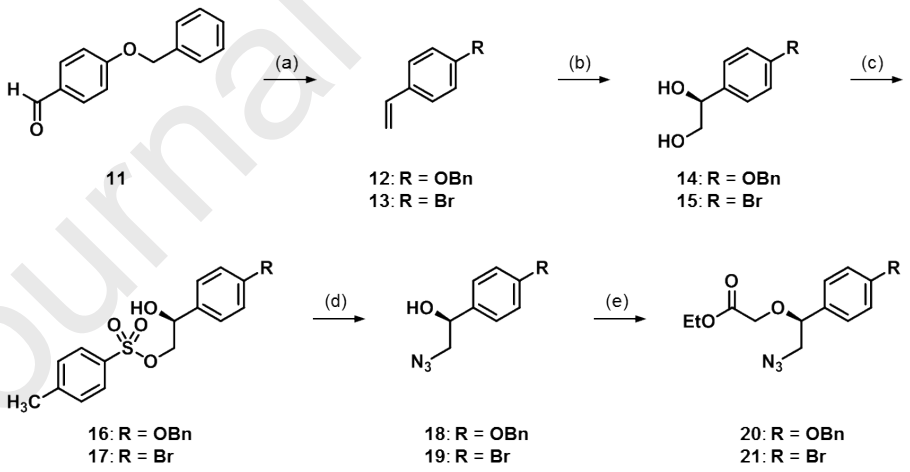


Figure 2: Structures of reported and envisaged LpxC inhibitors.

2. Results and Discussion

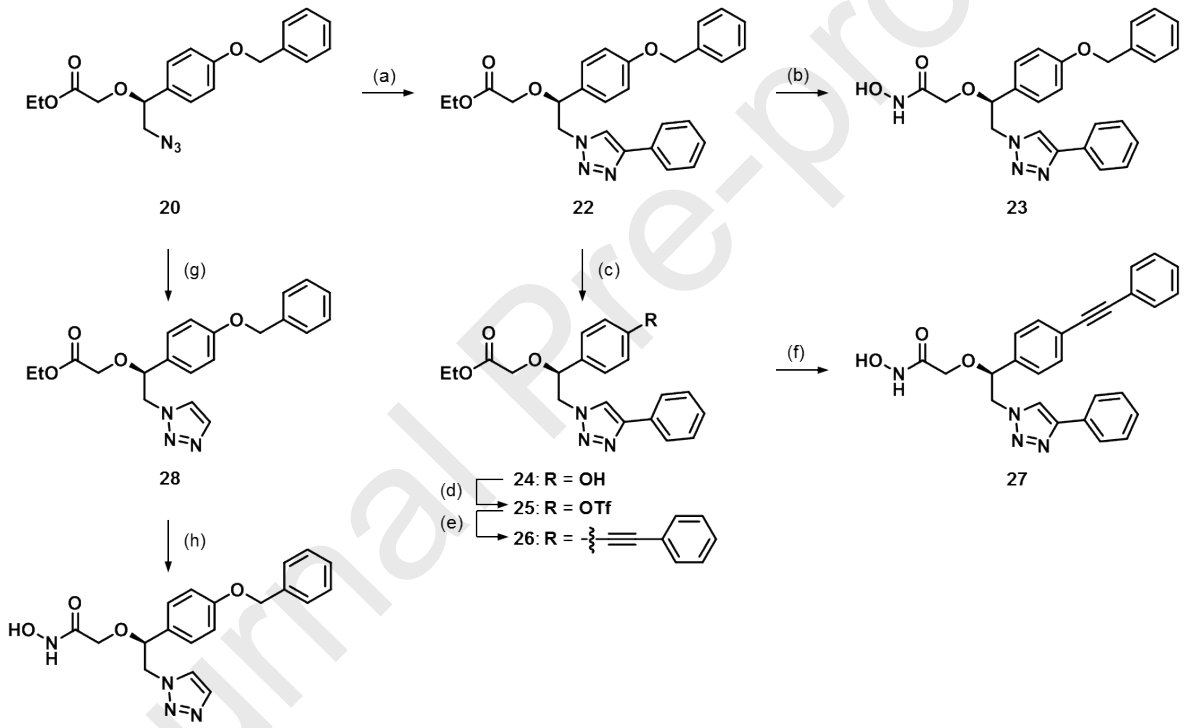
2.1. Chemistry

The envisaged triazole derivatives were synthesized from 4-bromostyrene (**13**) and 4-benzyloxystyrene (**12**). The latter compound could be easily accessed from 4-(benzyloxy)benzaldehyde (**11**) via a Wittig reaction with methyltriphenylphosphonium bromide and potassium *tert*-butoxide (Scheme 1). To obtain the (*S*)-configured vicinal diols **14** and **15**, styrene derivatives **12** and **13** were subjected to asymmetric Sharpless dihydroxylation reactions employing AD-mix-α. Subsequently, the primary alcohol groups were transformed into better leaving groups by performing Bu_2SnO-catalyzed regioselective tosylations of diols **14** and **15**. Then, the thereby obtained tosylates **16** and **17** were transformed into azides **18** and **19** via nucleophilic substitutions with sodium azide. Alkylation of the secondary alcohols **18** and **19** with ethyl bromoacetate gave the benzyloxyacetic acid derivatives **20** and **21**, representing the central intermediates of the envisaged syntheses. *ent*-**21**, the (*R*)-configured enantiomer of **21**, was obtained from 4-bromostyrene (**13**) in principally the same way by using AD-mix-β in the dihydroxylation step.



Scheme 1: Reagents and conditions: (a) methyltriphenylphosphonium bromide, KO*t*Bu, THF, -10 °C → rt, **12** 94%; (b) AD-mix-α, *t*BuOH/H_2O (1:1), 0 °C, **14** 80%, **15** 88%; (c) *p*-TsCl, Bu_2SnO, NEt_3, THF, rt, **16** 76%, **17** 85%; (d) NaN_3, DMSO, 80 °C, **18** 70%, **19** 85%; (e) ethyl bromoacetate, base, NBu_4I, DMF, **20** 68%, **21** 64%.

In order to obtain the 4-phenyl-substituted 1,2,3-triazole derivatives **23** and **27**, azide **20** was subjected to a Cu(I)-catalyzed azide-alkyne cycloaddition with phenylacetylene (Scheme 2). The resulting triazole derivative **22** could be transformed into hydroxamic acid **23** by performing an aminolysis with hydroxylamine. Additionally, ester **22** was used to access diphenylacetylene derivative **27**. At first, its benzyl protective group was hydrogenolytically cleaved to obtain phenol **24**. Subsequently, phenol **24** was treated with trifluoromethanesulfonic anhydride to give triflate **25**, which was then subjected to a Sonogashira coupling with phenylacetylene yielding diphenylacetylene derivative **26**. A final aminolysis of ester **26** with hydroxylamine gave hydroxamic acid **27**.

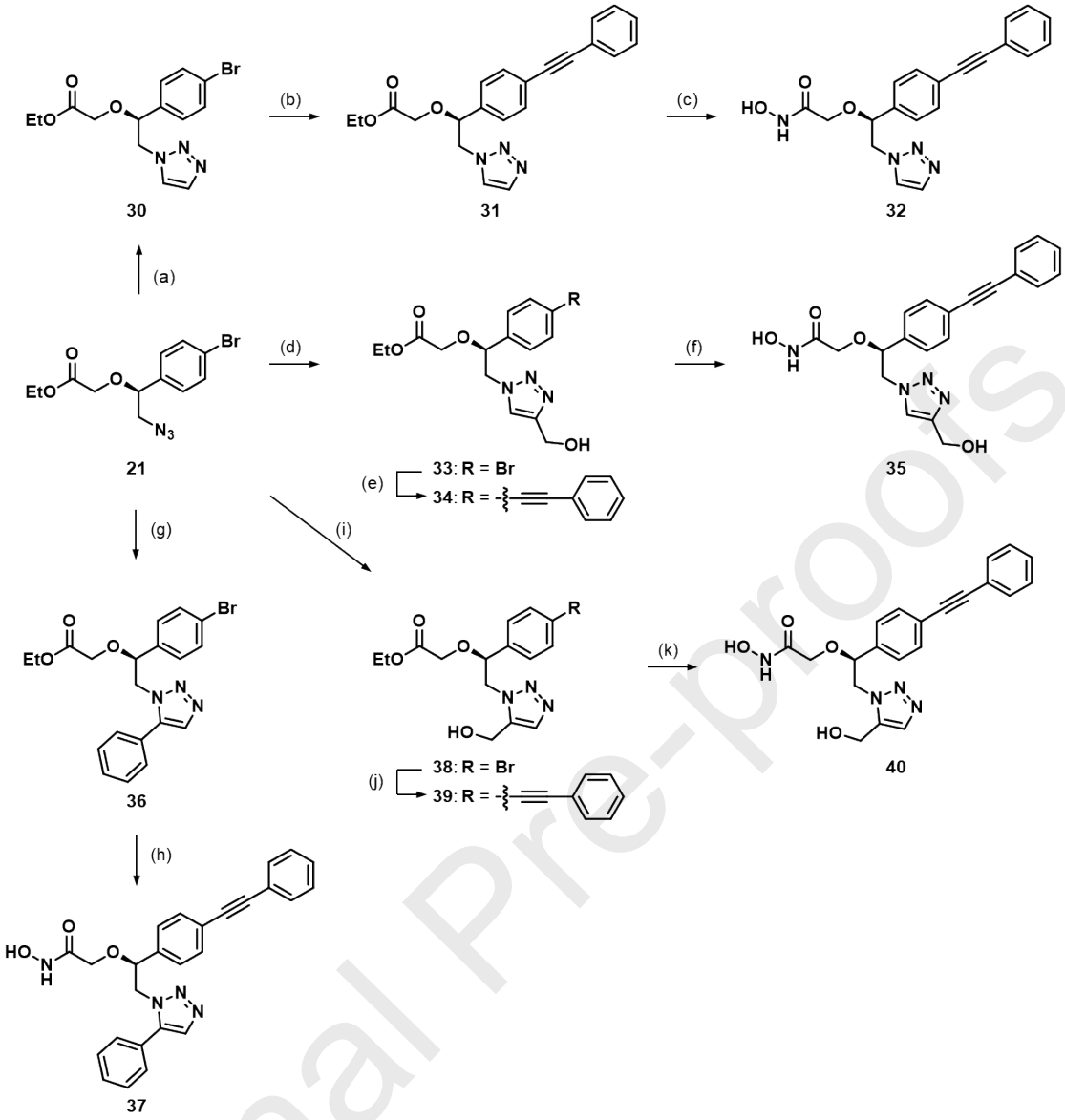


Scheme 2: Reagents and conditions: (a) phenylacetylene, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 92%; (b) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 26%; (c) H_2, Pd/C, MeOH, rt, 78%; (d) Tf_2O, NEt_3, CH_2Cl_2, -20 °C, 92%; (e) phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 63%; (f) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 54%; (g) trimethylsilylacetylene, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 38%; (h) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 64%.

Benzyl ether **29** was also accessed from azide **20** (Scheme 2). In order to obtain the respective triazole derivative, a Cu(I)-catalyzed azide-alkyne cycloaddition with azide **20** and trimethylsilylacetylene was performed. However, under the conditions of the cycloaddition, additionally the cleavage of the trimethylsilyl protective group occurred yielding the 1-monosubstituted triazole derivative **28**. Subsequently, ester **28** was transformed into hydroxamic acid **29** by treating the compound with hydroxylamine.

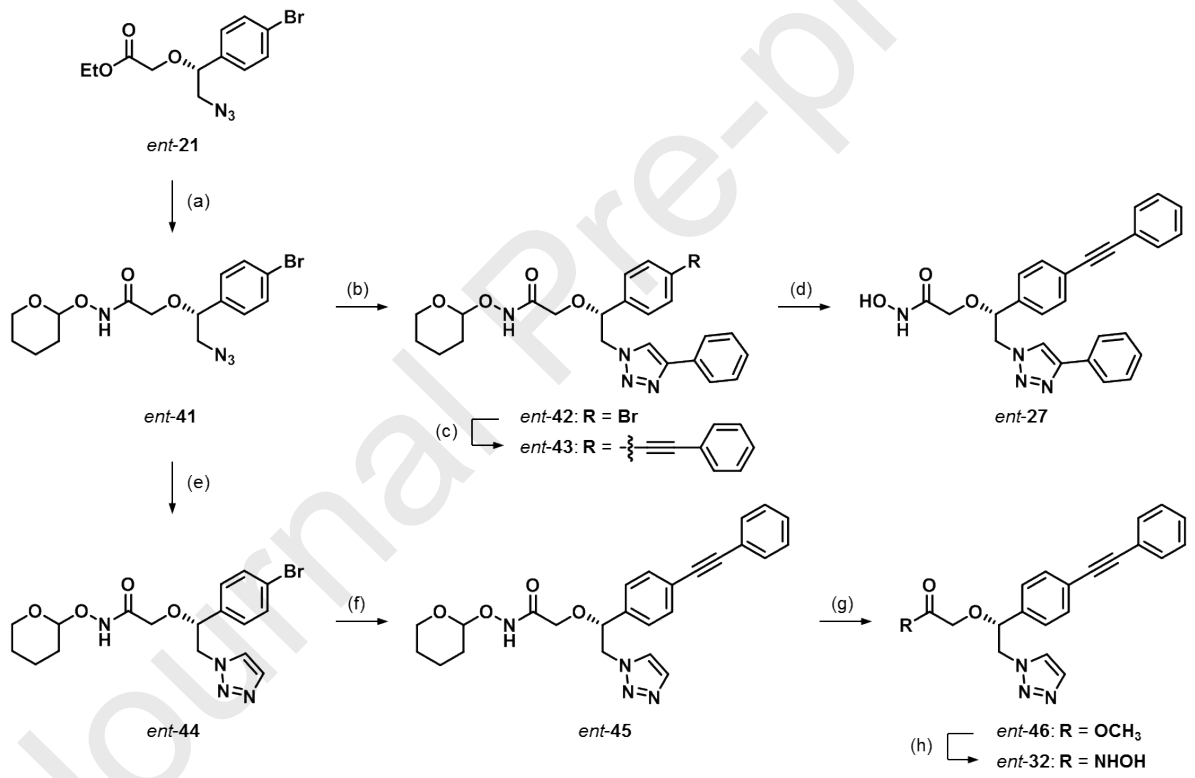
In order to shorten the synthesis of the respective diphenylacetylene derivative **32**, the hydroxamic acid was synthesized from 4-bromophenyl derivative **21** (Scheme 3). After reacting azide **21** with trimethylsilylacetylene in a Cu(I)-catalyzed azide-alkyne cycloaddition, the resulting crude triazole derivative was directly treated with tetrabutylammonium fluoride to completely cleave the trimethylsilyl protective group. The thereby obtained 1-monosubstituted triazole derivative **30** was subjected to a Sonogashira coupling with phenylacetylene. The reaction was performed in refluxing triethylamine using tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide as catalysts. The resulting diphenylacetylene derivative **31** was finally transformed into hydroxamate **32** by performing an aminolysis with hydroxylamine.

The 4-hydroxymethyl-substituted 1,2,3-triazole derivative **35** was obtained in principally the same way. After a Cu(I)-catalyzed azide-alkyne cycloaddition of azide **21** and propargyl alcohol, the resulting triazole derivative **33** was coupled with phenylacetylene. In the Sonogashira coupling of aryl bromide **33** bis(triphenylphosphine)palladium(II) dichloride and copper(I) iodide were used as catalysts and the transformation was conducted in a refluxing mixture of diisopropylamine and THF. Under these conditions, a higher yield could be obtained compared to the respective C-C coupling of aryl bromide **30**. The obtained diphenylacetylene derivative **34** was subjected to a final aminolysis with hydroxylamine, yielding hydroxamic acid **35**.



Scheme 3: Reagents and conditions: (a) 1. trimethylsilylacetylene, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 2. NBu_4F, rt, 92%; (b) phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 58%; (c) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 72%; (d) propargyl alcohol, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 93%; (e) phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, diisopropylamine, THF, Δ, 93%; (f) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 52%; (g) phenylacetylene, $Cp^*RuCl(PPh_3)_2$, THF, Δ, 61%; (h) 1. phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, diisopropylamine, THF, Δ, 2. $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 25%; (i) propargyl alcohol, $Cp^*RuCl(PPh_3)_2$, dioxane, 60 °C (microwave), 26%; (j) phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, THF, DIPA, 80 °C, 71%; (k) NH_2OH aq., *i*PrOH/THF, rt, 73%.

The 1,5-disubstituted 1,2,3-triazole derivatives **37** and **40** were finally obtained via ruthenium-catalyzed azide-alkyne cycloadditions. The cycloaddition of azide **21** and phenylacetylene gave the 5-phenyl-substituted 1,2,3-triazole **36**, which was subjected to a Sonogashira coupling and a subsequent aminolysis with hydroxylamine to yield hydroxamic acid **37**. The 5-hydroxymethyl-substituted 1,2,3-triazole derivative **38** was obtained via a ruthenium-catalyzed azide-alkyne cycloaddition of azide **21** and propargyl alcohol. A subsequent Sonogashira coupling of aryl bromide **38** with phenylacetylene yielded diphenylacetylene derivative **39**. The performance of the final aminolysis in a mixture of isopropanol and THF, employing a 50% aqueous solution of hydroxylamine, gave the desired hydroxamic acid **40**.



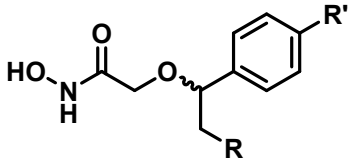
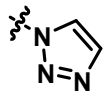
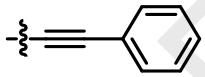
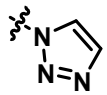
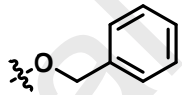
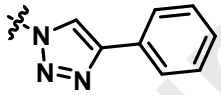
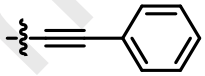
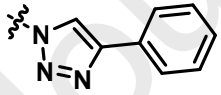
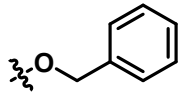
Scheme 4: Reagents and conditions: (a) *O*-THP-hydroxylamine, LiHMDS, THF, -78 °C, 90%; (b) phenylacetylene, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 82%; (c) phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 64%; (d) HCl, MeOH, rt, 38%; (e) acetylene, sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O (1:1), rt, 48%; (f) phenylacetylene, $Pd(PPh_3)_4$, CuI, NEt_3, Δ, 24%; (g) HCl, MeOH, rt, 62%; (h) $NH_2OH{\cdot}HCl$, NaOMe, MeOH, rt, 42%.

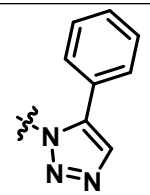
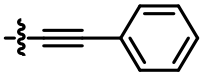
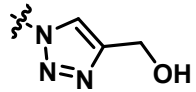
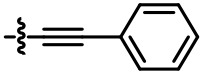
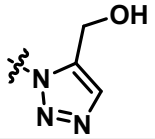
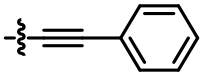
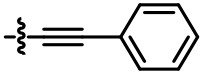
ent-**27** and *ent*-**32**, the (*R*)-configured enantiomers of **27** and **32**, were synthesized from *ent*-**21** via a different synthetic route to test the feasibility of establishing the hydroxamate moiety at an earlier reaction step. At first, ester *ent*-**21** was reacted with *O*-THP-hydroxylamine yielding the tetrahydropyranyl-protected hydroxamic acid *ent*-**41**. After the establishment of the 4-phenyl-substituted 1,2,3-triazole ring and the diphenylacetylene side chain, the THP protective group of compound *ent*-**43** was cleaved under acidic conditions yielding hydroxamic acid *ent*-**27**. Similarly, in order to obtain hydroxamic acid *ent*-**32**, azide *ent*-**41** was reacted with gaseous acetylene in a Cu(I)-catalyzed azide-alkyne cycloaddition. Subsequently, a Sonogashira coupling of the resulting aryl bromide *ent*-**44** with phenylacetylene was performed. Finally, the THP protective group of the obtained diphenylacetylene derivative *ent*-**45** should be cleaved. However, the reaction of the 1-monosubstituted triazole derivative in HCl-saturated methanol led to a solvolysis yielding methyl ester *ent*-**46**. From this compound, the desired hydroxamic acid *ent*-**32** could be accessed via an aminolysis with hydroxylamine.

2.2. Biological evaluation

Table 1: Antibacterial and LpxC inhibitory activities of the investigated hydroxamic acids.

* The compound could not be tested at a concentration of 200 µM due to solubility issues.

cmpd.	config.	 R	R'	zone of inhibition [mm]		MIC [µg/mL]		IC_{50} [µM]
				E. coli BL21(DE3)	*E. coli* D22	*E. coli* BL21(DE3)	*E. coli* D22	*E. coli* LpxCC63A
32	(*S*)			<6	21.0 ± 1.0	>64	1	36.0 ± 1.7
ent-**32**	(*R*)			<6	11.0 ± 1.4	>64	≥32	>200
29	(*S*)			<6	15.0 ± 2.0	>64	>64	>200
27	(*S*)			<6	12.3 ± 0.6	>64	0.25	10.4 ± 9.7
ent-**27**	(*R*)			<6	8.7 ± 1.2	>64	32	>200
23	(*S*)			<6	10.0 ± 1.0	>64	32	>200

37	(*S*)			<6	10.7 ± 0.6	>64	8	>20*
35	(*S*)			<6	20.7 ± 0.6	>64	4	8.5 ± 1.3
40	(*S*)			10.3 ± 1.2	29.5 ± 3.9	>64	1	23.4 ± 5.8
9	(*S*)	**OH**		9.5 ± 0.4	20.5 ± 0.2	64	1	86.2 ± 1.9
ent-**9**	(*R*)			<6	13.0 ± 1.7	>64	32	>200
CHIR-090 (**5**)				31.7 ± 1.7	37.5 ± 1.5	0.25	0.032	0.076 ± 0.003

The antibacterial properties of lead compound **9** and the synthesized triazole derivatives were evaluated by performing broth microdilution susceptibility tests as well as disk diffusion assays against *E. coli* BL21(DE3) and the defective *E. coli* D22 strain, which due to an impaired lipid A biosynthesis is more sensitive towards various antibiotics (Table 1).[41] Additionally, a fluorescence-based *in vitro* enzyme assay was carried out to determine the inhibitory activity of the hydroxamic acids toward LpxC.[29]

The biological evaluation of the triazole derivatives revealed that the (*S*)-configured hydroxamic acids **32** and **27** show superior antibacterial as well as LpxC inhibitory activities compared to their (*R*)-configured enantiomers *ent*-**32** and *ent*-**27**. The same trend can be also observed in case of lead compound **9** and its enantiomer *ent*-**9**. Additionally, it was found that triazole derivatives **29** and **23**, possessing a flexible 4-(benzyloxy)phenyl-based lipophilic side chain, show diminished antibacterial and LpxC inhibitory activities compared to the respective diphenylacetylene derivatives **32** and **27**, which exhibit a linear and rigid side chain. Both observations are in agreement with our previous findings.[40, 42]

The reevaluation of the LpxC inhibitory activity of lead compound **9** revealed an IC_{50}-value being considerably higher than the one observed in previous investigations.[40] The comparison of the biological activities of the 1-monosubstituted triazole derivative **32** with the ones of lead compound **9** gave contradictory results. Whereas the replacement of the hydroxy group of compound **9** by a triazole ring leads to an increase in LpxC inhibitory activity, no change in the antibacterial activity against *E. coli* D22 and a decrease in the diameter of the zone of growth inhibition in the disk diffusion assays against *E. coli* BL21(DE3) can be observed.

The introduction of a phenyl substituent in position 4 of the triazole ring, leading to compound **27**, seems to be beneficial as it causes an increase in inhibitory activity toward LpxC and a reduction of the MIC against *E. coli* D22 in comparison with the 1-monosubstituted triazole derivative **32**. The decreased diameter of the zone of growth inhibition against the latter strain might result from a decreased diffusion rate of the substituted compound in the agar medium. In contrast, the 5-phenyl-substituted derivative **37** was found to be unable to inhibit the enzymatic activity of LpxC at the highest concentration tested and to exhibit a higher MIC-value against *E. coli* D22 than the 1-monosubstituted triazole derivative **32** as well as the 1,4-disubstituted derivative **27**.

With respect to the inhibitory activity toward LpxC, similar trends can be observed for the hydroxymethyl-substituted triazole derivatives **35** and **40**, with the 1,4-disubstituted compound **35** being a more potent LpxC inhibitor than its 1,5-disubstituted regioisomer **40**. Both hydroxymethyl-substituted compounds exceed the inhibitory activity of the 4,5-unsubstituted triazole derivative **32**. When assaying the antibacterial activities of regioisomers **35** and **40** contradictory results were observed. Whereas the 1,5-disubstituted triazole derivative **40** was found to exhibit the same MIC-value against *E. coli* D22 as compound **32**, the more potent LpxC inhibitor **35** was shown to exhibit an increased MIC-value. Both hydroxymethyl-substituted compounds caused larger halos of growth inhibition in the disc diffusion assays than the respective phenyl-substituted compounds, indicating that the presence of a polar hydroxy group leads to a higher diffusion rate in the agar medium.

Most of the synthesized (*S*)-configured triazole derivatives exhibiting a diphenylacetylene-based side chain are more potent LpxC inhibitors than lead compound **9**. Among those compounds, the 4-phenyl-substituted derivative **27** shows a particularly lower MIC-value against *E. coli* D22, whereas the 5-hydroxymethyl-substituted triazole derivative **40** caused considerably larger halos of growth inhibition in the disc diffusion assays against *E. coli* D22 as well as *E. coli* BL21(DE3) than lead compound **9**. However, all triazole derivatives were significantly less potent with respect to antibacterial and inhibitory activity than reference compound CHIR-090 (**5**).

2.3. Molecular docking studies

In order to rationalize the experimentally obtained IC_{50}-values for *E. coli* LpxC, molecular docking of all synthesized compounds into the LpxC structure (PDB ID: 3P3G) co-crystallized with the potent inhibitor LPC-009 (**6**, Figure 2) was performed.[36] All ligands except the two inactive compounds **23** and **29** could be docked into the binding pocket of LpxC. Only **23** and **29** possess a 4-(benzyloxy)phenyl group instead of a diphenylacetylene moiety, which did not fit to the hydrophobic tunnel, although different docking protocols were tried. The lipophilic distal parts of all other compounds are placed in the hydrophobic tunnel formed by L18, F192, I198, F212, and V217. The hydroxamic acid group of the docked inhibitors shows a bidentate chelation of the Zn^{2+}-ion. The hydroxy and carbonyl group of the hydroxamate moiety in all compounds interact with the side chains of E78 and T191, respectively. Different interactions, however, are observed for the middle part of the inhibitors, which together with calculated binding free energy values allow to discriminate active and inactive compounds.

The (*S*)-configured hydroxamic acid **32** having a monosubstituted triazole ring and its enantiomer *ent*-**32** exhibit a similar binding mode in LpxC (Fig. S1, Supporting information). In both cases, the triazole ring is directed to the UDP-binding site and is placed in a small pocket formed by conserved hydrophobic patch residue F192 and basic patch residue K239. The triazole ring of the active compound **32** is turned toward F192 and is able to make an edge-to-face aromatic interaction similar to the potent *E. coli* LpxC inhibitor LPC-051 (**7**, Figure 2) in its crystal structure of LpxC from *Pseudomonas aeruginosa* (PDB ID: 4LCH).[39] In contrast, the triazole ring of the inactive compound *ent*-**32** is oriented less favorably and the aromatic interaction with F192 is not observed. The other difference observed is the solvent exposure of the carbon-oxygen-carbon (C-O-C) linker. In the active compound **32** the oxygen atom is directed toward T191 while the carbon atoms are buried in the hydrophobic part of the pocket. In the inactive compound *ent*-**32**, the orientation of these atoms is reversed. That is, the oxygen atom is directed toward the hydrophobic C63 residue, while the carbon atoms are solvent-exposed.

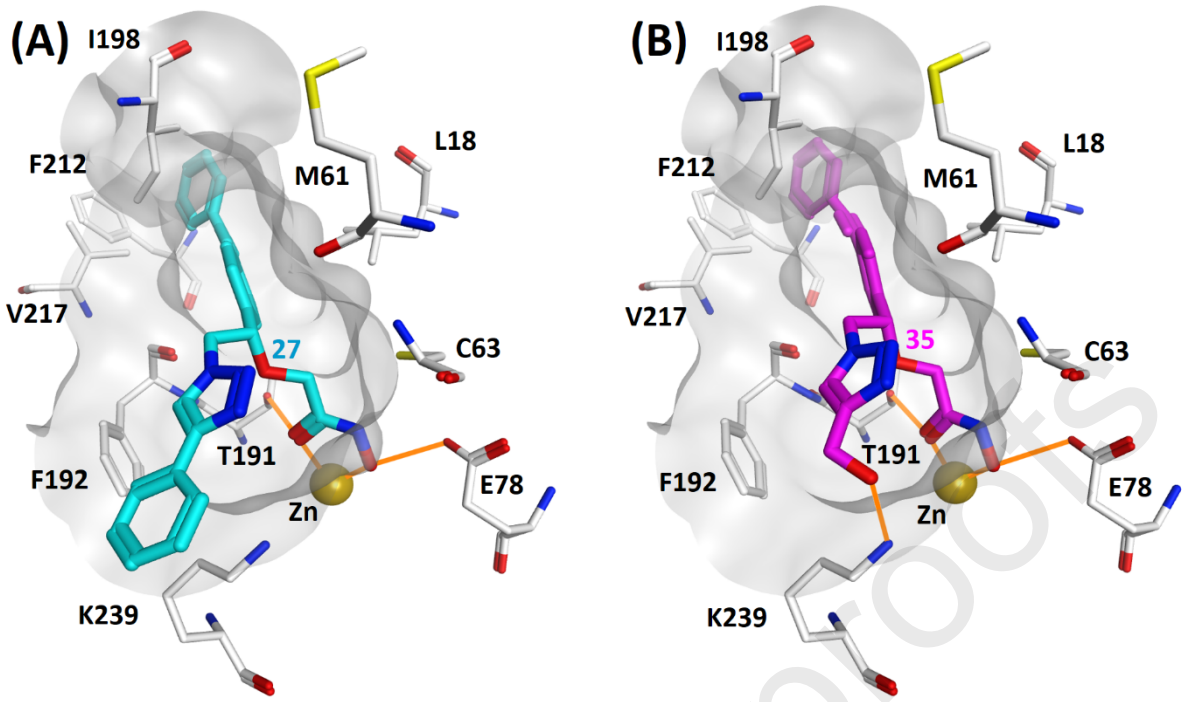


Figure 3: Predicted binding mode of LpxC inhibitors **27** (colored cyan, A) and **35** (colored magenta, B). The surface of the binding pocket is displayed and colored grey. LpxC-inhibitor hydrogen bonds and Zinc-coordination are shown as orange colored lines.

The active inhibitor **27** having a 4-(phenyl)triazole group and its (*R*)-isomer *ent*-**27** demonstrate docking poses similar to their analogues **32** and *ent*-**32**. The C-O-C linker and the triazole ring of **27** are placed in the same region as observed for **32**. The triazole ring is making aromatic interactions with F192, while the attached phenyl ring is additionally involved in cation-π interactions with K239. These interactions might contribute to the improved activity of **27** which is also reflected by a more favourable calculated binding energy (-159.1 kcal/mol) (Figure 3, Table 2). The triazole ring of the inactive compound *ent*-**27** is flipped due to the bulky phenyl substituent and the aromatic interaction with F192 is lost (Fig. S1, Supporting information). The aromatic interaction with F192 seems to play a crucial role for the activity of the studied inhibitors. This also explains why the replacement of the hydroxy group of **9** by a triazole ring in **32** increased the inhibitory activity. Due to the lack of an aromatic substituent in **9**, the interaction with F192 is missing, the UDP-binding site remains unoccupied, and the calculated interaction energy value is increased (-143.6 kcal/mol).

The hydroxy group of **9** forms hydrogen bonds with the backbone carbonyl oxygen of M61 and the backbone nitrogen of C63 as reported previously.[33]

In **37** the shifted position of the phenyl ring from position 4 to 5 of the triazole ring prevents the ligand from making an interaction with F192, which results in decreased activity. The triazole ring of **37** is turned toward C63 and is not engaged in any direct interaction (Fig. S1, Supporting information). The binding energy calculation confirms the less favourable binding mode of **37** (-138.1 kcal/mol).

The same trend is observed for the hydroxymethyl-substituted compounds when shifting the hydroxymethyl group from position 4 in compound **35** to position 5 in **40** which slightly reduces the activity (Figure 3). The orientation of the triazole ring in the most active compound from this series, **35**, is similar to the other active triazole containing derivatives **32** and **27**. In addition, the hydroxy group attached to the triazole ring in **35** is engaged in a hydrogen bond interaction with K239. An interaction with this residue is also observed for the phosphate and glucosamine moieties of the deacetylated substrate **3** (Figure 1) as well as for the potent reference inhibitor LPC-009 (**6**, Figure 2).[25, 36] In contrast, the triazole ring of **40** is flipped by 180 degrees (Fig. S1, Supporting information). The triazole orientation observed for other active compounds leading to an interaction with F192 is not possible due to the hydroxymethyl substituent at position 5. Nevertheless, the triazole ring of **40** stays in the UDP-binding site. Its hydroxy group interacts with the backbone carbonyl of M61 and backbone nitrogen of C63 like in case of **9**. Additionally, the nitrogen of the triazole ring is interacting with K239. These three hydrogen bond interactions are probably compensating for the lost aromatic interaction with F192. Favourable interaction energies have been calculated for both active compounds **35** (-150.6 kcal/mol) and **40** (-159.1 kcal/mol).

The calculated interaction energy values and docking scores are listed in Table 2. Whereas the docking scores were not able to discriminate between active and inactive inhibitors, the calculated binding energies worked well. More favourable binding free energies (< -150 kcal/mol) were observed for the active inhibitors compared to the inactive ones. The only exception is the moderately active non-triazole inhibitor **9** (-143.6 kcal/mol), which, however gave better binding energy value than its inactive enantiomer *ent*-**9** (-136.7 kcal/mol).

Table 2: Calculated docking scores and protein-ligand interaction energies (Amber12:EHT/MOE) of the studied compounds.

compound	experimental pIC_{50}	binding free energy ΔG [kcal/mol]	Glide docking score	calculated ligand interaction energy ΔH [kcal/mol]
32	4.4	-6.07	-6.7	-150.2
ent-**32**	< 3.7	> -5.10	-6.8	-141.7
29	< 3.7	> -5.10	n.a.	n.a.
27	5.0	6.89	-6.3	-159.1
ent-**27**	< 3.7	> -5.10	-6.4	-144.1
23	< 3.7	> -5.10	n.a.	n.a.
37	< 4.7	> -6.48	-5.4	-138.1
35	5.1	-7.03	-6.7	-150.6
40	4.6	-6.34	-6.7	-159.1
9	4.1	-5.65	-6.5	-143.6
ent-**9**	< 3.7	> -5.10	-6.4	-136.7

3. Discussion and conclusions

In divergent syntheses, a series of triazole-based LpxC inhibitors was accessed. Starting from the enantiomerically pure azides **20**, **21**, and *ent*-**21**, which represent the central intermediates of the synthetic routes, via copper- or ruthenium-catalyzed azide-alkyne cycloadditions, Sonogashira couplings with phenylacetylene and final aminolyses with hydroxylamine, most of the synthesized hydroxamic acids were obtained. When synthesizing the various hydroxamic acids, different reaction conditions for analogous reaction steps were employed.

Thus, it was observed, that when performing the Sonogashira coupling of the aryl bromides in the presence of bis(triphenylphosphine)palladium(II) chloride in a mixture of THF and diisopropylamine (cf. syntheses of **34** and **39**) higher yields were obtained compared to the usage of tetrakis(triphenylphosphine)palladium(0) as catalyst and triethylamine as solvent (cf. syntheses of *ent*-**43**, *ent*-**45**, and **31**). The route via triflate **25** was found to be disadvantageous. Although the triflate should be more reactive than the respective aryl bromides, the Sonogashira coupling of compound **25** gave the desired diphenylacetylene derivative in a similar yield as the analogous coupling of aryl bromide *ent*-**42**. Additionally, this synthetic route required further reaction steps, including the debenzylation of ether **22**, the triflation of phenol **24**, and the synthesis of styrene derivative **12**, which in contrast to 4-bromostyrene (**13**) was not commercially available.

Most of the performed aminolyses with hydroxylamine were performed employing hydroxylamine hydrochloride and sodium methoxide in methanol and gave the final hydroxamic acids in variable yields ranging from 26% to 72%. However, when the reaction was performed in a mixture of isopropanol and THF using an aqueous solution of hydroxylamine, hydroxamic acid **40** was obtained in a relatively high yield (73%). In an alternative strategy, a THP-protected hydroxamate moiety was established in an early reaction step and the protective group should be cleaved in the end. Whereas the reaction of ester *ent*-**21** with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine gave THP-protected hydroxamic acid *ent*-**41** in high yield (90%), the subsequent acid-catalyzed cleavage of the protective group in case of triazole derivative *ent*-**43** gave hydroxamic acid *ent*-**27** in only 38% yield and completely failed in case of triazole derivative *ent*-**45** yielding ester *ent*-**46** instead.

When synthesizing the disubstituted triazole derivatives, the performed Cu(I)-catalyzed azide-alkyne cycloadditions gave the 1,4-disubstituted regioisomers in higher yields than the ruthenium-catalyzed cycloadditions leading to the respective 1,5-disubstituted triazole derivatives. When synthesizing the 1-monosubstituted triazole derivatives, it was found that a two-step sequence including a Cu(I)-catalyzed azide-alkyne cycloaddition with trimethylsilylacetylene and a subsequent desilylation (cf. syntheses of **30**) gave the desired triazole derivative in a higher yield than the direct coupling with acetylene (cf. syntheses of *ent*-**44**).

The biological evaluation of the triazolyl-substituted benzyloxyacetohydroxamic acids revealed that (*S*)-configuration at the stereocenter as well as a linear and rigid diphenylacetylene-based side chain lead to superior antibacterial as well as LpxC inhibitory activities.

The replacement of the hydroxy group of lead compound **9** by a triazole ring generally led to an increase in inhibitory activity against LpxC. Particularly, the 1,4-disubstituted triazole derivatives **27** and **35**, bearing a phenyl ring and a hydroxymethyl group in position 4, respectively, showed relatively high inhibitory activities, exhibiting lower IC_{50} values than their 1,5-disubstituted regioisomers as well as the 4,5-unsubstituted triazole derivative **32**.

With respect to antibacterial activities, the 4-phenyl substituted triazole derivative **27** exhibited a considerably low MIC value against the defective *E. coli* D22 strain, whereas the 5-hydroxymethyl-substituted compound **40** was found to cause the largest halos of growth inhibition in the disc diffusion assays against *E. coli* BL21(DE3) and the D22 strain.

Molecular docking studies revealed that aromatic interactions between the triazole ring of the synthesized compounds and F192, which is part of a highly conserved hydrophobic patch within the active site of LpxC, play a crucial role for the activity of the studied inhibitors. In the predicted binding modes, the triazole ring of the active compounds **27**, **32**, and **35** is turned toward this important residue and is able to make edge-to-face aromatic interactions with it. Additionally, interactions of the substituent at the triazole ring with the basic patch residue K239 (cation-π or hydrogen bond interactions) seem to enhance activity.

Finally, in case of the studied benzyloxyacetohydroxamic acids, active inhibitors could be discriminated from inactive ones based on their calculated protein-ligand interaction energy values.

In conclusion, the triazole moiety was found to be a suitable functional group to replace the hydroxy group of hydroxamic acid **9** and to link various substituents to its main scaffold, which can address additional binding sites of LpxC.

4. Experimental Section

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). Reversed phase thin layer chromatography (RP-TLC): Silica gel 60 RP-18 $F_{254}S$ 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. Automatic flash column chromatography: Isolera™ One (Biotage®); brackets include: eluent, cartridge-type; product-containing fractions were freeze-dried using a Christ Alpha 2-4 LDplus freeze-dryer. Microwave assisted reactions were conducted in a CEM discover system in closed-vessel mode. 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): Mercury plus 400 spectrometer (Varian); 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); LiChroCART® 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%.

Synthetic procedures

1-(Benzyloxy)-4-vinylbenzene (12)

Under N_2 atmosphere, a solution of methyltriphenylphosphonium bromide (10 g, 28 mmol) in dry THF (100 mL) was cooled to -10 °C and potassium *tert*-butoxide (4.0 g, 35 mmol) was added. After 5 min, 4-(benzyloxy)benzaldehyde (5.0 g, 24 mmol) was added and the reaction 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 over Na_2SO_4, filtered, and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (Ø = 6 cm, h = 15 cm, V = 50 mL, cyclohexane/ethyl acetate = 95/5, R_f = 0.29) to give **12** as colorless solid (4.6 g, 22 mmol, 94%). m.p. = 67 °C; ^{1}H NMR (D_3COD): δ [ppm] = 5.07 (s, 2H, PhC*H*$_2$OPh), 5.08 (dd, *J* = 10.9/1.1 Hz, 1H, CH=C*H*$_2$), 5.61 (dd, *J* = 17.6/1.1 Hz, 1H, CH=C*H*$_2$), 6.66 (dd, *J* = 17.6/10.9 Hz, 1H, C*H*=CH$_2$), 6.92 – 6.96 (m, 2H, $H_{arom.}$), 7.27 – 7.39 (m, 5H, $H_{arom.}$), 7.41 – 7.44 (m, 2H, $H_{arom.}$); ^{13}C NMR (D_3COD): δ [ppm] = 71.0 (1C, Ph*C*H$_2$O), 111.7 (1C, CH=*C*H$_2$), 115.9 (2C, $C_{arom.}$), 128.4 (2C, $C_{arom.}$), 128.5 (2C, $C_{arom.}$), 128.9 (1C, $C_{arom.}$), 129.5 (2C, $C_{arom.}$), 132.1 (1C, $C_{arom.}$), 137.6 (1C, *C*H=CH$_2$), 138.7 (1C, $C_{arom.}$), 160.0 (1C, $C_{arom.}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3036, 2859, 1605, 1508,

1454, 1381, 1238, 1169, 1022, 995, 899, 833, 729, 694; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{15}H_{15}O$: 211.1117, found: 211.1113; HPLC (method 1): t_R = 21.5 min, purity 97.1%.

(*S*)-1-[4-(Benzyloxy)phenyl]ethane-1,2-diol (14)

AD-mix-α (28 g) was added to a mixture of *tert*-butyl alcohol (100 mL) and water (100 mL). The mixture was cooled to 0 °C, **12** (4.2 g, 20 mmol) and TBME (23.7 mL) were added. After stirring at 0 °C for 16 h, sodium sulfite (30 g, 240 mmol) was added and the reaction mixture was stirred for 1 h. Ethyl acetate was added and after the separation of the layers, the aqueous phase was further extracted with ethyl acetate (3×). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was removed in vacuo. The residue was purified by flash column chromatography (*Ø* = 6 cm, h = 15 cm, V= 50 mL, cyclohexane/ethyl acetate = 1/2, R_f = 0.48) to give **14** as colorless solid (3.9 g, 16 mmol, 80%). m.p. = 130 °C; $[\alpha]_D^{20}$ = +27.5 (7.8, methanol); ^{1}H NMR (D_3COD): δ [ppm] = 3.59 (d, *J* = 6.1 Hz, 2H, HOCHC*H*$_2$OH), 4.62 (t, *J* = 6.1 Hz, 1H, HOC*H*CH_2OH), 5.07 (s, 2H, PhC*H*$_2$OPh), 6.94 – 6.98 (m, 2H, $H_{arom.}$), 7.26 – 7.31 (m, 3H, $H_{arom.}$), 7.33 – 7.38 (m, 2H, $H_{arom.}$), 7.40 – 7.44 (m, 2H, $H_{arom.}$); ^{13}C NMR (D_3COD): δ [ppm] = 68.7 (1C, HOCH*C*H_2OH), 71.0 (1C, Ph*C*H_2OPh), 75.5 (1C, HO*C*HCH_2OH), 115.7 (2C, $C_{arom.}$), 128.5 (2C, $C_{arom.}$), 128.6 (2C, $C_{arom.}$), 128.8 (1C, $C_{arom.}$), 129.5 (2C, $C_{arom.}$), 135.6 (1C, $C_{arom.}$), 138.8 (1C, $C_{arom.}$), 159.7 (1C, $C_{arom.}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3302, 2905, 2866, 1609, 1508, 1454, 1377, 1238, 1165, 1072, 1042, 1007, 818, 741, 694, 629; HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{15}H_{16}NaO_3$: 267.0992, found: 267.0996; HPLC (method 1): t_R = 15.8 min, purity 99.8%.

(*S*)-2-[4-(Benzyloxy)phenyl]-2-hydroxyethyl 4-methylbenzenesulfonate (16)

Dibutyltin oxide (0.20 g, 0.8 mmol), *p*-toluenesulfonyl chloride (3.7 g, 19 mmol) and triethylamine (6.5 mL, 47 mmol) were added to a solution of **14** (3.5 g, 14 mol) in THF (75 mL). After stirring the mixture for 16 h at ambient temperature, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over Na_2SO_4, 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 = 0.49) to give **16** as colorless solid (4.3 g, 11 mmol, 76%). m.p. = 89 °C; $[\alpha]_D^{20}$ = +22.1 (3.4, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.45 (s, 3H, PhC*H*$_3$), 4.03 (dd, *J* = 10.4/8.5 Hz, 1H, HOCHC*H*$_2$OSO$_2$), 4.11 (dd, *J* = 10.4/3.4 Hz, 1H, HOCHC*H*$_2$OSO$_2$), 4.92 (dd, *J* = 8.5/3.4 Hz, 1H, HOC*H*CH$_2$OSO$_2$), 5.05 (s, 2H, PhC*H*$_2$OPh), 6.91 – 6.96 (m, 2H, $H_{arom.}$), 7.21 – 7.24 (m, 2H, $H_{arom.}$), 7.30 – 7.35 (m, 3H, $H_{arom.}$), 7.36 – 7.44 (m, 4H, $H_{arom.}$), 7.75 – 7.79 (m, 2H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 21.8 (1C, PhCH$_3$), 70.2 (1C, PhCH$_2$OPh), 71.7 (1C, HOCHCH$_2$OSO$_2$), 74.5 (1C, HOCHCH$_2$OSO$_2$), 115.2 (2C, $C_{arom.}$), 127.6 (2C, $C_{arom.}$), 127.7 (2C, $C_{arom.}$), 128.1 (2C, $C_{arom.}$), 128.2 (1C, $C_{arom.}$), 128.8 (2C, $C_{arom.}$), 130.1 (2C, $C_{arom.}$), 130.7 (1C, $C_{arom.}$), 132.8 (1C, $C_{arom.}$), 136.9 (1C, $C_{arom.}$), 145.2 (1C, $C_{arom.}$), 159.1 (1C, $C_{arom.}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3480, 3429, 1612, 1512, 1377, 1323, 1246, 1169, 1096, 1011, 914, 814, 745, 691; HRMS (*m*/*z*): $[M+NH_4]^+$ calcd for $C_{22}H_{26}NO_5S$: 416.1526, found: 416.1567; HPLC (method 1): t_R = 20.7 min, purity 96.0%.

(*S*)-2-Azido-1-[4-(benzyloxy)phenyl]ethan-1-ol (18)

Sodium azide (1.4 g, 21 mmol) was added to a solution of **16** (4.2 g, 11 mmol) in DMSO (53 mL) and the reaction mixture was heated to 80 °C for 3 h. Then, the reaction mixture was cooled to ambient temperature, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over

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 = 8/2, R_f = 0.40) to give **18** as colorless solid (2.0 g, 7.4 mmol, 70%). m.p. = 65 °C; $[\alpha]_D^{20}$ = +37.8 (2.6, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.41 (dd, *J* = 12.6/4.0 Hz, 1H, HOCHC*H*$_2$N$_3$), 3.48 (dd, *J* = 12.6/8.2 Hz, 1H, HOCHC*H*$_2$N$_3$), 4.83 (dd, *J* = 8.2/4.0 Hz, 1H, HOC*H*CH$_2$N$_3$), 5.07 (s, 2H, PhC*H*$_2$OPh), 6.96 – 7.00 (m, 2H, $H_{arom.}$), 7.27 – 7.35 (m, 3H, $H_{arom.}$), 7.36 – 7.45 (m, 4H, $H_{arom.}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 58.2 (1C, HOCH*C*H$_2$N$_3$), 70.2 (1C, Ph*C*H$_2$OPh), 73.2 (1C, HO*C*HCH$_2$N$_3$), 115.2 (2C, $C_{arom.}$), 127.4 (2C, $C_{arom.}$), 127.6 (2C, $C_{arom.}$), 128.2 (1C, $C_{arom.}$), 128.8 (2C, $C_{arom.}$), 133.1 (1C, $C_{arom.}$), 137.0 (1C, $C_{arom.}$), 159.0 (1C, $C_{arom.}$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3414, 2978, 2920, 2087, 1609, 1508, 1454, 1385, 1304, 1234, 1173, 1115, 1080, 1057, 1015, 880, 864, 826, 748, 698, 625; HRMS (*m*/*z*): [M+Na]$^+$ calcd for $C_{15}H_{15}N_3NaO_2$: 292.1056, found: 292.1057; HPLC (method 1): t_R = 19.0 min, purity 99.0%.

Ethyl (*S*)-2-{2-azido-1-[4-(benzyloxy)phenyl]ethoxy}acetate (20)

Under N_2 atmosphere, sodium hydride (55% suspension in paraffin oil, 0.10 g, 2.3 mmol), tetrabutylammonium iodide (0.13 g, 0.35 mmol) and ethyl bromoacetate (0.78 mL, 7.0 mmol) were added to a solution of **18** (0.94 g, 3.5 mmol) in DMF (30 mL). After stirring at ambient temperature for 16 h, water was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over 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 = 20/1 → 9/1) to give **20** as colorless oil (0.84 g, 2.4 mmol, 68%). TLC (cyclohexane/ethyl acetate = 8:2): R_f = 0.54; $[\alpha]_D^{20}$ = +91.9 (5.4, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.27 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 3.25 (dd, *J* = 12.9/4.1 Hz, 1H,

OCHC*H*$_2$N$_3$), 3.59 (dd, *J* = 12.9/8.1 Hz, 1H, OCHC*H*$_2$N$_3$), 3.91 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.07 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.14 – 4.24 (m, 2H, OC*H*$_2$CH$_3$), 4.62 (dd, *J* = 8.1/4.1 Hz, 1H, OC*H*CH$_2$N$_3$), 5.07 (s, 2H, PhC*H*$_2$OPh), 6.96 – 7.00 (m, 2H, H$_{arom.}$), 7.23 – 7.28 (m, 2H, H$_{arom.}$), 7.31 – 7.45 (m, 5H, H$_{arom.}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 14.3 (1C, OCH$_2$*C*H$_3$), 56.5 (1C, OCH*C*H$_2$N$_3$), 61.2 (1C, O*C*H$_2$CH$_3$), 65.9 (1C, O*C*H$_2$CO$_2$Et), 70.2 (1C, Ph*C*H$_2$OPh), 81.3 (1C, O*C*HCH$_2$N$_3$), 115.3 (2C, C$_{arom.}$), 127.6 (2C, C$_{arom.}$), 128.2 (1C, C$_{arom.}$), 128.4 (2C, C$_{arom.}$), 128.7 (2C, C$_{arom.}$), 129.9 (1C, C$_{arom.}$), 136.9 (1C, C$_{arom.}$), 159.3 (1C, C$_{arom.}$), 170.1 (1C, *C*O$_2$Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2982, 2099, 1751, 1732, 1609 1508, 1454, 1377, 1285, 1238, 1204, 1173, 1123, 1022, 918, 864, 829, 737, 698; HRMS (*m*/*z*): [M+NH$_4$]$^+$ calcd for $C_{19}H_{25}N_4O_4$: 373.1870, found: 373.1907; HPLC (method 1): t_R = 21.1 min, purity 95.6%.

Ethyl (*S*)-2-{1-[4-(benzyloxy)phenyl]-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy}acetate (22)

Phenylacetylene (0.11 mL, 1.0 mmol), sodium ascorbate (20 mg, 0.1 mmol) and copper(II) sulfate (10 mg, 0.06 mmol) were added to a solution of **20** (200 mg, 0.56 mmol) in a *t*BuOH/H_2O mixture (1:1; 45 mL). After stirring at ambient temperature for 24 h, a saturated aqueous solution of $NaHCO_3$ was added and the mixture was extracted with ethyl acetate (3×). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated 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 = 0.30) to give **22** as colourless solid (240 mg, 0.52 mmol, 92%). m.p. = 106 °C; $[\alpha]_D^{20}$ = +42.2 (2.1, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.18 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 3.83 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.03 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.08 – 4.16 (m, 2H, OC*H*$_2$CH$_3$), 4.60 (dd, *J* = 14.2/8.5 Hz, 1H,

OCHC*H*$_2$Ar), 4.67 (dd, *J* = 14.2/3.6 Hz, 1H, OCHC*H*$_2$Ar), 4.81 (dd, *J* = 8.5/3.6 Hz, 1H, OC*H*CH$_2$Ar), 5.07 (s, 2H, PhC*H*$_2$OPh), 6.98 – 7.02 (m, 2H, H$_{arom.}$), 7.24 – 7.27 (m, 2H, H$_{arom.}$), 7.30 – 7.36 (m, 2H, H$_{arom.}$), 7.37 – 7.45 (m, 6H, H$_{arom.}$), 7.83 – 7.86 (m, 2H, H$_{arom.}$), 8.10 (m, 1H, 5-H$_{triazole}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 14.2 (1C, OCH$_2$*C*H$_3$), 56.1 (1C, OCH*C*H$_2$Ar), 61.1 (1C, O*C*H$_2$CH$_3$), 66.1 (1C, O*C*H$_2$CO$_2$Et), 70.2 (1C, PhCH$_2$OPh), 80.8 (1C, O*C*HCH$_2$Ar), 115.5 (2C, C$_{arom.}$), 121.7 (1C, C-5$_{triazole}$), 125.9 (2C, C$_{arom.}$), 127.6 (2C, C$_{arom.}$), 128.1 (1C, C$_{arom.}$), 128.2 (1C, C$_{arom.}$), 128.3 (2C, C$_{arom.}$), 128.8 (2C, C$_{arom.}$), 128.9 (3C, C$_{arom.}$), 131.0 (1C, C$_{arom.}$), 136.8 (1C, C$_{arom.}$), 147.6 (1C, C-4$_{triazole}$), 159.6 (1C, C$_{arom.}$), 169.8 (1C, *C*O$_2$Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2924, 1721, 1609, 1512, 1454, 1292, 1242, 1103, 1015, 768, 741, 694; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{27}H_{28}N_3O_4$: 458.2074, found: 458.2083; HPLC (method 1): t_R = 24.0 min, purity 98.5%.

(*S*)-2-{1-[4-(Benzyloxy)phenyl]-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy}-*N*-hydroxyacetamide (23)

Hydroxylamine hydrochloride (48 mg, 0.7 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.14 mL, 0.8 mmol) were added to a solution of **22** (50 mg, 0.11 mmol) in methanol (25 mL). After stirring for 16 h at ambient temperature, the reaction mixture was acidified with HCl 1.0 M until a pH 5-6 was reached. The reaction mixture was extracted with ethyl acetate (3×) and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, dichloromethane/methanol = 98/2, R_f = 0.25) to give **23** as colorless solid (13 mg, 0.03 mmol, 26%). m.p. = 139 °C; $[\alpha]_D^{20}$ = -8.8 (2.5, methanol); ^{1}H NMR (D_3COD): δ [ppm] = 3.81 (d, *J* = 14.4 Hz, 1H, OC*H*$_2$CONHOH), 3.87 (d, *J* = 14.4 Hz, 1H, OC*H*$_2$CONHOH), 4.63 – 4.71 (m, 1H,

OCHC*H*$_2$Ar), 4.74 – 4.79 (m, 1H, OCHC*H*$_2$Ar), 4.84 – 4.90 (m, 1H, OC*H*CH$_2$Ar), 5.09 (s, 2H, PhC*H*$_2$OPh), 7.00 – 7.05 (m, 2H, H$_{arom.}$), 7.24 – 7.46 (m, 10H, H$_{arom.}$), 7.76 – 7.80 (m, 2H, H$_{arom.}$), 8.31 (m, 1H, 5-H$_{triazole}$); ^{13}C NMR (D_3COD): δ [ppm] = 56.7 (1C, OCH*C*H$_2$Ar), 67.7 (1C, O*C*H$_2$CONHOH), 71.0 (1C, Ph*C*H$_2$OPh), 82.0 (1C, O*C*HCH$_2$Ar), 116.4 (2C, C$_{arom.}$), 123.4 (1C, C-5$_{triazole}$), 126.7 (2C, C$_{arom.}$), 128.5 (2C, C$_{arom.}$), 128.9 (1C, C$_{arom.}$), 129.3 (1C, C$_{arom.}$), 129.4 (2C, C$_{arom.}$), 129.5 (2C, C$_{arom.}$), 130.0 (2C, C$_{arom.}$), 130.1 (1C, C$_{arom.}$), 131.7 (1C, C$_{arom.}$), 138.5 (1C, C$_{arom.}$), 148.7 (1C, C-4$_{triazole}$), 160.9 (1C, C$_{arom.}$), 168.4 (1C, *C*ONHOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3383, 2967, 2924, 2874, 1678, 1609, 1512, 1443, 1385, 1242, 1180, 1115, 1007, 845, 814, 764, 745, 691; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{25}H_{25}N_4O_4$: 445.1870, found: 445.1905; HPLC (method 2): t_R = 17.2 min, purity 97.6%.

Ethyl (*S*)-2-[1-(4-hydroxyphenyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy]acetate (24)

22 (70 mg, 0.15 mmol) was dissolved in methanol (10 mL) and 10% Pd/C (7.0 mg) was added to the solution. The reaction mixture was stirred under H_2 atmosphere (balloon) at ambient temperature for 2 d. Then, the reaction mixture was filtered through Celite® and the filtrate was evaporated in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, cyclohexane/ethyl acetate = 8/2) to give **24** as colorless oil (44 mg, 0.12 mmol, 78%). TLC (cyclohexane/ethyl acetate = 2/1): R_f = 0.25; $[\alpha]_D^{20}$ = +55.8 (2.1, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.19 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 3.85 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.03 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.10 – 4.17 (m, 2H, OC*H*$_2$CH$_3$), 4.62 (dd, *J* = 14.2/8.0 Hz, 1H, OCHC*H*$_2$Ar), 4.68 (dd, *J* = 14.2/3.8 Hz, 1H, OCHC*H*$_2$Ar), 4.79 (dd, *J* = 8.0/3.8 Hz, 1H, OC*H*CH$_2$Ar), 6.85 – 6.89 (m, 2H, 3-H$_{4\text{-hydroxyphenyl}}$, 5-H$_{4\text{-}}$

$_{hydroxyphenyl}$), 7.11 – 7.15 (m, 2H, 2-H$_{4\text{-hydroxyphenyl}}$, 6-H$_{4\text{-hydroxyphenyl}}$), 7.30 – 7.35 (m, 1H, 4''-H$_{phenyl}$), 7.40 – 7.45 (m, 2H, 3''-H$_{phenyl}$, 5''-H$_{phenyl}$), 7.82 – 7.86 (m, 2H, 2''-H$_{phenyl}$, 6''-H$_{phenyl}$), 8.12 (m, 1H, 5'-H$_{triazole}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 14.2 (1C, $OCH_2\mathit{C}H_3$), 56.2 (1C, $OCH\mathit{C}H_2Ar$), 61.2 (1C, $O\mathit{C}H_2CH_3$), 65.9 (1C, $O\mathit{C}H_2CO_2Et$), 80.6 (1C, $O\mathit{C}HCH_2Ar$), 116.2 (2C, C-3'$_{4\text{-hydroxyphenyl}}$, C-5'$_{4\text{-hydroxyphenyl}}$), 121.9 (1C, C-5'$_{triazole}$), 125.9 (2C, C-2''$_{phenyl}$, C-6''$_{phenyl}$), 127.8 (1C, C$_{arom.}$), 128.3 (1C, C-4''$_{phenyl}$), 128.4 (2C, C-3''$_{phenyl}$, C-5''$_{phenyl}$), 129.0 (2C, C-2'$_{4\text{-hydroxyphenyl}}$, C-6'$_{4\text{-hydroxyphenyl}}$), 130.7 (1C, C$_{arom.}$), 147.6 (1C, C-4'$_{triazole}$), 157.4 (1C, C-4'$_{4\text{-hydroxyphenyl}}$), 170.1 (1C, $\mathit{C}O_2Et$); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2978, 1740, 1612, 1516, 1443, 1373, 1207, 1119, 1026, 837, 764, 694; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{20}H_{22}N_3O_4$: 368.1605, found: 368.1618; HPLC (method 1): t_R = 19.4 min, purity 99.1%.

Ethyl (*S*)-2-[2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-(4-{[(trifluoromethyl)sulfonyl]oxy}phenyl)ethoxy]acetate (25)

Under N_2 atmosphere, triethylamine (0.14 mL, 1.0 mmol) was added to a solution of **24** (100 mg, 0.28 mmol) in dichloromethane (10 mL) at -20 °C. Then, a solution of trifluoromethanesulfonic anhydride (0.07 mL, 0.42 mmol) in dichloromethane (0.5 mL) was added slowly. The reaction mixture was stirred at -20 °C for 30 min. Afterwards, a saturated aqueous solution of $NaHCO_3$ was added. The reaction mixture was extracted with dichloromethane (3×) and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, cyclohexane/ethyl acetate = 8/1) to give **25** as yellowish solid (130 mg, 0.26 mmol, 92%). TLC (cyclohexane/ethyl acetate = 3/1): R_f = 0.33; m.p. = 90 °C; $[\alpha]_D^{20}$ = +39.3 (4.3, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.19 (t, *J* = 7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 3.86 (d, *J* = 16.2 Hz, 1H, $OC\mathit{H}_2CO_2Et$), 4.07 (d,

J = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.10 – 4.16 (m, 2H, OC*H*$_2$CH$_3$), 4.60 (dd, *J* = 14.2/8.2 Hz, 1H, OCHC*H*$_2$Ar), 4.71 (dd, *J* = 14.2/3.2 Hz, 1H, OCHC*H*$_2$Ar), 4.95 (dd, *J* = 8.2/3.2 Hz, 1H, OC*H*CH$_2$Ar), 7.31 – 7.37 (m, 3H, $H_{arom.}$), 7.41 – 7.49 (m, 4H, $H_{arom.}$), 7.83 – 7.87 (m, 2H, $H_{arom.}$), 8.08 (s, 1H, 5'-$H_{triazole}$); ^{13}C NMR ($CDCl_3$): δ [ppm] = 14.2 (1C, OCH$_2$*C*H$_3$), 56.1 (1C, OCH*C*H$_2$Ar), 61.4 (1C, O*C*H$_2$CH$_3$), 66.6 (1C, O*C*H$_2$CO$_2$Et), 80.4 (1C, O*C*HCH$_2$Ar), 118.9 (q, *J* = 320 Hz, 1C, *C*F$_3$), 121.8 (1C, C-$5_{triazole}$), 122.4 (2C, $C_{arom.}$), 126.0 (2C, $C_{arom.}$), 128.5 (1C, $C_{arom.}$), 128.8 (2C, $C_{arom.}$), 129.0 (2C, $C_{arom.}$), 130.3 (1C, $C_{arom.}$), 137.5 (1C, $C_{arom.}$), 147.6 (1C, $C_{arom.}$), 149.9 (1C, $C_{arom.}$), 169.3 (1C, OCH$_2$*C*O$_2$Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1751, 1501, 1466, 1420, 1246, 1207, 1134, 1018, 895, 841, 764, 694; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{21}H_{21}F_3N_3O_6S$: 500.1098, found: 500.1109; HPLC (method 1): t_R = 23.5 min, purity 96.4%.

Ethyl (*S*)-2-{2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-[4-(phenylethynyl)phenyl]ethoxy}acetate (26)

Under N_2 atmosphere, tetrakis(triphenylphosphine)palladium(0) (18 mg, 0.02 mmol) and copper(I) iodide (3 mg, 0.02 mmol) were added to the a solution of **25** (78 mg, 0.16 mmol) in dry triethylamine (10 mL). Then, a solution of phenylacetylene (0.03 mL, 0.27 mmol) in dry triethylamine (1.5 mL) was added dropwise over a period of 1 h. After heating the reaction mixture to reflux overnight, the solvent was removed in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, cyclohexane/ethyl acetate = 8/2) to give **26** as colorless oil (44 mg, 0.10 mmol, 63%). TLC (cyclohexane/ethyl acetate = 1/1): R_f = 0.20; $[\alpha]_D^{20}$ = +74.4 (2.6, methanol); ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.19 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 3.86 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.08 (d, *J* = 16.2 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.10 – 4.17 (m, 2H, OC*H*$_2$CH$_3$), 4.61 (dd, *J* = 14.3/8.4 Hz, 1H, OCHC*H*$_2$Ar), 4.71 (dd, *J* = 14.3/3.6 Hz, 1H, OCHC*H*$_2$Ar),

4.89 (dd, *J* = 8.4/3.6 Hz, 1H, OC*H*CH$_2$Ar), 7.31 – 7.39 (m, 6H, H$_{arom.}$), 7.41 – 7.46 (m, 2H, H$_{arom.}$), 7.51 – 7.56 (m, 4H, H$_{arom.}$), 7.84 – 7.88 (m, 2H, H$_{arom.}$), 8.10 (s, 1H, 5'-H$_{triazole}$); ^{13}C NMR (CD$_3$OD): δ [ppm] = 14.4 (1C, OCH$_2$*C*H$_3$), 56.6 (1C, OCH*C*H$_2$Ar), 62.1 (1C, O*C*H$_2$CH$_3$), 67.1 (1C, O*C*H$_2$CO$_2$Et), 81.7 (1C, O*C*HCH$_2$Ar), 89.6 (1C, *C*≡C), 90.9 (1C, *C*≡C), 123.7 (1C, C-5'$_{triazole}$), 124.4 (1C, C$_{arom.}$), 125.3 (1C, C$_{arom.}$), 126.7 (2C, C$_{arom.}$), 128.3 (2C, C$_{arom.}$), 129.3 (1C, C$_{arom.}$), 129.5 (2C, C$_{arom.}$), 129.6 (1C, C$_{arom.}$), 130.0 (2C, C$_{arom.}$), 131.8 (1C, C$_{arom.}$), 132.6 (2C, C$_{arom.}$), 133.0 (2C, C$_{arom.}$), 138.7 (1C, C$_{arom.}$), 148.6 (1C, C-4'$_{triazole}$), 171.5 (1C, OCH$_2$*C*O$_2$Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 1751, 1466, 1443, 1207, 1126, 1042, 1022, 976, 837, 760, 691; HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{28}$H$_{26}$N$_3$O$_3$: 452.1969, found: 452.1985; HPLC (method 1): t$_R$ = 24.8 min, purity 95.4%.

(*S*)-*N*-Hydroxy-2-{2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-[4-(phenylethynyl)phenyl]ethoxy}acetamide (27)

Hydroxylamine hydrochloride (81 mg, 1.2 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.46 mL, 2.5 mmol) were added to a solution of **26** (84 mg, 0.19 mmol) in methanol (25 mL). After stirring for 16 h at ambient temperature, the reaction mixture was acidified with 1.0 M HCl until pH 5-6 was reached and extracted with ethyl acetate (3×). The combined organic layers were dried over Na$_2$SO$_4$, filtered and the solvent removed in vacuo. The residue was purified by flash column chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, dichloromethane/methanol = 98/2, R$_f$ = 0.15) to give **27** as colorless solid (44 mg, 0.10 mmol, 54%). m.p. = 118 °C; $[\alpha]_D^{20}$ = +41.5 (2.3, methanol); ^{1}H NMR (CD$_3$OD): δ [ppm] = 3.86 (d, *J* = 14.3 Hz, 1H, OC*H*$_2$CONHOH), 3.93 (d, *J* = 14.3 Hz, 1H, OC*H*$_2$CONHOH), 4.73 (dd, *J* = 14.2/4.2 Hz, 1H, OCHC*H*$_2$Ar), 4.80 (dd, *J* = 14.2/7.5 Hz, 1H, OCHC*H*$_2$Ar), 4.98 (dd, *J* = 7.5/4.2 Hz,

1H, OC*H*CH_2Ar), 7.32 – 7.46 (m, 8H, $H_{arom.}$), 7.49 – 7.53 (m, 2H, $H_{arom.}$), 7.54 – 7.58 (m, 2H, $H_{arom.}$), 7.78 – 7.83 (m, 2H, $H_{arom.}$), 8.36 (s, 1H, 5'-$H_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 56.5 (1C, OCH*C*H_2Ar), 68.1 (1C, O*C*H_2CONHOH), 82.1 (1C, O*C*HCH$_2$Ar), 89.5 (1C, *C*≡C), 91.0 (1C, *C*≡C), 123.5 (1C, C-5'$_{triazole}$), 124.3 (1C, $C_{arom.}$), 125.4 (1C, $C_{arom.}$), 126.7 (2C, $C_{arom.}$), 128.3 (2C, $C_{arom.}$), 129.4 (1C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 129.7 (1C, $C_{arom.}$), 130.0 (2C, $C_{arom.}$), 131.6 (1C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 133.1 (2C, $C_{arom.}$), 138.4 (1C, $C_{arom.}$), 148.8 (1C, C-4'$_{triazole}$), 168.2 (1C, OCH$_2$*C*ONH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3129, 2924, 1740, 1651, 1609, 1512, 1443, 1369, 1234, 1204, 1119, 1088, 1042, 980, 849, 756, 691; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{23}N_4O_3$: 439.1765, found: 439.1780; HPLC (method 2): t_R = 17.1 min, purity 99.3%.

Ethyl (*S*)-2-[1-(4-bromophenyl)-2-(1*H*-1,2,3-triazol-1-yl)ethoxy]acetate (30)

Trimethylsilylacetylene (0.15 mL, 1.1 mmol), sodium ascorbate (30 mg, 0.15 mmol), and copper sulfate pentahydrate (14 mg, 0.06 mmol) were added to a solution of **21** (170 mg, 0.52 mmol) in a 1:1 mixture of *t*BuOH and H_2O (20 mL). The reaction mixture was stirred at ambient temperature for 16 h. Then, tetrabutylammonium fluoride trihydrate (250 mg, 0.78 mmol) was added and the reaction mixture was stirred at ambient temperature for 72 h. Then, the reaction mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography (Ø = 3 cm, h = 19 cm, V = 20 mL, dichloromethane/methanol = 20/1, R_f = 0.44) to give **30** as brown oil (170 mg, 0.47 mmol, 92%). $[\alpha]_D^{20}$ = +89.3 (5.7, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.20 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 3.90 (d, *J* = 16.4 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.05 (d, *J* = 16.4 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.12 (q, *J* = 7.2 Hz, 2H, OC*H*$_2$CH$_3$), 4.70 (dd, *J* = 14.2/4.4 Hz, 1H, OCHC*H*$_2$Ar), 4.74 (dd, *J* = 14.2/7.5 Hz, 1H,

OCHC*H*$_2$Ar), 4.95 (dd, *J* = 7.5/4.4 Hz, 1H, OC*H*CH$_2$Ar), 7.25 – 7.27 (m, 2H, 2'-H$_{4\text{-bromophenyl}}$, 6'-H$_{4\text{-bromophenyl}}$), 7.52 – 7.55 (m, 2H, 3'-H$_{4\text{-bromophenyl}}$, 5'-H$_{4\text{-bromophenyl}}$), 7.69 – 7.70 (m, 1H, 4''-H$_{\text{triazole}}$), 8.03 – 8.04 (m, 1H, 5''-H$_{\text{triazole}}$); ^{13}C NMR (CD_3OD): δ [ppm] = 14.4 (1C, OCH$_2$*C*H$_3$), 56.2 (1C, OCH*C*H$_2$Ar), 62.0 (1C, O*C*H$_2$CH$_3$), 67.0 (1C, O*C*H$_2$CO$_2$Et), 81.3 (1C, O*C*HCH$_2$Ar), 123.8 (1C, C-4'$_{\text{4-bromophenyl}}$), 127.3 (1C, C-5''$_{\text{triazole}}$), 130.0 (2C, C-2'$_{\text{4-bromophenyl}}$, C-6'$_{\text{4-bromophenyl}}$), 133.0 (2C, C-3'$_{\text{4-bromophenyl}}$, C-5'$_{\text{4-bromophenyl}}$), 134.1 (1C, C-4''$_{\text{triazole}}$), 137.7 (1C, C-1'$_{\text{4-bromophenyl}}$), 171.4 (1C, *C*O$_2$Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2982, 1748, 1593, 1485, 1281, 1207, 1123, 1072, 1011, 822, 783, 748; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{14}H_{17}{}^{79}BrN_3O_3$: 354.0448, found: 354.0423; HPLC (method 1): t_R = 19.9 min, purity 96.7%.

Ethyl (*S*)-2-{1-[4-(phenylethynyl)phenyl]-2-(1*H*-1,2,3-triazol-1-yl)ethoxy}acetate (31)

Under N_2 atmosphere, copper(I) iodide (21 mg, 0.11 mmol), tetrakis(triphenylphosphine)palladium(0) (104 mg, 0.09 mmol) and phenylacetylene (0.17 mL, 1.5 mmol) were added to a solution of **30** (160 mg, 0.45 mmol) in triethylamine (15 mL). The mixture was heated to reflux for 48 h. After evaporation of the solvent, the residue was purified by flash column chromatography (Ø = 3 cm, h = 20 cm, V = 20 mL, dichloromethane/methanol = 20/1, R_f = 0.44) to give **31** as brown oil (99 mg, 0.26 mmol, 58%). $[\alpha]_D^{20}$ = +99.9 (1.3, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.20 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$), 3.91 (d, *J* = 16.4 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.06 (d, *J* = 16.4 Hz, 1H, OC*H*$_2$CO$_2$Et), 4.13 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 4.69 – 4.83 (m, 2H, OCHC*H*$_2$Ar), 4.96 – 5.03 (m, 1H, OC*H*CH$_2$Ar), 7.32 – 7.41 (m, 5H, H$_{\text{arom.}}$), 7.48 – 7.56 (m, 4H, H$_{\text{arom.}}$), 7.82 – 8.02 (m br, 1H, H$_{\text{triazole}}$), 8.17 – 8.31 (m br, 1H, H$_{\text{triazole}}$); ^{13}C NMR (CD_3OD): δ [ppm] = 14.4 (1C, OCH$_2$*C*H$_3$), 56.7 (1C, OCH*C*H$_2$Ar), 62.0 (1C, O*C*H$_2$CH$_3$),

67.0 (1C, O*C*H$_2$CO$_2$Et), 81.5 (1C, O*C*HCH$_2$Ar), 89.5 (1C, *C*≡C), 90.9 (1C, *C*≡C), 124.3 (1C, C$_{arom.}$), 125.2 (1C, C$_{arom.}$), 128.3 (2C, C$_{arom.}$), 129.5 (2C, C$_{arom.}$), 129.6 (1C, C$_{arom.}$), 132.6 (2C, C$_{arom.}$) 132.9 (2C, C$_{arom.}$), 138.7 (1C, C$_{arom.}$), 171.4 (1C, *C*O$_2$Et), the signals for the two carbon atoms of the triazole moiety could not be observed in the spectrum; IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2982, 1748, 1609, 1508, 1462, 1443, 1381, 1285, 1207, 1123, 1072, 1026, 841, 787, 756, 691; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{22}H_{22}N_3O_3$: 376.1656, found: 376.1680; HPLC (method 1): t_R = 22.7 min, purity 96.2%.

(*S*)-*N*-Hydroxy-2-{1-[4-(phenylethynyl)phenyl]-2-(1*H*-1,2,3-triazol-1-yl)ethoxy}acetamide (32)

Hydroxylamine hydrochloride (190 mg, 2.7 mmol) and a 5.4 M solution of sodium methoxide in methanol (0.50 mL, 2.7 mmol) were added to a solution of **31** (95 mg, 0.25 mmol) in methanol (5.5 mL). After stirring for 16 h at ambient temperature, the reaction mixture was acidified with a 1.0 M solution of HCl until pH 5-6 was reached. The reaction mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was purified by automated flash column chromatography (100% water → 100% acetonitrile, Biotage® SNAP KP-C18-HS 12 g) to give **32** as colorless solid (66 mg, 0.18 mmol, 72%). RP-TLC (acetonitrile/water = 1/1): R_f = 0.26; m.p. = 148 °C; $[\alpha]_D^{20}$ = +51.8 (1.2, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 3.83 (d, *J* = 14.3 Hz, 1H, OC*H*$_2$CONHOH), 3.90 (d, *J* = 14.3 Hz, 1H, OC*H*$_2$CONHOH), 4.73 (dd, *J* = 14.2/4.3 Hz, 1H, OCHC*H*$_2$Ar), 4.80 (dd, *J* = 14.2/7.7 Hz, 1H, OCHC*H*$_2$Ar), 4.93 (dd, *J* = 7.7/4.3 Hz, 1H, OC*H*CH$_2$Ar), 7.35 – 7.40 (m, 5H, H$_{arom.}$), 7.50 – 7.55 (m, 4H, H$_{arom.}$), 7.70 – 7.71 (m, 1H, 4'-H$_{triazole}$), 7.99 – 8.01 (m, 1H, 5'-H$_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 56.1 (1C, OCH*C*H$_2$Ar), 68.1 (1C, O*C*H$_2$CONHOH), 82.1 (1C, O*C*HCH$_2$Ar), 89.5 (1C, *C*≡C),

90.9 (1C, *C*≡C), 124.3 (1C, $C_{arom.}$), 125.4 (1C, $C_{arom.}$), 127.1 (1C, C-5'$_{triazole}$), 128.2 (2C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 129.7 (1C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 133.0 (2C, $C_{arom.}$), 134.2 (1C, C-4'$_{triazole}$), 138.4 (1C, $C_{arom.}$), a signal for OCH_2*C*ONH could not be observed; IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3337, 3129, 2955, 2886, 1678, 1466, 1447, 1289, 1219, 1211, 1180, 1107, 1084, 1045, 1015, 964, 891, 837, 799, 756, 691; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{20}H_{19}N_4O_3$: 363.1452, found: 363.1453; HPLC (method 2): t_R = 16.1 min, purity 95.1%.

Ethyl (*S*)-2-{1-(4-bromophenyl)-2-[5-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]ethoxy}acetate (38)

$Cp^*RuCl(PPh_3)_2$ (30 mg, 0.04 mmol) was added to a solution of **21** (250 mg, 0.76 mmol) in 1,4-dioxane (2 mL) and the mixture was stirred for 20 min at ambient temperature. Then, propargyl alcohol (0.05 mL, 48 mg, 0.86 mmol) was added and the mixture was heated to 60 °C for 1 h under microwave irradiation. After removing the solvent *in vacuo*, the residue was purified by flash column chromatography (Ø = 3 cm, h = 26.5 cm, V = 20 mL, dichloromethane/methanol = 20/1) to give **38** as yellowish oil (76 mg, 0.20 mmol, 26% yield). TLC (ethyl acetate): R_f = 0.53; $[\alpha]_D^{20}$ = +60.6 (7.2, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.19 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 3.85 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.00 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.10 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 4.65 (dd, *J* = 14.4/4.2 Hz, 1H, $OCHCH_2Ar$), 4.71 – 4.83 (m, 3H, $OCHCH_2Ar$ (1H), $ArCH_2OH$), 4.96 (dd, *J* = 8.1/4.2 Hz, 1H, $OCHCH_2Ar$), 7.26 – 7.32 (m, 2H, 2'-$H_{4\text{-bromophenyl}}$, 6'-$H_{4\text{-bromophenyl}}$), 7.53 – 7.59 (m, 2H, 3'-$H_{4\text{-bromophenyl}}$, 5'-$H_{4\text{-bromophenyl}}$), 7.62 (s, 1H, 4''-$H_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 14.4 (1C, OCH_2CH_3), 53.9 (1C, $ArCH_2OH$), 54.6 (1C, $OCHCH_2Ar$), 62.0 (1C, OCH_2CH_3), 67.1 (1C, OCH_2CO_2Et), 82.0 (1C, $OCHCH_2Ar$), 123.8 (1C, C-4'$_{4\text{-bromophenyl}}$), 130.1 (2C, C-2'$_{4\text{-}}$

bromophenyl, C-6'$_{4\text{-bromophenyl}}$), 133.1 (2C, C-3'$_{4\text{-bromophenyl}}$, C-5'$_{4\text{-bromophenyl}}$), 133.2 (1C, C-4''$_{triazole}$), 137.9 (1C, C-1'$_{4\text{-bromophenyl}}$), 140.2 (1C, C-5''$_{triazole}$), 171.2 (1C, *C*O_2Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3337, 2980, 2908, 1742, 1592, 1486, 1451, 1408, 1380, 1284, 1206, 1119, 1070, 1010, 823, 770, 741, 709, 642, 584, 557, 529; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{15}H_{19}{}^{79}BrN_3O_4$: 384.0553, found: 384.0545; HPLC (method 1): t_R = 19.2 min, purity 96.0%.

Ethyl (*S*)-2-(2-[5-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]-1-[4-(phenylethynyl)phenyl]ethoxy}acetate (39)

Under N_2 atmosphere, copper(I) iodide (6 mg, 0.032 mmol) and bis(triphenylphosphine)palladium(II) chloride (19 mg, 0.027 mmol) were added to a solution of **38** (100 mg, 0.27 mmol) in a mixture of dry THF (10 mL) and diisopropylamine (3 mL). The obtained mixture was stirred at ambient temperature for 20 min. Then, phenylacetylene (0.06 mL, 56 mg, 0.55 mmol) was added and the mixture was heated to 80 °C. After 1 h, additional phenylacetylene (0.06 mL, 56 mg, 0.55 mmol) was added and heating was continued for 45 h. Then, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (Ø = 3 cm, h = 30 cm, V = 20 mL, dichloromethane/methanol = 20/1, R_f = 0.40) to give **39** as yellow oil (77 mg, 0.19 mmol, 71%). $[\alpha]_D^{20}$ = +71.2 (2.6, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.19 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 3.87 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.02 (d, *J* = 16.3 Hz, 1H, OCH_2CO_2Et), 4.10 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 4.67 (dd, *J* = 14.4/4.2 Hz, 1H, $OCHCH_2Ar$), 4.73 (d, *J* = 14.0 Hz, 1H, $ArCH_2OH$), 4.75 – 4.82 (m, 2H, $OCHCH_2Ar$ (1H), $ArCH_2OH$ (1H)), 5.00 (dd, *J* = 8.2/4.2 Hz, 1H, $OCHCH_2Ar$), 7.36 – 7.41 (m, 5H, 2''-$H_{4\text{-(phenylethynyl)phenyl}}$, 6''-$H_{4\text{-(phenylethynyl)phenyl}}$,3'-$H_{phenyl}$, 4'-$H_{phenyl}$, 5'-$H_{phenyl}$), 7.50 – 7.53 (m, 2H, 2'-H_{phenyl}, 6'-H_{phenyl}),

7.53 – 7.57 (m, 2H, 3''-$H_{4\text{-(phenylethynyl)phenyl}}$, 5''-$H_{4\text{-(phenylethynyl)phenyl}}$), 7.62 (s, 1H, 4'''-$H_{triazole}$); ^{13}C NMR ($CD_3OD$): δ [ppm] = 14.4 (1C, OCH_2CH_3), 53.9 (1C, $ArCH_2OH$), 54.7 (1C, $OCHCH_2Ar$), 62.0 (1C, OCH_2CH_3), 67.1 (1C, OCH_2CO_2Et), 82.3 (1C, $OCHCH_2Ar$), 90.0 (1C, C≡C), 90.0 (1C, C≡C), 124.4 (1C, C-1'$_{phenyl}$), 125.3 (1C, C-4''$_{4\text{-(phenylethynyl)phenyl}}$), 128.3 (2C, C-2''$_{4\text{-(phenylethynyl)phenyl}}$, C-6''$_{4\text{-(phenylethynyl)phenyl}}$), 129.57 (2C, C-3'$_{phenyl}$, C-5'$_{phenyl}$), 129.62 (1C, C-4'$_{phenyl}$), 132.6 (2C, C-2'$_{phenyl}$, C-6'$_{phenyl}$), 133.0 (2C, C-3''$_{4\text{-(phenylethynyl)phenyl}}$, C-5''$_{4\text{-(phenylethynyl)phenyl}}$), 133.2 (1C, C-4'''$_{triazole}$), 138.9 (1C, C-1''$_{4\text{-(phenylethynyl)phenyl}}$), 140.2 (1C, C-5'''$_{triazole}$), 171.3 (1C, CO_2Et); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3295, 3059, 2954, 2913, 1737, 1608, 1509, 1441, 1412, 1374, 1300, 1225, 1120, 1019, 837, 755, 690, 556, 523; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{23}H_{24}N_3O_4$: 406.1761, found: 406.1807; HPLC (method 1): t_R = 22.6 min, purity 99.8%.

(*S*)-*N*-Hydroxy-2-{2-[5-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]-1-[4-(phenylethynyl)phenyl]ethoxy}acetamide (40)

Under N_2 atmosphere, **39** (72 mg, 0.18 mmol) was dissolved in a mixture of isopropanol (5 mL) and dry THF (5 mL). After stirring the mixture for 10 min at 0 °C, a 50% aqueous solution of hydroxylamine (3.0 mL) was added and the reaction mixture was stirred at ambient temperature for 16 h. Then, the solvent was removed *in vacuo* and the residue was purified by automated flash column chromatography (100% water → 100% acetonitrile, Biotage® SNAP KP-C18-HS 12 g) to give **40** as colorless solid (51 mg, 0.13 mmol, 73%). TLC (ethyl acetate): R_f = 0.06; m.p. = 124 °C; $[\alpha]_D^{20}$ = +49.3 (2.7, methanol); ^{1}H NMR (DMSO-d_6): δ [ppm] = 3.62 (d, *J* = 13.3 Hz, 1H, $OCH_2CONHOH$), 3.69 (d, *J* = 13.3 Hz, 1H, $OCH_2CONHOH$), 4.50 (s, 2H, $ArCH_2OH$), 4.58 (dd, *J* = 14.3/4.8 Hz, 1H, $OCHCH_2Ar$), 4.71 (dd, *J* = 14.3/7.4 Hz, 1H, $OCHCH_2Ar$), 4.92 (dd, *J* = 7.0/5.0 Hz, 1H, $OCHCH_2Ar$), 7.32 – 7.40 (m, 2H, 2''-$H_{4\text{-(phenylethynyl)phenyl}}$,

6''-$H_{4\text{-(phenylethynyl)phenyl}}$), 7.40 – 7.48 (m, 3H, 3'-H_{phenyl}, 4'-H_{phenyl}, 5'-H_{phenyl}), 7.52 – 7.61 (m, 5H, 2'-H_{phenyl}, 6'-H_{phenyl}, 3''-$H_{4\text{-(phenylethynyl)phenyl}}$, 5''-$H_{4\text{-(phenylethynyl)phenyl}}$, 4'''-$H_{triazole}$); ^{13}C NMR (DMSO-$d_6$): δ [ppm] = 52.2 (1C, Ar*C*H_2OH), 52.6 (1C, OCH*C*H_2Ar), 67.3 (1C, O*C*H_2CONHOH), 79.9 (1C, O*C*HCH$_2$Ar), 89.1 (1C, *C*≡C), 99.6 (1C, $C_{arom.}$), 122.2 (1C, $C_{arom.}$), 122.3 (1C, $C_{arom.}$), 127.4 (2C, C-2''$_{4\text{-(phenylethynyl)phenyl}}$, C-6''$_{4\text{-(phenylethynyl)phenyl}}$), 128.9 (2C, C-3'$_{phenyl}$, C-5'$_{phenyl}$), 129.0 (1C, C-4'$_{phenyl}$), 131.5 (2C, $C_{arom.}$), 131.6 (2C, $C_{arom.}$), 132.3 (1C, C-4'''$_{triazole}$), 138.56 (1C, $C_{arom.}$), 138.64 (1C, $C_{arom.}$), 164.7 (1C, *C*ONHOH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3225, 2875, 1665, 1509, 1442, 1413, 1338, 1300, 1243, 1208, 1112, 1038, 988, 841, 755, 689, 557, 524; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{21}H_{21}N_4O_4$: 393.1557, found: 393.1583; HPLC (method 2): t_R = 15.0 min, purity 95.4%.

2-[(*R*)-2-Azido-1-(4-bromophenyl)ethoxy]-*N*-{[(*RS*)-tetrahydro-2*H*-pyran-2-yl]oxy}acetamide (*ent*-41)

Under N_2 atmosphere, *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.4 g, 12 mmol) was added to a solution of *ent*-**21** (3.9 g, 12 mmol) in dry tetrahydrofuran (150 mL) and the reaction mixture was cooled down to -78 °C. Then, a 1 M solution of LiHMDS in THF (25 mL, 25 mmol) was added and the reaction mixture was stirred for 1 h. The mixture was then quenched with a saturated aqueous solution of NH_4Cl. Once at ambient temperature, the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and 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) to give *ent*-**41** as colorless oil (4.2 g, 11 mmol, 90%). TLC (cyclohexane/ethyl acetate = 1/2): R_f = 0.57; $[\alpha]_D^{20}$ = -83.8 (1.5, dichloromethane); ^{1}H NMR (CD_3OD): δ [ppm] = 1.53 – 1.87 (m, 6H,

OCHC*H*$_2$C*H*$_2$C*H*$_2$CH$_2$O), 3.36 – 3.42 (m, 1H, OCHC*H*$_2$N$_3$), 3.56 – 3.63 (m, 2H, OCHC*H*$_2$N$_3$ (1H), OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O (1H)), 3.89 – 3.99 (m, 2H, OC*H*$_2$CONH), 4.01 – 4.09 (m, 1H, OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O), 4.62 (dd, *J* = 7.7/4.0 Hz, 1H, OC*H*CH$_2$N$_3$), 4.91 – 4.94 (m, 1H, OC*H*CH$_2$CH$_2$CH$_2$CH$_2$O), 7.32 – 7.35 (m, 2H, 2'-H$_{4\text{-bromophenyl}}$, 6'-H$_{4\text{-bromophenyl}}$), 7.54 – 7.57 (m, 2H, 3'-H$_{4\text{-bromophenyl}}$, 5'-H$_{4\text{-bromophenyl}}$); ^{13}C NMR (CD$_3$OD): δ [ppm] = 19.4 (1C, OCHCH$_2$*C*H$_2$CH$_2$CH$_2$O), 26.6 (1C, OCHCH$_2$CH$_2$*C*H$_2$CH$_2$O), 29.0 (1C, OCH*C*H$_2$CH$_2$CH$_2$CH$_2$O), 57.0 (1C, OCH*C*H$_2$N$_3$), 63.2 (1C, OCHCH$_2$CH$_2$CH$_2$*C*H$_2$O), 68.2 (1C, O*C*H$_2$CONH), 82.7 (1C, O*C*HCH$_2$N$_3$), 103.4 (1C, O*C*HCH$_2$CH$_2$CH$_2$CH$_2$O), 123.6 (1C, C-4'$_{4\text{-bromophenyl}}$), 130.1 (2C, C-2'$_{4\text{-bromophenyl}}$, C-6'$_{4\text{-bromophenyl}}$), 133.0 (2C, C-3'$_{4\text{-bromophenyl}}$, C-5'$_{4\text{-bromophenyl}}$), 138.4 (1C, C-1'$_{4\text{-bromophenyl}}$), 168.2 (1C, OCH$_2$*C*ONH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3217, 2943, 2099, 1686, 1485, 1439, 1273, 1258, 1204, 1111, 1069, 1034, 1011, 945, 872, 818; HRMS (*m*/*z*): [M+H]$^+$ calcd for C$_{15}$H$_{20}$^{79}BrN$_4$O$_4$: 399.0662, found: 399.0707; HPLC (method 1): t_R = 18.8 min, purity 99.5%.

2-[(*R*)-1-(4-Bromophenyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy]-*N*-{[(*RS*)-tetrahydro-2*H*-pyran-2-yl]oxy}acetamide (*ent*-42)

Phenylacetylene (0.11 mL, 1.0 mmol), sodium ascorbate (20 mg, 0.1 mmol), and copper sulfate pentahydrate (13 mg, 0.05 mmol) were added to a solution of *ent*-**41** (200 mg, 0.5 mmol) in a 1:1 mixture of *t*BuOH and H$_2$O (30 mL). The reaction 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 phases were dried (Na$_2$SO$_4$), filtered and evaporated. The residue was purified by flash column chromatography (Ø = 2 cm, h = 15 cm, V = 10 mL, cyclohexane/ethyl acetate = 2/1, R_f= 0.06) to give *ent*-**42** as colourless solid (210 mg,

0.41 mmol, 82%). m.p. = 157 °C; $[\alpha]_D^{20}$ = -58.3 (1.2, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.49 – 1.85 (m, 6H, OCHC*H*$_2$C*H*$_2$C*H*$_2$CH$_2$O), 3.49 – 3.57 (m, 1H, OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O), 3.85 (d, *J* = 14.4 Hz, 1H, OC*H*$_2$CONH), 3.91 – 4.05 (m, 2H, OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O (1H), OC*H*$_2$CONH (1H)), 4.69 – 4.80 (m, 2H, OCHC*H*$_2$Ar), 4.86 – 4.89 (m, 1H, OC*H*CH$_2$CH$_2$CH$_2$CH$_2$O), 4.94 – 4.99 (m, 1H, OC*H*CH$_2$Ar), 7.30 – 7.37 (m, 3H, 2'-H$_{4\text{-bromophenyl}}$, 6'-H$_{4\text{-bromophenyl}}$, 4'''-H$_{phenyl}$), 7.41 – 7.46 (m, 2H, 3'''-H$_{phenyl}$, 5'''-H$_{phenyl}$), 7.55 – 7.58 (m, 2H, 3'-H$_{4\text{-bromophenyl}}$, 5'-H$_{4\text{-bromophenyl}}$), 7.79 – 7.83 (m, 2H, 2'''-H$_{phenyl}$, 6'''-H$_{phenyl}$), 8.35 – 8.38 (m, 1H, 5''-H$_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 19.4 (1C, OCHCH$_2$*C*H$_2$CH$_2$CH$_2$O), 26.1 (1C, OCHCH$_2$CH$_2$*C*H$_2$CH$_2$O), 28.9 (1C, OCH*C*H$_2$CH$_2$CH$_2$CH$_2$O), 56.4 (1C, OCH*C*H$_2$Ar), 63.2 (1C, OCHCH$_2$CH$_2$CH$_2$*C*H$_2$O), 68.1 (1C, O*C*H$_2$CONH), 81.8 (1C, O*C*HCH$_2$Ar), 103.3 (1C, O*C*HCH$_2$CH$_2$CH$_2$CH$_2$O), 123.5 (1C, C-5''$_{triazole}$), 124.0 (1C, C-4'$_{4\text{-bromophenyl}}$), 126.7 (2C, C-2'''$_{phenyl}$, C-6'''$_{phenyl}$), 129.4 (1C, C-4'''$_{phenyl}$), 129.96 (2C, C-3'''$_{phenyl}$, C-5'''$_{phenyl}$), 130.04 (2C, C-2'$_{4\text{-bromophenyl}}$, C-6'$_{4\text{-bromophenyl}}$), 131.7 (1C, C-1'''$_{phenyl}$), 133.1 (2C, C-3'$_{4\text{-bromophenyl}}$, C-5'$_{4\text{-bromophenyl}}$), 137.5 (1C, C-1'$_{4\text{-bromophenyl}}$), 148.7 (1C, C-4''$_{triazole}$), a signal for OCH$_2$*C*ONH could not be observed; IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3129, 2943, 1686, 1485, 1443, 1339, 1204, 1111, 1038, 1011, 872, 822, 764, 694; HRMS (*m*/*z*): [M+H]$^+$ calcd for $C_{23}H_{26}{}^{79}BrN_4O_4$: 501.1132, found: 501.1137; HPLC (method 1): t_R = 19.2 min, purity 99.9%.

2-{(*R*)-2-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-1-[4-(phenylethynyl)phenyl]ethoxy}-*N*-{[(*RS*)-tetrahydro-2*H*-pyran-2-yl]oxy}acetamide (*ent*-43)

Under N_2 atmosphere, copper(I) iodide (14 mg, 0.07 mmol), tetrakis(triphenylphosphine)palladium(0) (41 mg, 0.04 mmol) and phenylacetylene (0.12 mL, 1.1 mmol) were added to a solution of *ent*-**42** (180 mg, 0.36 mmol) in triethylamine (50 mL). The mixture was heated to reflux for 16 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 = 1/1, R_f = 0.30) to give *ent*-**43** as colorless solid (120 mg, 0.23 mmol, 64%). m.p. = 160 °C; $[\alpha]_D^{20}$ = -52.9 (0.4, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 1.51 – 1.82 (m, 6H, OCHC*H*$_2$C*H*$_2$C*H*$_2$CH$_2$O), 3.51 – 3.57 (m, 1H, OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O), 3.85 – 3.89 (m, 1H, OC*H*$_2$CONH), 3.94 – 4.05 (m, 2H, OCHCH$_2$CH$_2$CH$_2$C*H*$_2$O (1H), OC*H*$_2$CONH (1H)), 4.72 – 4.82 (m, 2H, OCHC*H*$_2$Ar), 4.87 – 4.90 (m, 1H, OC*H*CH$_2$CH$_2$CH$_2$CH$_2$O), 4.99 – 5.03 (m, 1H, OC*H*CH$_2$Ar), 7.33 – 7.39 (m, 3H, $H_{arom.}$), 7.42 – 7.46 (m, 4H, $H_{arom.}$), 7.51 – 7.53 (m, 2H, $H_{arom.}$), 7.55 – 7.58 (m, 2H, $H_{arom.}$), 7.62 – 7.67 (m, 1H, $H_{arom.}$), 7.81 – 7.83 (m, 2H, $H_{arom.}$), 8.37 – 8.39 (m, 1H, 5'-$H_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 19.4 (1C, OCHCH$_2$*C*H$_2$CH$_2$CH$_2$O), 26.2 (1C, OCHCH$_2$CH$_2$*C*H$_2$CH$_2$O), 29.0 (1C, OCH*C*H$_2$CH$_2$CH$_2$CH$_2$O), 56.5 (1C, OCH*C*H$_2$Ar), 63.2 (1C, OCHCH$_2$CH$_2$CH$_2$*C*H$_2$O), 68.1 (1C, O*C*H$_2$CONH), 82.1 (1C, O*C*HCH$_2$Ar), 89.5 (1C, *C*≡C), 90.9 (1C, *C*≡C), 103.3 (1C, O*C*HCH$_2$CH$_2$CH$_2$CH$_2$O), 123.5 (1C, C-5'$_{triazole}$), 124.3 (1C, $C_{arom.}$), 125.4 (1C, $C_{arom.}$), 126.7 (2C, $C_{arom.}$), 128.3 (2C, $C_{arom.}$), 129.4 (1C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 130.0 (2C, $C_{arom.}$), 131.7 (1C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 133.0 (2C, $C_{arom.}$), 133.8 (1C, $C_{arom.}$), 138.5 (1C, $C_{arom.}$), 148.8 (1C, C-4'$_{triazole}$), 168.0 (1C, OCH$_2$*C*ONH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3356, 2951, 1709, 1485, 1439, 1204, 1115, 1038, 872, 837, 760, 691; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{31}H_{31}N_4O_4$: 523.2340, found: 523.2357; HPLC (method 1): t_R = 21.8 min, purity 96.4%.

(*R*)-*N*-Hydroxy-2-{2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-[4-(phenylethynyl)phenyl]ethoxy}acetamide (*ent*-27)

HCl-saturated methanol (0.5 mL) was added to a solution of *ent*-**43** (50 mg, 0.10 mmol) in methanol (10 mL). The reaction mixture was stirred at ambient temperature for 16 h. Then the solvent was removed in vacuo and the residue was purified by flash column

chromatography (Ø = 1 cm, h = 15 cm, V = 5 mL, dichloromethane/methanol = 9.5/0.5, R_f = 0.29) to give *ent*-**27** as colourless solid (16 mg, 0.04 mmol, 38%). m.p. = 184 °C; $[\alpha]_D^{20}$ = -61.0 (0.7, methanol); ^{1}H NMR (CD_3OD): δ [ppm] = 3.87 (d, *J* = 14.2 Hz, 1H, OC*H*$_2$CONHOH), 3.93 (d, *J* = 14.2 Hz, 1H, OC*H*$_2$CONHOH), 4.74 (dd, *J* = 14.3/4.3 Hz, 1H, OCHC*H*$_2$Ar), 4.80 (dd, *J* = 14.3/7.5 Hz, 1H, OCHC*H*$_2$Ar), 4.98 (dd, *J* = 7.5/4.3 Hz, 1H, OC*H*CH$_2$Ar), 7.32 – 7.46 (m, 8H, $H_{arom.}$), 7.49 – 7.54 (m, 2H, $H_{arom.}$), 7.54 – 7.58 (m, 2H, $H_{arom.}$), 7.78 – 7.83 (m, 2H, $H_{arom.}$), 8.36 (s, 1H, 5'-$H_{triazole}$); ^{13}C NMR (CD_3OD): δ [ppm] = 56.5 (1C, OCH*C*H$_2$Ar), 68.1 (1C, O*C*H$_2$CONHOH), 82.1 (1C, O*C*HCH$_2$Ar), 89.5 (1C, *C*≡C), 91.0 (1C, *C*≡C), 123.5 (1C, C-5'$_{triazole}$), 124.3 (1C, $C_{arom.}$), 125.4 (1C, $C_{arom.}$), 126.7 (2C, $C_{arom.}$), 128.3 (2C, $C_{arom.}$), 129.4 (1C, $C_{arom.}$), 129.6 (2C, $C_{arom.}$), 129.7 (1C, $C_{arom.}$), 130.0 (2C, $C_{arom.}$), 131.7 (1C, $C_{arom.}$), 132.6 (2C, $C_{arom.}$), 133.1 (2C, $C_{arom.}$), 138.4 (1C, $C_{arom.}$), 148.8 (1C, C-4'$_{triazole}$), 168.3 (1C, OCH$_2$*C*ONH); IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3132, 2920, 2851, 1651, 1512, 1439, 1234, 1200, 1119, 1088, 1042, 980, 849, 833, 752, 691; HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{26}H_{23}N_4O_3$: 439.1765, found: 439.1796; HPLC (method 2): t_R = 17.5 min, purity 96.0%.

Biological evaluation

Disk diffusion assay

The antibiotic activity of the synthesized inhibitors was determined by disk diffusion assays. Liquid cultures of *E. coli* BL21(DE3) and the defective strain *E. coli* D22[41] were grown overnight in LB broth[43] 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, GE Healthcare). Pure DMSO, serving as a negative and CHIR-090, 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. Each assay was performed at least three times on separate days.

Minimum Inhibitory Concentration (MIC)

The MIC values of the compounds were determined by means of the microdilution method 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.

10 µL of a twofold dilution series of the compounds in DMSO and 90 µL of LB broth were dispensed to each well of a 96-well plate. Then 100 µL of the inoculated medium were added, resulting in $5 \cdot 10^6$ cfu $\cdot$ mL^{-1} and a final concentration range of the test compounds between 64 µg $\cdot$ mL^{-1} and 0.25 µg $\cdot$ mL^{-1}. The plates were incubated for 20 h at 37 °C. The MIC was defined as the lowest concentration of the compounds that prevented visible growth after incubation. Each assay was performed at least three times on separate days.

LpxC assay

Protein purification

The plasmid for the expression of LpxCC63A (pETEcLpxCC63A) was kindly provided by Carol Fierke.[44] The C63A mutation lowers the undesired influence of Zn^{2+}-concentration on enzymatic activity. The purification of LpxC was performed essentially as previously described.[45] Strong anion exchange was performed with a column containing 20 mL of quaternary ammonium-sepharose (Q-Sepharose) fast flow media (GE Healthcare). The fractions containing LpxC were concentrated and desalted using molecular weight cut off (MWCO) spin columns (10 kDa). 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 stored 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 a Nanodrop 2000C.

Enzyme inhibition assay

A fluorescence-based microplate assay for LpxC activity was performed as described by Clements *et al.*[29] The wells in a black, non-binding, 96 well fluorescence microplate (Greiner Bio One, Frickenhausen) were filled with 93 µL of 26.9 µM UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine in assay buffer (40 mM sodium

morpholinoethanesulfonic acid, 80 µM dithiothreitol, 0.02% Brij 35 (pH 6.0)). In order to assay the inhibitors at final concentrations from 2 µM up to 200 µM, 2 µL of a respective dilution of the compounds in DMSO were added. After addition of 5 µL of a solution of purified LpxC (50 µg · mL^{-1}) in assay buffer, 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 nmol *o*-phthaldialdehyde-2-mercaptoethanol in 0.1 M borax[46] and detected by a Tristar[2] plate reader (Berthold, Bad Wildbad) at 340 nm excitation and 460 nm emission wavelengths. Each assay was performed at least three times on separate days. The IC_{50} values were calculated via Probit-log concentration graphs with the aid of the software Origin.

Computational methods

To conduct the computational studies, a similar docking protocol as in our previous studies was implemented.[33] The crystal structure of *E. coli* LpxC in complex with inhibitor LPC-009 (PDB ID: 3P3G) was downloaded from the Protein Data Bank (www.rcsb.org) and used as a protein model.[47] Protein preparation process was carried out using the Protein Preparation Wizard tool in Schrödinger Suite.[48] Bond orders were assigned. Hydrogen atoms and missing amino acid side chains were automatically added, solvent particles were removed, protonation states and tautomeric forms were optimized with PROPKA tools at pH 7.0. Energy minimization of the complex was performed using the OPLS3e force-field and default settings to remove the steric clashes such as bad contacts and unsuitable torsional angles.[49]

Ligands were prepared using the LigPrep tool of Schrödinger Suite applying the OPLS3e force-field. The neutral form of the hydroxamic acids was utilized for all inhibitors. Conformational enrichment of molecules was done using Confgen by adjusting 25 conformers per each compound and performing force-field minimization on conformers.

Molecular docking studies were done using the Glide program in Schrödinger Suite. Grid files were generated by centroid of ligand and box size 20*20*20 Å. Metal constraint to the Zn^{2+}-ion as well as H-bond constraints to the E78 and T191 residues and positional constraint placed on the diphenylacetylene linker group were set. Docking studies were run in standard precision (SP) mode with flexible ligand sampling and enhanced planarity of conjugated π-groups. To validate the docking protocol, redocking of the inhibitor LPC-009 to *E. coli* LpxC crystal structure (PDB ID: 3P3G) was carried out and good agreement to the crystal structure was observed (RMSD 0.30 Å).

The docking poses were visually analyzed using the molecular modeling program MOE[50] and used for protein-ligand binding calculations. An in-house script implemented in MOE was used to carry out the interaction energy calculations using the Amber12:EHT force-field and default distance dependent dielectric implicit solvation model (GB/SA). The obtained LpxC-inhibitor complexes were energy minimized.[51-52] During the minimization protein backbone atoms were tethered using a force constant of (3/2) kT / 2 (σ = 0.5 Å). Complexes showing the most favorable interaction energy values were selected.

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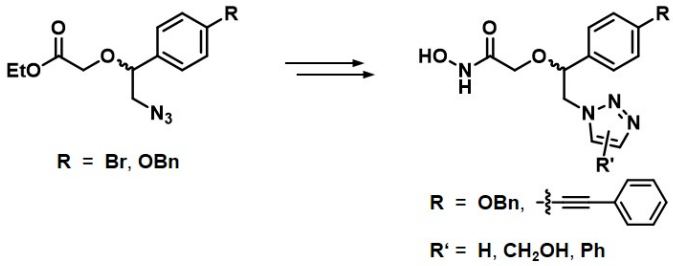

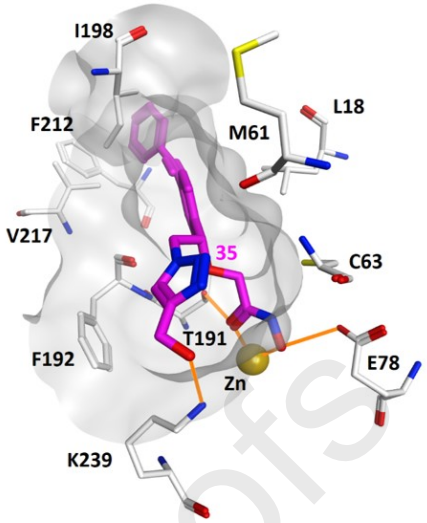

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: