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Phenyltriazole-functionalized sulfamate inhibitors targeting tyrosyl- or isoleucyl-tRNA synthetase

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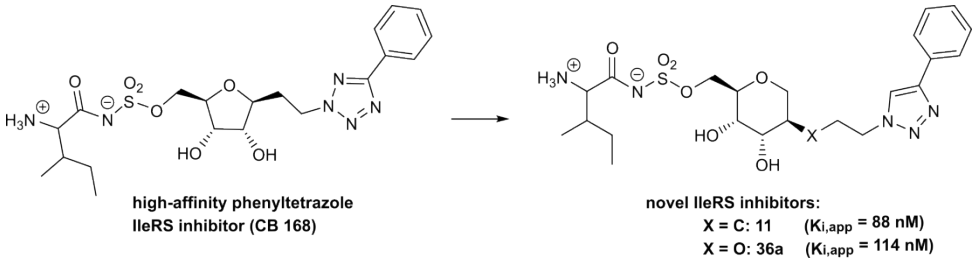
Abstract

Antimicrobial resistance is considered as one of the major threats for the near future as the lack of effective treatments for various infections would cause more deaths than cancer by 2050. The development of new antibacterial drugs is considered as one of the cornerstones to tackle this problem. Aminoacyl-tRNA synthetases (aaRSs) are regarded as good targets to establish new therapies. Apart from being essential for cell viability, they are clinically validated. Indeed, mupirocin, an isoleucyl-tRNA synthetase (IleRS) inhibitor, is already commercially available as a topical treatment for MRSA infections. Unfortunately, resistance developed soon after its introduction on the market, hampering its clinical use. Therefore, there is an urgent need for new cellular targets or improved therapies. Follow-up research by Cubist Pharmaceuticals led to a series of selective and *in vivo* active aminoacyl-sulfamoyl aryltetrazole inhibitors targeting IleRS (e.g. CB 168).

Here, we describe the synthesis of new IleRS and TyrRS inhibitors based on Cubist Pharmaceuticals compounds, whereby the central ribose was substituted for a tetrahydropyran ring. Various linkers were evaluated connecting the six-membered ring with the base-mimicking part of the synthesized analogues. Out of eight novel molecules, a three-atom spacer to the phenyltriazole moiety, which was established using azide-alkyne click chemistry, appeared to be the optimized linker to inhibit IleRS. However, **11** ($K_{i,app}$ = 88 ± 5.3 nM) and **36a** ($K_{i,app}$ = 114 ± 13.5 nM) did not reach the same level of inhibitory activity as for the known high-affinity natural adenylate-intermediate analogue isoleucyl-sulfamoyl adenosine (IleSA, CB 138; $K_{i,app}$ = 1.9 ± 4.0 nM) and CB 168, which exhibit a comparable inhibitory activity as the native ligand. Therefore, **11** was docked into the active site of IleRS using a known crystal structure of *T. thermophilus* in complex with mupirocin. Here, we observed the loss of the crucial 3'- and 4'- hydroxyl group interactions with the target enzyme compared to CB 168 and mupirocin, which we suggest to be the reason for the limited decrease in enzyme affinity. Despite the lack of antibacterial activity, we believe that structurally optimizing these novel mupirocin analogues via a structure-based approach could ultimately result in aaRS inhibitors which would help to tackle the antibiotic resistance problem.

Graphical abstract



Keywords

Antibiotic resistance, aminoacyl-tRNA synthetases, isoleucyl-tRNA synthetase inhibitors, mupirocin, aryl-tetrazole derivatives.

Highlights

- Eight new CB 168 analogues targeting isoleucyl- or tyrosyl-tRNA synthetase
- New IleRS-targeting compounds with 3-carbon linker showed good inhibitory activity
- Docking approach for **11** revealed a different binding mode compared to CB 168
- Activity decrease was due to loss of crucial pyran hydroxyl group interactions
- Enhanced lipophilic/hydrophilic balance suggested an improved bacterial uptake

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes for protein synthesis as they are required for a correct attachment of amino acids to their corresponding tRNA molecule.[1] These enzymes are divided in two classes based on their different overall fold.[2] Class I aaRSs contain a Rossmann binding fold, which is characterized by the conserved KMSKS and HIGH sequence motifs.[3] On the contrary, the active site of Class II aaRS synthetases have a unique seven-stranded β-sheet flanked by α-helices. Despite these conserved features, it was shown that a selective targeting of bacterial isoleucyl-tRNA synthetase (IleRS) is feasible. Polyketide IleRS inhibitor mupirocin (Figure 1A)[4] was approximately 8000 times more active against Gram-positive bacteria versus human cells.[1] Unfortunately, its activity spectrum is in practice restricted to Gram-positive bacteria due to poor penetration through the outer membrane of Gram-negative variants.[5] Nevertheless, mupirocin exhibits a remarkable activity towards the ESKAPE pathogen MRSA in particular. The ESKAPE pathogens are bacteria that are often resistant to many of the available antibiotics, and mupirocin therefore is included on the WHO list of essential medicines in 2019.[6]

However, low- and high-level resistance in Gram-positive bacteria against mupirocin appeared quickly after it was clinically introduced in 1985 because of decolonization failure and its widespread use led to an increase in resistance.[7] Low-level resistance (Minimal Inhibitory Concentration (MIC) < 64 μg/mL) arises when point mutations emerge during bacterial proliferation affecting the Rossmann fold in the synthetase.[8] However, the main concern is about the high-level resistance (MIC > 512 μg/mL), originating from bacteria expressing a novel IleRS[9] of which the plasmid encoding for the resistant enzyme (*mupA* gene) can be passed on via conjugal mating.[10] Nonetheless, the emergence of cross-resistance is quite unlikely due to the rather unexplored target. Halofuginone and tavaborole (Figure 1A) are the only other two commercially available aaRS inhibitors. Halofuginone is a competitive inhibitor competing with proline in the active site of ProRS and is solely used in cattle breeding.[11] It is a non-toxic derivative of the plant alkaloid febrifugine, which was historically recognized for its antiprotozoal activity. Tavaborole on the other hand is a topical antifungal drug for the treatment of onychomycosis which targets the editing site of LeuRS.[12] Its boron atom makes a covalently bound complex with the ribose hydroxyl groups of the 3'-terminal adenosine of a $tRNA_{Leu}$ molecule, prohibiting the charging with leucine. Also, resistance to tavaborole after repeated exposure has not been demonstrated,[13] unlike mupirocin.

Therefore, research started at the end of the 1990s in order to find new IleRS inhibitors. SmithKline Beecham Pharmaceuticals (now GSK) synthesized analogues of mupirocin whereby they replaced the labile ester functional group in the long aliphatic chain of mupirocin with various heterocyclic groups in order to avoid systemic breakdown to monic acid A.[14,15] Most of these new compounds could not reach the same antibacterial activity due to poor cell wall penetration, but increased polarity in subsequent research resulted in a compound which had potential to be systemically used *in vivo*.[16] Despite these promising results, no further follow-up was reported. Cubist Pharmaceuticals (since 2015 acquired by Merck & Co.) used the high-affinity aminoacyl-sulfamate adenosines (aaSAs, Figure 1B) as a starting point to synthesize IleRS inhibitors with improved chemical stability and bacterial selectivity. Substituted thiazoles were directly attached to the ribose ring as well, replacing the adenine group.[17] No antibacterial activity was reported for these selective molecules in contrast to another compound series in which the sugar ring was connected with aryl-substituted tetrazoles via a two-carbon atom linker (Figure 1B).[18] This approach gave rise to selective IleRS inhibitors of which the phenoxyphenyltetrazole (CB 432, Figure 1B) in particular had an antibacterial activity. However, research was halted due to its low bioavailability *in vivo*.

A recent report by the WHO stated that 10 million people would decease every year by 2050 due to antibacterial resistance.[19] Therefore, antibiotic research awakened with several global action plans as a result (e.g. Joint programming initiative on AntiMicrobial Resistance (AMR), Transatlantic task force on AMR, Global action plan on AMR, New drugs for bad bugs and only very recently the AWaRe campaign) and triggered academic groups to find new cellular targets or improve existing therapies. For example, Oxford Drug Design synthesized *N*-leucinyl benzenesulfonamides, based on previously reported benzenesulfonamide aaRS inhibitors,[20,21] which selectively and strongly inhibited *E. coli* LeuRS.[22] More specifically for mupirocin, it recently was shown by Lounsbury et al. that a combination therapy could resensitize mupirocin-resistant bacteria.[23]

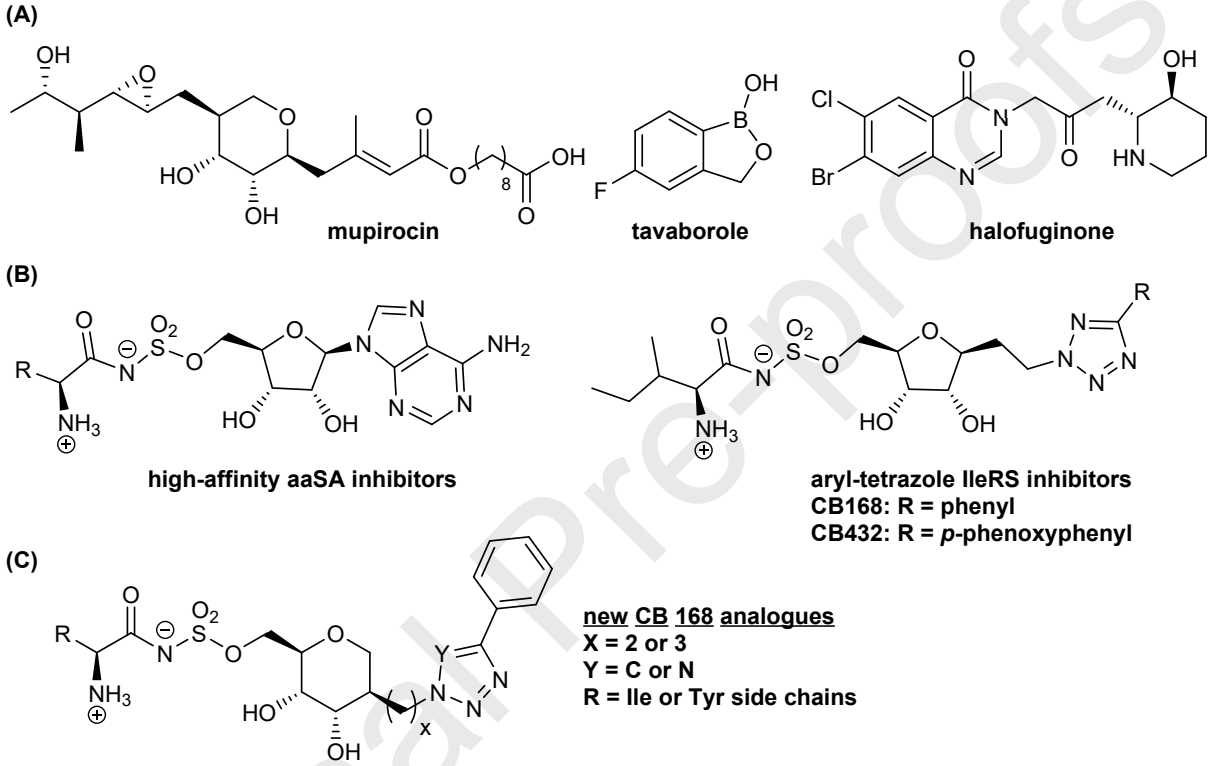


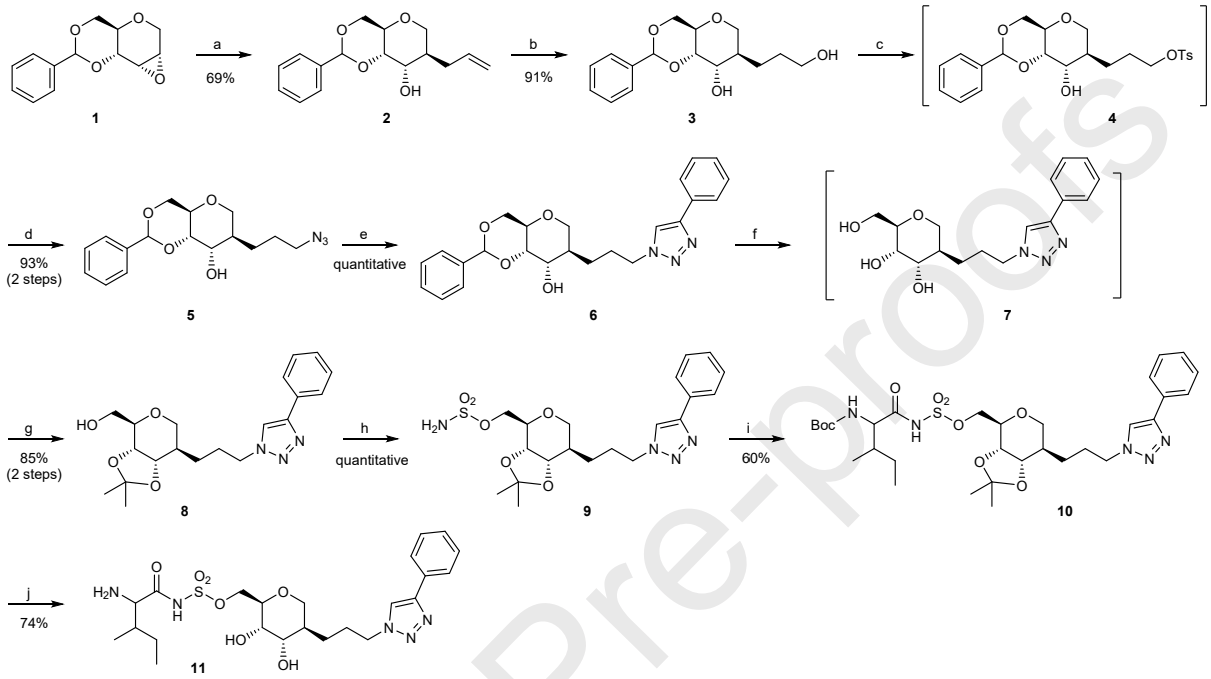
Figure 1: (A) Structure of all marketed aaRS inhibitors; (B) (Left) High-affinity but non-selective competitive aaSA inhibitors of the active intermediate, (Right) Cubist Pharmaceuticals aryl-tetrazole IleRS inhibitors; (C) Novel proposed structures targeting IleRS and TyrRS.

Following this trend, we envisioned to design novel inhibitors based on the tetrazole approach of Cubist Pharmaceuticals (Figure 1C). The ribose of the latter compounds was replaced by a tetrahydropyran ring as in mupirocin, however with a different implantation of both side chains on this ring. The base-mimicking chain was moved to the C2 position in analogy with the base connection in 1,5-anhydrohexitol nucleosides (HNA), which have been extensively studied in our research group for other purposes.[24-26] The sulfamate-containing chain was consequently shifted to C5 next to the ring oxygen, preserving a 1,4-cis relationship of both substituents on the tetrahydropyran ring. The sulfamate was further coupled to isoleucine or tyrosine to target IleRS and Class I TyrRS, respectively. Additionally, the importance of the epoxide functional group in mupirocin was investigated by introducing an epoxide-mimicking chain in our compounds because in the mupirocin-IleRS complex structure (PDB entry 1JZS) no significant interactions with this part of the molecule are noted. The optimal distance between the tetrahydropyran ring and the heterocycle needed evaluation as well because our six-membered ring is less flexible than the ribose ring used by Cubist Pharmaceuticals.

Following synthesis of all compounds, the enzymatic and antibacterial activity was evaluated. We here show that using this altered mupirocin scaffold it is feasible to ultimately develop new aaRS inhibitors which can contribute in the fight against the antibiotic resistance problem.

2. Results

2.1. Synthesis of phenyltriazoles/tetrazoles with different linkers

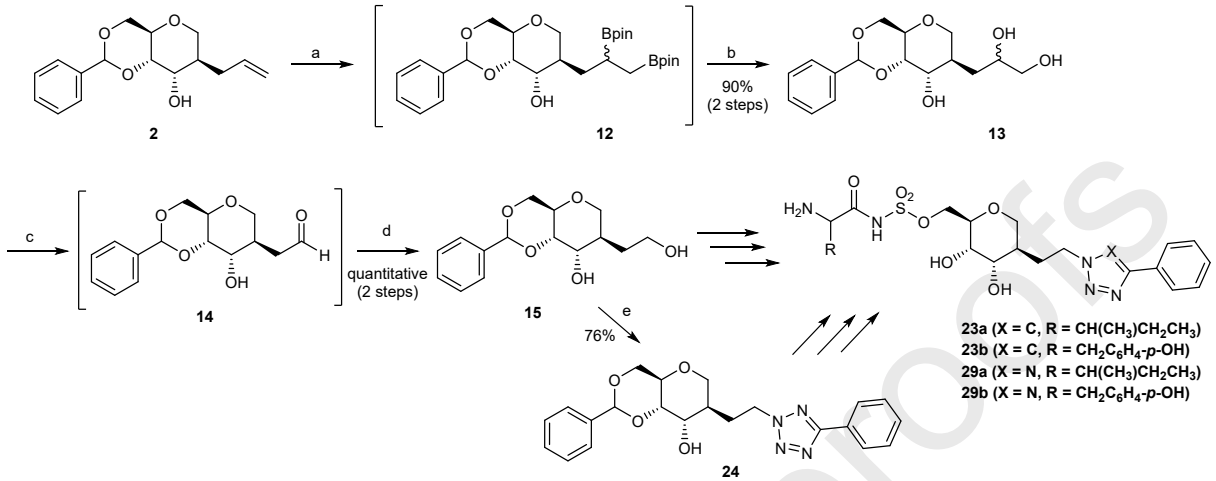


Reagents and conditions: (a) (1) CuI, allyl-MgCl, anh. THF, -30 °C, 15 min.; (2) **1**, anh. THF, -30 °C, 1.5 h; (b) BH_3·THF, anh. THF, rt, 1 h; (2) NaOH, H_2O_2, H_2O, 0 °C - rt, overnight; (c) (1) TEA, anh. DCM, -20 °C; (2) TsCl, anh. DCM, -20 °C - rt, overnight; (d) NaN_3, anh. DMF, 55 °C, overnight; (e) CuI, TEA, phenylacetylene, DMF, 50 °C, overnight; (f) PTSA·H_2O, THF/H_2O, 40 °C, overnight; (g) PTSA·H_2O, DMP, acetone, rt, overnight; (h) (1) CSI, HCOOH, 0 °C, 30 min.; (2) anh. MeCN, rt, 5 h; (3) **8**, DMA, rt, overnight; (i) Boc-Ile-OSu, Cs_2CO_3, anh. DMF, 0 °C - rt, 2 d; (j) TFA/H_2O, rt, 4 h.

*Scheme 1: General procedure towards isoleucine coupled C3 linked phenyltriazole **11**.*

The new implantation of both side chains on the pyran ring provoked some uncertainty regarding how the molecules would bind inside the active site of the synthetase. Consequently, the optimal aliphatic linker between the six-membered ring and the heterocycle for obtaining the best ligand-enzyme interactions needed to be determined. We started out synthesizing a C3 spacer linking the pyran ring to a 1,4-phenyl-substituted 1,2,3-triazole (Scheme 1). This heterocyclic scaffold was chosen because one of the most potent inhibitors of Cubist Pharmaceuticals in terms of enzymatic activity (and selectivity) contained a phenyltetrazole substituent (CB 168).[18] The amino acid coupled sulfamate moiety was likewise retained because it is known for its high binding affinity.[27] The synthetic route started from commercially available allitol epoxide **1** which opened regio- and stereoselectively at the C2-position in a trans-diaxial manner with the Gilman-reagent of allylmagnesium chloride.[28] Hydroboration-oxidation of the obtained alkene **2**, followed by selective tosylation of the resulting primary alcohol and subsequent *in situ* azide substitution, afforded compound **5**.

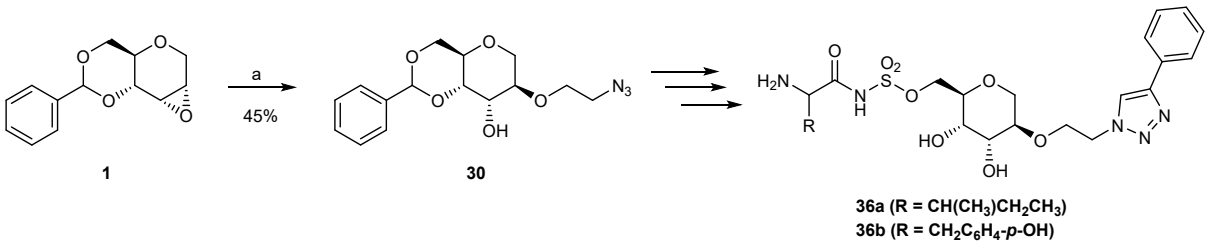
Azide-alkyne click chemistry gave phenyltriazole **6**, which was subsequently de- and re-acetalized into the isopropylidene protected alcohol **8**. Sulfamoylation of the latter compound was followed by coupling of isoleucine to the acquired sulfamate functional group. Finally, acidic removal of all protecting groups gave the desired compound **11** in a nice overall yield of 22%.



Reagents and conditions: (a) Cs_2CO_3, B_2pin_2, anh. MeOH, anh. THF, 70 °C, overnight; (b) (1) H_2O_2, NaOH, H_2O, 0 °C - rt, 1.5 h; (2) $Na_2S_2O_3$, H_2O, 0 °C - rt, 1.5 h; (c) $NaIO_4$, MeOH, H_2O, rt, overnight; (d) $NaBH_4$, anh. MeOH, 0 °C - rt, overnight; (e) (1) PPh_3, DEAD, anh. THF, 0 °C; (2) 5-phenyltetrazole, anh. THF/DMF, 0 °C - rt, overnight.

*Scheme 2: Synthetic route towards isoleucine and tyrosine coupled C2 linked phenyltriazoles and phenyltetrazoles **23** and **29** respectively (see supplemental schemes S1 and S2 for complete synthetic procedures).*

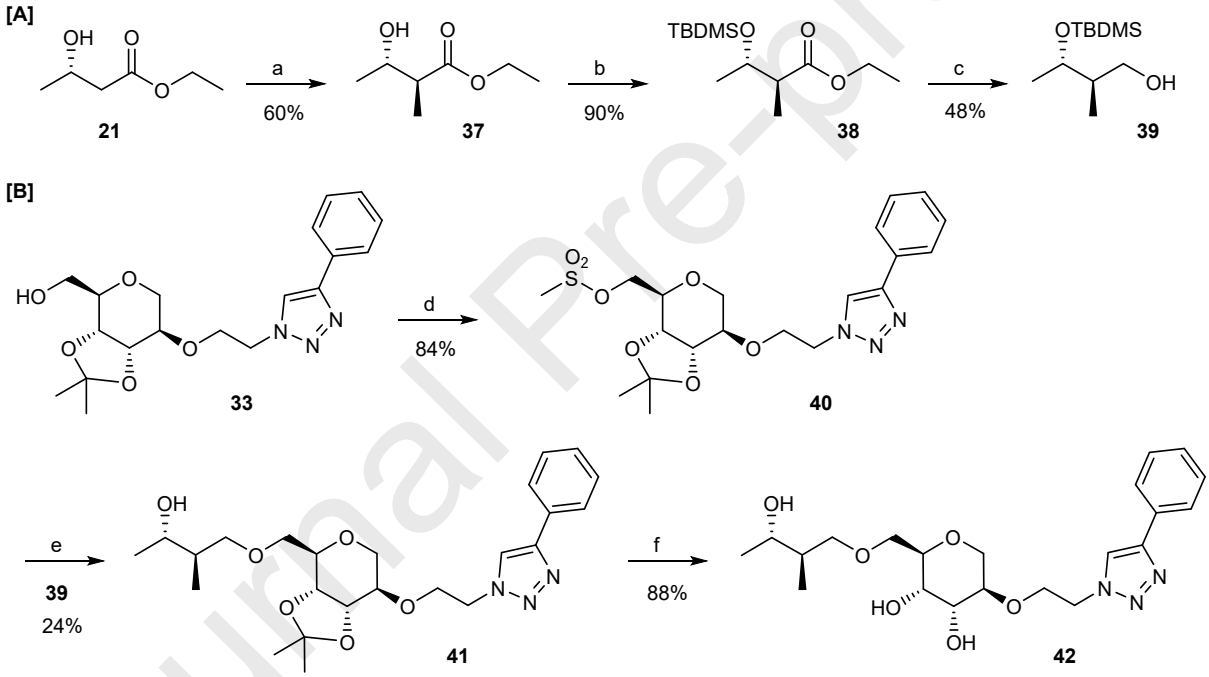
To compare the effect of different linkers on binding and subsequent synthetase affinities, we synthesized potential IleRS inhibitors **23a** and **29a** having one carbon atom less in the spacer between the pyran and heterocyclic ring (Scheme 2). The epoxide opening using vinylmagnesium chloride would have been the obvious choice to obtain the desired linker, but a previous report attained both inseparable stereoisomers attributable to the expected inversion and unusual retention of configuration at C2 due to the presence of the produced magnesium halide salt in the reaction solution.[28] Therefore, a racemic mixture of vicinal diol **13** was prepared using periodate, followed by oxidative cleavage and reduction of the obtained aldehyde, giving C2 linked diol **15**. The same general reaction procedure (see Scheme 1) was then pursued to afford phenyltriazole **23a**, or alternatively, the primary alcohol was subjected to a Mitsunobu reaction with 5-phenyltetrazole, leading ultimately to analogue **29a**. In addition, to expand our evaluation also protected tyrosine was coupled to both sulfamate compounds. Following conventional hydrogenolysis for removal of the benzyl group and TFA treatment, the analogues **23b** and **29b** were obtained in acceptable yields.



Reagents and conditions: (a) (1) NaH, 2-azidoethan-1-ol, anh. DMF, 0 °C, 30 min.; (2) **1**, anh. DMF, 0 °C - 80 °C, overnight.

*Scheme 3: Preparation of isoleucine and tyrosine coupled O-C2 linked phenyltriazoles **36** (see supplemental scheme S3 for complete synthetic procedure).*

A third type of linker envisaged a more polar O-C2 fragment to connect the tetrahydropyran ring and the heterocyclic fragment. Freshly-made 2-azidoethan-1-ol was utilized to open allitol epoxide **1** in moderate yield (Scheme 3). This led to the O-C2 linked compounds **36a-b** following the above described general synthetic procedure (see Scheme 1).



Reagents and conditions: [A] (a) (1) $(i\text{Pr})_2\text{NH}$, *n*-BuLi, anh. THF, -78 °C - 0 °C, 1 min.; (2) ethyl-(*S*)-3-hydroxybutyrate, DMPU, anh. THF, -78 °C - -60 °C, 45 min.; (3) MeI, anh. THF, 0 °C, 2.5 h; (b) (1) imidazole, anh. DMF; (2) TBDMS-Cl, anh. DMF, rt, overnight; (c) DIBAL (hexanes), anh. DCM, 0 °C, 1 h; [B] (d) (1) TEA, anh. DCM, 0 °C; (2) MsCl, anh. DCM, 0 °C - rt, 2 h; (e) (1) NaH, **39**, anh. DMF, 0 °C, 30 min.; (2) **40**, anh. DMF, 0 °C - 40 °C, overnight; (f) $\text{TFA}/\text{H}_2\text{O}$, rt, 5 h.

*Scheme 4: Procedure towards ether coupled and O-C2 linked mupirocin analogue **42**, in view of targeting IleRS (see supplemental scheme S4 for complete synthetic procedure).*

A totally different approach was envisioned for the introduction of an ether linked epoxide-mimicking side chain of mupirocin, while preserving the O-C2 spacer (Scheme 4) and targeting solely IleRS. Commercially available ethyl-(*S*)-3-hydroxybutyrate was therefore methylated in *anti* in high diastereoselectivity, followed by TBDMS protection affording compound **39**

following ester reduction with DIBAL.[29] Alcohol **33**, which was obtained during preparation of inhibitors **36**, was first mesylated allowing **39** to be coupled to the central scaffold, albeit in poor yield due to elimination reaction on **40** affording the major undesired product. In addition, we observed as well cleavage of the O-C2 linker under the applied reaction conditions. Likewise, the TBDMS group appeared unstable during the ether coupling reaction leading to **41** in lower amounts, while further isopropylidene deprotection afforded the desired compound **42**.

2.2. Inhibitory activity tests

The ability of all isoleucine-coupled compounds **11**, **23a**, **29a** and **36a**, together with **42**, to inhibit their target synthetase was determined by a radiolabeled *in vitro* aminoacylation assay (Figures 2 and 3).[30] The compounds **11** and **36a** having three atoms in their linker (almost) fully inhibited IleRS at concentrations of respectively 0.5 µM and 1.0 µM (Figure 2). As only the latter two compounds were clearly able to inhibit IleRS to a greater extent than the other three, we decided to perform a serial dilution analysis for **11** and **36a** in order to compare their activity with the high-affinity inhibitor IleSA (Figure 3). As calculated apparent inhibitory constants being independent of enzyme concentrations provide a better basis for comparative evaluations, a $K_{i,app}$ of 88 ± 5.3 nM was obtained for **11**, slightly better than for its O-C2 linked analogue **36a** having a $K_{i,app}$ of 114 ± 13.5 nM. However, these values are approximately two orders of magnitude higher than for IleSA which possesses a $K_{i,app}$ of 1.9 nM ± 4.0 nM.[31] In contrast to **11** and **36a**, the C2 linked heterocyclic molecules **23a** and **29a** displayed an inhibitory activity of around 1 µM only (Figure 2), with the ether-linked compound **42** being devoid of activity even at a concentration of 200 µM (data not shown).

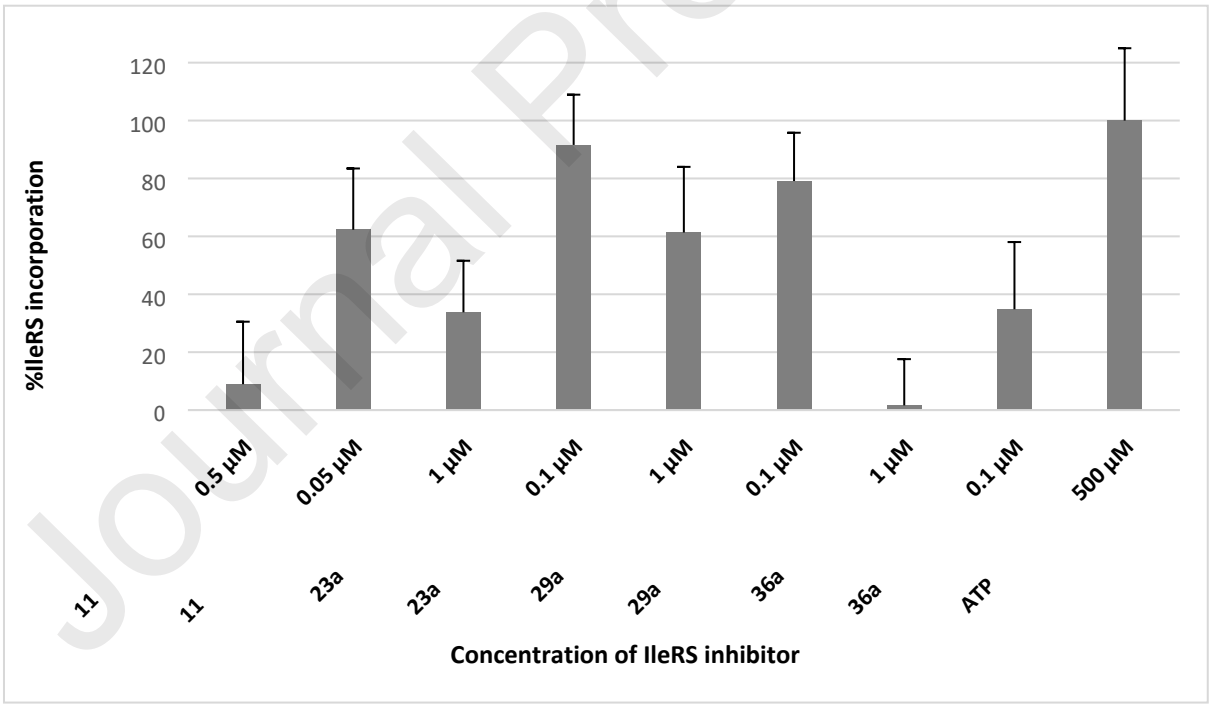


Figure 2: Aminoacylation activity of IleRS in presence of the indicated inhibitor. Each reaction was carried out with 10 nM IleRS, using 25 µM ^{14}C-labelled isoleucine and 500 µM ATP and a total E. coli tRNA preparation at a final concentration of 2 mg/mL. Values were normalized based on the measured activity of each enzyme in the absence of inhibitor. Experiments were done in triplicate; the mean and standard deviation are visualized.

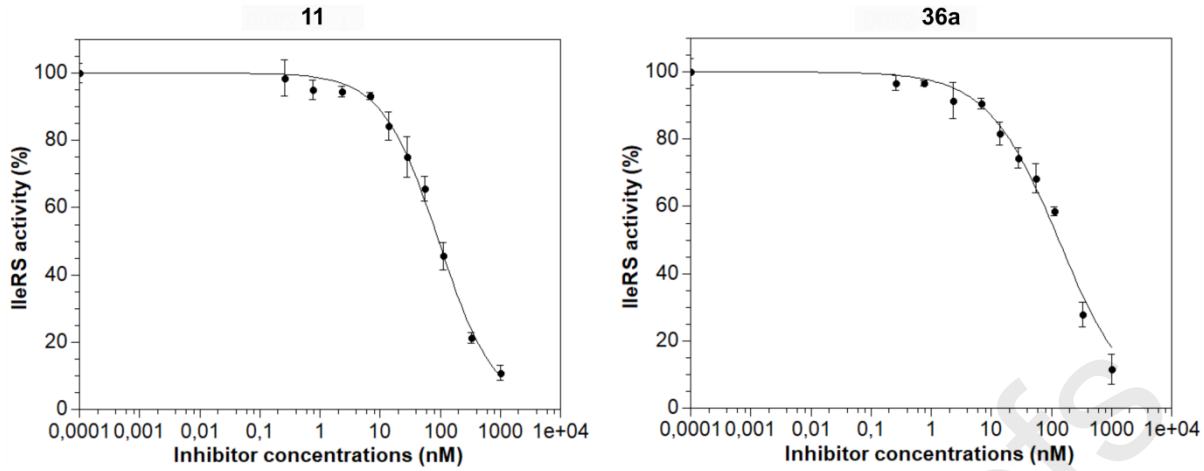


*Figure 3: Aminoacylation activity of E. coli IleRS in the presence of different concentrations of the inhibitors **11** and **36a**. The presented fits of the measured points were calculated using the Hill equation with fixed enzyme concentration. All data points were normalized to the measured activity of IleRS in the absence of inhibitor. Measurements were performed in triplicate; the mean and standard error are presented.*

The same assay was used for all TyrRS-targeting compounds **23b**, **29b** and **36b**, but unfortunately none of these compounds displayed enzyme inhibitory activity at 5 µM, even not the phenyltriazole **36b** functionalized with the O-C2 spacer (data not presented). Hence, the TyrRS active site seems less tolerant to large deviations of the regular nucleoside scaffold.

2.3. Docking results

The docking of **11** was performed in a *Thermus thermophilus* IleRS structure (PDB entry 1JZS for mupirocin) using Autodock Vina (Figure 4).[32] The conformation of the pyran ring in the highest affinity binding mode of **11** does not correspond to the pyran ring as seen in mupirocin since the position of the ring oxygen relative to the side chains is different in both compounds (Figure 4A). As a result, this orientation of **11** prevents the 3'- and 4'-OH groups from interacting with the synthetase residues D553 and E550 respectively. The same conclusion can be drawn with regard to the difference in binding mode with an isoleucyl-adenylate derivative (PDB entry 1JZQ, Figure 4B). Respecting the conserved amino acid part of the latter sulfamide high-affinity inhibitor, we clearly observe the altered orientations of the pyran hydroxyl groups between both compounds. However, additional π-π stacking interaction between the triazole/phenyl groups of **11** and enzyme residue H54 compensates the lack of these pyran ring interactions. Though, this might explain the small decrease in IleRS activity of **11** compared to mupirocin and the sulfamide isoleucyl-adenylate analogue which both display a similar binding mode (Figure 4C).

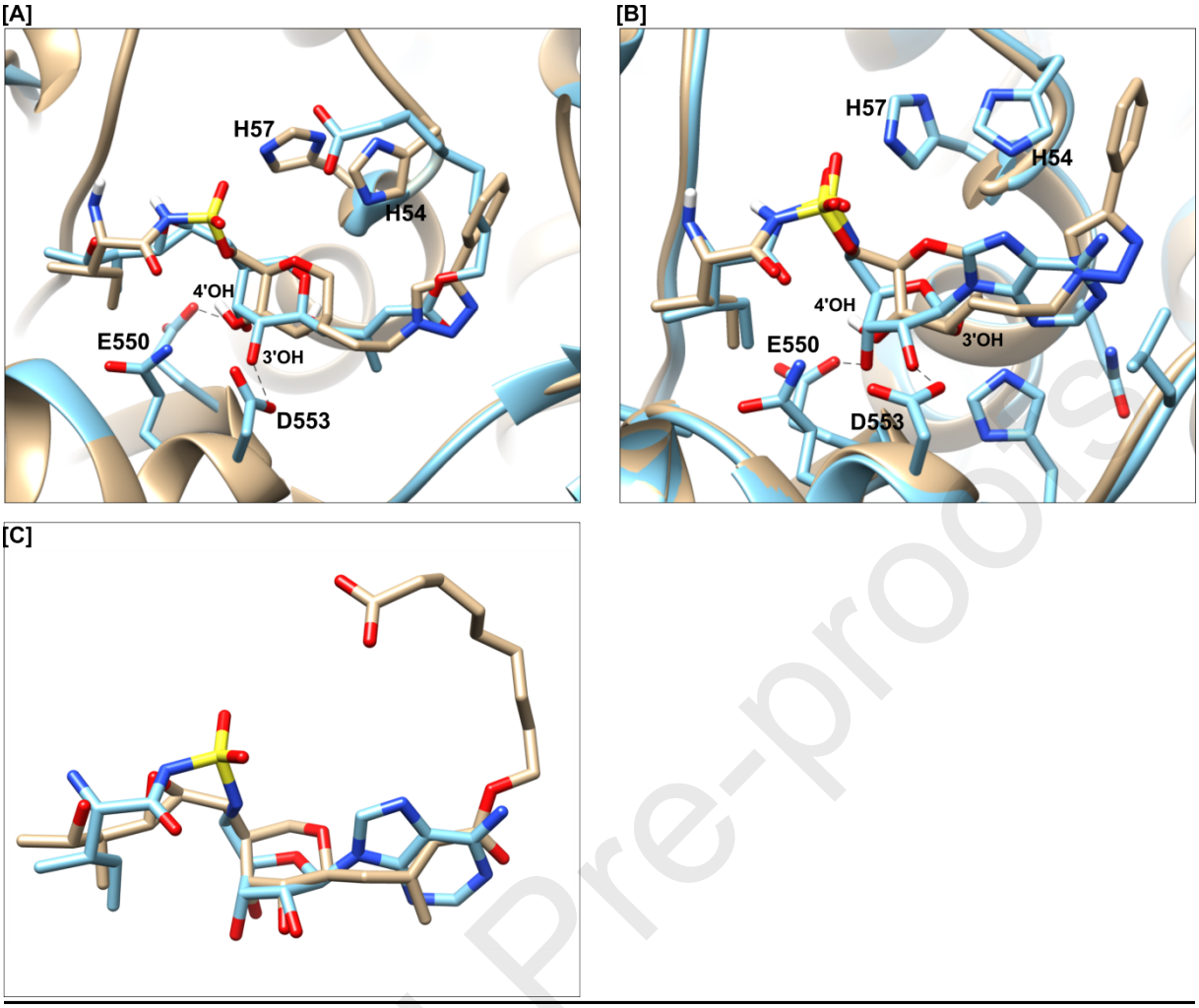


Figure 4: Docking of the highest affinity binding mode of **11** in a *T. thermophilus* IleRS structure using Autodock Vina. The isoleucine conformation remained conserved and crucial interactions of the pyran ring are shown by black dashed lines. The structures with **11** are displayed in grey and with mupirocin or the isoleucyl-adenylate analogue are presented in light blue. [A] Overlay with the crystal structure of IleRS in complex with mupirocin (PDB entry 1JZS); [B] Superposition with the crystal structure containing a high-affinity sulfamide inhibitor (PDB entry 1JZQ). [C] Comparison of binding mode between mupirocin (grey) and the adenylate derivative (light blue).

2.4. Antibacterial tests

All eight final compounds were submitted for screening for their *in vitro* antimicrobial activity at the Laboratory for Microbiology, Parasitology and Hygiene (LMPH) in Antwerp, using a resazurin assay, against Gram-negative bacterium *E. coli*, Gram-positive bacterium *S. aureus*, yeast *C. candida* and a non-pathogenic strain of *Mycobacterium tuberculosis*. Unfortunately, no antibacterial activity was observed at the maximum concentration of 64 µM tested (data not shown).

3. Discussion and conclusion

Mupirocin has become the most prescribed topical antibiotic and is used as a last resort for treating MRSA infections of open wounds owing to its rather unexplored mechanism of action,

the inhibition of isoleucyl-tRNA synthetase (IleRS).[33] Unfortunately, the emergence of high-level resistance against mupirocin appeared soon after it was clinically introduced.[7] Therefore, pharmaceutical companies have tried to develop analogues of mupirocin based on monic acid A or bisubstrate inhibitors targeting IleRS.[14-18] However, none of them was commercialized due to mostly low bacterial uptake or bioavailability and research was discontinued. In view of a recent WHO report stating that antibiotic resistance is nowadays one of the biggest threats to global health, food security and development, we intended to tackle the resistance problem of mupirocin by synthesizing novel inhibitors, based on the Cubist Pharmaceuticals compound CB 168 (Figure 1B). Our compounds however, exhibit a different implantation of both side chains on the newly introduced tetrahydropyran ring while preserving a 1,4-cis relationship, as is the case for the ring implantation in mupirocin (Figure 1C). The sulfamate linker ended up on the C6 position of the six-membered ring while the phenyltriazole/tetrazole moieties were moved to position C2 and were connected via different linkers to the pyran ring (Scheme 1 and supplemental schemes S1-S3). The importance of the epoxide in mupirocin structure was additionally investigated by synthesizing an ether-linked side chain while retaining the same length (Supplemental scheme S4).

Eight CB 168 analogues **11**, **23a-b**, **29a-b**, **36a-b** and **42** were prepared with a generally good overall yield (Schemes 1-4; for complete synthetic procedures, see also supplemental schemes S1-S4). The difference in their molecular structure was the linker between the pyran ring and the heterocycle because initially we had no idea which length would be optimal for proper binding with IleRS and TyrRS, respectively. Precursors functionalized with a C2, C3 and O-C2 linker were consequently made and were coupled with isoleucine and/or tyrosine. They were subsequently tested for their inhibitory activity against their corresponding enzyme using an *in vitro* aminoacylation assay (Figures 2 and 3). All analogues were furthermore broadly evaluated for their antimicrobial properties.

It is clear from the inhibitory activity results of the isoleucine coupled compounds **11**, **23a**, **29a** and **36a** that the optimal length between the pyran ring and the heterocycle is a C3 spacer. Compounds **11** and **36a** still showed significant inhibitory activity with a $K_{i,app}$ of 88 and 114 nM, respectively (Figure 3), whereas the C2 linked compounds **23a** and **29a** were almost unable to inhibit IleRS at 1 μM (Figure 2). These values are still higher than the one obtained for an analogue of the natural adenylate intermediate, IleSA ($K_{i,app}$ = 1.9 nM), which resembles CB 168 because the latter high-affinity inhibitor retained the isoleucine-ribose core.

In order to understand this decrease for the most potent inhibitor, an in silico docking with **11** was carried out (Figure 4) to identify differences in binding mode between mupirocin and an isoleucyl-adenylate analogue, a bioisostere of the active intermediate. In the conserved conformation of the isoleucyl-sulfamate moiety, the side chains of **11** occupy a comparable position in the binding site as mupirocin (Figure 4A). However, the position of the 3'-OH group is oriented differently in **11**, which prevents hydrogen bond interaction with enzyme residue D553. The location of the 4'-OH group is comparable but not identical to its equivalent in mupirocin, resulting in a less pronounced interaction with E550. For the high-affinity sulfamide inhibitor, both hydroxyl groups are ideally located for interacting with D553 and E550 (Figure 4B), analogous as for mupirocin (Figure 4C). From this comparison, it seems that a proper interaction with protein residues D553 and E550, as present in both mupirocin and the native ligand derivative, is crucial for inhibiting IleRS. Despite the lack of these pyran ring interactions, **11** still shows strong inhibitory activity towards the enzyme. This could be explained by the additional π-π stacking interaction between the heterocyclic/phenyl groups of **11** and histidine residue H54, which seem to compensate for the loss of the 3'- and 4'-OH hydrogen bond interactions.

Intrigued by this docking result, we wanted to investigate whether we could obtain the desired pyran ring interactions by synthesizing an ether linked side chain instead of the strongly binding amino acid containing chain (Scheme 4). Apparently, the conformation dictated by the epoxide functional group in mupirocin is necessary for optimal binding because **42** proved devoid of inhibitory activity. This lack of interaction was likewise found for all tyrosine coupled compounds **23b**, **29b** and **36b**, suggesting that even within the same synthetase class, the active site is sufficiently different so that structurally diverse molecules have to be prepared for inhibiting a specific synthetase.

In order to explain the lack of activity for the TyrRS-targeting compounds and the latter hypothesis on diversity of the active sites within the same aaRS class, docking of these molecules was performed. However, this proved difficult and ambiguous results were obtained. A possible explanation could be the narrower pocket of TyrRS compared to IleRS so that these TyrRS-targeting compounds are most likely too big to appropriately bind in the TyrRS active site as highlighted by comparing both sites (Figure 5). The IleRS pocket is more open at the base-mimicking part of mupirocin (Figure 5A) in contrast with the TyrRS active site where the adenine ring of the adenylate intermediate is tightly enclosed (Figure 5B). This results in the newly introduced triazole part in our compounds not to be tolerated by the latter enzyme.

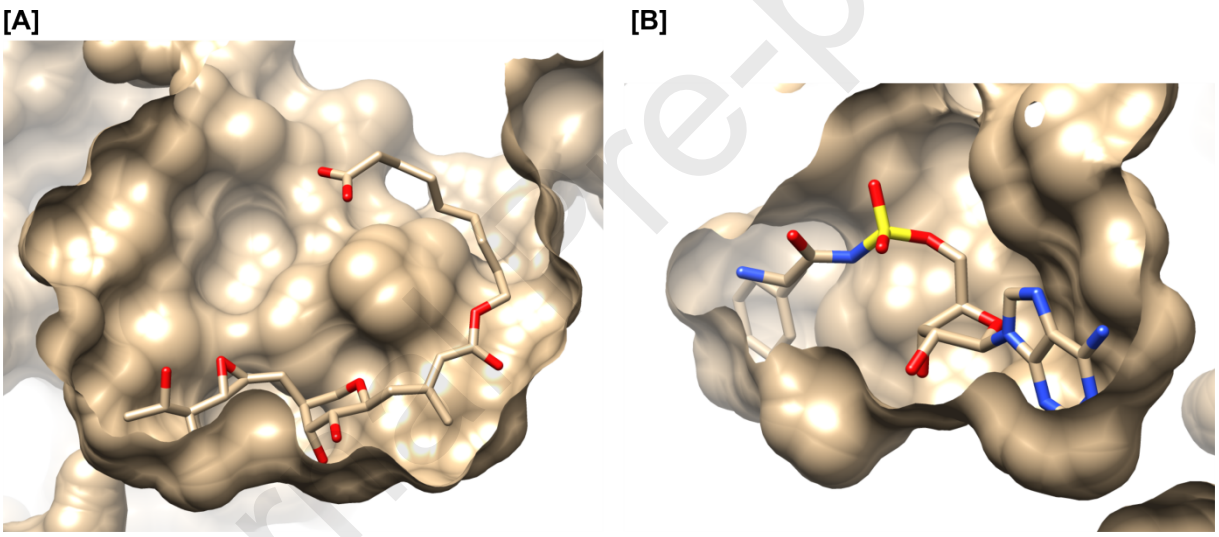


Figure 5: Size comparison between the IleRS and TyrRS active site; [A] *T. thermophilus* IleRS crystal structure in complex with mupirocin (PDB entry 1JZS); [B] *E. coli* TyrRS structure in complex with a Tyr-AMP analogue (PDB entry 1VBM).

For the sake of completeness, all compounds were tested for their whole-cell activity on different pathogens, but unfortunately even **11** and **36a** proved devoid of inhibitory activity. This insinuates that the compounds are not able to diffuse through the bacterial cell wall, most probably because of their high polarity (especially at the sulfamate part). Altering this feature by attaching more hydrophobic groups on the heterocycle could be exploited to increase uptake. Another possibility would be to stimulate an active transport which was the case for a polar antitubercular compound investigated by the group of C.C. Aldrich.[34] This particular inhibitor is actively taken up by the ATP-binding cassette transporters, which integrate nucleosides and cofactors exclusively in *M. tuberculosis*.

Despite the antibacterial inactivity of these new CB 168 analogues, we nevertheless conclude that optimizing this altered scaffold would result in aaRS inhibitors with optimal binding

interactions with their synthetase. Further research for targeting different aaRSs via this structure-based drug design approach is therefore ongoing in our research group.

4. Experimental section

4.1. Reagents and analytical procedures

Reagents and solvents were purchased from commercial suppliers (Acros, Sigma-Aldrich) and used as provided, unless indicated otherwise. DMF, THF, DCM and MeOH were of analytical grade and were stored over 4 Å molecular sieves. All other solvents used for reactions were analytical grade and used as provided. Reactions were carried out in oven-dried glassware under a nitrogen atmosphere with stirring at room temperature, unless indicated otherwise. ^{14}C-radiolabeled amino acids and scintillation liquid were purchased from Perkin Elmer.
^{1}H and ^{13}C NMR spectra of the compounds dissolved in $CDCl_3$, CD_3OD or DMSO-d_6 were recorded on a Bruker UltraShield Avance 300 MHz and 400 MHz or when needed on a 500 MHz and 600 MHz spectrometer. The chemical shifts are expressed as δ values in parts per million (ppm), using the residual solvent peaks ($CDCl_3$: ^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm; DMSO: ^{1}H, 2.50 ppm; ^{13}C, 39.52 ppm; CD_3OD: ^{1}H, 3.31 ppm; ^{13}C, 49.00 ppm) as a reference. Coupling constants are reported in Hertz (Hz). The peak patterns are indicated by the following abbreviations: br s = broad singlet, d = doublet, m = multiplet, q = quadruplet, s = singlet and t = triplet. High resolution mass spectra were recorded on a quadruple time-of-flight mass spectrometer (SYNAPT G2 HDMS, Waters, Milford, US) equipped with a standard ESI interface; samples were infused in acetonitrile/H_2O (1:1) at 5 µL/min. For TLC, precoated aluminum sheets were used (Merck, Silica gel 60 F254). The spots were visualized by UV light at 254 nm or when needed with 5% H_2SO_4 in EtOH or $KMnO_4$. Chromatography was performed on ICN silica gel 60Å 60-200. Eluent compositions are expressed as v/v. The characterization of the most important intermediates and all final compounds by NMR and mass spectrometry and complete synthetic schemes are provided in the supplementary file.

4.2. Chemical synthesis and analysis of intermediates and final compounds

4.2.1. (4*aR*,7*R*,8*S*,8*aS*)-7-allyl-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**2**).

Copper(I) iodide (0.163 g, 0.854 mmol, 0.2 eq.) was put under an atmosphere of nitrogen and anhydrous tetrahydrofuran (THF, 15.7 mL) was added. The suspension was cooled to -30 °C and allylmagnesium chloride (10.7 mL, 5.0 eq., 2.0 M in THF) was added dropwise. Epoxide **1** (1.0 g, 4.27 mmol, 1.0 eq.) was dissolved in anhydrous THF (9.5 mL, 0.45 M) and was added dropwise to the Gilman-like reagent. The mixture was stirred for 1.5 h at -30 °C. After completion, the reaction was quenched with aqueous saturated ammonium chloride (50 mL) at -30 °C. The mixture was then stirred for 30 min. at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 50:50) to give compound **2** (0.810 g, 69%) with R_f = 0.70 (heptane:ethyl acetate 50:50) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53 – 7.44 (m, 2H), 7.42 – 7.31 (m, 3H), 5.77 (m, 1H), 5.62 (s, 1H), 5.11 (m, 2H), 4.30 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.04 (s, 1H), 3.98 (dd, *J* = 11.7, 2.8 Hz, 1H), 3.94 – 3.85 (m, 1H), 3.76 – 3.67 (m, 2H), 3.64 (d, *J* = 11.6 Hz, 1H), 2.43 – 2.17 (m, 3H), 1.92 (dd, *J* = 8.7, 6.4 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.63, 136.31, 129.53, 128.64, 126.47, 117.42, 102.33, 77.95, 69.65, 68.73, 67.60, 66.11, 41.53, 34.48 ppm. HRMS (ESI): calcd. for $C_{16}H_{20}O_4Na$ $[M + Na]^+$ 299.1254; found 299.1253.

4.2.2. (4*aR*,7*R*,8*S*,8*aS*)-7-(3-hydroxypropyl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**3**).

Alkene **2** (0.266 g, 0.963 mmol, 1.0 eq.) was put under an atmosphere of nitrogen and anhydrous THF (3.9 mL, 0.25 M) was added. Borane tetrahydrofuran complex (1.1 mL, 1.1 eq., 1.0 M in THF) was added and the mixture was stirred for 1 h at room temperature. After completion of the reaction (TLC), the solution was cooled to 0 °C. Aqueous sodium hydroxide (1.4 mL, 1.5 eq., 1.0 M) and aqueous hydrogen peroxide (0.1 mL, 1.5 eq., 35 wt.%) were added dropwise. The mixture was allowed to warm to room temperature and was stirred overnight at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with aqueous saturated sodium bicarbonate and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 50:50 → 0:100) to afford compound **3** (0.258 g, 91%) with R_f = 0.12 (heptane:ethyl acetate 50:50) as a white solid. ^{1}H NMR (400 MHz, DMSO): δ = 7.45 (m, 2H), 7.35 (m, 3H), 5.64 (s, 1H), 4.98 (d, *J* = 3.5 Hz, 1H), 4.41 (t, *J* = 5.1 Hz, 1H), 4.13 (dd, *J* = 9.9, 4.8 Hz, 1H), 3.87 – 3.81 (m, 2H), 3.76 (td, *J* = 9.9, 4.9 Hz, 1H), 3.67 (dd, *J* = 9.4, 2.4 Hz, 1H), 3.62 (t, *J* = 10.1 Hz, 1H), 3.50 (d, *J* = 11.3 Hz, 1H), 3.41 (q, *J* = 5.7 Hz, 2H), 1.64 – 1.58 (m, 1H), 1.58 – 1.38 (m, 4H) ppm. ^{13}C NMR (101 MHz, DMSO): δ = 138.49, 129.18, 128.38, 126.86, 101.47, 77.86, 68.96, 67.78, 67.15, 65.57, 61.16, 43.49, 31.30, 26.27 ppm. HRMS (ESI): calcd. for $C_{16}H_{22}O_5Na$ $[M + Na]^+$ 317.1360; found 317.1360.

4.2.3. (4*aR*,7*R*,8*S*,8*aS*)-7-(3-azidopropyl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**5**).

Diol **3** (0.216 g, 0.734 mmol, 1.0 eq.) and triethylamine (0.2 mL, 1.101 mmol, 1.5 eq.) were put under nitrogen and anhydrous dichloromethane (DCM, 1.8 mL) was added. The solution was then cooled to -20 °C. *p*-Toluenesulfonyl chloride (0.140 g, 0.734 mmol, 1.0 eq.) was dissolved in anhydrous DCM (1.8 mL) and was added dropwise, resulting in a final 0.2 M solution. The mixture was allowed to warm to room temperature and was stirred overnight at room temperature. The reaction was quenched in cold water. The aqueous layer was extracted with DCM (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure to yield crude compound **4** (HRMS). HRMS (ESI): calcd. for $C_{23}H_{29}O_7S$ $[M + H]^+$ 449.1628; found 449.1628.
Sodium azide (0.072 g, 1.101 mmol, 1.5 eq.) was placed in a round bottom flask under nitrogen. Crude tosylate **4** was dissolved in anhydrous *N,N*-dimethylformamide (DMF, 3.7 mL, 0.2 M) and was added to the sodium azide. The mixture was stirred overnight at 55 °C after which the solution was cooled to room temperature and water was added. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 50:50) to obtain compound **5** (0.218 g, 93%) with R_f = 0.58 (heptane:ethyl acetate 50:50) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 – 7.46 (m, 2H), 7.40 – 7.35 (m, 3H), 5.63 (s, 1H), 4.30 (dd, *J* = 10.3, 5.0 Hz, 1H), 4.02 (dd, *J* = 11.7, 2.6 Hz, 2H), 3.92 (td, *J* = 10.1, 4.9 Hz, 1H), 3.74 – 3.67 (m, 2H), 3.62 (d, *J* = 11.7 Hz, 1H), 3.39 – 3.27 (m, 2H), 2.35 (s, 1H), 1.86 – 1.80 (m, 1H), 1.78 – 1.50 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.27, 129.26, 128.36, 126.14, 102.06, 77.72, 69.29, 68.85, 67.41, 65.90, 51.36, 41.47, 27.31, 26.78 ppm. HRMS (ESI): calcd. for $C_{16}H_{21}N_3O_4Na$ $[M + Na]^+$ 342.1424; found 342.1426.

4.2.4. (4*aR*,7*R*,8*S*,8*aS*)-2-phenyl-7-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)hexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**6**).

The obtained azide **5** (0.120 g, 0.376 mmol, 1.0 eq.), copper(I) iodide (0.007 g, 0.038 mmol, 0.1 eq.) and triethylamine (0.2 mL, 1.127 mmol, 3.0 eq.) were dissolved in DMF (1.9 mL, 0.2 M) and the solution was put under an atmosphere of nitrogen. Phenylacetylene (0.1 mL, 0.526 mmol, 1.4 eq.) was added and the mixture was stirred overnight at 50 °C. Water was added and the aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 25:75) to afford compound **6** (0.158 g, 100%) with R_f = 0.67 (ethyl acetate) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.87 – 7.82 (m, 2H), 7.77 (s, 1H), 7.48 – 7.32 (m, 8H), 5.60 (s, 1H), 4.48 – 4.40 (m, 2H), 4.28 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.04 – 3.99 (m, 2H), 3.91 (td, *J* = 10.0, 5.0 Hz, 1H), 3.70 – 3.65 (m, 2H), 3.62 (d, *J* = 11.7 Hz, 1H), 2.48 (s, 1H), 2.13 – 1.97 (m, 2H), 1.85 (ddd, *J* = 8.0, 5.4, 2.6 Hz, 1H), 1.75 – 1.45 (m, 2H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 147.87, 137.15, 130.47, 129.19, 128.83, 128.29, 128.17, 126.08, 125.64, 119.40, 101.95, 77.53, 69.15, 68.61, 67.36, 65.70, 50.18, 41.36, 28.67, 26.41 ppm. HRMS (ESI): calcd. for $C_{24}H_{28}N_3O_4$ $[M + H]^+$ 422.2074; found 422.2079.

4.2.5. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methanol (**8**).

Triazole **6** (0.161 g, 0.382 mmol, 1.0 eq.) and *p*-toluenesulfonic acid monohydrate (0.073 g, 0.382 mmol, 1.0 eq.) were put under an atmosphere of nitrogen and were dissolved in a 1:1 mixture of THF:water (2.6 mL:2.6 mL, 0.075 M). The mixture was stirred overnight at 40 °C, after which the solvents were removed under reduced pressure to give crude compound **7** (HRMS). HRMS (ESI): calcd. for $C_{17}H_{24}N_3O_4$ $[M + H]^+$ 334.1761; found 334.1766.
The obtained crude mixture and *p*-toluenesulfonic acid monohydrate (0.249 g, 1.31 mmol, 3.4 eq.) were dissolved in acetone (15 mL, 0.025 M). 2,2-Dimethoxypropane (0.3 mL, 2.4 mmol, 6.3 eq.) was added and the mixture was stirred overnight at room temperature under nitrogen, after which the reaction was quenched with aqueous saturated sodium bicarbonate. Acetone was removed under reduced pressure and the remaining aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (ethyl acetate) to give compound **8** (0.121 g, 85%) with R_f = 0.38 (ethyl acetate) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (m, 2H), 7.76 (s, 1H), 7.39 (m, 3H), 4.43 (t, *J* = 7.0 Hz, 1H), 4.08 (dd, *J* = 4.9, 2.6 Hz, 1H), 3.88 (dd, *J* = 9.1, 5.2 Hz, 1H), 3.78 (dd, *J* = 12.0, 2.9 Hz, 1H), 3.67 (dd, *J* = 11.6, 1.4 Hz, 1H), 3.58 (dt, *J* = 11.7, 5.9 Hz, 1H), 3.38 (ddd, *J* = 9.0, 6.3, 2.8 Hz, 1H), 2.06 (m, 3H), 1.93 (t, *J* = 6.2 Hz, 1H), 1.69 – 1.52 (m, 2H), 1.49 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 147.83, 130.43, 128.85, 128.26, 125.68, 119.58, 108.99, 78.30, 75.79, 70.06, 65.56, 63.18, 50.27, 36.42, 28.32, 28.20, 27.04, 26.16 ppm. HRMS (ESI): calcd. for $C_{20}H_{28}N_3O_4$ $[M + H]^+$ 374.2074; found 374.2069.

4.2.6. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl sulfamate (**9**).

A flask was charged with nitrogen and was cooled to 0 °C. Chlorosulfonyl isocyanate (0.1 mL, 1.00 mmol, 3.1 eq.) was added and then formic acid (0.04 mL, 1.02 mmol, 3.1 eq.) was added dropwise. The mixture was stirred for 30 min. at 0 °C. The resulting white solid was dissolved in anhydrous acetonitrile (1.0 mL, 1.0 M) and the solution was stirred for 5 h at room temperature. Alcohol **8** (0.121 g, 0.324 mmol, 1.0 eq.) was dissolved in *N,N*-dimethylacetamide

(1.6 mL) and was added dropwise to sulfamoyl chloride. The mixture was stirred overnight at room temperature, after which triethylamine (0.7 mL, 4.86 mmol, 15 eq.) was added and the mixture was stirred for 10 min. at room temperature. Methanol (0.7 mL, 16.20 mmol, 50 eq.) was then added, resulting in a transparent solution and the mixture was stirred for another 15 min. at room temperature. The solvents were then removed under reduced pressure, whereafter the residue was dissolved in aqueous saturated sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (ethyl acetate) to yield compound **9** (0.147 g, 100%) with R_f = 0.67 (ethyl acetate) as a transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (s, 2H), 7.80 (s, 1H), 7.46 – 7.27 (m, 3H), 5.82 (s, 2H), 4.37 (dd, *J* = 13.5, 7.8 Hz, 3H), 4.20 (dd, *J* = 11.2, 5.9 Hz, 1H), 4.07 (d, *J* = 3.7 Hz, 1H), 3.87 (dd, *J* = 9.1, 4.9 Hz, 1H), 3.76 – 3.64 (m, 2H), 3.53 (dd, *J* = 8.1, 5.9 Hz, 1H), 2.13 – 1.90 (m, 3H), 1.63 – 1.36 (m, 5H), 1.32 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.05, 130.75, 129.19, 128.52, 126.01, 120.36, 109.67, 76.60, 75.98, 70.44, 69.59, 65.99, 50.52, 36.37, 28.45, 28.39, 27.28, 26.48 ppm. HRMS (ESI): calcd. for $C_{20}H_{29}N_4O_6S$ $[M + H]^+$ 453.1802; found 453.1801.

4.2.7. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl ((tert-butoxycarbonyl)isoleucyl)sulfamate (**10**).

Cesium carbonate (0.194 g, 0.597 mmol, 3.0 eq.) was placed in a round bottom flask charged with nitrogen and was cooled to 0 °C. Compound **9** (0.090 g, 0.199 mmol, 1.0 eq.) and *t*-butyloxycarbonyl-*L*-isoleucine hydroxysuccinimide ester (0.131 g, 0.398 mmol, 2.0 eq.) were dissolved in anhydrous DMF (6.6 mL, 0.03 M) and were added. The mixture was gradually warmed to room temperature and was stirred for 2 days at room temperature. The solvent was then removed under reduced pressure and the residue was resuspended in DCM. The suspension was filtered over celite, whereafter the solvent was removed under reduced pressure. The obtained residue was purified by silica gel chromatography (DCM:methanol 100:0 → 90:10) to afford the isoleucine coupled compound **10** (0.079 g, 60%) with R_f = 0.37 (DCM:methanol 90:10) as a yellow oil. HRMS (ESI): calcd. for $C_{31}H_{46}N_5O_9S$ $[M - H]^-$ 664.3021; found 664.3014.

4.2.8. ((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)tetrahydro-2*H*-pyran-2-yl)methyl isoleucylsulfamate (**11**).

The isoleucine containing compound **10** (0.079 g, 0.119 mmol, 1.0 eq.) was placed under nitrogen and an 1:1 mixture of trifluoroacetic acid:water (0.5 mL:0.5 mL) was added. The mixture was stirred for 4 h at room temperature, after which more water was added and the reaction mixture was lyophilized. The residue was purified by silica gel chromatography (DCM:methanol 90:10 → 85:15) to provide the desired isoleucine containing product **11** (0.046 g, 74%) with R_f = 0.07 (DCM:methanol 90:10) as a light-yellow solid. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.36 (s, 1H), 7.84 – 7.80 (m, 2H), 7.43 (m, 2H), 7.36 – 7.31 (m, 1H), 4.47 (t, *J* = 7.2 Hz, 2H), 4.30 (dd, *J* = 10.8, 2.0 Hz, 1H), 4.21 (dd, *J* = 10.9, 5.3 Hz, 1H), 3.89 – 3.84 (m, 2H), 3.75 (ddd, *J* = 9.7, 5.3, 2.0 Hz, 1H), 3.62 (dd, *J* = 9.7, 3.1 Hz, 1H), 3.58 – 3.53 (m, 2H), 3.35 (s, 1H), 2.02 (m, 3H), 1.76 (m, 1H), 1.65 – 1.55 (m, 2H), 1.46 – 1.39 (m, 1H), 1.32 – 1.24 (m, 1H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, CD_3OD): δ = 175.03, 148.84, 131.73, 129.97, 129.32, 126.67, 122.33, 112.06, 98.80, 76.23, 71.67, 70.32, 65.92, 65.62, 61.38, 51.38, 43.22, 38.10, 29.41, 26.79, 25.72, 15.46, 12.16 ppm. HRMS (ESI): calcd. for $C_{23}H_{34}N_5O_7S$ $[M - H]^-$ 524.2184; found 524.2185.

4.2.9. 3-((4*aR*,7*R*,8*S*,8*aS*)-8-hydroxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-7-yl)propane-1,2-diol (**13**).

Alkene **2** (0.753 g, 2.72 mmol, 1.0 eq.), cesium carbonate (0.266 g, 0.817 mmol, 0.3 eq.) and bis(pinacolato)diboron (1.384 g, 5.45 mmol, 2.0 eq.) were put under an atmosphere of nitrogen and were dissolved in anhydrous THF (11 mL, 0.25 M). Anhydrous methanol (MeOH, 0.6 mL, 0.792 mmol, 5.0 eq.) was added and the mixture was refluxed (70 °C) overnight to give *in situ* crude compound **12** with R_f = 0.57 (heptane:ethyl acetate 50:50).
The solution was cooled to 0 °C. Aqueous sodium hydroxide (5.5 mL, 5.0 eq., 2.5 M) and aqueous hydrogen peroxide (1.4 mL, 5.0 eq., 30 wt%) were added dropwise. The mixture was allowed to warm to room temperature and was stirred for 1.5 h at room temperature. After completion of the reaction (TLC), the solution was cooled to 0 °C and the reaction was quenched with aqueous saturated sodium thiosulfate (15 mL) and water (5.0 mL). The mixture was then stirred for 1.5 h at room temperature. The aqueous layer was extracted with ethyl acetate (4x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate:MeOH 95:5) to afford racemic compound **13** (0.761 g, 90%) with R_f = 0.18 (ethyl acetate) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.56 – 7.48 (m, 2H), 7.39 – 7.32 (m, 3H), 5.66 (s, 1H), 4.22 (dd, *J* = 9.9, 4.8 Hz, 1H), 4.11 – 3.86 (m, 3H), 3.81 – 3.64 (m, 4H), 3.50 (d, *J* = 5.5 Hz, 2H), 2.14 – 2.00 (m, 1H), 1.76 (m, 2H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 137.67, 128.10, 127.26, 125.81, 101.62, 77.47, 77.34, 70.15, 69.29, 68.85, 68.56, 67.22, 66.84, 66.77, 66.71, 66.04, 65.81, 64.48, 39.48, 38.97, 33.22, 32.51 ppm. HRMS (ESI): calcd. for $C_{16}H_{22}O_6Na$ $[M + Na]^+$ 333.1309; found 333.1305.

4.2.10. (4*aR*,7*R*,8*S*,8*aS*)-7-(2-hydroxyethyl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**15**).

Racemic vicinal diol **13** (0.846 g, 2.73 mmol, 1.0 eq.) was dissolved in MeOH (182 mL, 0.015 M). Sodium periodate (0.875 g, 4.09 mmol, 1.5 eq.) was dissolved in water (41 mL) and was added to the solution containing **13**. The mixture was put under an atmosphere of nitrogen and was stirred overnight at room temperature. The solvents were concentrated under reduced pressure and water was added. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure to obtain crude compound **14** (HRMS) with R_f = 0.78 (ethyl acetate). HRMS (ESI): calcd. for $C_{15}H_{18}O_5Na$ $[M + Na]^+$ 301.1047; found 301.1038.
Crude aldehyde **14** was put under an atmosphere of nitrogen and was dissolved in anhydrous MeOH (14 mL, 0.2 M). The solution was cooled to 0 °C and sodium borohydride (0.227 g, 6.00 mmol, 2.2 eq.) was added portionwise. The mixture was allowed to warm to room temperature and was stirred overnight. The solvent was removed under reduced pressure and water was added. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate) to afford compound **15** (0.764 g, 100%) with R_f = 0.42 (ethyl acetate) as a transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53 – 7.44 (m, 2H), 7.43 – 7.34 (m, 3H), 5.63 (s, 1H), 4.31 (dd, *J* = 10.1, 4.8 Hz, 1H), 4.03 (dd, *J* = 11.7, 2.3 Hz, 2H), 3.98 – 3.87 (m, 1H), 3.81 – 3.69 (m, 4H), 3.65 (d, *J* = 10.7 Hz, 1H), 2.42 (s, 1H), 2.06 (m, 1H), 1.97 – 1.67 (m, 2H), 1.65 (s, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.58, 129.55, 128.66, 126.44, 102.36, 78.12, 69.62, 69.24, 67.65, 66.50, 61.08, 38.69, 32.76 ppm. HRMS (ESI): calcd. for $C_{15}H_{20}O_5Na$ $[M + Na]^+$ 303.1203; found 303.1196.

4.2.11. (4*aR*,7*R*,8*S*,8*aS*)-7-(2-azidoethyl)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**17**).

The same procedure (step c and d in Scheme 1) was followed using 0.531 g (1.894 mmol, 1.0 eq.) of diol **15** to afford its tosylate **16** with R_f = 0.73 (heptane:ethyl acetate 25:75) as an oil. HRMS (ESI): calcd. for $C_{22}H_{26}O_7SNa$ $[M + Na]^+$ 457.1292; found 457.1286; and subsequent azide substituted product **17** (0.183 g, 32%) with R_f = 0.74 (heptane:ethyl acetate 25:75) as a transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.55 – 7.43 (m, 2H), 7.42 – 7.31 (m, 3H), 5.58 (s, 1H), 4.27 (dd, *J* = 10.1, 4.9 Hz, 1H), 4.05 – 3.92 (m, 2H), 3.88 (td, *J* = 9.9, 4.9 Hz, 1H), 3.74 – 3.61 (m, 2H), 3.56 (d, *J* = 11.8 Hz, 1H), 3.46 – 3.24 (m, 2H), 2.66 (s, 1H), 1.97 – 1.79 (m, 2H), 1.74 – 1.58 (m, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.58, 129.56, 128.65, 126.52, 102.33, 78.01, 69.52, 68.83, 67.66, 65.94, 49.84, 39.54, 29.00 ppm. HRMS (ESI): calcd. for $C_{15}H_{19}N_3O_4Na$ $[M + Na]^+$ 328.1268; found 328.1271.

4.2.12. (4*aR*,7*R*,8*S*,8*aS*)-2-phenyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)hexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**18**).

The same procedure (step e in Scheme 1) was followed using 0.183 g (0.599 mmol, 1.0 eq.) of the azide compound to yield triazole **18** (0.208 g, 85%) with R_f = 0.50 (heptane:ethyl acetate 25:75) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88 – 7.80 (m, 2H), 7.78 (s, 1H), 7.53 – 7.31 (m, 8H), 5.63 (s, 1H), 4.52 (t, *J* = 7.4 Hz, 2H), 4.32 (dd, *J* = 10.1, 4.8 Hz, 1H), 4.10 – 4.02 (m, 2H), 3.95 (td, *J* = 9.9, 4.8 Hz, 1H), 3.78 – 3.64 (m, 3H), 2.42 – 2.08 (m, 3H), 1.97 – 1.88 (m, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 129.62, 129.18, 128.68, 128.56, 126.42, 126.04, 119.74, 102.43, 77.88, 69.50, 68.91, 67.83, 65.72, 48.76, 39.24, 30.51 ppm. HRMS (ESI): calcd. for $C_{23}H_{26}N_3O_4$ $[M + H]^+$ 408.1918; found 408.1922.

4.2.13. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methanol (**20**).

The same procedure (step f and g in Scheme 1) was followed using 0.208 g (0.510 mmol, 1.0 eq.) of the triazole product to obtain its triol **19** as a transparent oil; and subsequent isopropylidene protected compound **20** (0.183 g, 100%) with R_f = 0.37 (ethyl acetate) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87 – 7.80 (m, 2H), 7.78 (s, 1H), 7.49 – 7.30 (m, 3H), 4.62 – 4.46 (m, 2H), 4.11 (dd, *J* = 4.8, 2.6 Hz, 1H), 3.94 (dd, *J* = 9.0, 5.3 Hz, 1H), 3.89 – 3.78 (m, 2H), 3.73 (dd, *J* = 11.7, 1.9 Hz, 1H), 3.61 (dt, *J* = 11.7, 5.9 Hz, 1H), 3.42 (ddd, *J* = 8.7, 6.1, 2.6 Hz, 1H), 2.28 – 1.99 (m, 3H), 1.93 (t, *J* = 6.2 Hz, 1H), 1.49 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.31, 130.77, 129.17, 128.54, 126.01, 119.79, 109.59, 78.58, 76.08, 70.52, 65.45, 63.55, 48.41, 34.44, 31.18, 28.41, 26.49 ppm. HRMS (ESI): calcd. for $C_{19}H_{26}N_3O_4$ $[M + H]^+$ 360.1918; found 360.1912.

4.2.14. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl sulfamate (**21**).

The same procedure (step h in Scheme 1) was followed using 0.216 g (0.601 mmol, 1.0 eq.) of the primary alcohol to give the sulfonamide **21** (0.120 g, 46%) with R_f = 0.68 (ethyl acetate) as a transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (s, 1H), 7.82 – 7.74 (m, 2H), 7.46 – 7.29 (m, 3H), 5.96 (s, 2H), 4.46 (t, *J* = 6.3 Hz, 2H), 4.35 (d, *J* = 9.6 Hz, 1H), 4.22 (dd, *J* = 11.2, 6.0 Hz, 1H), 4.09 – 4.03 (m, 1H), 3.90 (dd, *J* = 9.1, 5.1 Hz, 1H), 3.72 (s, 2H), 3.55 (dd, *J* = 7.9, 5.5 Hz, 1H), 2.17 – 1.93 (m, 3H), 1.44 (s, 3H), 1.31 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.07, 130.61, 129.23, 128.59, 125.99, 120.54, 109.87, 76.49, 75.76, 70.33, 69.56, 65.57,

48.37, 34.13, 30.79, 28.38, 26.43 ppm. HRMS (ESI): calcd. for $C_{19}H_{27}N_4O_6S$ $[M + H]^+$ 439.1646; found 439.1639.

4.2.15. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl ((tert-butoxycarbonyl)isoleucyl)sulfamate (**22a**).

The same procedure (step i in Scheme 1) was followed using 0.060 g (0.137 mmol, 1.0 eq.) of the sulfonamide to afford the isoleucine coupled compound **22a** (0.089 g, 100%) with R_f = 0.45 (DCM:MeOH 90:10) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 (s, 1H), 7.88 – 7.78 (m, 2H), 7.47 – 7.29 (m, 3H), 5.45 (d, *J* = 7.5 Hz, 1H), 4.51 (t, *J* = 6.4 Hz, 2H), 4.43 – 4.20 (m, 2H), 4.14 – 4.05 (m, 1H), 4.04 – 3.90 (m, 2H), 3.79 – 3.66 (m, 2H), 3.58 – 3.48 (m, 1H), 2.24 – 1.91 (m, 3H), 1.91 – 1.77 (m, 1H), 1.44 (s, 3H), 1.41 (s, 9H), 1.31 (s, 3H), 1.17 – 1.06 (m, 1H), 0.97 – 0.79 (m, 7H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.65, 148.05, 130.79, 129.13, 128.44, 125.99, 120.49, 109.70, 76.35, 75.84, 69.65, 65.37, 48.28, 34.12, 30.76, 28.60, 28.40, 26.44, 24.84, 15.91, 11.63 ppm. HRMS (ESI): calcd. for $C_{30}H_{46}N_5O_9S$ $[M + H]^+$ 652.3011; found 652.3019.

4.2.16. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl (3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)sulfamate (**22b**).

The same procedure (step i in Scheme 1) was followed using 0.060 g (0.137 mmol, 1.0 eq.) of the sulfonamide and *t*-butyloxycarbonyl-*O*-benzyl-*L*-tyrosine hydroxysuccinimide ester (0.128 g, 0.274 mmol, 2.0 eq.) to afford the tyrosine coupled compound **22b** (0.104 g, 96%) with R_f = 0.55 (DCM:MeOH 90:10) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86 – 7.76 (m, 3H), 7.46 – 7.23 (m, 8H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.83 (d, *J* = 8.1 Hz, 2H), 5.44 (s, 1H), 4.94 (s, 2H), 4.48 – 4.37 (m, 2H), 4.35 – 4.23 (m, 2H), 4.19 – 4.09 (m, 1H), 4.06 – 3.98 (m, 1H), 3.95 – 3.87 (m, 1H), 3.68 (s, 2H), 3.54 – 3.44 (m, 1H), 3.13 (d, *J* = 11.0 Hz, 1H), 2.13 – 1.87 (m, 3H), 1.40 (s, 3H), 1.37 – 1.22 (m, 12H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.87, 148.02, 137.34, 130.87, 130.82, 129.16, 128.84, 128.45, 128.22, 127.73, 126.00, 120.46, 115.04, 109.67, 76.41, 75.81, 70.20, 69.65, 65.41, 48.23, 34.11, 30.71, 28.58, 28.42, 26.47 ppm. HRMS (ESI): calcd. for $C_{40}H_{50}N_5O_{10}S$ $[M + H]^+$ 792.3273; found 792.3288.

4.2.17. ((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-2*H*-pyran-2-yl)methyl isoleucylsulfamate (**23a**).

The same procedure (step j in Scheme 1) was followed using 0.089 g (0.137 mmol, 1.0 eq.) of the isoleucine coupled compound to provide final product **23a** (0.070 g, 100%) with R_f = 0.06 (DCM:MeOH 90:10) as a transparent solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.41 (s, 1H), 7.87 – 7.79 (m, 2H), 7.49 – 7.40 (m, 2H), 7.40 – 7.30 (m, 1H), 4.56 (t, *J* = 5.8 Hz, 2H), 4.38 – 4.21 (m, 2H), 3.96 – 3.86 (m, 2H), 3.83 – 3.75 (m, 1H), 3.74 – 3.66 (m, 1H), 3.66 – 3.57 (m, 2H), 2.28 – 1.98 (m, 3H), 1.76 (s, 1H), 1.68 – 1.55 (m, 1H), 1.35 – 1.24 (m, 1H), 1.05 (d, *J* = 6.9 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.70, 147.23, 129.96, 128.27, 127.65, 124.96, 120.72, 74.51, 69.80, 68.51, 64.09, 63.46, 59.69, 47.87, 39.12, 36.42, 28.75, 24.00, 13.75, 10.44 ppm. HRMS (ESI): calcd. for $C_{22}H_{32}N_5O_7S$ $[M - H]^-$ 510.2028; found 510.2022.

4.2.18. ((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)tetrahydro-2*H*-pyran-2-yl)methyl tyrosylsulfamate (**23b**).

Palladium on carbon (10 wt.% loading, 0.014 g, 0.131 mmol, 1.0 eq.) was placed under a nitrogen blanket, while the tyrosine coupled compound (0.104 g, 0.131 mmol, 1.0 eq.) was

dissolved in anhydrous MeOH (13 mL, 0.01 M) and added to the catalyst. The atmosphere was exchanged for hydrogen gas and the reaction mixture was vigorously stirred overnight at room temperature. The mixture was then filtered over celite. The filtrate was evaporated under reduced pressure, giving the crude debenzylated compound (HRMS) with R_f = 0.24 (DCM:MeOH 90:10) as a transparent oil. HRMS (ESI): calcd. for $C_{33}H_{42}N_5O_{10}S$ $[M - H]^-$ 700.2658; found 700.2670.

In analogy with compound **23a**, the crude debenzylated product was deprotected to afford the tyrosine coupled final compound **23b** (0.020 g, 27% over 2 steps) with R_f = 0.13 (DCM:MeOH 80:20) as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.34 (s, 1H), 7.82 – 7.73 (m, 2H), 7.44 – 7.34 (m, 2H), 7.33 – 7.25 (m, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 6.72 (d, *J* = 8.3 Hz, 2H), 4.49 (t, *J* = 6.6 Hz, 2H), 4.30 – 4.12 (m, 2H), 3.89 – 3.77 (m, 3H), 3.72 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.63 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.55 (d, *J* = 11.6 Hz, 1H), 3.18 (dd, *J* = 14.4, 4.6 Hz, 1H), 2.94 (dd, *J* = 14.5, 8.3 Hz, 1H), 2.19 – 1.96 (m, 2H), 1.69 (s, 1H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.71, 156.18, 147.23, 130.04, 129.94, 128.27, 127.65, 125.26, 124.97, 120.72, 115.05, 74.56, 69.75, 68.65, 64.09, 63.46, 56.72, 47.87, 39.10, 35.94, 28.69 ppm. HRMS (ESI): calcd. for $C_{25}H_{30}N_5O_8S$ $[M - H]^-$ 560.1820; found 560.1820.

4.2.19. (4*aR*,7*R*,8*S*,8*aS*)-2-phenyl-7-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)hexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**24**).

Diol **15** (0.667 g, 2.379 mmol, 1.0 eq.) and triphenylphosphine (0.749 g, 2.86 mmol, 1.2 eq.) were put under an atmosphere of nitrogen and dissolved in anhydrous THF (6.0 mL). The solution was cooled to 0 °C and diethyl azodicarboxylate (1.3 mL, 1.2 eq., 40 wt% in toluene) was added dropwise. 5-Phenyltetrazole (0.417 g, 2.86 mmol, 1.2 eq.) was dissolved in a 1:1 mixture of anhydrous THF:DMF (1.8 mL:1.8 mL, 0.25 M final diol concentration) and was added dropwise to the reaction mixture, which was allowed to warm to room temperature. The mixture was then stirred overnight. The reaction was quenched with water at 0 °C and the mixture was stirred for 15 min. at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 55:45) to give compound **24** (0.743 g, 76%) with R_f = 0.42 (heptane:ethyl acetate 50:50) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.19 – 8.08 (m, 2H), 7.55 – 7.43 (m, 5H), 7.41 – 7.30 (m, 3H), 5.63 (s, 1H), 4.76 (t, *J* = 7.3 Hz, 2H), 4.32 (dd, *J* = 10.2, 4.7 Hz, 1H), 4.12 – 4.01 (m, 2H), 3.93 (dd, *J* = 9.6, 5.0 Hz, 1H), 3.72 (m, 3H), 2.49 – 2.18 (m, 3H), 1.92 (dd, *J* = 5.9, 3.5 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.42, 130.71, 129.60, 129.23, 128.67, 127.57, 127.15, 126.42, 102.42, 77.87, 69.51, 68.79, 67.84, 65.65, 51.41, 39.11, 29.45 ppm. HRMS (ESI): calcd. for $C_{22}H_{25}N_4O_4$ $[M + H]^+$ 409.1870; found 409.1866.

4.2.20. ((3*aS*,4*R*,7*R*,7a*S*)-2,2-dimethyl-7-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methanol (**26**).

The same procedure (step f and g in Scheme 1) was followed using 0.728 g (1.782 mmol, 1.0 eq.) of the tetrazole to obtain its triol **25** as a transparent oil; and subsequent isopropylidene protected compound **26** (0.642 g, 100%) with R_f = 0.18 (heptane:ethyl acetate 50:50) as a light-yellow transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.18 – 8.10 (m, 2H), 7.53 – 7.44 (m, 3H), 4.80 (t, *J* = 6.9 Hz, 2H), 4.13 (dd, *J* = 5.0, 2.6 Hz, 1H), 3.95 (dd, *J* = 8.9, 5.2 Hz, 1H), 3.88 – 3.75 (m, 3H), 3.61 (dt, *J* = 11.8, 5.9 Hz, 1H), 3.47 – 3.37 (m, 1H), 2.34 – 2.16 (m, 2H), 2.08 – 1.99 (m, 1H), 1.89 (t, *J* = 6.3 Hz, 1H), 1.49 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 130.69, 129.21, 127.14, 109.62, 78.74, 75.95, 70.45, 65.32, 63.58, 61.93, 51.11,

34.32, 30.68, 30.10, 28.43, 26.53 ppm. HRMS (ESI): calcd. for $C_{18}H_{25}N_4O_4$ $[M + H]^+$ 361.1870; found 361.1870.

4.2.21. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-4-yl)methyl sulfamate (**27**).

The same procedure (step h in Scheme 1) was followed using 0.642 g (1.781 mmol, 1.0 eq.) of the primary alcohol to give the sulfonamide **27** (0.738 g, 94%) with R_f = 0.25 (heptane:ethyl acetate 50:50) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 – 8.10 (m, 2H), 7.53 – 7.45 (m, 3H), 4.86 (br s, 2H), 4.80 (t, *J* = 6.7 Hz, 2H), 4.42 (dd, *J* = 11.4, 2.3 Hz, 1H), 4.28 (dd, *J* = 11.4, 6.2 Hz, 1H), 4.16 (dd, *J* = 3.1, 2.1 Hz, 1H), 3.93 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.81 (d, *J* = 2.7 Hz, 2H), 3.61 (ddd, *J* = 8.7, 6.3, 2.3 Hz, 1H), 2.35 – 2.18 (m, 2H), 2.10 – 2.01 (m, 1H), 1.49 (s, 3H), 1.35 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 130.75, 129.24, 127.14, 77.51, 75.74, 70.93, 69.70, 65.62, 50.98, 34.04, 29.99, 28.36, 26.44 ppm. HRMS (ESI): calcd. for $C_{18}H_{26}N_5O_6S$ $[M + H]^+$ 440.1598; found 440.1593.

4.2.22. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-4-yl)methyl ((tert-butoxycarbonyl)isoleucyl)sulfamate (**28a**).

The same procedure (step i in Scheme 1) was followed using 0.170 g (0.387 mmol, 1.0 eq.) of the sulfonamide to afford the isoleucine coupled compound **28a** (0.252 g, 100%) with R_f = 0.47 (DCM:MeOH 90:10) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.16 – 8.06 (m, 2H), 7.54 – 7.40 (m, 3H), 4.80 (t, *J* = 6.0 Hz, 2H), 4.12 (dd, *J* = 6.2, 2.9 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.79 (td, *J* = 8.0, 2.6 Hz, 2H), 3.67 – 3.46 (m, 2H), 2.35 – 2.12 (m, 2H), 2.02 – 1.93 (m, 1H), 1.83 – 1.77 (m, 2H), 1.49 – 1.41 (m, 12H), 1.33 (s, 3H), 1.01 – 0.83 (m, 8H) ppm. HRMS (ESI): calcd. for $C_{29}H_{43}N_6O_9S$ $[M - H]^-$ 651.2817; found 651.2823.

4.2.23. ((3*aS*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-4-yl)methyl (3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)sulfamate (**28b**).

The same procedure (step i in Scheme 1) was followed using 0.183 g (0.416 mmol, 1.0 eq.) of the sulfonamide and *t*-butyloxycarbonyl-*O*-benzyl-*L*-tyrosine hydroxysuccinimide ester (0.390 g, 0.833 mmol, 2.0 eq.) to afford the tyrosine coupled compound **28b** (0.330 g, 100%) with R_f = 0.42 (DCM:MeOH 90:10) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 – 8.07 (m, 2H), 7.53 – 7.28 (m, 8H), 7.11 (d, *J* = 6.4 Hz, 2H), 6.88 (d, *J* = 5.2 Hz, 2H), 5.00 (s, 2H), 4.81 – 4.66 (m, 2H), 4.34 – 4.13 (m, 2H), 4.11 – 4.03 (m, 1H), 3.97 – 3.88 (m, 1H), 3.84 – 3.68 (m, 2H), 3.65 – 3.47 (m, 2H), 3.21 – 3.07 (m, 1H), 2.29 – 2.04 (m, 2H), 1.90 (s, 1H), 1.48 – 1.24 (m, 15H) ppm. HRMS (ESI): calcd. for $C_{39}H_{47}N_6O_{10}S$ $[M - H]^-$ 791.3080; found 791.3080.

4.2.24. ((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-2*H*-pyran-2-yl)methyl isoleucylsulfamate (**29a**).

The same procedure (step j in Scheme 1) was followed using 0.252 g (0.386 mmol, 1.0 eq.) of the isoleucine coupled compound to provide final product **29a** (0.198 g, 100%) with R_f = 0.06 (DCM:MeOH 90:10) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.14 – 8.04 (m, 2H), 7.55 – 7.46 (m, 3H), 4.82 (t, *J* = 7.2 Hz, 2H), 4.38 (dd, *J* = 10.7, 1.6 Hz, 1H), 4.29 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.98 – 3.87 (m, 2H), 3.85 – 3.77 (m, 1H), 3.74 – 3.61 (m, 3H), 2.38 – 2.09 (m, 2H), 2.08 – 1.94 (m, 1H), 1.79 (s, 1H), 1.70 – 1.55 (m, 1H), 1.38 – 1.22 (m, 1H), 1.06 (d, *J* = 7.0 Hz, 3H), 0.96 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 172.86, 164.52, 129.89, 128.39, 126.83, 126.02, 74.48, 69.68, 68.96, 64.11, 63.46, 59.45, 50.76, 39.06, 36.34,

27.83, 24.02, 13.72, 10.45 ppm. HRMS (ESI): calcd. for $C_{21}H_{31}N_6O_7S$ [M - H]$^-$ 511.1980; found 511.1994.

4.2.25. ((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(2-(5-phenyl-2*H*-tetrazol-2-yl)ethyl)tetrahydro-2*H*-pyran-2-yl)methyl tyrosylsulfamate (**29b**).

Palladium on carbon (10 wt.% loading, 0.044 g, 0.416 mmol, 1.0 eq.) was placed under a nitrogen blanket, while the tyrosine coupled compound (0.330 g, 0.416 mmol, 1.0 eq.) was dissolved in anhydrous MeOH (41 mL, 0.01 M) and added to the catalyst. The atmosphere was exchanged for hydrogen gas and the reaction mixture was vigorously stirred overnight at room temperature. The mixture was then filtered over celite. The filtrate was evaporated under reduced pressure, giving the crude debenzylated compound (HRMS) with R_f = 0.23 (DCM:MeOH 90:10) as a transparent solid. HRMS (ESI): calcd. for $C_{32}H_{41}N_6O_{10}S$ [M - H]$^-$ 701.2610; found 701.2610.
In analogy with compound **29a**, the crude debenzylated product was deprotected to afford the tyrosine coupled final compound **29b** (0.234 g, 100% over 2 steps) with R_f = 0.03 (DCM:MeOH 90:10) as a light-yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.12 – 8.02 (m, 2H), 7.53 – 7.44 (m, 3H), 7.16 (d, *J* = 8.5 Hz, 2H), 6.79 (d, *J* = 8.5 Hz, 2H), 4.79 (t, *J* = 7.1 Hz, 2H), 4.34 (dd, *J* = 10.8, 1.5 Hz, 1H), 4.24 (dd, *J* = 10.9, 5.2 Hz, 1H), 3.97 – 3.85 (m, 3H), 3.83 – 3.75 (m, 1H), 3.69 (dd, *J* = 9.7, 2.9 Hz, 1H), 3.63 (d, *J* = 11.7 Hz, 1H), 3.25 (dd, *J* = 14.5, 4.9 Hz, 1H), 3.02 (dd, *J* = 14.5, 8.1 Hz, 1H), 2.36 – 2.09 (m, 2H), 1.81 – 1.71 (m, 1H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.30, 164.50, 156.19, 130.10, 129.88, 128.39, 126.80, 126.03, 125.15, 115.10, 74.55, 69.64, 68.98, 64.15, 63.46, 56.60, 50.75, 38.98, 35.88, 27.76 ppm. HRMS (ESI): calcd. for $C_{24}H_{29}N_6O_8S$ [M - H]$^-$ 561.1773; found 561.1783.

4.2.26. 2-Azidoethan-1-ol.

Sodium azide (4.68 g, 72.0 mmol, 3.0 eq.) was weighted in a flask and was put under an atmosphere of nitrogen. Water (30 mL, 0.8 M) was added and then 2-bromoethanol (1.7 mL, 24.01 mmol, 1.0 eq.). The mixture was stirred overnight at 80 °C. The reaction solution was extracted with diethyl ether (4x). The combined organic layers were dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure to give 2-azidoethan-1-ol (2.091 g, 100%) with R_f = 0.37 (heptane:ethyl acetate 50:50) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.79 (dd, *J* = 10.0, 5.1 Hz, 2H), 3.53 – 3.39 (m, 2H), 2.01 (t, *J* = 5.6 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 61.82, 53.83 ppm.

4.2.27. (4*aR*,7*R*,8*S*,8*aS*)-7-(2-azidoethoxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**30**).

Sodium hydride (0.529 g, 13.23 mmol, 3.1 eq., 60 wt% dispersion in mineral oil) was weighted in a flask and was put under an atmosphere of nitrogen. The flask was cooled to 0 °C and a solution of 2-azidoethan-1-ol (1.115 g, 12.81 mmol, 3.0 eq.) in anhydrous DMF (12.8 mL, 1.0 M) was added dropwise. The mixture was stirred for 30 min. at 0 °C. Epoxide **1** (1.0 g, 4.27 mmol, 1.0 eq.) was dissolved in anhydrous DMF (9.5 mL, 0.45 M) and was added dropwise. The temperature was gradually raised to 80 °C and the mixture was stirred overnight. After completion, the reaction was quenched with aqueous saturated ammonium chloride (10 mL) at 0 °C. The mixture was then stirred for 15 min. at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 50:50) to give compound **30** (0.620 g, 45%) with R_f = 0.34 (heptane:ethyl acetate 50:50) as a transparent oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53 – 7.43 (m, 2H), 7.42 – 7.31 (m, 3H), 5.63

(s, 1H), 4.30 (dd, *J* = 9.9, 4.5 Hz, 1H), 4.20 (d, *J* = 2.6 Hz, 1H), 4.00 – 3.82 (m, 4H), 3.81 – 3.60 (m, 3H), 3.56 – 3.49 (m, 1H), 3.39 (m, 2H), 2.50 (br s, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.27, 129.16, 128.27, 126.12, 101.97, 77.91, 77.34, 69.02, 68.50, 66.32, 64.89, 50.68 ppm. HRMS (ESI): calcd. for $C_{15}H_{19}N_3O_5Na$ $[M + Na]^+$ 344.1217; found 344.1218.

4.2.28. (4*aR*,7*R*,8*S*,8*aS*)-2-phenyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)hexahydropyrano[3,2-*d*][1,3]dioxin-8-ol (**31**).

The same procedure (step e in Scheme 1) was followed using 0.459 g (1.428 mmol, 1.0 eq.) of the azide **30** to yield the triazole **31** (0.605 g, 100%) with R_f = 0.44 (ethyl acetate) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98 (s, 1H), 7.89 – 7.81 (m, 2H), 7.45 – 7.31 (m, 8H), 5.44 (s, 1H), 4.62 (t, *J* = 5.0 Hz, 2H), 4.27 (dd, *J* = 10.1, 4.9 Hz, 1H), 4.09 – 4.05 (m, 1H), 4.03 – 3.93 (m, 2H), 3.89 – 3.80 (m, 3H), 3.72 – 3.61 (m, 2H), 3.52 – 3.47 (m, 1H), 2.38 (d, *J* = 1.4 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.41, 130.87, 129.51, 129.22, 128.58, 128.53, 126.42, 125.95, 121.15, 102.37, 78.01, 77.79, 69.35, 68.14, 66.68, 66.23, 65.54, 50.71 ppm. HRMS (ESI): calcd. for $C_{23}H_{26}N_3O_5$ $[M + H]^+$ 424.1867; found 424.1863.

4.2.29. ((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methanol (**33**).

The same procedure (step f in Scheme 1) was followed using 0.605 g (1.429 mmol, 1.0 eq.) of the triazole product to obtain its triol **32** (HRMS) with R_f = 0.09 (ethyl acetate) as a transparent oil. HRMS (ESI): calcd. for $C_{16}H_{22}N_3O_5$ $[M + H]^+$ 336.1554; found 336.1552; and subsequent isopropylidene protected compound **33** (0.416 g, 78%) with R_f = 0.22 (ethyl acetate) was obtained as a white solid (step g). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.99 (s, 1H), 7.86 – 7.77 (m, 2H), 7.44 – 7.35 (m, 2H), 7.34 – 7.26 (m, 1H), 4.53 (t, *J* = 5.0 Hz, 2H), 4.16 – 4.10 (m, 1H), 3.94 (dd, *J* = 9.4, 5.0 Hz, 3H), 3.82 (t, *J* = 13.4 Hz, 2H), 3.65 – 3.53 (m, 3H), 3.33 (ddd, *J* = 8.9, 6.5, 2.6 Hz, 1H), 3.12 (br s, 1H), 1.44 (s, 3H), 1.30 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 147.80, 130.86, 129.11, 128.35, 125.89, 121.41, 109.55, 78.54, 74.62, 73.52, 70.69, 68.07, 65.33, 63.11, 50.58, 28.36, 26.47 ppm. HRMS (ESI): calcd. for $C_{19}H_{26}N_3O_5$ $[M + H]^+$ 376.1867; found 376.1897.

4.2.30. ((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl sulfamate (**34**).

The same procedure (step h in Scheme 1) was followed using 0.416 g (1.108 mmol, 1.0 eq.) of primary alcohol **33** to give the sulfonamide **34** (0.370 g, 74%) with R_f = 0.51 (ethyl acetate) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98 (s, 1H), 7.87 – 7.76 (m, 2H), 7.46 – 7.35 (m, 2H), 7.34 – 7.25 (m, 1H), 6.07 (br s, 2H), 4.54 (s, 2H), 4.39 (d, *J* = 11.1 Hz, 1H), 4.21 (dd, *J* = 11.4, 5.6 Hz, 1H), 4.16 – 4.06 (m, 2H), 4.04 – 3.92 (m, 3H), 3.89 (d, *J* = 12.9 Hz, 1H), 3.56 (d, *J* = 11.0 Hz, 2H), 3.50 – 3.40 (m, 1H), 1.42 (s, 3H), 1.28 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 147.92, 130.66, 129.22, 128.50, 125.98, 121.47, 110.09, 76.26, 74.08, 73.02, 70.08, 69.55, 68.38, 65.86, 50.59, 28.33, 26.39 ppm. HRMS (ESI): calcd. for $C_{19}H_{27}N_4O_7S$ $[M + H]^+$ 455.1595; found 455.1596.

4.2.31. ((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl ((tert-butoxycarbonyl)isoleucyl)sulfamate (**35a**).

The same procedure (step i in Scheme 1) was followed using 0.110 g (0.242 mmol, 1.0 eq.) of the sulfonamide to afford the isoleucine coupled compound **35a** (0.162 g, 100%) with R_f = 0.29 (DCM:MeOH 90:10) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (s, 1H), 7.89

– 7.81 (m, 2H), 7.46 – 7.36 (m, 2H), 7.31 (dd, *J* = 12.0, 4.6 Hz, 1H), 5.51 (d, *J* = 7.4 Hz, 1H), 4.61 (t, *J* = 4.5 Hz, 2H), 4.41 (d, *J* = 10.3 Hz, 1H), 4.28 (dd, *J* = 10.6, 5.9 Hz, 1H), 4.11 (m, 1H), 4.06 – 3.94 (m, 3H), 3.90 (d, *J* = 12.5 Hz, 1H), 3.64 (m, 1H), 3.56 (d, *J* = 10.4 Hz, 2H), 3.51 – 3.41 (m, 1H), 1.88 (s, 1H), 1.57 – 1.47 (m, 1H), 1.42 (s, 12H), 1.29 (s, 3H), 1.12 (m, 1H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.19, 147.87, 130.82, 129.11, 128.31, 125.96, 121.58, 109.86, 76.01, 74.41, 73.37, 70.23, 69.93, 68.39, 65.60, 50.61, 28.57, 28.34, 26.40, 24.67, 15.86, 11.74 ppm. HRMS (ESI): calcd. for $C_{30}H_{44}N_5O_{10}S$ $[M - H]^-$ 666.2814; found 666.2817.

4.2.32. ((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-*c*]pyran-4-yl)methyl (3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)sulfamate (**35b**).

The same procedure (step i in Scheme 1) was followed using 0.140 g (0.308 mmol, 1.0 eq.) of the sulfonamide and *t*-butyloxycarbonyl-*O*-benzyl-*L*-tyrosine hydroxysuccinimide ester (0.289 g, 0.616 mmol, 2.0 eq.) to afford the tyrosine coupled compound **35b** (0.235 g, 94%) with R_f = 0.49 (DCM:MeOH 90:10) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.43 – 7.23 (m, 8H), 7.11 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 2H), 5.46 (s, 1H), 4.95 (s, 2H), 4.54 (t, 2H), 4.44 – 4.24 (m, 2H), 4.23 – 4.13 (m, 1H), 4.13 – 3.83 (m, 5H), 3.63 – 3.37 (m, 3H), 3.16 (dd, *J* = 13.1, 4.0 Hz, 1H), 1.45 – 1.20 (m, 15H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.84, 155.97, 147.85, 137.35, 129.14, 128.80, 128.33, 128.19, 127.72, 125.98, 121.65, 114.96, 109.89, 76.13, 74.54, 73.26, 70.17, 69.84, 68.62, 65.86, 53.83, 50.54, 26.43 ppm. HRMS (ESI): calcd. for $C_{40}H_{48}N_5O_{11}S$ $[M - H]^-$ 806.3076; found 806.3087.

4.2.33. ((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-2*H*-pyran-2-yl)methyl isoleucylsulfamate (**36a**).

The same procedure (step j in Scheme 1) was followed using 0.162 g (0.243 mmol, 1.0 eq.) of the isoleucine coupled compound to provide final product **36a** (0.128 g, 100%) with R_f = 0.06 (DCM:MeOH 90:10) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.39 (s, 1H), 7.90 – 7.80 (m, 2H), 7.49 – 7.39 (m, 2H), 7.38 – 7.29 (m, 1H), 4.71 – 4.57 (m, 2H), 4.44 – 4.35 (m, 1H), 4.23 (dd, *J* = 10.9, 5.6 Hz, 1H), 4.06 – 3.94 (m, 3H), 3.83 – 3.63 (m, 5H), 3.47 (d, *J* = 3.2 Hz, 1H), 2.08 – 1.93 (m, 1H), 1.70 – 1.53 (m, 1H), 1.37 – 1.20 (m, 1H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.94 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 172.72, 147.08, 129.95, 128.35, 127.62, 125.07, 121.42, 77.96, 73.50, 69.24, 67.33, 67.01, 64.55, 63.32, 59.41, 50.05, 36.33, 24.02, 13.71, 10.45 ppm. HRMS (ESI): calcd. for $C_{22}H_{32}N_5O_8S$ $[M - H]^-$ 526.1977; found 526.1979.

4.2.34. ((2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-2*H*-pyran-2-yl)methyl tyrosylsulfamate (**36b**).

Palladium on carbon (10 wt.% loading, 0.031 g, 0.291 mmol, 1.0 eq.) was placed under a nitrogen blanket, while the tyrosine coupled compound (0.235 g, 0.291 mmol, 1.0 eq.) was dissolved in anhydrous MeOH (28 mL, 0.01 M) and added to the catalyst. The atmosphere was exchanged for hydrogen gas and the reaction mixture was vigorously stirred overnight at room temperature. The mixture was then filtered over celite. The filtrate was evaporated under reduced pressure, giving the crude debenzylated compound (HRMS) with R_f = 0.24 (DCM:MeOH 90:10) as a transparent oil. HRMS (ESI): calcd. for $C_{33}H_{42}N_5O_{11}S$ $[M - H]^-$ 716.2607; found 716.2617.

In analogy with compound **36a**, the crude debenzylated product was further deprotected to afford the tyrosine coupled final compound **36b** (0.145 g, 86%) with R_f = 0.05 (DCM:MeOH

85:15) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.36 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 6.79 (d, *J* = 8.2 Hz, 2H), 4.62 (m, 2H), 4.36 (d, *J* = 10.5 Hz, 1H), 4.19 (dd, *J* = 10.8, 5.5 Hz, 1H), 4.04 – 3.94 (m, 3H), 3.91 (dd, *J* = 7.6, 5.3 Hz, 1H), 3.82 – 3.66 (m, 4H), 3.45 (d, *J* = 2.3 Hz, 1H), 3.23 (dd, *J* = 14.4, 4.7 Hz, 1H), 3.01 (dd, *J* = 14.5, 8.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.15, 156.21, 147.05, 130.12, 129.92, 128.35, 127.63, 125.11, 125.08, 121.45, 115.12, 78.00, 73.55, 69.18, 67.33, 67.05, 64.56, 63.32, 56.55, 50.04, 35.84 ppm. HRMS (ESI): calcd. for $C_{25}H_{30}N_5O_9S$ $[M - H]^-$ 576.1770; found 576.1772.

4.2.35. Ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate (**37**).

Diisopropylamine (5.9 mL, 41.6 mmol, 2.2 eq.) was put under an atmosphere of nitrogen and was dissolved in anhydrous THF (41.6 mL, 1.0 M). The solution was cooled to -78 °C and *n*-BuLi (2.5 M solution in hexanes, 15.9 mL, 39.7 mmol, 2.1 eq.) was added dropwise. The mixture was warmed to 0 °C for 1 min. and then recooled again to -78 °C, whereafter it was stirred for 15 min. Ethyl (*S*)-3-hydroxybutanoate (2.5 mL, 18.92 mmol, 1.0 eq.) was dissolved in anhydrous THF (12.6 mL, 1.5 M) and was added dropwise to the LDA solution. 1,3-Dimethyl-1,3-diazinan-2-one (DMPU, 3.9 mL, 32.2 mmol, 1.7 eq.) was also added dropwise, whereafter the mixture was stirred for 45 min. at a temperature range between -78 and -60 °C. Methyl iodide (1.5 mL, 23.65 mmol, 1.25 eq.) was then added at 0 °C and the reaction mixture was stirred for 2.5 h at this temperature. The reaction was quenched with aqueous saturated ammonium chloride at 0 °C and the mixture was then stirred for 15 min. at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 75:25) affording compound **37** (1.662 g, 60%) as a colourless liquid. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.18 (q, *J* = 7.1 Hz, 2H), 3.92 – 3.85 (m, 1H), 2.76 (d, *J* = 5.7 Hz, 1H), 2.47 – 2.41 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.22 (d, *J* = 6.4 Hz, 3H), 1.19 (d, *J* = 7.2 Hz, 3H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 175.89, 69.38, 60.54, 46.88, 20.69, 14.14, 14.05 ppm. HRMS (ESI): calcd. for $C_7H_{14}O_3Na$ $[M + Na]^+$ 169.0835; found 169.0836.

4.2.36. Ethyl (2*S*,3*S*)-3-((tert-butyldimethylsilyl)oxy)-2-methylbutanoate (**38**).

Ethyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate **37** (1.662 g, 11.37 mmol, 1.0 eq.) and imidazole (2.58 g, 37.9 mmol, 3.33 eq.) were combined and put under an atmosphere of nitrogen. The reactants were dissolved in anhydrous DMF (28.4 mL, 0.4 M) and *t*-butyldimethylsilyl chloride (2.86 g, 18.99 mmol, 1.67 eq.) was added portionwise at room temperature. The mixture was stirred overnight at ambient temperature, whereafter the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and aqueous saturated bicarbonate. The aqueous phase was extracted two times more with ethyl acetate. The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure yielding compound **38** (2.96 g, 90%) as a light-yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.15 – 4.08 (m, 2H), 4.04 – 3.98 (m, 1H), 2.52 – 2.44 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.13 (d, *J* = 6.2 Hz, 3H), 1.08 (d, *J* = 7.1 Hz, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 175.27, 70.16, 60.15, 48.15, 25.66, 20.53, 14.11, 12.69, -4.37, -5.17 ppm. HRMS (ESI): calcd. for $C_{13}H_{28}O_3SiNa$ $[M + Na]^+$ 283.1700; found 283.1701.

4.2.37. (2*R*,3*S*)-3-((tert-butyldimethylsilyl)oxy)-2-methylbutan-1-ol (**39**).

Compound **38** (0.250 g, 0.960 mmol, 1.0 eq.) was put under an atmosphere of nitrogen and dissolved in anhydrous DCM (3.2 mL, 0.3 M). Diisobutylaluminum hydride (1.0 M solution in

hexanes, 2.4 mL, 2.4 mmol, 2.5 eq.) was added dropwise at 0 °C, whereafter the mixture was stirred for 1 h at this temperature. The reaction was quenched with MeOH (0.3 mL) and aqueous saturated sodium tartrate (Rochelle's salt, 1.0 mL). The mixture was filtered through celite and the aqueous layer was extracted with DCM (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 90:10) to give compound **39** (0.100 g, 48%) with R_f = 0.45 (heptane:ethyl acetate 75:25) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.87 – 3.66 (m, 2H), 3.61 – 3.44 (m, 1H), 2.93 (br s, 1H), 1.67 – 1.49 (m, 1H), 1.19 (d, *J* = 6.2 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H), 0.88 (s, 9H), 0.07 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 74.04, 66.06, 42.10, 26.07, 22.29, 18.16, 14.75, -4.01, -4.71 ppm. HRMS (ESI): calcd. for $C_{11}H_{27}O_2Si$ $[M + H]^+$ 219.1775; found 219.1780.

4.2.38. ((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-4-yl)methyl methanesulfonate (**40**).

Compound **33** (0.1 g, 0.266 mmol, 1.0 eq.) and triethylamine (0.07 mL, 0.533 mmol, 2.0 eq.) were combined and put under an atmosphere of nitrogen. The reactants were dissolved in anhydrous DCM (0.8 mL, 0.35 M) and the solution was cooled to 0 °C. Methanesulfonyl chloride (0.03 mL, 0.400 mmol, 1.5 eq.) was added dropwise and the reaction mixture was stirred for 2 h while slowly raising the solution to room temperature. After completion (TLC), the reaction was quenched with water at 0 °C and the mixture was then stirred for 15 min. at room temperature. The aqueous layer was extracted with DCM (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate) to give compound **40** (0.101 g, 84%) with R_f = 0.46 (ethyl acetate) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95 (s, 1H), 7.88 – 7.80 (m, 2H), 7.49 – 7.39 (m, 2H), 7.37 – 7.30 (m, 1H), 4.59 (dd, *J* = 5.5, 4.4 Hz, 2H), 4.39 (dd, *J* = 11.4, 2.0 Hz, 1H), 4.25 – 4.14 (m, 2H), 4.00 (t, *J* = 5.1 Hz, 2H), 3.96 – 3.84 (m, 2H), 3.66 – 3.56 (m, 2H), 3.49 – 3.41 (m, 1H), 2.97 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 147.97, 130.90, 129.19, 128.45, 125.97, 121.18, 110.10, 76.28, 74.27, 73.42, 69.87, 69.78, 68.30, 65.82, 50.66, 37.77, 28.39, 26.43 ppm. HRMS (ESI): calcd. for $C_{20}H_{28}N_3O_7S$ $[M + H]^+$ 454.1642; found 454.1639.

4.2.39. (2*S*,3*R*)-4-(((3*aR*,4*R*,7*R*,7*aS*)-2,2-dimethyl-7-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-4*H*-[1,3]dioxolo[4,5-c]pyran-4-yl)methoxy)-3-methylbutan-2-ol (**41**).

Sodium hydride (60 wt% dispersion in mineral oil, 0.018 g, 0.458 mmol, 2.5 eq.) was put under an atmosphere of nitrogen and cooled to 0 °C. Alcohol **39** (0.100 g, 0.458 mmol, 2.5 eq.) was dissolved in anhydrous DMF (1.5 mL) and added dropwise to the base. The mixture was stirred for 30 min. at 0 °C. Mesylate **40** (0.083 g, 0.183 mmol, 1.0 eq.) was dissolved in anhydrous DMF (1.5 mL) and added dropwise to the alkoxide at 0 °C. The reaction solution was slowly warmed to 40 °C and reacted overnight at this temperature. The reaction was quenched with aqueous saturated ammonium chloride at 0 °C and the mixture was then stirred for 15 min. at room temperature. The aqueous layer was extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Following filtration, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane:ethyl acetate 25:75 → 0:100) affording compound **41** (0.020 g, 24%) with R_f = 0.21 (ethyl acetate) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.02 (s, 1H), 7.90 – 7.79 (m, 2H), 7.48 – 7.38 (m, 2H), 7.37 – 7.28 (m, 1H), 4.68 – 4.57 (m, 2H), 4.17 – 4.08 (m, 1H), 4.03 – 3.86 (m, 4H), 3.73 – 3.29 (m, 10H), 1.79 – 1.68 (m, 1H), 1.46 (s,

3H), 1.33 (s, 3H), 1.16 (d, J = 6.2 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 129.08, 128.32, 126.07, 121.38, 109.72, 77.03, 74.64, 73.71, 72.57, 71.58, 70.92, 68.23, 65.43, 50.73, 40.35, 28.43, 26.51, 21.41, 14.02 ppm. HRMS (ESI): calcd. for $C_{24}H_{36}N_3O_6$ $[M + H]^+$ 462.2598; found 462.2594.

4.2.40. (2*R*,3*S*,4*R*,5*R*)-2-(((2*R*,3*S*)-3-hydroxy-2-methylbutoxy)methyl)-5-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethoxy)tetrahydro-2*H*-pyran-3,4-diol (**42**).

Compound **41** (0.020 g, 0.043 mmol, 1.0 eq.) was put under an atmosphere of nitrogen and a 1:1 mixture of trifluoroacetic acid:water (0.5 mL: 0.5 mL) was added. The mixture was stirred for 5 h at room temperature. After completion, more water was added and the solution was lyophilized. The residue was purified by silica gel chromatography (DCM:MeOH 90:10) yielding final compound **42** (0.016 g, 88%) with R_f = 0.23 (DCM:MeOH 90:10) as a colourless oil. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.42 (s, 1H), 7.89 – 7.80 (m, 2H), 7.50 – 7.41 (m, 2H), 7.41 – 7.32 (m, 1H), 4.66 (m, 2H), 4.07 – 3.95 (m, 2H), 3.94 – 3.90 (m, 1H), 3.83 – 3.72 (m, 2H), 3.72 – 3.57 (m, 6H), 3.55 – 3.37 (m, 4H), 1.73 (m, 1H), 1.11 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.9 Hz, 3H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 128.21, 127.54, 125.06, 121.40, 77.99, 74.60, 74.20, 70.84, 69.34, 67.55, 66.60, 65.14, 62.70, 50.07, 39.72, 18.52, 11.67 ppm. HRMS (ESI): calcd. for $C_{21}H_{32}N_3O_6$ $[M + H]^+$ 422.2285; found 422.2282.

4.3. Inhibition assays with *E. coli* aaRS

Cloning, expression and purification of *E.coli* aminoacyl-tRNA synthetases and purification of tRNA was performed as reported.[31] *In vitro* inhibitory activity determination with purified *E. coli* aaRS was carried out as described by Nautiyal et al.[35]

4.4. Computational docking

Docking of **11** was performed in the protein structure from PDB entry 1JZS using Autodock Vina.[32] Applying Autodock Tools,[36,37] polar hydrogens were added to the protein and a grid box was defined for docking the ligand. The *x*-, *y*- and *z*-dimensions of the grid were 20, 20 and 22 Å respectively. The isoleucine moiety in **11** was restrained during the docking protocol since the binding of this motif is conserved in the previously discussed crystal structure with PDB entry 1JZQ.

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Conflicts of interest

No conflicts of interest are declared.

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Supplementary data

Supplementary Data related to this article can be found online.

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