Scientific paper

Design and Synthesis of Novel UDP-Mur-*N*Ac, UDP-Mur-*N*Ac-L-Ala and UDP-Mur-*N*Ac-L-Ala-D-Glu mimetics

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Dedicated to Professor Branko Stanovnik on the occasion of his 70th birthday

Abstract

A series of novel phenylsulfonylcarbamates were designed and synthesized as mimetics of UDP-Mur-*N*Ac, UDP-Mur-*N*Ac-L-Ala and UDP-Mur-*N*Ac-L-Ala-D-Glu with the potential to inhibit ATP-dependent amide-forming ligases MurB, C, D and E, involved in the biosynthesis of bacterial cell wall. A detailed synthesis of these compounds is presented.

Keywords: MurB**,** MurC, MurD, MurE, inhibitors, antibacterial

1. Introduction

The discovery and use of antibiotics was one of the most significant factors in controlling infectious diseases caused by bacteria in the 20th century. However, the subsequent emergence and spread of antibiotic resistance in pathogenic microorganisms has rendered several available antibiotics ineffective, and consequently several infectious diseases, such as tuberculosis, are re-emerging and posing a serious clinical threat.[1] This situation has created an urgent need for the development of new antibacterial agents directed towards novel targets.[2]

One of the best known and most validated targets for antibacterial therapy is the peptidoglycan biosynthetic pathway. Peptidoglycan is an essential component of the bacterial cell wall that confers a defined cell shape and preserves cell integrity by compensating internal osmotic pressure. Any perturbation of the multi-step peptidoglycan biosynthesis may lead to cell lysis.[3] Peptidoglycan is formed as a linear chain of repeating *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) units, connected by short peptide chains. Four ATP-dependent ligases (MurC, MurD, MurE and MurF) catalyze the assembly of the peptide moiety by the successive addition of L-alanine, D-glutamate, *meso*-diaminopimelate (or L-lysine) and D-alanyl-D-alanine to UDP-Mur*N*Ac which is synthesized from UDP-*N*Ac enolpyruvate by a reductase MurB (Fig. 1). A divalent cation Mg^{2+} or Mn^{2+}, is essential to the formation of an amide bond.[4] Through their ε-amino group, *meso*-A_2pm and L-Lys are involved in cross-linking the peptide units linked to the glycan chains, and they thus play a key role in the stability of the macromolecule and the integrity of the bacterial cell.[5]

These essential cytoplasmic enzymes are highly specific and are present only in eubacteria, thus making them attractive targets for the development of new therapeutic antibacterial agents. However, research on these targets has been limited, due perhaps to the very few natural antibiotics that are known to inhibit them (bacilysin, fosfomycin, D-cycloserine).[4]

The reaction mechanism[6] of Mur ligases Mur C through F is believed to be similar to that of other ATP-dependent amide-forming enzymes such as glutamine synthetase,[7–10] glutathione synthetase[11–12] and D-Ala-D-

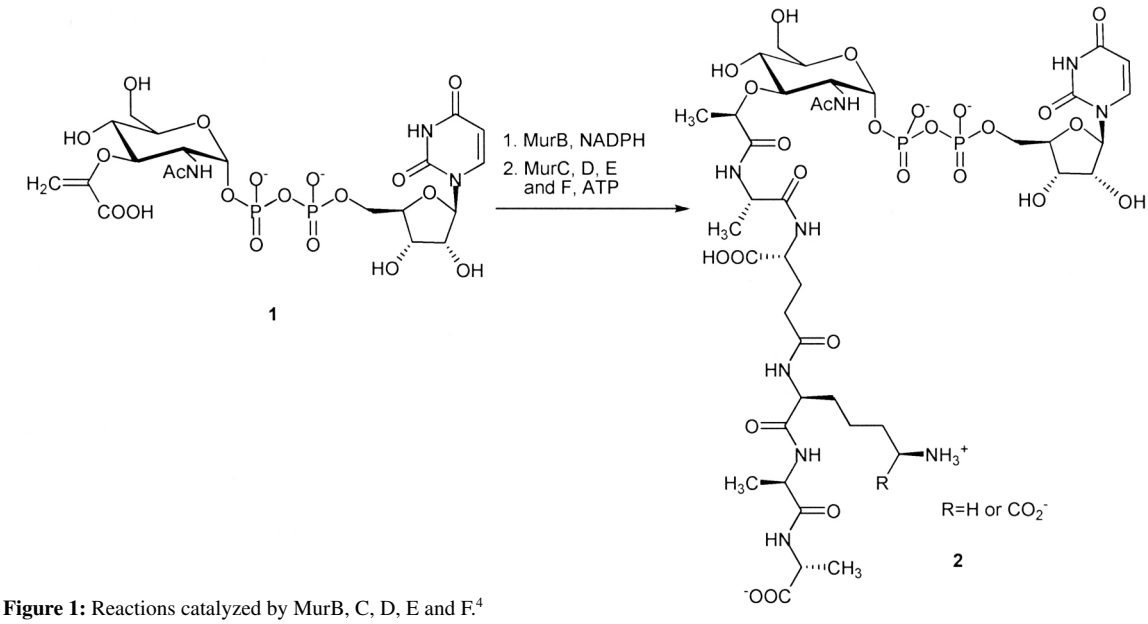

Figure 1: Reactions catalyzed by MurB, C, D, E and F.[4]

Ala ligase.[13,14] These enzymes all catalyze the formation of an acyl phosphate that is attacked by the incoming amino acid to form a tetrahedral intermediate that collapses to the amide product.

Several peptidomimetic compounds have been prepared that are structurally related to substrates, intermediates and products of the reactions catalyzed by MurC-F enzymes. Phosphinate transition state analogues are the most potent inhibitors, with IC_{50} in the low nanomolar range. However, none of these peptide-like compounds exhibit significant antibacterial activity, because of their high polarity and the resultant low cell permeability.[4] Furthermore, Babič and colleagues also prepared *O*- and *N*-sulfamoyl amides as diphosphate analogues of UDP-*N*-Ac-glucosamine and UDP-*N*-Ac-muramic acid. However, no inhibitory activity at Mur ligases have been reported so far for these compounds.[15]

In a search for new compounds with the potential to inhibit MurB, MurC, MurD and MurE, we focused on initially on an analysis of the active site within the X-ray crystal structures of MurB-F complexed with the reaction substrates or products.[16–19] Initial docking of small peptide fragments using E-Hits[20] showed that it was possible to omit the sugar residue in substrates/products while still retaining binding affinity, due to the interactions of other moieties with the active sites of these enzymes. In addition, a new diphosphate mimetic, ethyl (2-oxoethylamino) phenylsulfonylcarbamate, was introduced using molecular modelling. A general structure for good enzyme inhibition was proposed, which consisted of peptide mimetic, diphosphate mimetic and phenyl substitute for sugar residue, which orients both mimetics towards the proper conformation and, to some level restricts the molecule's degrees of freedom. (Fig. 2).

The hydroxamic acid functionality, a well-known metal complex forming group,[21] was also introduced into some of the mimetics, in order to form strong interactions with the Mg^{2+} in the enzymes' active sites. This functional group was used previously in the inhibition of a variety of enzymes, including ureases, peroxidases, and matrix metalloproteinases using the mechanism of metal complexation. This functional group was also used in the design of therapeutics targeting cancer, cardiovascular disease, HIV, Alzheimer's, malaria, allergic diseases, tuberculosis, metal poisoning, and iron overload disease.[22] However, to our knowledge, it has not used for inhibiting ligases.

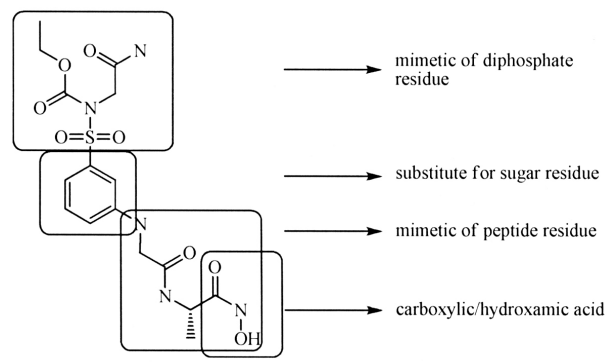

Figure 2: General structure of substituted phenylsulfonylcarbamates.

2. Results and Discussion

The overall synthetic approach is outlined in **Figs. (3),** (**4**) and (**5**). The synthesis of UDP-Mur-*N*Ac mimetics (Fig. (**3**)) was started from 3-nitrobenzenesulfonyl chlori-

de (**4**),[23] which was coupled with GlyOMe hydrochloride[24] to give sulfonamide **5,** followed by the transformation of the methyl ester into the corresponding amide **6** *via* aminolysis.[25] Compound **9** was synthesized from **6** in three steps. Compound **6** readily reacted with ethylchloroformate in the presence of NaH as a base to give **7**, followed by catalytic hydrogenation of the nitro functional group[26] to give the aromatic amine **8** in 58% yield upon crystallization**.** The white crystals turned brown upon longer exposure to light, possibly due to the oxidation of the amino functional group. Compound **8** was then treated with glyoxyllic acid monohydrate under the conditions of reductive amination using $NaCNBH_3$.[27] The reaction was monitored with TLC until the starting material had disappeared and the product obtained was used for further reactions with amino acid derivatives (Fig. (**3**)).

The synthesis of compounds **13a** and **13b** is outlined in **Fig**. (**4**). These compounds were synthesized starting from Gly (**12a**) and L-Ala (**12b**) which were first protected with Boc-anhydride[28] followed by coupling with commercially available O-benzylhydroxylamine hydrochloride in the presence of EDC and HOBt.[29] The Boc-protective group was removed by deprotection using CF_3COOH[30] or HCl[31] to give **11a** and **11b**, respectively.

Coupling of **9** with various amines **13a-13d** yielded compounds **14a**-**14d** followed by hydrogenolysis of the benzyl ester moiety under conditions of heterogeneous catalytic transfer hydrogenation using Pd/C as catalyst and ammonium formate as hydrogen donor[32] to give UDP-Mur-*N*Ac-L-Ala mimetics **15a**-**15d** (Fig. 5). At this stage H_2/Pd/C was also used for hydrogenolysis of benzyl protective group. However, with some compounds (**15a** and **15b**), the sample decomposed and therefore ammonium formate was preferentially used.

15d was further used for the synthesis of UDP-Mur-*N*Ac-L-Ala-D-Glu mimetic, which was obtained by coupling **14d** with Glu(OBzl)Obzl · $pTolSO_3H$ to give **16**, followed by hydrogenolysis with H_2/Pd/C to give **17**.

Our modelling procedure for **15d** revealed that the mode of binding for one of the conformations of the molecule is similar to that of UMA(UDP-Mur*N*Ac-L-Ala), which is also presented in Fig. 7. Comparison of

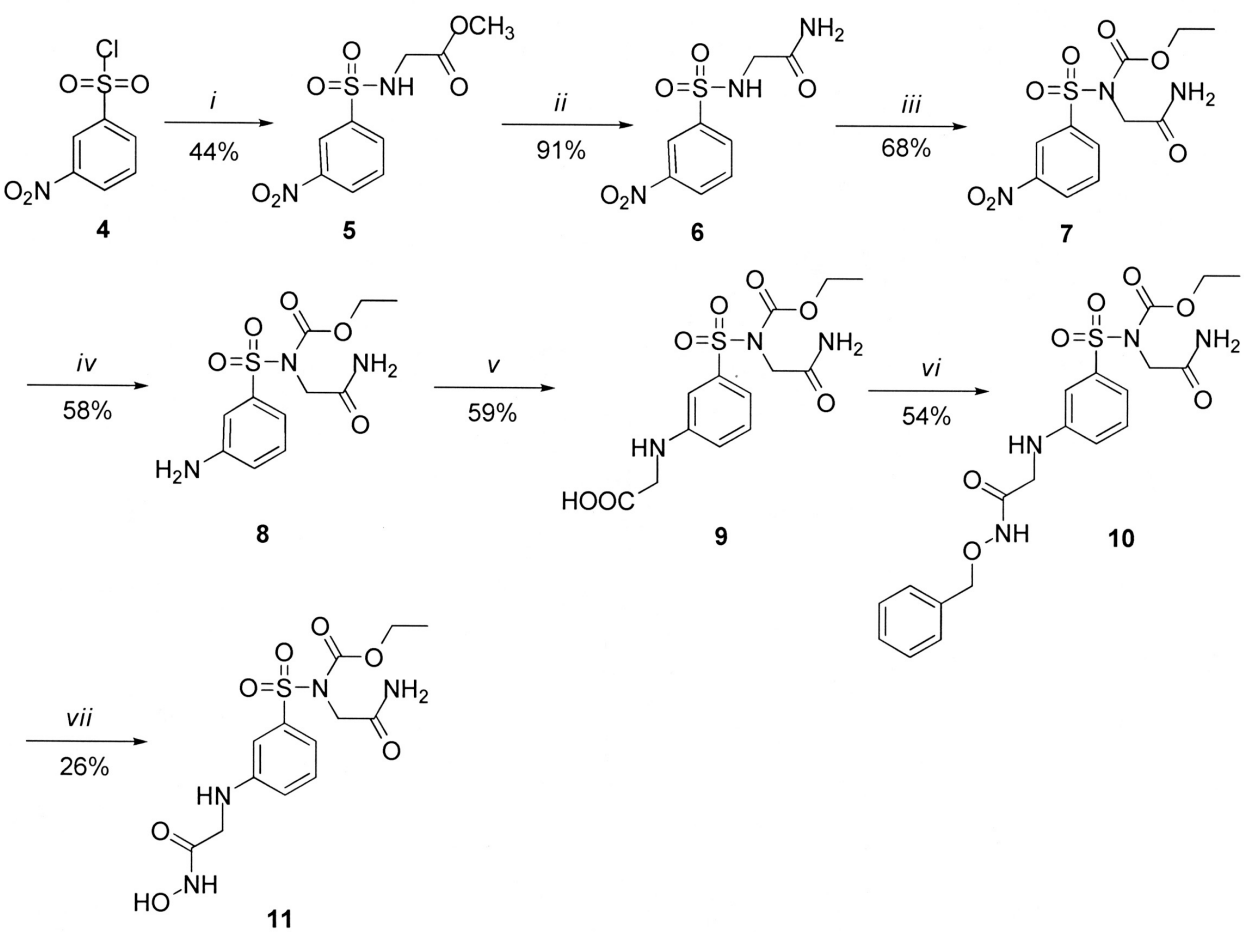

Figure 3: Synthesis of UDP-Mur-*N*Ac mimetic compounds **9** and **11**. *i.* H_2NCH_2COOMe,[24] Et_3N, CH_2Cl_2, *ii.* $NH_{3(g)}$, CH_3OH, *iii.* $ClCOOCH_2CH_3$, NaH, DMF, *iv.* H_2/Pd/C, MeOH:THF=1:1, *v.* OHC–COOH · H_2O, MeOH, $NaCNBH_3$, *vi.* H_2NOBz · HCl, EDC, HOBt, NMM, CH_2Cl_2, *vii.* NH_4HCO_2, Pd/C, MeOH.

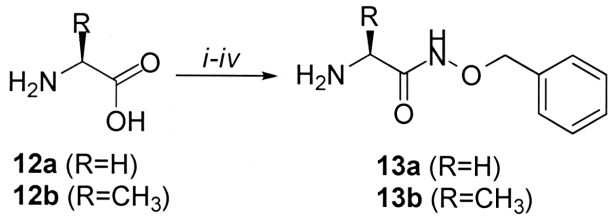

Figure 4: Synthesis of peptidomimetic building blocks **13a** and **13b**. *i.* $(Boc)_2O$, dioxan, water, *ii.* $H_2NOBz \cdot HCl$, EDC, HOBt, NMM, DMF, *iii*. CF_3COOH/ CH_2Cl_2 (1:9) for **13a**, $HCl_{(g)}$, diethyl ether for **13b**, *iv*. $NaHCO_3$, H_2O.

ver, inhibitory activity has to be measured, and will be reported in due course before any firm conclusions can be made.

In conclusion, 7 new compounds with the potential to inhibit MurB, C, D and E have been synthesized. The peptide fragments, which strongly resemble the peptide chains of UDP-Mur-Nac, UDP-Mur-*N*Ac-L-Ala and UDP-Mur-*N*Ac-L-Ala were introduced, and linked to a phenyl fragment attached to a sulfonocarbamoyl mimetic of diphosphate. The biological activity of the synthesized compounds is under investigation.

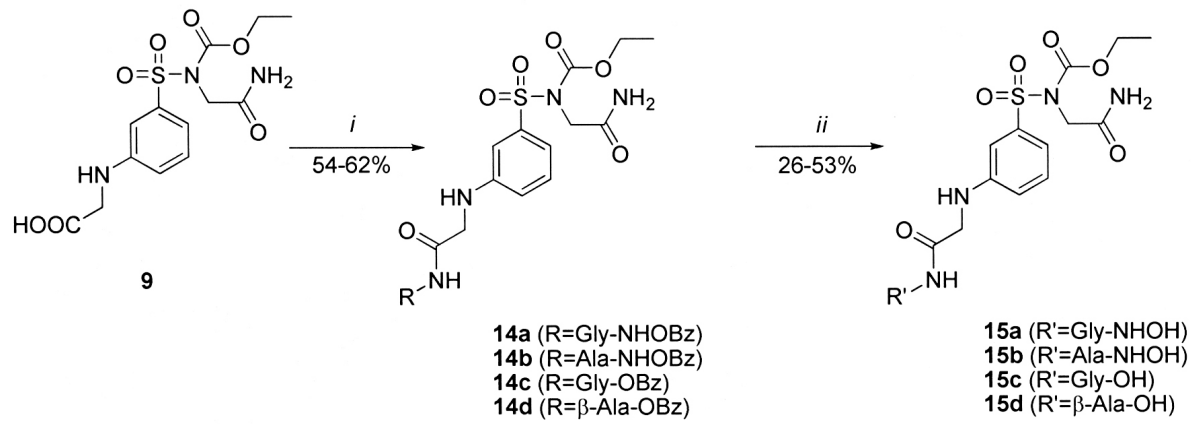

Figure 5: Synthesis of UDP-Mur-*N*Ac-L-Ala mimetics **15a-e**. *i*. Suitable amino acid derivative, EDC, HOBt, NMM, DMF, 0 °C → r.t., *ii*. NH_4HCO_2, Pd/C, MeOH.

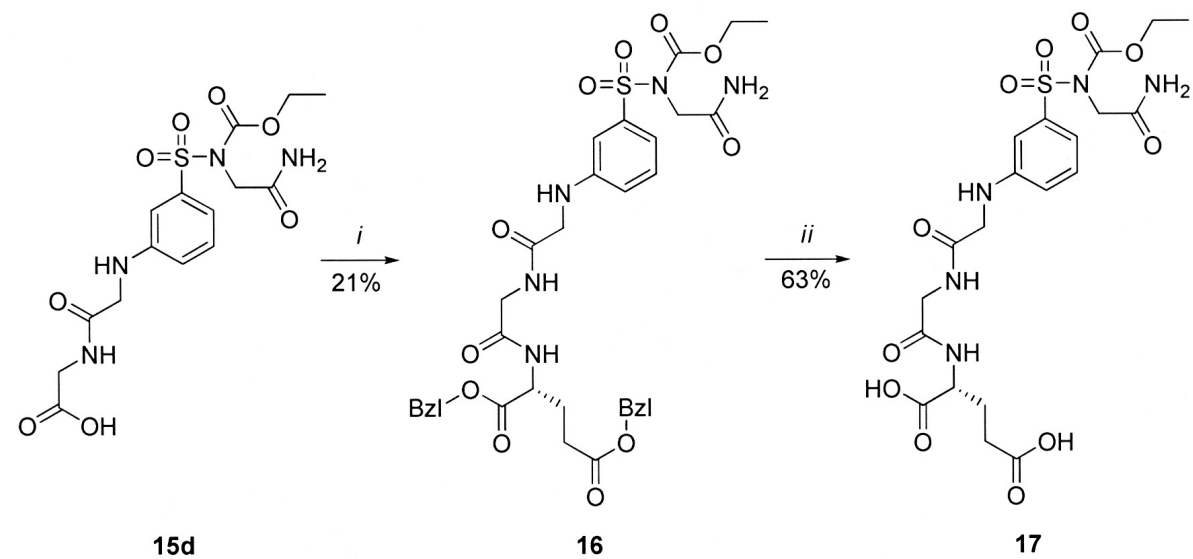

Figure 6: Synthesis of UDP-Mur-*N*Ac-LAla-D-Glu mimetic **17**. *i*. Glu(OBzl)Obz · $pTolSO_3H$, EDC, HOBt, NMM, DMF, 0 °C → r.t., *ii.* H_2/Pd/C, MeOH.

UMA and **15d** reveals that the sulfonocarbamate moiety forms 6 H-bonds with the active site, according to our model, and could mimic diphosphate. Furthermore, an additional interaction with Asp 175, which is not present in the binding mode of UMA, is also seen. Howe-

3. Acknowledgements

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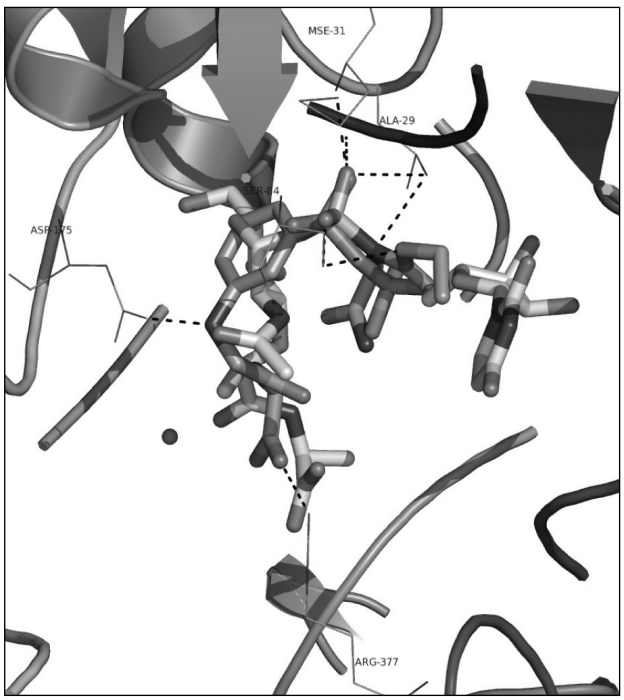

Figure 7: Proposed binding mode of **15d** (green) into the active site of MurC. The binding mode of UMA (grey) is also presented for comparison.

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4. Experimental

4. 1. General Experimental Methods

Chemicals from Fluka and Sigma-Aldrich Chemical Co. were used without further purification. Anhydrous tetrahydrofuran, dichloromethane and Et_3N were dried and purified by distillation over Na, K_2CO_3 and KOH, respectively. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel ($60F_{254}$) plates (0.25 mm). Column chromatography was performed on silica gel 60 (Merck, particle size 240–400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H-, COSY-, HMQC- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ or DMSO-d_6 solution with TMS as internal standard. Chemical shifts were reported in ppm (δ) downfield from TMS. All the coupling constants (*J*) are in hertz. IR spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer. Mass spectra were obtained with a VG-Analytical Autospec Q mass spectrometer with EI or FAB ionization (MS Centre, Jožef Stefan Institute, Ljubljana). Elemental analyses were performed by the Department of Organic Chemistry, Faculty of Chemistry and Chemical Technology, Ljubljana, on a Perkin Elmer elemental analyzer 240 C. All reported yields are yields of purified products.

4. 2. Molecular Modelling

The three dimensional structure of the synthesized **15d** was generated with Pymol v0.99 (DeLano Scientific LLC). The geometry of compound **15d** was minimized using MM^+ force field, where a Polak-Ribiere (conjugate gradient) algorithm was applied until the gradient value was smaller than 0.001 kcal/Åmol. The initial crude optimization was followed by further minimization by a semi-empirical AM1 method where the same algorithm was applied again using the same parameters as for the initial crude optimization. The X-ray structures of UMA bound into the MurC active site was obtained from the Protein Data Bank (PDB, 1p3d). E-Hits molecular docking tool[20] was used to determine possible binding modes. UMA was taken as a reference molecule and MurC was clipped by 6 Å around UMA. All docking solutions were inspected by using Pymol v0.99 and compared to the experimentally determined structure of the ligand-enzyme complex. **Fig. (7)**, which represents binding mode with the closest resemblance to the binding mode of UMA (pKi = –2.7), was then generated.

Synthesis of methyl 2-(3-nitrophenylsulfonamido)acetate (5) To a solution of triethylamine (21.3 mL, 153.5 mmol) in dichloromethane (200 mL) glycine methyl ester hydrochloride[24] was added and the mixture stirred for 15 minutes at 0 °C. A solution of 3-nitrobenzensulfonyl chloride (22.7 g, 102.3 mmol) (**4**) in dichloromethane (50 mL) was added dropwise and then stirred for 24 hours at room temperature. The solution was extracted with 1M HCl (2 × 50 mL), saturated $NaHCO_3$ solution (30 mL) and brine (30 mL), and the organic phase was dried over anhydrous Na_2SO_4. After evaporation of solvent *in vacuo*, the crude product was crystallized from diethyl ether and filtered to give white crystals. Yield = 44%. mp 125–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H, CH_3OCO), 3.90 (d, *J* 5.5 Hz, 2H, CH_2COO), 5.23 (t, *J* = 5.5 Hz, 1H, $\underline{NH}CH_2$), 7.73–7.76 (m, 1H, ArH), 8.18–8.22 (m, 1H, ArH), 8,42–8,46 (m, 1H, ArH), 8.69–8,74 (m, 1H, ArH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 43.6 ($\underline{CH_2}C(O)NH_2$), 51.8 (OCH_3), 121.3 (ArC), 126.9 (ArC), 131.0 (ArC), 132.4 (ArC), 142.4 (ArC), 147.7 (ArC), 169.2 ppm. MS (m/z): 275 (M+H, 29), 297 (MNa^+, 100). IR (KBr): ν 3458, 3209, 2361, 1735, 1609, 1529, 1437, 1310, 1355, 1233, 1171, 1124, 974, 881, 839, 734, 670, 581 cm^{-1}. Anal. Calcd for $C_9H_{10}N_2O_6S$: C 39.42, H 3.68, N 10.21. Found: C 39.63, H 3.58, N 10.20

Synthesis of 2-(3-nitrophenylsulfonamido)acetamide (6). $NH_{3(g)}$ was bubbled into a solution of **5** in a mixed solvent of methanol and THF (1:2, 150 mL) for 45 minutes and the solution then stirred for 24 hours. After evaporation of the solvent *in vacuo*, the crude product was crystallized from diethyl ether to give a white solid. Yield = 91%. mp 178–180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.50 (s, 2H, $\underline{CH_2}CONH_2$), 7.05 (s, 1H, $CONH_2$), 7.31 (s, 1H, $CONH_2$), 7.85–7.91 (m, 1H, ArH), 8.20–8.23 (d, 1H, Ar-H, *J* 8.0 Hz), 8.27 (s, 1H, SO_2NH), 8.45–8.48 (m, 1H, Ar-H), 8.55–8.59 (m, 1H, ArH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 47.8 ($\underline{CH_2}C(O)NH_2$), 121.7, 127.0, 131.0, 132.7, 142.2, 147.8, 169.4 ppm. MS (m/z): 260 (M+H, 92), 282 (M+Na, 100). IR (KBr): ν 3449, 3364, 3098, 3363, 1929, 1670, 1596, 1525, 1421, 1357, 1179, 1131, 1087, 912, 881, 839, 811, 764, 736, 697, 580 cm^{-1}. Anal. Calcd for $C_8H_9N_3O_5S$: C 37.06, H 3.50, N 16.21. Found: C 37.29, H 3.50, N 16.19

Synthesis of ethyl 2-amino-2-oxoethyl(3-nitrophenylsulfonyl)carbamate (7). Sodium hydride (60% in mineral oil) (1.11 g, 27.76 mmol) was suspended in DMF under argon atmosphere and cooled to –10 °C. **6** (6.485 g, 25.0 mmol) was then added slowly in portions over 30 minutes and stirring of the suspension was continued for an additional hour at room temperature. The reaction mixture was cooled to 0 °C and ethyl chloroformate (2.51 ml, 26.3 mmol) was added dropwise. After stirring the reaction mixture for 24 hours, dichloromethane was added (100 mL) and then extracted with 10% citric acid (3 × 25 mL), water (15 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, evaporated *in vacuo* and the crude product crystallized from diethyl ether to give white crystals. Yield = 68%. mp 180–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 4.06 (q, 2H, $CH_3\underline{CH_2}$), 4.44 (s, 2H, $\underline{CH_2}CONH_2$), 7.27 (s, 1H, $CH_2CO\underline{NH_2}$), 7.63 (s, 1H, $CH_2CO\underline{NH_2}$), 7.90–7.98 (m, 1H, ArH), 8.40–8.46 (m, 1H, ArH), 8.54–8.59 (m, 1H, ArH), 8.78–8.84 (m, 1H, ArH) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 13.3, 47.6, 63.3, 123.4, 128.1, 130.4, 134.4 ppm. MS (m/z): 332 (M+H, 100). IR (KBr): ν 3445, 3192, 3005, 2362, 1727, 1686, 1617, 1532, 1475, 1368, 1332, 1251, 1173, 1125, 1016, 944, 881, 849, 803, 766, 733, 671, 588, 569 cm^{-1}. Anal. Calcd for $C_{11}H_{13}N_3O_7S$: C 39.88, H 3.69, N 12.68. Found: C 40.02, H 4.15, N 12.39.

Synthesis of ethyl 2-amino-2-oxoethyl(3-aminophenylsulfonyl)carbamate (8). Argon was bubbled into a solution of **7** (5.811 g, 17.6 mmol) in a mixed solvent of THF and methanol (1:1, 200 mL) for 30 minutes. 10% Pd/C, unreduced, was then added and H_2 was bubbled into the resulting mixture which was stirred for 30 minutes. Hydrogenation and stirring of the resulting mixture were continued for 24 hours under hydrogen atmosphere. Pd/C was filtered off and the solution concentrated *in vacuo* to yield crude product which was crystallized from mixed solution of diethyl ether and petroleum ether (5:1, 60 mL) to yield pure white crystals. Yield 58%. mp 193–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.31 (s, 2H, $\underline{CH_2}CONH_2$), 5.61 (s, 2H, Ar–NH), 6.80–6.85 (m, 1H, Ar-H), 7.05–7.10 (m, 2H, ArH + $CH_2CO\underline{NH_2}$), 7.12–7.16 (m, 1H, ArH), 7.17–7.25 (m, 1H, Ar–H), 7.48 (s, 1H, $CH_2CO\underline{NH_2}$) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 13.3, 47.4, 62.6, 112.2, 114.2, 118.0, 128.9 ppm. MS (m/z): 302 (M+H, 100). IR (KBr): ν 3479, 3425, 3348, 3006, 2360, 1714, 1687, 1644, 1596, 1487, 1355, 1283, 1180, 1079, 1023, 937, 805, 770, 681, 626, 566 cm^{-1}. Anal. Calcd for $C_{11}H_{15}N_3O_5S$: C 43.85, H 5.02, N 13.95. Found: C 44.04, H 4.78, N 13.97.

Synthesis of 2-(3-[N-(2-amino-2-oxoethyl)-N-(ethoxycarbonyl)sulfamoyl]phenylamino)acetic acid (9). To a solution of **8** (2.838 g, 9.42 mmol) and of glyoxylic acid monohydrate (1.04 g, 11.3 mmol) in methanol (70 mL), $NaCNBH_3$ was added in portions over 20 minutea at 0 °C. After the mixture was stirred at room temperature for 24 hours, the solvent was evaporated *in vacuo*. The crude reaction product was dissolved in dichloromethane (100 mL) and extracted with 0.5M HCl (50 mL). When the aqueous phase was extracted with dichloromethane (4 × 20mL), yellow crystals appeared, which were filtered off and the filtrate was washed with dichloromethane (3 × 20 mL). To the combined organic extracts petrolether was added and they were left at 0 °C overnight. Pure yellow crystals were filtered off and found to be the same as those that crystallized from water phase. Yield = 59%. mp 160–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 3.71 (s, 2H, $NH\underline{CH_2}COOH$), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.33 (s, 2H, $\underline{CH_2}CONH_2$), 6.45 (br, 1H, Ar–NH), 6.83–7.87 (m, 1H, ArH), 7.11–7.17 (m, 3H, ArH + $CONH_2$), 7.25–7.29 (m, 1H, ArH), 7.53 (s, 1H, $CH_2CO\underline{NH_2}$), 12.62 (br, 1H, $NHCH_2\underline{COOH}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7 ($\underline{CH_3}CH_2$), 44.4 ($NH\underline{CH_2}COOH$), 47.7 ($\underline{CH_2}C(O)NH_2$), 63.1 ($CH_3\underline{CH_2}$), 110.8 (ArC), 115.2 (ArC), 116.9 (ArC), 129.2, 139.9, 148.5, 151.6, 168.6, 172.1 ppm. MS (m/z): 360 (M+H, 100). IR (KBr): ν 3407, 3193, 3360, 1742, 1674, 1602, 1526, 1488, 1350, 1269, 1178, 1136, 1084, 1004, 931, 771, 676, 568 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_3O_7S$: C 43.45, H 4.77, N 11.69. Found: C 43.46, H 4.68, N 11.64.

Synthesis of ethyl 2-amino-2-oxoethyl{3-[2-(benzyloxyamino)-2-oxoethylamino]phenylsulfonyl}carbamate (10). To a solution of **9** (975 mg, 2.72 mmol) and O-benzylhydroxylamine hydrochloride (434 mg, 2.72 mmol) in dichloromethane (40 mL) was added *N*-methylmorpholine (0.90 mL, 8.16 mmol) at 0 °C. The mixture was stirred for 10 minutes followed by the addition of HOBt (367 mg, 2.72 mmol) and EDC (521 mg, 2.72 mmol). The reaction mixture was allowed to warm to

room temperature and stirred for 24 hours under argon atmosphere. The solvent was removed under reduced pressure and the residue dissolved in dichloromethane (120 mL), extracted with 10% citric acid (3 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL) and brine (15 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was crystallized from diethyl ether to yield white crystals. Yield 54%. mp 140–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 3.67 (d, *J* 5.1 Hz, 2H, NH$\underline{CH_2}$CO), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.33 (s, 2H, $\underline{CH_2}CONH_2$), 4.79 (s, 2H, $\underline{CH_2}$Ph), 6.49 (s, 1H, ArH), 6,82 (d, *J* 7.2 Hz, 1H, Ar-H), 7.13–7.37 (m, 8H, ArH + CO$\underline{NH_2}$), 7.52 (s, 1H, $CH_2CO\underline{NH_2}$), 12.25 (s, 1H, CO$\underline{NH}OCH_2$Ph) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 13.4, 43.7, 47.4, 76.7, 110.8, 116.5, 128.0, 128.5, 128.9 ppm. MS (m/z): 465 (M+H, 100). IR (KBr): ν 3414, 3197, 2976, 2361, 1742, 1678, 1603, 1516, 1425, 1366, 1235, 1166, 1011, 939, 867, 801, 764, 701, 681, 612, 579, 530 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_4O_7S$: C 51.72, H 5.21, N 12.06. Found: C 51.55, H 5.37, N 12.07.

Synthesis of 2-amino-*N*-(benzyloxy)acetamide (13a). To a solution of Boc-Gly[35] (552 mg, 3.15 mmol) (**12a**) and O-benzylhydroxylamine hydrochloride (503 mg, 3.15 mmol) in DMF (40 mL), *N*-methylmorpholine (1.0 mL, 9.42 mmol) was added at 0 °C. The mixture was stirred for 10 minutes followed by the addition of HOBt (425 mg, 3.15 mmol) and EDC (0.604 mg, 3.15 mmol). The reaction mixture was warmed to room temperature and stirred for 24 hours under argon atmosphere. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (80 mL), washed with 10% citric acid (3 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was purified by flash chromatography on a silica gel column using hexane/ethyl acetate (1/1) as eluent (R_f = 0.17) to give pure white crystals which were used for the next reaction. Yield = 33%. mp 68–70 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42(s, 9H, *t*-Bu) 3.68 (s, 2H, NH$\underline{CH_2}$CONH), 4.87 (s, 2H, CH_2Ph), 5.41 (s, 1H, OCONH), 7.36 (s, 5H, ArH), 9.50 (s, 1H, $\underline{NH}OCH_2$Ph) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 27.8, 40.7, 76.6, 127.9, 128.4 ppm. MS (m/z): 281 (M+H, 49), 225 (100). IR (KBr): ν 3412, 3234, 2976, 2360, 1721, 1683, 1530, 1417, 1364, 1291, 1165, 1033, 949, 919, 900, 860, 786, 742, 698, 608, 503 cm^{-1}. Anal. Calcd for $C_{14}H_{20}N_2O_4$: C 59.99, H 7.19, N 9.99. Found: C 60.24, H 7.06, N 10.15.

The product of the previous step (2.260 g, 8.07 mmol) was dissolved in a mixed solvent of dichloromethane and trifluoroacetic acid (9:1, 78 mL) and stirred for 1 hour. After the removal of solvent *in vacuo* the residue was dissolved in water and treated with dichloromethane (2 × 20 mL). Water phase was alkalized to pH 8 using $NaHCO_3$. The white crystals obtained were filtered, washed with water and dried to afford compound **13a**. Yield = 58%. mp 171–173 °C. ^{1}H NMR (300 MHz, DMSO-d_d) δ 3.02 (s, 2H, $\underline{CH_2}NH_2$), 4.79 (s, 2H, CH_2Ar), 7.35–7.39 (m, 5H, ArH) ppm. DEPT-135 NMR (300 MHz, D_2O + D_2SO_4): δ 38.6, 78.8, 129.1, 129.6, 123.0 ppm. MS (m/z): 181 (M+H, 100). IR (KBr): ν 2989, 2365, 1991, 1686, 1617, 1492, 1428, 1400, 1373, 1319, 1252, 1215, 1110, 1064, 1016, 952, 896, 837, 742, 696, 606, 545, 468 cm^{-1}. Anal. Calcd for $C_9H_{12}N_2O_4$: C 59.99, H 6.71, N 15.55. Found: C 59.72, H 6.57, N 15.17.

Synthesis of (*S*)-2-amino-*N*-(benzyloxy)propionamide (13b). To a solution of Boc-L-Ala[36] (1.112 g, 5.88 mmol) (**12b**) and O-benzylhydroxylamine hydrochloride (935 mg, 5.88 mmol) in DMF (50 mL) *N*-methylmorpholine (1.95 mL, 17.75 mmol) was added at 0 °C. The mixture was stirred for 10 minutes followed by the addition of HOBt (794 mg, 5.88 mmol) and EDC (1.128 g, 5.88 mmol). The reaction mixture was warmed to room temperature and stirred for 24 hours under argon atmosphere. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (80 mL), washed with 10% citric acid (3 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL) and brine (20 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was purified by crystallization from diethyl ether/petroleum ether to give white crystals which were used in the next reaction. Yield = 60%. mp 102–105 °C. $[\alpha]^{20}_D$ –55,769 (c 0.260, MeOH). ^{1}H NMR (300 MHz, DMSO-d_d) δ 1.31 (d, *J* 7.2 Hz 3H, CH$\underline{CH_3}$), 1.41 (s, 9H, $(CH_3)_3C$), 3.98–4.03 (m, 1H, CO$\underline{CH}$), 4.87 (s, 2H, CH_2Ph), 5.21 (d, *J* 7.5 Hz, 1H, COCH$\underline{NH}$), 7.35–7.40 (m, 5H, Ar–H), 9.49 (s, 1H, CONHO) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 17.7, 27.8, 47.2, 76.4, 127.9, 128.5 ppm. MS (m/z): 295 (M+H, 7), 317 (M+Na, 100). IR (KBr): ν 3337, 3256, 2988, 2875, 2363, 1672, 1520, 1450, 1367, 1320, 1255, 1163, 1072, 942, 905, 853, 787, 749, 699, 640, 570, 523, 498 cm^{-1}. Anal. Calcd for $C_{15}H_{22}N_2O_4$: C 61.21, H 7.53, N 9.52. Found: C 61.39, H 7.43, N 9.55.

The product of the previous reaction (2.78 g, 9.56 mmol) was dissolved in diethyl ether (50 mL) and treated with $HCl_{(g)}$ at 0 °C for 30 minutes. Solvent was evaporated *in vacuo*, the residue dissolved in methanol/dichloromethane = 1/4 (50 mL) and extracted with saturated $NaHCO_3$ solution (3 × 10 mL). The aqueous phase was acidified to pH 8 with 1M HCl and extracted with dichloromethane (3 × 10 mL). The combined extracts were quenched with NaCl, dried over anhydrous Na_2SO_4 and evaporated to yield a pure brown powder. Yield = 66%. mp 90–94 °C. $[\alpha]^{20}_D$ = –15.405 (c 0.185, MeOH). ^{1}H NMR (300 MHz, DMSO-d_d) δ 1.08 (d, *J* 6.8 Hz, 3H, CH$\underline{CH_3}$), 3.17 (q, 1H, $\underline{CH}CH_3$), 4.79 (s, 2H, CH_2Ph), 7.35–7.40 (m, 5H, ArH) ppm. MS (m/z): 195 (M+H, 100). IR (KBr): ν 3670, 3069, 2853, 2102, 1606, 1508, 1452, 1361, 1281, 1205, 1181,

1085, 1052, 921, 857, 830, 753, 733, 702, 672, 613, 494 cm^{-1}. Anal. Calcd for $C_{10}H_{14}N_2O_2$: C 61.84, H 7.27, N 14.42. Found: C 61.65, H 7.20, N 14.34.

4. 3. General Procedure for the Synthesis of 14a-d

To a solution of **9** (1 mmol) and the corresponding amino acid derivative (**14a**-**14d**) (1.1 mmol) in DMF (10 mL) *N*-methylmorpholine (3 mmol) was added. The solution was stirred at 0 °C for 10 minutes followed by addition of EDC (1 mmol) and HOBt (1 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 hours under argon atmosphere. Solvent was removed under reduced pressure and the residue dissolved in dichloromethane (50 mL), extracted with 10% citric acid (3 × 5mL), saturated $NaHCO_3$ (2 × 5 mL) and brine (5 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude product was purified by flash column chromatography or by crystallization.

Synthesis of ethyl 2-amino-2-oxoethyl{3-[2-(2-(benzyloxyamino)-2-oxoethylamino]-2-oxoethylamino}phenylsulfonyl)carbamate (14a). Compound **14a** was prepared by the reaction of compound **9** with (S)-2-amino-*N*-2-amino-*N*-(benzyloxy)acetamide (**13a**) following the procedure described above and crystallized from diethyl ether to give white crystals. Yield = 51%. mp 138–140 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 3.67 (d, *J* 5.5 Hz, 2H, NH$\underline{CH_2}$CO), 3.76 (s, 2H, NH$\underline{CH_2}$CONH), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.32 (s, 2H, $\underline{CH_2}CONH_2$), 4.78 (s, 2H, $\underline{CH_2}$Ph), 6.48 (t, *J* 5.5 Hz, 1H, $\underline{NH}CH_2CO$), 6.80–6.89 (d, *J* 6.3 Hz, 1H, Ar–H), 7.13–7.28 (m, 4H, Ar–H + CO$\underline{NH_2}$), 7.32–7.40 (m, 5H, Ar–H), 7.51 (s, 1H, $CH_2CO\underline{NH_2}$), 8.18–8.22 (m, 1H, CO$\underline{NH}CH_2$), 11.15 (s, 1H, CO$\underline{NH}$O) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6 (CH_3), 46.2 (NH$\underline{CH_2}$CO), 47.7 ($\underline{CH_2}C(O)NH_2$), 63.0 ($\underline{CH_2}CH_3$), 76.2 (CH_2Ar), 111.1 (ArC), 115.2 (ArC), 116.8 (ArC), 128.2 (ArC), 128.1 (ArC), 128.7 (ArC), 129.2 (ArC), 135.8, 139.8, 148.5, 151.5, 165.9, 168.4, 169.9 ppm. MS (m/z): 522 (M+H, 100), 558 (M+Na, 100). IR (KBr): ν 3373, 1658, 1600, 1504, 1371, 1272, 1233, 1173, 686, 572 cm^{-1}. Anal. Calcd for $C_{22}H_{27}N_5O_8S$: C 50.66, H 5.22, N 13.43. Found: C 50.53, H 5.13, N 13.54.

Synthesis of (*S*)-ethyl 2-amino-2-oxoethyl(3-{2-[1-(benzyloxyamino)-1-oxopropan-2-ylamino]-2-oxoethylamino}phenylsulfonyl)carbamate (14b). Compound **14b** was prepared by the reaction of compound **9** with (S)-2-amino-*N*-(benzyloxy)propanamide (**13b**) following the procedure described above. The crude product was purified by flash chromatography using dichloromethane:methanol = 9:1 (R_f = 0.32) as eluent to give a product which was purified again with preparative chromatography using dichloromethane:methanol:acetone = 10:1:1 (R_f = 0.47) as eluent to give a pure, yellow solid. Yield = 57%. mp 132–135 °C. $[\alpha]^{20}_D$ –18.358 (c 0.335, 100 ml, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 1.17 (d, *J* 5.4 Hz, 3H, $\underline{CH_3}CH$), 3.76 (d, *J* 5.8 Hz, 2H, NH$\underline{CH_2}$CO), 4.01 (q, 2H, $CH_3\underline{CH_2}$), 4.2 (m, 1H, $CH_3\underline{CH}NH$), 4.33 (s, 2H, $\underline{CH_2}CONH_2$), 4.77 (s, 2H, CH_2Ph), 6.44 (t, *J* 5.8 Hz, 1H, $\underline{NH}CH_2CO$), 6.83–6.87 (m,1H, ArH), 7.14–7.31 (m, 4H, ArH + CO$\underline{NH_2}$), 7.36–7.40 (m, 5H, ArH), 7.52 (s, 1H, $CH_2CO\underline{NH_2}$), 8.14 (d, *J* 7.6 Hz, 1H, $\underline{CH}$NHC(O)), 11.24 (s, 1H, CO$\underline{N}$-$\underline{H}OCH_2$Ph) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 14.6, 19.2, 46.9, 47.0, 48.6, 63.9, 77.7, 112.0, 116.1, 117.8, 129.2, 129.8, 130.1 ppm. MS (m/z): 536 (M+H, 25), 558 (M+Na, 100). IR (KBr): ν 3436, 3209, 2982, 2360, 1742, 1678, 1605, 1511, 1239, 1164, 1081, 1023, 939, 866, 765, 703, 677, 575, 532 cm^{-1}. Anal. Calcd for $C_{23}H_{29}N_5O_8SxH_2O$: C 49.90, H 5.64, N 12.65. Found: C 49.87, H 5.18, N 13.05.

Synthesis of benzyl 2-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)acetate (14c). Compound **14c** was prepared by the reaction of compound **9** with glycine benzyl ester *p*-toluenesulfonate,[33] following the procedure described above, and crystallized from diethyl ether to give white crystals. Yield = 55%. mp 90–92 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 3.76 (d, *J* 5.7 Hz, 2H, NH$\underline{CH_2}$CO), 3.92 (d, *J* 6.0 Hz, 2H, NH$\underline{CH_2}$COO), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.32 (s, 2H, $\underline{CH_2}CONH_2$), 5.13 (s, 2H, CH_2Ph), 6.56 (t, *J* = 5.9 Hz, 1H, $\underline{NH}CH_2CO$), 6.86 (d, *J* 7.8 Hz, 1H, ArH), 7.12–7.45 (m, 9H, ArH + $CH_2CO\underline{NH_2}$), 7.50 (s, 1H, $CH_2CO\underline{NH_2}$), 8.40 (t, *J* 6.0 Hz, 1H, $\underline{NH}CH_2COO$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7 (CH_3), 46.4 (NH$\underline{CH_2}$CO), 47.9 ($\underline{CH_2}C(O)NH_2$), 63.1 ($\underline{CH_2}CH_3$), 65.9 (CH_2Ar), 111.5 (ArC), 115.6 (ArC), 116.8 (ArC), 127.9 (ArC), 128.1 (ArC), 128.4 (ArC), 129.3 (ArC), 135.9, 139.8, 148.6, 151.6, 168.7, 169.6, 170.5 ppm. MS (m/z): 507 (M+H, 73), 529 (M+Na, 100). IR (KBr): ν 3394, 3198, 2975, 1740, 1674, 1603, 1526, 1344, 1364, 1316, 1233, 1165, 1011, 939, 869, 765, 683, 577, 535 cm^{-1}. Anal. Calcd for $C_{22}H_{26}N_4O_8S$: C 52.17, H 5.17, N 11.06. Found: C 52.20, H 5.06, N 11.05.

Synthesis of benzyl 2-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)acetate (14d). Compound **14d** was prepared by the reaction of compound **9** with benzyl β-alanine benzyl ester *p*-toluenesulfonate[34] following the procedure described above. The product was recrystallized from diethyl ether to give a yellow powder (62%), mp 81–83 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, *J* 7.1 Hz, 3H, $\underline{CH_3}CH_2$), 2.49–2.55 (m, 2H, $\underline{CH_2}COOCH_2$Ph), 3.37 (m, 2H, NH$\underline{CH_2}CH_2$), 3.67 (d, *J* 5.7 Hz, 2H, NH$\underline{CH_2}$CO), 4.02 (q, 2H, $CH_3\underline{CH_2}$), 4.32 (s, 2H, $\underline{CH_2}CONH_2$), 5.07 (s, 2H, $\underline{CH_2}$Ar), 6.48 (t, *J* 5.7 Hz, 1H, $\underline{NH}CH_2CO$), 6,77–6.83

(m, 1H, ArH), 7.12–7.26 (m, 4H, ArH + $CONH_2$), 7.33–7.37 (m, 5H, ArH), 7.52 (s, 1H, CH_2CONH_2), 8.05 (t, *J* 5.6 Hz, 1H, $CONHCH_2CH_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6 (CH_3), 33.6 (CH_2COO), 34.5 ($NHCH_2CH_2$), 46.3 ($NHCH_2CO$), 47.6 ($CH_2C(O)NH_2$), 62.9 (CH_2CH_3), 65.4 (CH_2Ar), 111.1 (ArC), 115.2 (ArC), 116.7 (ArC), 127.8 (ArC), 127.9 (ArC), 128.3 (ArC), 129.1 (ArC), 135.9, 139.7, 148.4, 151.4, 168.4, 169.4, 171.0 ppm. MS (m/z): 521 (M+H, 100). IR (KBr): ν 3422, 3326, 3184, 1734, 1685, 1601, 1515, 1486, 1349, 1315, 1268, 1177, 1082, 1010, 937, 856, 750, 729, 701, 682, 626, 566 cm^{-1}. Anal. Calcd for $C_{23}H_{28}N_4O_8S$: C 53.07, H 5.42, N 10.76. Found: C 52.82, H 5.11, N 10.38.

4. 3. General Procedure for the Synthesis of 11 and 15a-d

Into a solution of **10** or **14a-d** (1 mmol) in methanol (10 mL), argon was bubbled for 10 minutes. 10% Pd/C, unreduced (10% wt/wt), was added followed by ammonium formate (3 mmol) and the mixture stirred for 24 hours. The mixture was filtered, solvent was evaporated *in vacuo* and crude product was purified by flash column chromatography followed by crystallization from diethyl ether.

Synthesis of ethyl 2-amino-2-oxoethyl{3-[2-(hydroxyamino)-2-oxoethylamino]phenylsulfonyl}carbamate (11). Compound **11** was prepared from compound **10** following the procedure described above. The product was purified by flash chromatography using dichloromethane:methanol = 9:1 as eluent (R_f = 0.17) and recrystallized from diethyl ether to give white crystals. Yield = 26%. mp 85–87 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 3.64 (d, *J* 5.5 Hz, 2H, $NHCH_2CO$), 4.03 (q, 2H, CH_3CH_2), 4.32 (s, 2H, CH_2CONH_2), 6.43 (t, *J* 5.5 Hz, 1H, $NHCH_2CO$), 6.85 (d, *J* 7.2 Hz, 1H, ArH), 7.15–7.28 (m, 4H, ArH + $CONH_2$), 7.52 (s, 1H, CH_2CONH_2), 8.84 (s, 1H, CONHOH), 10.61 (s, 1H, CONHOH) ppm. DEPT-135 NMR (300 MHz, DMSO-d_6): δ 13.2, 47.4, 62.6, 73.5 110.9, 115.0, 116.8, 128.8 ppm. MS (m/z): 375 (M+H, 60), 324 (100). IR (KBr): ν 3364, 1693, 1601, 1335, 1158, 1009, 936, 766, 682 cm^{-1}. Anal. Calcd for $C_{13}H_{18}N_4O_7S \cdot 7/6\ H_2O$: C 37.79, H 4.85, N 14.69. Found: C 38.02, H 5.24, N 14.25.

Synthesis of ethyl 2-amino-2-oxoethyl(3-{2-[2-(hydroxyamino)-2-oxoethylamino]-2-oxoethylamino}phenylsulfonyl)carbamate (15a). Compound **15a** was prepared from compound **14a** following the procedure described above. The product was purified by flash chromatography (dichloromethane:methanol =5:1 followed by dichloromethane:methanol =1:1) and recrystallized from diethyl ether to give white crystals. Yield = 44%. R_f (dichloromethane:methanol=1:1) = 0.70. mp 158–160 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 3.65 (d, *J* 5.4 Hz, 2H, $NHCH_2CH_2$), 3.67 (d, *J* 5.5 Hz, 2H, $NHCH_2CONH$,), 4.03 (q, 2H, CH_3CH_2), 4.32 (s, 2H, CH_2CONH_2), 6.49 (t, *J* 5.5 Hz, 1H, $NHCH_2CO$), 6.85 (d, *J* 7.8 Hz, 1H, ArH,), 7.14–7.16 (m, 3H, ArH + $CONH_2$), 7.26–7.34 (m, 1H, ArH), 7.52 (s, 1H, CH_2CONH_2), 8.19 (t, *J* 4.9 Hz, 1H, $NHCH_2CH_2$), 8.89 (s, 1H, CONHOH), 10.56 (s, 1H, CONHOH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6 (CH_3), 46.1 ($NHCH_2CO$), 47.7 ($CH_2C(O)NH_2$), 63.0 (CH_2CH_3), 111.0 (ArC), 115.2 (ArC), 116.8 (ArC), 129.2 (ArC), 139.8, 148.6, 151.5, 168.4, 169.8 ppm. MS (m/z): 454 (M+H, 100). IR (KBr): ν 3466, 3391, 3345, 3282, 2996, 2918, 2343, 1732, 1681, 1635, 1605, 1528, 1510, 1479, 1431, 1410, 1374, 1359, 1334, 1301, 1247, 1185, 1161, 1090, 1036, 1011, 989, 937, 877, 844, 790, 766, 701, 674, 622, 571, 528 cm^{-1}. Anal. Calcd for $C_{15}H_{21}N_4O_8S$: C 40.63, H 5.08, N 15.79. Found: C 40.42, H 4.68, N 15.47.

Synthesis of (*S*)-ethyl 2-amino-2-oxoethyl(3-{2-[1-(hydroxyamino)-1-oxopropan-2-ylamino]-2-oxoethylamino}phenylsulfonyl)carbamate (15b). Compound **15b** was prepared from compound **14b** following the procedure described above. The resulting crude yellow oil was purified by flash chromatography using dichloromethane:methanol = 4:1 (R_f = 0.19) as eluent and recrystallized from diethyl ether to give white crystals. Yield = 52%. mp 119–122 °C. [α] $^{20}_D$ –11.725 (c 0.255, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 1.18 (d, *J* 5.4 Hz, 3H, CH_3CHNH), 3.75 (d, *J* 5.8 Hz, 2H, $NHCH_2CO$), 4.02 (q, 2H, CH_3CH_2), 4.25 (m, 1H, CH_3CHNH), 4.33 (s, 2H, CH_2CONH_2), 6.45 (t, *J* 5.8 Hz, 1H, $NHCH_2CO$), 6.85 (d, *J* 7.2 Hz, 1H, ArH), 7.15–7.17 (m, 3H, Ar–H), 7.25–7.31 (m, 1H, CH_2CONH_2), 7.53 (s, 1H, CH_2CONH_2), 8.08 (d, *J* 7.2 Hz, 1H, CH_3CHNH), 8.87 (s, 1H, CONHOH) 10.65 (s, 1H, CONHOH) ppm. MS (m/z): 446 (M+H, 18), 468 (MNa^+, 100). IR (KBr): ν 3417, 2357, 1684, 1601, 1519, 1349, 1268, 1177, 1013, 937, 858, 765, 682, 567 cm^{-1}. Anal. Calcd for $C_{16}H_{23}N_5O_8S$: C 43.14, H 5.20, N 15.72. Found: C 43.43, H 5.52, N 15.32.

Synthesis of 2-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)acetic acid (15c). Compound **15c** was prepared from compound **14c** following the procedure described above. The product was purified by flash chromatography (dichloromethane:methanol =5:1 followed by dichloromethane:methanol =1:1) and recrystallized from diethyl ether to give white crystals Yield = 53%. R_f (dichloromethane:methanol) = 0.73. mp 162–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, *J* 7.0 Hz, 3H, CH_3CH_2), 3.57 (d, *J* 3.64 Hz, 2H, $NHCH_2COOH$), 3.74 (s, 2H, $NHCH_2CONH$), 4.00 (q, 2H, CH_3CH_2), 4.35 (s, 2H, CH_2CONH_2), 6.21 (br, 1H, $NHCH_2CO$), 6.85 (d, *J* 7.8 Hz, 1H, ArH), 7.11–7.20 (m, 3H, Ar–H + $CONH_2$), 7.27–7.32 (m, 1H, ArH), 7.62 (s, 1H, $CONH_2$), 7.69 (s, 1H,

CH_2CONH_2), 7.81 (s, 1H, NHCH2COOH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7 (CH_3), 42.7 (CH_2COO), 46.5 (NHCH_2CO), 47.8 (CH_2C(O)NH_2), 63.1 (CH_2CH_3), 111.1 (ArC), 115.4 (ArC), 117.0 (ArC), 129.3 (ArC), 139.9, 148.7, 151.6, 168.7, 169.7, 171.8 ppm. MS (m/z): 417 (M+H, 100). IR (KBr): ν 3398, 2977, 2362, 1734, 1669, 1604, 1396, 1373, 1342, 1244, 1165, 1077, 1013, 986, 941, 867, 798, 767, 680, 608, 575, 528 cm^{-1}. Anal. Calcd for $C_{15}H_{20}N_4O_8S \cdot 7/3H_2O$: C 39.30, H 5.42, N 12.22. Found: C 39.63, H 5.18, N 11.86.

Synthesis of 3-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)propanoic acid (15d). Compound **15d** was prepared from compound **14d** following the procedure described above. The product was purified by flash chromatography (dichloromethane:methanol =5:1 followed by dichloromethane:methanol =1:1) and recrystallized from diethyl ether to give white crystals (Yield 53%). R_f (dichloromethane:methanol=1:1) = 0.53. mp 122–124 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 2.35 (t, *J* 6.9 Hz, 3H, CH_2CH_2COOH), 3.29 (q, 2H, NHCH_2CH_2), 3.68 (d, *J* 4.0 Hz, 2H, NHCH_2CONH), 4.02 (q, 2H, CH_3CH_2), 4.32 (s, 2H, CH_2CONH_2), 6.48 (t, *J* 4.0 Hz, 1H, NHCH_2CO), 6.82 (d, *J* 8.7 Hz, 1H, ArH), 7.15–7.18 (m, 3H, Ar–H + CONH_2), 7.27–7.32 (m, 1H, ArH), 7.54 (s, 1H, CH_2CONH_2), 8.01 (t, *J* 5.5 Hz, 1H, NHCH_2CH_2) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7 (CH_3), 34.3 (CH_2COO), 34.9 (C(O)NHCH_2), 46.3 (NHCH_2CO), 47.7 (CH_2C(O)NH_2), 63.0 (CH_2CH_3), 111.0 (ArC), 115.3 (ArC), 116.9 (ArC), 129.2 (ArC), 139.8, 148.6, 151.5, 168.5, 169.4, 173.4 ppm. MS (m/z): 431 (M+H, 9), 453 (M+Na, 100). IR (KBr): ν 3524, 3418, 3323, 3177, 2936, 2363, 1732, 1685, 1601, 1528, 1487, 1407, 1375, 1349, 1320, 1275, 1245, 1183, 1138, 1085, 1018, 939, 876, 812, 768, 712, 683, 629, 569, 535 cm^{-1}. Anal. Calcd for $C_{16}H_{22}N_4O_8S \times 2/3\ H_2O$: C 34.43, H 5.32, N 12.66. Found: C 43.34, H 5.27, N 12.32.

Synthesis of (*R*)-dibenzyl 2-[2-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)acetamido]pentanedioate (16). To a solution of **15d** (505 mg, 1.214 mmol) and D-glutamic acid, dibenzyl ester *p*-toluenesulfonate (605 mg, 1.214 mmol) in DMF (5 mL) *N*-methylmorpholine (0.40 mL, 3.63 mmol) was added. A solution was stirred at 0 °C for 10 minutes followed by the addition of EDC (256 mg, 1.335 mmol) and HOBt (164 mg, 1.214 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 hours under argon atmosphere. Solvent was removed under reduced pressure and the residue dissolved in dichloromethane (50 mL), extracted with 10% citric acid (3 × 5 mL), saturated $NaHCO_3$ (2 × 5 mL) and brine (5 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent *in vacuo* the crude brown oil was purified by flash column chromatography using dichloromethane:methanol = 9:1 (R_f = 0.42) as eluent to obtain **16**, which was crystallized from diethyl ether to give a white solid. Yield = 21%. mp 85–87 °C. $[\alpha]^{20}_D$ +7.939 (c 0.330, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 1.85–1.83 (m, 1H, CHCH_2CH_2COO), 2.00–2.09 (m, 1H, CHCH_2CH_2COO), 2.44 (t, *J* 7.6 Hz, 2H, CH_2CH_2COO), 3.76–3.81 (m, 4H, ArNHCH_2CO + NHCH_2CONHCH_2), 4.02 (q, 2H, CH_3CH_2), 4.35 (s, 2H, CH_2CONH_2), 4.37–4.43 (m, 1H, NHCHC(O)), 5.06–5.15 (AB, *J* 12.5 Hz, ν 25 Hz, 4H, 2xCH_2Ph), 6.50 (t, *J* 5.8 Hz, 1H, NHCH_2CO), 6.86 (d, *J* 7.7 Hz, 1H, ArH), 7.15 (d, *J* 8.2 Hz, 1H, ArH), 7.20 (s, 2H, ArH), 7.24–7.42 (m, 11H, ArH + CONH_2), 7.52 (s, 1H, CH_2CONH_2), 8.15 (t, *J* 5.6 Hz, 1H, CONHCH$_2$), 8.33 (d, *J* 7.6 Hz, 1H, CONHCH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6 (CH_3), 26.0 (CH_2CH_2CO), 29.7 (CH_2CH_2CO), 41.5 (CH_2NHCO), 46.2 (NHCH_2CO), 47.7 (CH_2C(O)NH_2), 51.1 (NHCHC(O)), 62.9 (CH_2CH_3), 65.4 (CH_2Ar), 66.0 (CH_2Ar), 111.2 (ArC), 115.3 (ArC), 116.7 (ArC), 127.7 (ArC), 127.8 (ArC), 127.9 (ArC), 128.3 (ArC), 129.2 (ArC), 135.8, 136.0, 139.8, 148.5, 151.5, 168.4, 168.9, 169.8, 171.3, 171.9 ppm. MS (m/z): 726 (M+H, 100). IR (KBr): ν 3373, 1738, 1660, 1599, 1503, 1356, 1231, 1171, 685cm^{-1}. Anal. Calcd for $C_{34}H_{39}N_5O_{11}S$: C 56.27, H 5.42, N 9.65. Found: C 56.18, H 5.27, N 9.70.

Synthesis of (*R*)-2-[2-(2-{3-[*N*-(2-amino-2-oxoethyl)-*N*-(ethoxycarbonyl)sulfamoyl]phenylamino}acetamido)acetamido]pentanedioic acid (17). Into a solution of **16** (0.5 mmol) in methanol (50 mL) argon was bubbled for 10 minutes. 10% Pd/C, unreduced (10% wt/wt), was added and the mixture then bubbled with hydrogen for 30 minutes followed by stirring under hydrogen atmosphere for 24 hours. The mixture was filtered, solvent was evaporated *in vacuo* and crude product was crystallized from diethyl ether to yield a pure white solid. Yield = 63%. mp 78–80 °C. $[\alpha]^{20}_D$ –5.582 (c 0.335, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 1.70–1.83 (m, 1H, CH_2CH_2COO), 1.83–1.91 (m, 1H, CH_2CH_2COO), 2.25 (t, *J* 7.7 Hz, 2H, CH_2CH_2COO), 3.73–3.78 (m, 4H, ArNHCH_2CO + NHCH_2CONHCH), 4.03 (q, 2H, CH_3CH_2), 4.15–4.19 (m, 1H, NHCH), 4.33 (s, 2H, CH_2CONH_2), 6.51 (t, *J* 5.8 Hz, 1H, NHCH_2CO), 6.50 (t, *J* 5.3 Hz, 1H, NHCH_2CO), 6.84–6.88 (m, 1H, ArH), 7.15 (d, *J* 8.2 Hz, 1H, ArH), 7.13–7.33 (m, 3H, ArH + CONH_2), 7.35–7.40 (m, 1H, ArH), 7.52 (s, 1H, CH_2CONH_2), 8.01 (t, *J* 5.6 Hz, 1H, CONHCH$_2$), 8.33 (d, *J* 7.6 Hz, 1H, CONHCH), 8.15 (t, *J* 5.6 Hz, 1H, CONHCH), 8.33 (d, *J* 7.6 Hz 1H, CONHCH$_2$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6 (CH_3), 26.6 (CH_2CH_2CO), 30.2 (CH_2CH_2CO), 41.7 (CH_2NHCO), 46.2 (NHCH_2CO), 47.7 (CH_2C(O)NH_2), 51.3 (NHCHC(O)), 63.0 (CH_2CH_3), 111.2 (ArC), 115.3 (ArC), 116.7 (ArC), 129.3 (ArC), 139.8, 148.5, 151.5, 168.4, 168.5, 169.8, 173.0, 173.8 ppm. MS (m/z): 546 (M+H, 14), 568 (M+Na, 100). IR (KBr): ν 3372, 2979, 2363, 1734, 1684, 1604, 1534, 1413,

1374, 1344, 1244, 1167, 1012, 986, 938, 877, 766, 681, 623, 570, 538 cm^{-1}. Anal. Calcd for $C_{20}H_{27}N_5O_{11}Sx7/3$ H_2O: C 40.88, H 5.43, N 11.92. Found: C 40.84, H 5.65, N 11.63.

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Povzetek

Načrtovali in sintetizirali smo serijo novih fenilsulfonilkarbamatov, ki oponašajo strukturo UDP-Mur-*N*Ac, UDP-Mur-*N*Ac-L-Ala in UDP-Mur-*N*Ac-L-Ala-D-Glu in so kot takšni potencialni inhibitorji od ATP odvisnih ligaz MurB, C, D in E, udeleženih v biosintezi bakterijske celične stene. Predstavljena je podrobna sinteza omenjenih spojin