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A new approach towards peptidosulfonamides: synthesis of potential inhibitors of bacterial peptidoglycan biosynthesis enzymes MurD and MurE

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Dedicated to Professor Miha Tišler on the occasion of his 80th birthday

Abstract—Peptidosulfonamides are an emerging group of peptidomimetics with a variety of applications in medicinal chemistry. We present a novel approach to the synthesis of peptidosulfonamides, and apply it to a series of new potential inhibitors of the bacterial peptidoglycan biosynthesis enzymes MurD and MurE. The synthesis was conducted via *N*-phthalimido β-aminoethanesulfonyl chlorides, which are new building blocks for the synthesis of peptidosulfonamides. In the most crucial step, sulfonic acids or their sodium salts were converted into the corresponding sulfonyl chlorides using an excess of either $SOCl_2$ or $SOCl_2$/DMF, and then coupled to the *C*-protected amino acid. None of the compounds significantly inhibited MurD, however, some inhibited MurE; one had an IC_{50} below 200 μM, which constitutes a promising starting point for further development. Molecular modelling simulations were performed on two analogues to investigate the absence of inhibitory activity of the sulfonamide compounds on MurD.

1. Introduction

Infectious diseases are the second leading cause of death worldwide and the third leading cause of death in developed countries.[1] Due to the emergence and dissemination of resistant bacterial strains, there is an urgent need for the development of novel antibacterial agents.[2] The bacterial cell wall peptidoglycan[3] is an important target for antibiotic research. Many antibacterial agents, like bacitracin, vancomycin, penicillins and cephalosporins, act by inhibiting the late enzymatic steps of bacterial peptidoglycan biosynthesis.[4] On the other hand, the early intracellular steps, catalysed by a series of Mur enzymes (MurA to MurF), have been underexploited as antibacterial targets.[5–7]

Recently, we focused our attention on the D-glutamic acid-adding enzyme (UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase, or MurD), which catalyses the addition of D-Glu to UDP-Mur*N*Ac-L-Ala during the synthesis of the cytoplasmic precursor UDP-Mur*N*Ac-pentapeptide. MurD is an ATP-dependant, amide-forming enzyme that performs the initial phosphorylation of the carboxylic acid (Fig. 1). The resulting acyl-phosphate is then attacked by the incoming amino acid (D-Glu) to form a high-energy tetrahedral intermediate, which finally collapses into the amide product and inorganic phosphate. All Mur ligases act via this mechanism, which has been confirmed by X-ray diffraction analysis,[8] by isotope transfer[9] and rapid quench[10] experiments, and by the chemical trapping method.[11] To date, several phosphinates of general formula **1** have been developed as tetrahedral transition-state analogue inhibitors of MurD,[12–14] and a QSAR study has been done for some of them.[15] Although the most active inhibitors still retain UDP-Mur*N*Ac or structurally closely related fragments, some less complex molecules based on the key phosphinodipeptide L-Ala-Ψ[PO(OH)-CH_2]-D-Glu have been shown to possess good inhibitory activities.[12–14]

To prepare improved inhibitors of MurD, we sought an innovative tetrahedral functional group that could be used as a transition-state mimetic. Over the last decade, the peptidosulfonamides have been recognized as emerging building

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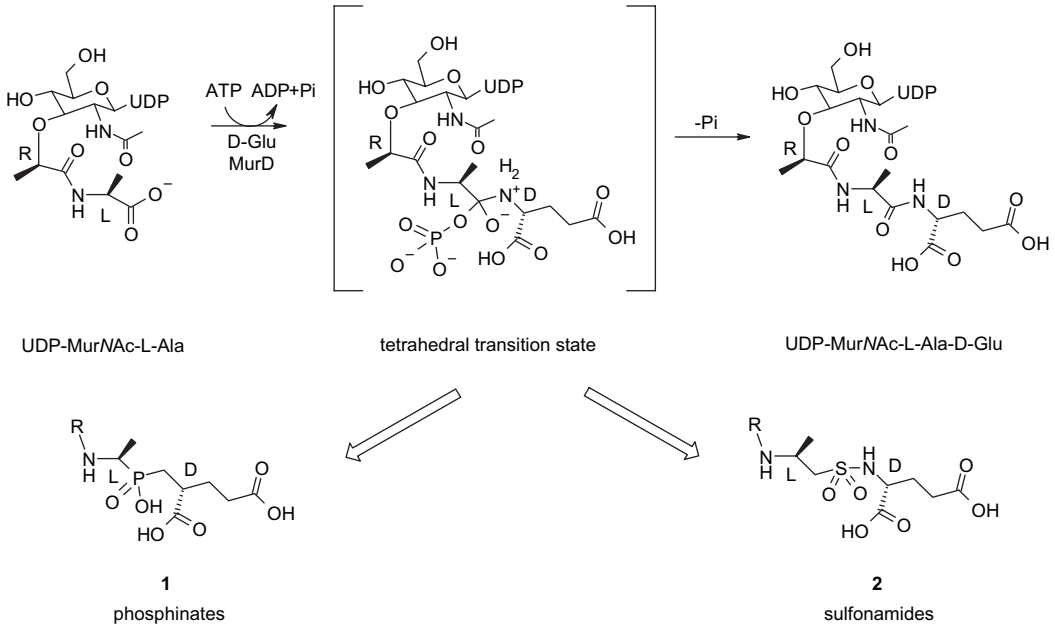


Figure 1. Reaction catalysed by MurD and design of transition-state analogue inhibitors.

blocks for preparing peptidomimetics and enzyme inhibitors.[16] Due to the intrinsic chemical instability of α-peptidosulfonamides, most of the studies of peptides containing the SO_2NH junction have been limited to β-peptidosulfonamides.[17] Sulfonamides possess a geometry similar to that of the tetrahedral intermediate formed during the peptide bond cleavage or formation.[18] Additionally, the stability of peptidosulfonamide peptidomimetics towards degradation by proteases is significantly increased.[19] As this type of transition-state mimetic has not yet been evaluated for inhibition of Mur enzymes, we prepared a series of peptidosulfonamides **2** of general formula R-L-Ala-Ψ(CH_2-SO_2)-D-Glu (Fig. 1) and assayed them for inhibition of MurD.

MurE is another cytoplasmic enzyme that is essential for the biosynthesis of bacterial peptidoglycan. It catalyses the attachment of the third amino acid residue to the product of the MurD reaction (UDP-Mur*N*Ac-L-Ala-D-Glu). Depending on the microorganism species, this amino acid is generally *meso*-diaminopimelic acid, L-lysine or L-ornithine.[20] All compounds designed as transition-state analogue inhibitors of MurD are thus highly interesting as potential inhibitors of MurE, for which they could act as substrate analogues.

2. Results and discussion

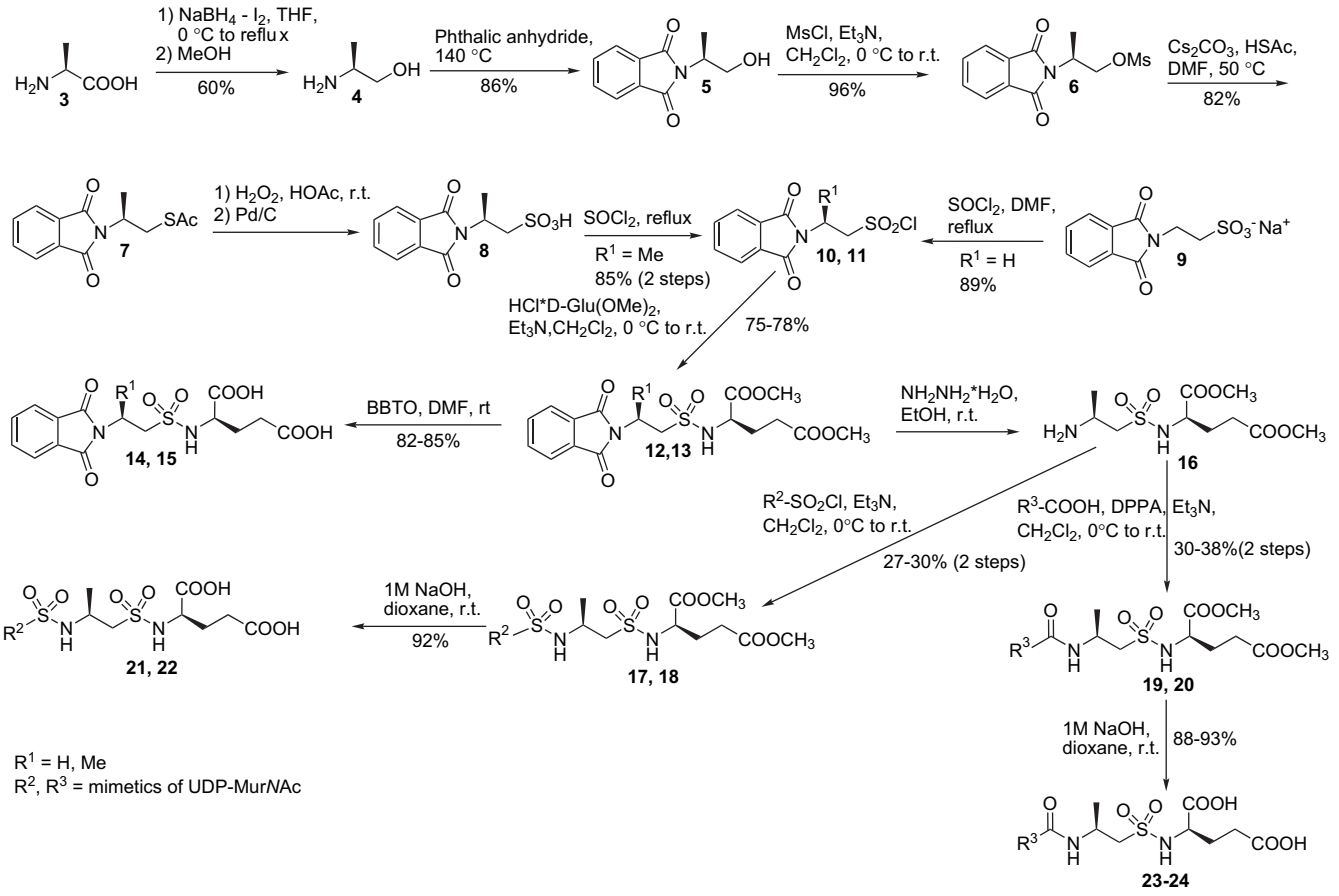
2.1. Synthesis

The crucial step in the synthesis of peptidosulfonamides is the conversion of sulfonic acids into the corresponding sulfonyl chlorides. β-Substituted β-aminoethanesulfonyl chlorides are usually obtained from sulfonic acids or their salts using triphosgene[21–23] or phosgene[24–26] as chlorinating agent. Recently, we developed a new method for the synthesis of *N*-phthalimido β-aminoethanesulfonyl chlorides using thionyl chloride.[27] In this paper we present the application of this method to the synthesis of potential inhibitors of the bacterial peptidoglycan biosynthesis enzymes MurD and MurE.

The synthesis of sulfonamide inhibitors **14**, **15** and **21**–**24** is presented in Scheme 1. We started the synthesis with free L-alanine **3**, which was reduced to amino alcohol **4** using the $NaBH_4/I_2$ system,[28] and phthaloylated with phthalic anhydride to give *N*-phthalimido-protected amino alcohol **5** in high yield. The protected amino alcohol **5** was mesylated with methanesulfonyl chloride and Et_3N in dichloromethane. In the next step, mesylate **6** was added to the mixture of thioacetic acid and Cs_2CO_3 in DMF and stirred at 50 °C for 24 h. Thioacetate **7** was then oxidized to the corresponding sulfonic acid **8** using aqueous hydrogen peroxide and acetic acid; after 24 h at rt, the excess peroxide was destroyed by adding 10% Pd/C. The resulting crude sulfonic acid **8** was finally refluxed in excess thionyl chloride to give sulfonyl chloride **10** in high yield. The sulfonyl chloride of taurine derivative **11** was obtained by a slight modification of the procedure, in which a catalytic amount of dry DMF was added to the reaction mixture to achieve clean and rapid chlorination of sodium salt **9**.

The corresponding sulfonyl chlorides **10** and **11** were coupled with *C*-protected D-glutamic acid to give methyl esters **12** and **13**, respectively, the selective deprotection of which with bis(tributyltin) oxide (BBTO)[29] yielded compounds **14** and **15**, respectively. We found that the reaction displays a high level of chemoselectivity between methyl esters and the phthalimido protecting group.

Hydrazinolysis of the phthalimido protecting group of compound **12** produced the crucial amine intermediate **16**, which was unstable to heat and prolonged storage at rt. Free amine **16** was immediately substituted by different carboxyl or sulfonyl moieties. The resulting compounds **17**–**20** were converted by alkaline hydrolysis into target sulfonamide inhibitors **21**–**24** (Table 1).



Scheme 1. Synthesis of β-sulfonopeptide inhibitors.

2.2. Inhibitory activities

Target compounds **14**, **15** and **21**–**24** were tested for inhibitory activity on MurD from *Escherichia coli* and on MurE from *Staphylococcus aureus*. The results are presented as residual activities (RA) of the enzymes in the presence of 1 mM compound (Table 1).

All target peptidosulfonamides (compounds **14**, **15** and **21**–**24**) proved to be poor inhibitors of MurD. Phosphinate **25** had previously been prepared and evaluated on MurD (IC_{50}=95 μM).[14] The RA of its structurally closely related sulfonamide analogue **24** was 80%, which makes the compound practically inactive against MurD. Compounds **24** and **25** were both designed with the purpose of mimicking the tetrahedral transition-state of the reaction catalysed by MurD. The substituted *trans*-cinnamoyl moiety present in both compounds was introduced to mimic the Mur*N*Ac part of the substrate. However, only phosphinate **25** inhibited MurD, in spite of the fact that it was tested as a mixture of four diastereoisomers, while the related sulfonamidopeptide **24** is diastereomerically pure. The reason for the poor inhibitory activity of peptidosulfonamides might be the elongation of the pseudopeptide backbone caused by the insertion of the additional methylene group, which may disrupt the active conformation of the molecule.

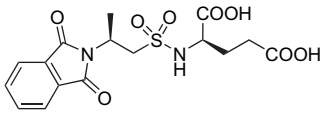
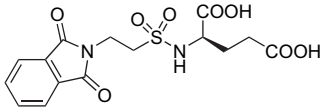
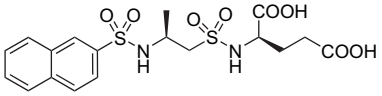
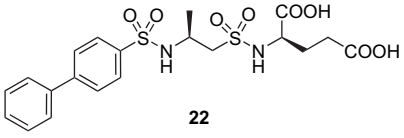
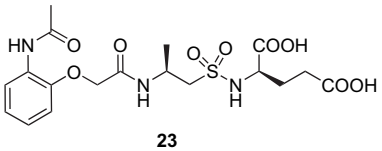
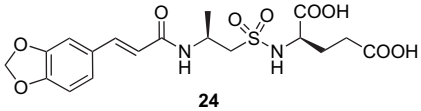
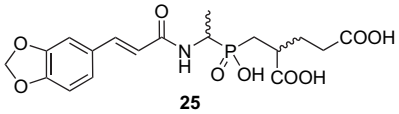
Although the compounds synthesized in this study were designed as potential transition-state analogue inhibitors of MurD, they turned out to be better inhibitors of MurE. In fact, biphenyl derivative **22** is a good inhibitor of MurE, with an IC_{50} in the micromolar range; it thus represents a promising starting point for further structural modifications. It is most likely that sulfonamidopeptide **22** inhibits MurE as a substrate analogue.

2.3. Molecular modelling

A molecular modelling study was performed to examine the differences in inhibitory activity between the sulfonamide (**24**) and phosphinate (**25**) types of inhibitors. To date, no crystallographic data of MurD inhibitors bound to the enzyme active site have been published. However, it is reasonable to assume that the inhibitors possessing the D-Glu functionality mimic the position occupied by the D-Glu moiety of the product UDP-Mur*N*Ac-L-Ala-D-Glu in the active site. Thus, we have considered only the situations where the D-Glu part was docked to the subpocket as defined in an analogous way to the experimental structure with bound UDP-Mur*N*Ac-L-Ala-D-Glu (pdb code 4uag[8]).

In Figures 2 and 3, the crystal structure of UDP-Mur*N*Ac-L-Ala in the active site of MurD from *E. coli* (pdb code 3uag[8]) is compared with modelled structures of compounds **25** and **24**, respectively. When the positions of both compounds in the active site are compared, one important difference can be observed. The phosphinic group of phosphinate inhibitor **25** is perfectly positioned to form a coordinative bond with the

Table 1. Residual activities of the enzymes in the presence of 1 mM inhibitor

Structure	RA (%) MurD	RA (%) MurE
 **14**	74	41
 **15**	77	ND[a]
 **21**	75	60
 **22**	70	12 (IC_{50}=181±18 μM)
 **23**	93	56
 **24**	80	64
 **25**	17[b] (IC_{50}=95±15 μM)[b]	ND[a]

Results represent the means of two independent experiments. Standard deviations were within ±10% of the means.
[a] ND=not determined.
[b] From Ref. 14.

Mn^{2+} ion, as expected for a transition-state analogue. In addition, the dicarboxylic moiety of the compound extents into the D-Glu binding pocket formed by Thr321, Lys348, Phe422 and Ser415. On the other hand, the $–SO_2–$ group of compound **24** can also form a coordinative bond with Mn^{2+}, but this results in an unfavourable position of the sulfonamide –NH– group. Consequently, the α-carboxyl group of D-Glu reorients itself by losing a strong hydrogen bond

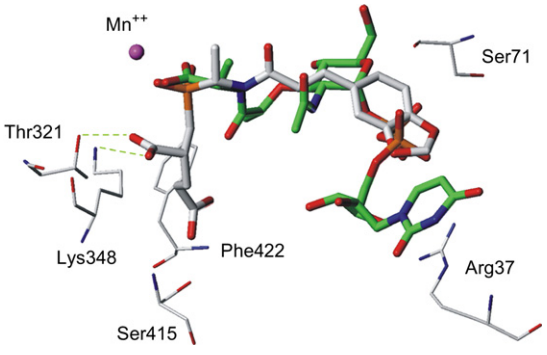


Figure 2. Superposition of phosphinate **25** (carbon atoms coloured grey) and UDP-Mur*N*Ac-L-Ala (carbon atoms coloured green) in the *E. coli* MurD active site. The subpocket into which the D-Glu part of the molecule is anchored is shown (Ser415 and Phe422).

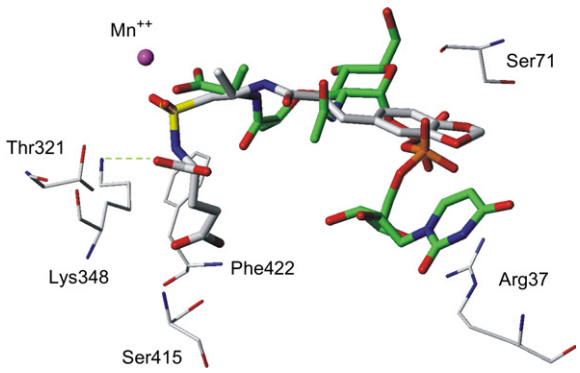


Figure 3. Superposition of sulfonamide **24** and UDP-Mur*N*Ac-L-Ala in the *E. coli* MurD active site. Colour representation as in Figure 2.

with Thr321 (see Figs. 2 and 3). This unfavourable interaction is also recognized in terms of the scoring, where the *F*-score ranked compound **24** (−17.6) much lower than compound **25** (−31.0). However, the sulfonamide bond has no influence on the orientation of 3-(1,3-benzodioxol-5-yl)-cinnamoyl part of compound **24**. This group, which is a good mimetic of the phospho-sugar part of UDP-Mur*N*Ac,[14] binds in a similar way in compounds **24** and **25**, and thus should not be responsible for the differences in biological activity observed.

It has to be pointed out that the geometry of the transition-state analogue at the peak of its free energy profile could be in variation with the transition structure, which is the point of highest potential energy of the molecule along the reaction pathway.[30] Thus, in the modelling of the transition-state structures, other contributions, such as entropic factors,[31] should in principle be considered. In addition, the substitution of the phosphinic group present in compound **25** with the sulfonamido group might result in a weaker coordination bond with the Mn^{2+}, which could consequently contribute to the lower inhibitory activity of compound **24**.

3. Conclusion

We have presented a simple and straightforward synthesis of new peptidosulfonamides as potential inhibitors of the bacterial peptidoglycan biosynthesis enzymes MurD and MurE. The synthesis was conducted via *N*-phthalimido β-aminoethanesulfonyl chlorides, which are new building blocks for the synthesis of peptidosulfonamides. In the most crucial step, sulfonic acids or their sodium salts were converted into the corresponding sulfonyl chlorides using either excess $SOCl_2$ or $SOCl_2$/DMF. From the inhibitory activity results and the molecular modelling study, we can conclude that β-peptidosulfonamides are not suitable for development of transition-state analogue inhibitors of MurD. However, compound **22** had a good inhibitory activity on MurE, and represents a promising starting point for further design of MurE inhibitors that act as substrate analogues.

4. Methods

4.1. Enzyme assays

4.1.1. MurD. Enzymatic assays were performed as previously described,[32] with slight modifications. The compounds were tested for their ability to inhibit the addition of D-[^{14}C]Glu to UDP-Mur*N*Ac-L-Ala in a mixture (final volume: 50 μL) containing 0.1 M Tris/HCl, pH 8.6, 5 mM $MgCl_2$, 25 μM UDP-Mur*N*Ac-L-Ala, 25 μM D-[^{14}C]Glu (50,000 cpm), 5% (v/v) DMSO, purified MurD from *E. coli*[33] (diluted with 20 mM potassium phosphate, pH 7.0, 1 mM dithiothreitol, 1 mg/mL BSA), and 1 mM test compound (all of the compounds were soluble in the enzyme assay mixture containing 5% DMSO). The mixture was incubated for 30 min at 37 °C, and the reaction stopped by adding 10 μL glacial acetic acid. The mixture was lyophilized and taken up in the HPLC elution buffer. The radioactive substrate and product were separated by reverse-phase HPLC with a Nucleosil $5C_{18}$ column (150×4.6 mm) as stationary phase, and isocratic elution at a flow rate of 0.6 mL/min with 50 mM ammonium formate, pH 4.7. The compounds were detected and quantified with an LB 506 C-1 HPLC radioactivity monitor (Berthold France, Thoiry, France) using Quickszint Flow 2 scintillator (Zinsser Analytic, Maidenhead, UK) at 0.6 mL/min. Residual activity was calculated with respect to a similar assay without inhibitor. Values are expressed as the means of two independent experiments. Standard deviations were within ±10% of the means.

4.1.2. MurE. The compounds were tested for their ability to inhibit the addition of L-[^{14}C]Lys to UDP-Mur*N*Ac-L-Ala-D-Glu in a mixture (final volume: 50 μL) containing 0.1 M Tris/HCl, pH 8.6, 15 mM $MgCl_2$, 100 μM UDP-Mur*N*Ac-L-Ala-D-Glu, 200 μM L-[^{14}C]Lys (50,000 cpm), 5% (v/v) DMSO, purified MurE from *S. aureus*[34] (diluted with 20 mM potassium phosphate, pH 7.0, 1 mM dithiothreitol) and 1 mM test compound (all of the compounds were soluble in the assay mixture containing 5% DMSO). The mixture was incubated for 30 min at 37 °C, and the reaction stopped by adding 10 μL glacial acetic acid. Separation and quantification were then performed as described for MurD. The IC_{50} value for compound **22** was determined from a range of inhibitor concentrations; value±standard deviation at 95% of confidence was calculated from the fitted regression equation using the logit/log plot.

4.2. Molecular modelling

Our modelling procedure was based on the crystal structure of the complex of the MurD enzyme from *E. coli* with its ligands UDP-Mur*N*Ac-L-Ala, ADP and Mn^{2+} (pdb entry 3uag[8]). Molecular modelling simulations were performed using the Sybyl7.1 (Tripos, Inc.) programme suite[35] and FlexX, a software package for incremental docking.[36] All of the compounds were initially modelled, then minimized for up to 1000 steps, and finally centred. Standard Gasteiger–Marsili charges[37] were used throughout. Docking of inhibitors into the *E. coli* MurD active site was performed in several independent runs. Residue Lys198 was included in the active site as the carbamoylated form[38] and all crystal water molecules were deleted. In addition, we defined the Mn^{2+} ion as an essential part of the active site since it makes a coordinative bond with the carboxylic functional group of the L-Ala part of UDP-Mur*N*Ac-L-Ala. We also defined residues Ser415 and Phe422 as a subpocket since in the experimentally determined structure of the complex MurD*UDP-Mur*N*Ac-L-Ala-D-Glu (pdb entry 4uag[8]), they bind the D-Glu part of UDP-Mur*N*Ac-L-Ala-D-Glu. For each compound, 100 positions (low energy conformations in the active site) were determined using FlexX as both docking and scoring functions.

5. Experimental

5.1. Materials

Chemicals from Sigma–Aldrich and Acros Organics were used without further purification. Analytical TLC was performed on Merck silica gel ($60F_{254}$) plates (0.25 mm); compounds were visualized with ultraviolet light. Column

chromatography was carried out on silica gel 60 (particle size 240–400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance DPX$_{300}$ spectrometer in $CDCl_3$ or DMSO-d_6 solution, with TMS as the internal standard. IR spectra were obtained on a Perkin–Elmer 1600 FTIR spectrometer. Optical rotation was measured on a Perkin–Elmer 1241 MC polarimeter. Microanalyses were performed on a Perkin–Elmer C, H, N analyzer 240 C. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer.

5.2. Synthesis of β-aminoethanesulfonyl chlorides 10, 11

5.2.1. (*S*)-2-Phthalimidopropanol (5). Phthalic anhydride (20.00 g, 135.0 mmol) and (*S*)-alaninol (9.66 g, 128.6 mmol) were fused at 140 °C for 7 h. The reaction mixture was cooled to rt and the resulting solid dissolved in EtOAc (200 mL). The solution was washed successively with saturated aqueous $NaHCO_3$ (60 mL), H_2O (60 mL), citric acid (10% w/w, 60 mL) and brine (60 mL). Drying (Na_2SO_4), followed by concentration in vacuo, produced compound **5** (22.70 g, 86%) as a white solid: R_f=0.48 ($CHCl_3$/MeOH=9/1); mp 79–82 °C (lit.[39] mp 77 °C); $[\alpha]_D^{23}$ +32.7 (*c* 0.312, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47 (d, 3H, *J*=7.1 Hz, CH_3), 2.70 (br s, 1H, OH), 3.91 (dd, 1H, *J*=11.8, 3.8 Hz, CH_2), 4.05 (dd, 1H, *J*=11.8, 7.5 Hz, CH_2), 4.45–4.63 (m, 1H, CH), 7.70–7.78 (m, 2H, Pht-H), 7.82–7.90 (m, 2H, Pht-H); FABMS: *m*/*z*=206 (M+H)$^+$.

5.2.2. (2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-propyl methanesulfonate (6). To a solution of alcohol **5** (9.76 g, 47.6 mmol) in CH_2Cl_2 (150 mL), Et_3N (8.0 mL, 57.0 mmol) was added. After cooling to 0 °C, methanesulfonyl chloride (4.5 mL, 57.0 mmol) was added dropwise. Stirring was continued overnight at rt, followed by addition of CH_2Cl_2 (100 mL). The mixture was washed with $NaHCO_3$ (5% w/w, 2×100 mL), H_2O (2×100 mL) and brine (80 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated. Mesylate **6** was crystallized from EtOAc/hexane. White crystals were obtained (12.90 g, 96%): R_f=0.64 ($CHCl_3$/MeOH=9/1); mp 71–74 °C; $[\alpha]_D^{23}$ +34.0 (*c* 0.315, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53 (d, 3H, *J*=6.8 Hz, CH_3), 2.99 (s, 3H, CH_3), 4.45 (dd, 1H, *J*=9.8, 4.4 Hz, CH_2), 4.68–4.90 (m, 2H, CH_2+CH), 7.71–7.80 (m, 2H, Pht-H), 7.82–7.91 (m, 2H, Pht-H); IR (KBr, cm^{-1}): 3012, 1771, 1709, 1467, 1354, 1170, 1042, 992, 821, 719, 517; FABMS: *m*/*z*=284 (M+H)$^+$; Anal. Calcd for $C_{12}H_{13}NO_5S$: C (50.87%), H (4.63%), N (4.94%). Found: C (51.16%), H (4.70%), N (4.96%).

5.2.3. *S*-[(2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]ethanethioate (7). Thioacetate (3.6 mL, 51.0 mmol) was added to a suspension of Cs_2CO_3 (15.25 g, 47.0 mmol) in DMF (70 mL). Mesylate **6** (12.05 g, 42.6 mmol) was added in one portion to the resulting solution and stirring was continued at 50 °C for 24 h, prior to which the reaction flask was covered with aluminium foil. The mixture was poured into distilled H_2O (250 mL), and the aqueous phase extracted with EtOAc (3×150 mL). The combined organic layers were washed with H_2O (150 mL), $NaHCO_3$ (5% w/w, 150 mL) and brine (150 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by column chromatography (EtOAc/hexane=1/1) to produce **7** as a white solid (9.20 g, 82%): R_f=0.40 (EtOAc/Hex=1/1); mp 54–57 °C; $[\alpha]_D^{23}$ +170.1 (*c* 0.332, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.58 (d, 3H, *J*=6.9 Hz, CH_3), 2.30 (s, 3H, CH_3), 3.40 (dd, 1H, *J*=13.9, 5.5 Hz, CH_2), 3.52 (dd, 1H, *J*=13.9, 9.7 Hz, CH_2), 4.42–4.58 (m, 1H, CH), 7.68–7.78 (m, 2H, Pht-H), 7.80–7.90 (m, 2H, Pht-H); IR (KBr, cm^{-1}): 3453, 2976, 1698, 1466, 1356, 1106, 944, 884, 714, 630; FABMS: *m*/*z*=264 (M+H)$^+$; Anal. Calcd for $C_{13}H_{13}NO_3S$: C (59.30%), H (4.98%), N (5.32%). Found: C (59.29%), H (4.89%), N (5.23%).

5.2.4. (2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-propanesulfonyl chloride (10). A mixture of H_2O_2 (30% w/w in H_2O, 30 mL) and HOAc (60 mL) was added to a solution of thioacetate **7** (9.05 g, 34.4 mmol) in HOAc (30 mL). After stirring for 24 h at rt, 10% Pd/C was added to destroy the excess peroxide. Filtration, concentration and co-evaporation with toluene (2×20 mL) and ether (2×20 mL) under reduced pressure produced crude sulfonic acid **8**. This compound was dried at 50 °C for 48 h in vacuo over P_2O_5 and NaOH, and afterwards refluxed in $SOCl_2$ (20 mL) for 7 h. Excess $SOCl_2$ was removed by evaporation, followed by co-evaporation with toluene and ether under reduced pressure. The resulting residue was purified through a silica plug (CH_2Cl_2) to give **10** as a white solid (8.41 g, 85%). An analytical sample was obtained by precipitation from CH_2Cl_2/hexane: R_f=0.65 (CH_2Cl_2/acetone=18/1); mp 83–85 °C; $[\alpha]_D^{23}$ +78.1 (*c* 0.310, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66 (d, 3H, *J*=7.2 Hz), 3.97 (dd, 1H, *J*=14.3, 3.6 Hz, CH_2), 4.77 (dd, 1H, *J*=14.3, 9.8 Hz, CH_2), 5.13–5.28 (m, 1H, CH), 7.72–7.81 (m, 2H, Pht-H), 7.84–7.93 (m, 2H, Pht-H); IR (KBr, cm^{-1}): 3467, 1776, 1711, 1374, 1169, 1062, 860, 724, 605, 525; EIMS: 287, 289 (M$^+$); Anal. Calcd for $C_{11}H_{10}ClNO_4S$: C (45.92%), H (3.50%), N (4.87%). Found: C (46.18%), H (3.52%), N (4.68%).

5.2.5. 2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethanesulfonyl chloride (11). To an ice-cooled mixture of sulfonic acid sodium salt **9** (5.00 g, 17.9 mmol), which was prepared as described,[40] and excess thionyl chloride (10 mL), DMF (1 mL) was added dropwise. The mixture was heated under reflux for 5 h. The chlorinating species was removed by evaporation, followed by co-evaporation with toluene and ether under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with H_2O (60 mL), saturated aqueous $NaHCO_3$ (60 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated, and the residue purified through a silica plug (CH_2Cl_2) to yield sulfonyl chloride **11** as a white solid (4.90 g, 89%): R_f=0.63 (CH_2Cl_2/acetone=18/1); mp 160–162 °C (lit.[40] mp 159–162 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.03–4.15 (m, 2H, CH_2SO_2), 4.38 (t, 2H, *J*=6.5 Hz, NCH_2), 7.74–7.83 (m, 2H, Pht-H), 7.86–7.96 (m, 2H, Pht-H); FABMS: *m*/*z*=274 (M+H)$^+$.

5.3. General procedure for the preparation of pseudodipeptides 12, 13

Sulfonyl chloride **10**, **11** (25.0 mmol) was dissolved in CH_2Cl_2 (40 mL) and added dropwise to an ice-cooled mixture of HCl*D-Glu(OMe)$_2$ (25.0 mmol) and Et_3N

(50.0 mmol) in CH_2Cl_2 (50 mL). The resulting mixture was stirred overnight allowing warming to rt. After dilution with CH_2Cl_2 (30 mL), the mixture was washed with ice-cold 2 M HCl (2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting residue was purified by column chromatography (CH_2Cl_2/acetone=15/1).

5.3.1. Dimethyl *N*-{[(2*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]sulfonyl}-D-glutamate (12). White solid (9.31 g, 78%): R_f=0.37 (CH_2Cl_2/acetone=15/1); mp 89–90 °C; $[\alpha]_D^{23}$ +42.9 (*c* 0.322, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.46 (d, 3H, *J*=7.2 Hz, CH_3), 1.69–1.84 (m, 1H, CH_2CH_2CO), 1.92–2.06 (m, 1H, CH_2CH_2CO), 2.36–2.46 (m, 2H, CH_2CH_2CO), 3.48 (dd, 1H, *J*=14.3, 4.5 Hz, CH_2SO_2), 3.59 (s, 3H, $COOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.80 (dd, 1H, *J*=14.3, 9.4 Hz, CH_2SO_2), 3.93–4.05 (m, 1H, CHCO), 4.64–4.79 (m, 1H, $CHCH_3$), 7.81–7.91 (m, 4H, Ar-H), 7.96 (d, 1H, *J*=9.0 Hz, NH); IR (KBr, cm^{-1}): 3282.9, 2962.5, 1714.7, 1440.7, 1381.5, 1305.2, 1156.1, 978.3, 716.5; FABMS: *m*/*z*=427 $(M+H)^+$; Anal. Calcd for $C_{18}H_{22}N_2O_8S$: C (50.70%), H (5.20%), N (6.57%). Found: C (50.96%), H (5.29%), N (6.39%).

5.3.2. Dimethyl *N*-{[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]sulfonyl}-D-glutamate (13). White solid (5.64 g, 75%): R_f=0.36 (CH_2Cl_2/acetone=15/1); mp 105–108 °C; $[\alpha]_D^{23}$ +14.9 (*c* 0.276, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90–2.10 (m, 1H, CH_2CH_2CO), 2.45–2.65 (m, 1H, CH_2CH_2CO), 2.36–2.46 (m, 2H, CH_2CH_2CO), 3.25–3.37 (m, 1H, CH_2SO_2), 3.40–3.55 (m, 1H, CH_2SO_2), 3.70 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 4.02–4.17 (m, 1H, NCH_2), 4.20–4.35 (m, 1H, CH), 4.37–4.50 (m, 1H, NCH_2), 5.55 (d, 1H, *J*=9.1 Hz, NH), 7.70–7.80 (m, 2H, Ar-H), 7.85–7.95 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 3485.8, 1641.6, 1438.1, 978.6, 720.4; FABMS: *m*/*z*=413 $(M+H)^+$; Anal. Calcd for $C_{17}H_{20}N_2O_8S$: C (49.51%), H (4.89%), N (6.79%). Found: C (49.46%), H (4.90%), N (6.67%).

5.4. General procedure for the hydrazinolysis of the phthalimido protecting group of pseudodipeptide 12

To a solution of pseudodipeptide **12** (15.0 mmol) in EtOH (40 mL), hydrazine monohydrate (17.0 mmol) was added, and the reaction mixture was stirred at rt for 96 h. The mixture was cooled to 0 °C and filtered to remove phthalhydrazide. The filtrate was concentrated to dryness and the resulting oil was redissolved in a minimum amount of EtOH, cooled to 0 °C, filtered and evaporated under reduced pressure. The resulting pale yellow oil **16** was immediately used for the next reaction step, without further purification.

5.5. General procedure for the preparation of *N*-sulfonyl peptidosulfonamides 17, 18

The required sulfonyl chloride (3.0 mmol) was dissolved in CH_2Cl_2 (15 mL) and added dropwise to an ice-cooled mixture of amine **16** (2.5 mmol) and Et_3N (6.0 mmol) in CH_2Cl_2 (25 mL). The resulting mixture was stirred overnight allowing warming to rt. After dilution with CH_2Cl_2 (50 mL), the mixture was washed with ice-cold 2 M HCl (2×30 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting residue was purified by column chromatography (CH_2Cl_2/acetone=10/1).

5.5.1. Dimethyl *N*-({(2*S*)-2-[(2-naphthylsulfonyl)amino]-propyl}sulfonyl)-D-glutamate (17). White solid (420 mg, 30%): R_f=0.32 (CH_2Cl_2/acetone=10/1); mp 97–99 °C; $[\alpha]_D^{23}$ −35.1 (*c* 0.297, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.06 (d, 3H, *J*=6.8 Hz, $CHCH_3$), 1.64–1.81 (m, 1H, CH_2CH_2CO), 1.85–2.01 (m, 1H, CH_2CH_2CO), 2.34 (t, 2H, *J*=7.4 Hz, CH_2CH_2CO), 3.00–3.23 (m, 2H, CH_2SO_2), 3.58 (s, 3H, $COOCH_3$), 3.60–3.73 (m, 4H, $CHCH_3$+$COOCH_3$), 3.83–3.95 (m, 1H, CHCO), 7.64–8.21 (m, 8H, Naph-H+2×NH), 8.46 (s, 1H, Naph-H); IR (KBr, cm^{-1}): 3299.4, 2949.2, 1734.7, 1439.0, 1330.6, 982.9, 820.3, 665.3; FABMS: *m*/*z*=487 $(M+H)^+$; Anal. Calcd for $C_{20}H_{26}N_2O_8S_2$: C (49.37%), H (5.39%), N (5.76%). Found: C (49.61%), H (5.40%), N (5.75%).

5.5.2. Dimethyl *N*-({(2*S*)-2-[([1,1′-biphenyl]-4-ylsulfonyl)-amino]propyl}sulfonyl)-D-glutamate (18). White solid (390 mg, 27%): R_f=0.36 (CH_2Cl_2/acetone=10/1); mp 136–138 °C; $[\alpha]_D^{23}$ −51.6 (*c* 0.295, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.11 (d, 3H, *J*=6.8 Hz, $CHCH_3$), 1.67–1.84 (m, 1H, CH_2CH_2CO), 1.87–2.04 (m, 1H, CH_2CH_2CO), 2.37 (t, 2H, *J*=7.5 Hz, CH_2CH_2CO), 3.08 (dd, 1H, *J*=13.9, 9.4 Hz, CH_2SO_2), 3.20 (dd, *J*=13.9, 3.8 Hz, CH_2SO_2), 3.58 (s, 3H, $COOCH_3$), 3.61–3.74 (m, 4H, $CHCH_3$+$COOCH_3$), 3.87–3.97 (m, 1H, CHCO), 7.41–7.96 (m, 11H, Ar-H+2×NH); IR (KBr, cm^{-1}): 3292.4, 2980.0, 1735.0, 1441.7, 1284.3, 1154.1, 983.5, 922.9, 763.7, 674.6, 577.9; FABMS: *m*/*z*=513 $(M+H)^+$; Anal. Calcd for $C_{22}H_{28}N_2O_8S_2$: C (51.55%), H (5.51%), N (5.47%). Found: C (51.68%), H (5.60%), N (5.45%).

5.6. General procedure for the preparation of *N*-acyl peptidosulfonamides 19, 20

DPPA coupling: to an ice-cooled mixture containing the amine **16** (2.5 mmol) and the required carboxylic acid (2.5 mmol) in dry DMF (20 mL), DPPA was slowly added (3.0 mmol), followed by dropwise addition of Et_3N (5.0 mmol). The reaction mixture was kept at 0 °C for another 2 h, and then allowed to warm up to rt. After 24 h, the reaction mixture was diluted with EtOAc (70 mL) and washed with an aqueous solution of citric acid (10% w/w, 50 mL), H_2O (50 mL), saturated aqueous $NaHCO_3$ (50 mL), H_2O (50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting residue was purified by column chromatography (CH_2Cl_2/acetone=10/1).

5.6.1. Dimethyl *N*-{[(2*S*)-2-({2-[2-(acetylamino)phenoxy]acetyl}amino)propyl]sulfonyl}-D-glutamate (19). Colourless oil (370 mg, 30%), used in the next reaction step without further purification: R_f=0.15 ($CHCl_3$/acetone=5/1); ^{1}H NMR (300 MHz, DMSO): δ 1.23 (d, 3H, *J*=6.8 Hz, $CHCH_3$), 1.71–1.87 (m, 1H, CH_2CH_2CO), 1.93–2.06 (m, 1H, CH_2CH_2CO), 2.11 (s, 3H, CH_3CO), 2.38–2.46 (m, 2H, CH_2CH_2CO), 3.10–3.29 (m, 2H, CH_2SO_2), 3.59 (s, 3H, $COOCH_3$), 3.65 (s, 3H, $COOCH_3$), 3.96–4.06 (m, 1H, CHCO), 4.25–4.37 (m, 1H, $CHCH_3$), 4.52 (ABq, 2H, *J*=15.1 Hz, OCH_2CO), 6.90–7.11 (m, 3H, Ar-H), 7.79–7.90 (m, 2H, Ar-H+SO_2NH), 8.28 (d, 1H, *J*=7.5 Hz,

*NH*CH), 9.35 (s, 1H, Ar-*NH*CO); IR (KBr, cm^{-1}): 3283.9, 1736.5, 1669.9, 1536.0, 1455.0, 1120.9, 753.4; FABMS: *m*/*z*=488 (M+H).

5.6.2. Dimethyl *N*-[((2*S*)-2-{[(*E*)-3-(1,3-benzodioxol-5-yl)-2-propenoyl]amino}propyl)sulfonyl]-D-glutamate (20). Colourless oil (150 mg, 38%): R_f=0.13 ($CHCl_3$/acetone=5/1); $[\alpha]_D^{23}$ +30.0 (*c* 0.140, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.25 (d, 3H, *J*=6.8 Hz, CH*CH*$_3$), 1.73–1.88 (m, 1H, *CH*$_2$CH$_2$CO), 1.92–2.05 (m, 1H, *CH*$_2$CH$_2$CO), 2.38–2.45 (m, 2H, CH$_2$*CH*$_2$CO), 3.09–3.29 (m, 2H, CH_2SO_2), 3.58 (s, 3H, $COOCH_3$), 3.66 (s, 3H, $COOCH_3$), 3.96–4.06 (m, 1H, CHCO), 4.24–4.36 (m, 1H, *CH*CH_3), 6.07 (s, 2H, OCH_2O), 6.41 (d, 1H, *J*=15.8 Hz, CH*CH*CO), 6.95 (d, 1H, *J*=7.9 Hz, Ar-H), 7.07 (dd, 1H, *J*=7.9, 1.5 Hz, Ar-H), 7.14 (d, 1H, *J*=1.5 Hz, Ar-H), 7.34 (d, 1H, *J*=15.8 Hz, *CH*CHCO), 7.86 (d, 1H, *J*=8.7 Hz, NH), 8.07 (d, 1H, *J*=7.9 Hz, NH); IR (KBr, cm^{-1}): 3276.8, 2953.3, 1735.8, 1654.6, 1616.2, 1491.1, 1447.4, 1251.2, 1148.1, 1037.5, 981.1; FABMS: *m*/*z*=471 $(M+H)^+$; Anal. Calcd for $C_{20}H_{26}N_2O_9S$: C (51.06%), H (5.57%), N (5.95%). Found: C (51.14%), H (5.76%), N (5.86%).

5.7. General procedure for the preparation of peptidosulfonamide inhibitors 21–24. Alkaline hydrolysis of esters

To a stirred solution of dimethyl-protected peptidosulfonamide **17**–**20** (0.4 mmol) in dioxane (2 mL), 1 M NaOH (2 mL) was added, and the reaction mixture was stirred overnight at rt. After the solvent was removed under reduced pressure, the oily residue was redissolved in H_2O (20 mL) and washed with EtOAc (2×20 mL). The aqueous phase was acidified to pH 1–2 using an aqueous solution of 2 M HCl, and extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine (1×20 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure.

5.7.1. *N*-({(2*S*)-2-[(2-Naphthylsulfonyl)amino]propyl}-sulfonyl)-D-glutamic acid (21). White solid (160 mg, 92%): R_f=0.72 (CH_3CN/MeOH/H_2O=3/1/1); mp 246–248 °C; $[\alpha]_D^{23}$ −44.5 (*c* 0.297, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.06 (d, 3H, *J*=6.4 Hz, CH*CH*$_3$), 1.62–1.77 (m, 1H, *CH*$_2$CH$_2$CO), 1.82–1.99 (m, 1H, *CH*$_2$CH$_2$CO), 2.26 (t, 2H, *J*=7.4 Hz, CH$_2$*CH*$_2$CO), 3.07 (dd, 1H, *J*=13.9, 9.0 Hz, CH_2SO_2), 3.20 (dd, 1H, *J*=13.9, 4.0 Hz, CH_2SO_2), 3.58–3.73 (m, 1H, *CH*CH_3), 3.80–3.91 (m, 1H, CHCO), 7.63–8.22 (m, 8H, Naph-H+2×NH), 8.47 (s, 1H, Naph-H), 12.55 (br s, 2H, 2×COOH); IR (KBr, cm^{-1}): 3288.9, 1706.1, 1420.5, 1314.5, 1216.7, 1156.2, 987.9, 821.0, 666.1; FABMS: *m*/*z*=457 $(M-H)^-$; Anal. Calcd for $C_{18}H_{22}N_2O_8S_2$: C (47.15%), H (4.84%), N (6.11%). Found: C (47.05%), H (4.94%), N (6.22%).

5.7.2. *N*-({(2*S*)-2-[([1,1′-Biphenyl]-4-yl-sulfonyl)amino]-propyl}sulfonyl)-D-glutamic acid (22). White solid (170 mg, 92%): R_f=0.76 (CH_3CN/MeOH/H_2O=3/1/1); mp 201–203 °C; $[\alpha]_D^{23}$ −50.1 (*c* 0.316, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.11 (d, 3H, *J*=6.8 Hz, CH*CH*$_3$), 1.63–1.81 (m, 1H, *CH*$_2$CH$_2$CO), 1.86–2.02 (m, 1H, *CH*$_2$CH$_2$CO), 2.23–2.33 (m, 2H, CH$_2$*CH*$_2$CO), 3.07 (dd, 1H, *J*=13.9, 9.4 Hz, CH_2SO_2), 3.23 (dd, 1H, *J*=13.9, 3.8 Hz, CH_2SO_2), 3.58–3.71 (m, 1H, *CH*CH_3), 3.79–3.89 (m, 1H, CHCO), 7.40–8.00 (m, 11H, Ar-H+2×NH); IR (KBr, cm^{-1}): 3288.0, 1714.9, 1312.9, 1159.4, 982.5, 765.1, 674.4, 574.8; FABMS: *m*/*z*=486 $(M+H)^+$; Anal. Calcd for $C_{20}H_{24}N_2O_8S_2$: C (49.58%), H (4.99%), N (5.78%). Found: C (49.20%), H (5.10%), N (5.50%).

5.7.3. *N*-{[(2*S*)-2-({2-[2-(Acetylamino)phenoxy]acetyl}-amino)propyl]sulfonyl}-D-glutamic acid (23). White solid (125 mg, 88%): R_f=0.71 (CH_3CN/MeOH/H_2O=3/1/1); mp 108–111 °C; $[\alpha]_D^{23}$ +22.6 (*c* 0.248, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.22 (d, 3H, *J*=6.8 Hz, CH*CH*$_3$), 1.66–1.82 (m, 1H, *CH*$_2$CH$_2$CO), 1.90–2.04 (m, 1H, *CH*$_2$CH$_2$CO), 2.11 (s, 3H, CH_3CO), 2.25–2.37 (m, 2H, CH$_2$*CH*$_2$CO), 3.10–3.29 (m, 2H, CH_2SO_2), 3.82–3.94 (m, 1H, CHCO), 4.24–4.39 (m, 1H, *CH*CH_3), 4.51 (s, 2H, OCH_2CO), 6.89–7.12 (m, 4H, Ar-H), 7.85 (d, 1H, *J*=7.5 Hz, SO_2NH), 8.45 (m, 1H, CONH), 9.45 (s, 1H, Ar-*NH*CO); IR (KBr, cm^{-1}): 3386.0, 3224.1, 1717.1, 1652.0, 1536.9, 1263.4, 1158.9, 1050.2, 746.9; FABMS: *m*/*z*=460 $(M+H)^+$; Anal. Calcd for $C_{18}H_{25}N_3O_9S$: C (47.05%), H (5.48%), N (9.15%). Found: C (47.18%), H (5.59%), N (8.76%).

5.7.4. *N*-[((2*S*)-2-{[(*E*)-3-(1,3-Benzodioxol-5-yl)-2-propenoyl]amino}propyl)sulfonyl]-D-glutamic acid (24). White solid (130 mg, 93%): R_f=0.71 (CH_3CN/MeOH/H_2O=3/1/1); mp 115–118 °C; $[\alpha]_D^{23}$ +51.6 (*c* 0.266, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.25 (d, 3H, *J*=6.8 Hz, CH*CH*$_3$), 1.73–1.88 (m, 1H, *CH*$_2$CH$_2$CO), 1.92–2.05 (m, 1H, *CH*$_2$CH$_2$CO), 2.38–2.45 (m, 2H, CH$_2$*CH*$_2$CO), 3.09–3.29 (m, 2H, CH_2SO_2), 3.96–4.06 (m, 1H, *CH*CO), 4.24–4.36 (m, 1H, *CH*CH_3), 6.07 (s, 2H, OCH_2O), 6.41 (d, 1H, *J*=15.8 Hz, CH*CH*CO), 6.95 (d, 1H, *J*=7.9 Hz, Ar-H), 7.07 (dd, 1H, *J*=7.9, 1.5 Hz, Ar-H), 7.14 (d, 1H, *J*=1.5 Hz, Ar-H), 7.34 (d, 1H, *J*=15.8 Hz), 7.86 (d, 1H, *J*=8.7, NH), 8.07 (d, 1H, *J*=7.9 Hz, NH); IR (KBr, cm^{-1}): 3314.7, 2965.9, 1717.1, 1653.6, 1525.9, 1448.1, 1338.5, 1252.6, 1123.9, 1038.2, 927.8; FABMS: *m*/*z*=443 $(M+H)^+$; Anal. Calcd for $C_{18}H_{22}N_2O_9S$: C (48.86%), H (5.01%), N (6.33%). Found: C (49.20%), H (5.41%), N (5.96%).

5.8. General procedure for the preparation of peptidosulfonamide inhibitors 14, 15

BBTO cleavage: to a stirred solution of BBTO (3.0 mmol) in toluene (20 mL), dimethyl-protected peptidosulfonamide **12**, **13** (1.0 mmol) was added. The mixture was refluxed for 48 h and the solvent evaporated under reduced pressure. The resulting oil was dissolved in EtOAc (30 mL) and washed with 5% aqueous $NaHCO_3$ (3×20 mL). The aqueous phase was acidified to pH 2–3 using an aqueous solution of 2 M HCl, and extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine (1×20 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure.

5.8.1. *N*-{[(2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]sulfonyl}-D-glutamic acid (14). Colourless oil (380 mg, 82%): R_f=0.60 (CH_3CN/MeOH/H_2O=3/1/1); $[\alpha]_D^{23}$ +34.5 (*c* 0.330, MeOH); ^{1}H NMR (300 MHz, DMSO): δ 1.46 (d, 3H, *J*=6.8 Hz, CH_3), 1.65–1.79 (m, 1H, *CH*$_2$CH$_2$CO), 1.92–2.05 (m, 1H, *CH*$_2$CH$_2$CO), 2.25–2.37 (m, 2H, CH$_2$*CH*$_2$CO), 3.46 (dd, 1H, *J*=14.3, 4.7 Hz, CH_2SO_2), 3.80 (dd, 1H, *J*=14.3, 9.0 Hz, CH_2SO_2), 3.85–3.93 (m, 1H, CHCO), 4.66–4.80 (m, 1H, *CH*CH_3), 7.75 (d,

1H, J=9.1 Hz, NH), 7.81–7.91 (m, 4H, Pht-H), 12.50 (br s, 2H, 2×COOH); IR (KBr, cm^{-1}): 3528.4, 1704.3, 1396.8, 1152.4, 1022.2, 722.7; FABMS: m/z=399 $(M+H)^+$; Anal. Calcd for $C_{16}H_{18}N_2O_8S$: C (48.24%), H (4.55%), N (7.03%). Found: C (48.50%), H (4.65%), N (6.80%).

5.8.2. *N*-{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethyl]sulfonyl}-D-glutamic acid (15). White solid (320 mg, 85%): R_f=0.60 ($CH_3CN/MeOH/H_2O$=3/1/1); mp 81–84 °C; $[\alpha]_D^{23}$ +7.8 (c 0.355, MeOH); ^1H NMR (300 MHz, DMSO): δ 1.70–1.85 (m, 1H, CH_2CH_2CO), 1.92–2.10 (m, 1H, CH_2CH_2CO), 2.20–2.30 (m, 2H, CH_2CH_2CO), 3.20–3.40 (m, 2H, CH_2SO_2), 3.95–4.05 (m, 3H, NCH_2+CH), 7.75–7.91 (m, 5H, NH+Pht-H), 12.45 (br s, 2H, 2×COOH); IR (KBr, cm^{-1}): 3288.8, 1691.6, 1442.8, 1406.7, 1142.6, 976.2, 720.8; FABMS: m/z=383 $(M-H)^-$; Anal. Calcd for $C_{16}H_{18}N_2O_8S$: C (46.87%), H (4.17%), N (7.29%). Found: C (47.20%), H (4.50%), N (7.05%).

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