Scientific paper

Design and Synthesis of New Peptidomimetics as Potential Inhibitors of MurE

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Received: 13-09-2010

Abstract

With the continuing emergence and spread of multidrug-resistant bacteria, there is an urgent need for the development of new antimicrobial agents. One possible source of new antibacterial targets is the biosynthesis of the bacterial cell-wall peptidoglycan. The assembly of the peptide stem is carried out by four essential enzymes, known as the Mur ligases (MurC, D, E and F). We have designed and synthesised a focused library of compounds as potential inhibitors of UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate:L-lysine ligase (MurE) from *Staphylococcus aureus*. This was achieved using two approaches: (i) synthesis of transition-state analogues based on the methyleneamino core; and (ii) synthesis of MurE reaction product analogues. Two methyleneamino-based compounds are identified as initial hits for inhibitors of MurE.

Keywords: MurE, peptidoglycan, methyleneamino, peptidomimetics

1. Introduction

The emergence and spread of multidrug resistant bacteria represents a serious threat for community health. Although more rational use of antibiotics might alleviate the problem, there is an undisputable need for new antimicrobial agents.[1–4] One possible source of new antibacterial targets is the biosynthesis of the bacterial cell-wall peptidoglycan.[5–7] Peptidoglycan is a heteropolymer that is composed of glycan chains that are cross-linked by short peptides. Its main functions are to preserve cell integrity, by withstanding the internal osmotic pressure, and to maintain a defined cell shape. Peptidoglycan is also involved in cell growth and division. Since peptidoglycan is found exclusively in eubacteria and has no known human counterpart, it represents an ideal target for selective toxicity.[8,9] The biosynthesis of peptidoglycan is a complex process that involves several reactions that take place in the cytoplasm and on the inner and outer leaflets of the membrane.[10–12] While most of the drugs that affect the bacterial cell wall target the enzymes involved in the late stages of this process, only a few agents target the enzymes involved in the cytoplasmic steps, making them underexploited as antibacterial targets.[5–7]

The assembly of the peptide stem of peptidoglycan is carried out by four essential enzymes, known as the Mur ligases (MurC, D, E and F). These are responsible for the sequential addition of L-Ala (MurC), D-Glu (MurD), a diamino acid (MurE) and dipeptide D-Ala-D-Ala onto the lactoyl group of UDP-MurNAc. The third amino acid is usually either *meso*-diaminopimelic acid (in most Gram-negative bacteria) or L-lysine (in most Gram-positive bacteria), although in some cases other amino acids are found at this position. The Mur ligases are ATP-dependent enzymes that catalyse the formation of an amide bond, with the concomitant formation of ADP and inorganic phosphate (P_i). Although they have only small overall sequence identities (between 15% and 22%), the Mur ligases share several common features. They are composed of three domains: the N-terminal domain that is responsible for the binding of the UDP precursor; a central ATP-binding domain; and a C-terminal domain that is involved in the binding of the amino acid or dipeptide. They also have the same reaction mechanisms, which consist of the acti-

vation of the carboxyl group of the UDP-precursor with ATP, which leads to the formation of an acyl phosphate intermediate and ADP; this is followed by nucleophilic attack of the α-amino group of the amino acid (or dipeptide), which results in the formation of a highly energetic tetrahedral intermediate that breaks down to form an amide and P_i.[10]

To assess the potential of the MurE enzymes (UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate:*meso*-diaminopimelate ligases) as antibacterial targets, several efforts have been made to define their structure and function. Studies of their substrate specificity have shown that the MurE enzymes are normally highly specific for their respective substrates. Although attempts have been made to crystallize various MurE enzymes, only the crystal structure of MurE from *E. coli* with its product, UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-A_2pm, has been defined to date, and the crystal structure of a lysine-adding enzyme has yet to be obtained.[13] The early search for inhibitors identified some A_2pm analogues and *N*-acyl-dipeptide derivatives with poor to moderate inhibitory activities against MurE from *E. coli*.[14–17] In 1998, a series of phosphinate-based transition-state analogues were synthesised the best inhibitor found was **1** (Figure 1) with an IC_{50} of 1.1 μM, which represents the best inhibitor of MurE to date. Interestingly, a derivative **2** (Figure 1), which is devoid of the UMP moiety, has an IC_{50} of only 700 μM.[18]

Later, some phosphinates and β-sulfonamides that were designed as transition-state analogues of the MurD enzyme were reported to have inhibitory activities against both MurD and MurE.[19,20] Similarly, a virtual screening investigation of a MurD crystal structure produced two benzene 1,3-dicarboxylic acid derivatives as inhibitors of MurD and MurE.[21] Recently, some compounds based on a phosphorylated hydroxyethylamine scaffold have been reported to be micromolar inhibitors of Mur ligases, including MurE.[22]

Our aim was to obtain new inhibitors of MurE that could be used as hit molecules in antibacterial drug discovery and as molecular tools for the exploration of L-Lys addition by MurE from Gram-positive bacteria. With no three-dimensional X-ray structural data available, we decided to design and synthesise a small focused library of peptidomimetics as potential inhibitors of MurE from *Staphylococcus aureus* (Figure 2). As can be seen from the co-crystal structure of MurE from *E. coli*, the peptide moiety of the product (UDP-MurNAc-L-Ala-γ-D-Glu-*meso*-A_2pm) forms a number of H-bonds with the enzyme, making the peptide part of the MurE product a good starting point for the design of peptidomimetic inhibitors. The library was designed based on two approaches: (i) synthesis of transition-state analogues that were based on the methyleneamino core, and (ii) synthesis of product analogues (Figure 2). In both approaches, we wanted to further increase the binding affinities of these inhibitors by the incorporation of rigid fragments: 4-piperidinecarboxylic acid was used as a replacement for D-Glu, while (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole was used as a rigid mimetic of L-Lys.

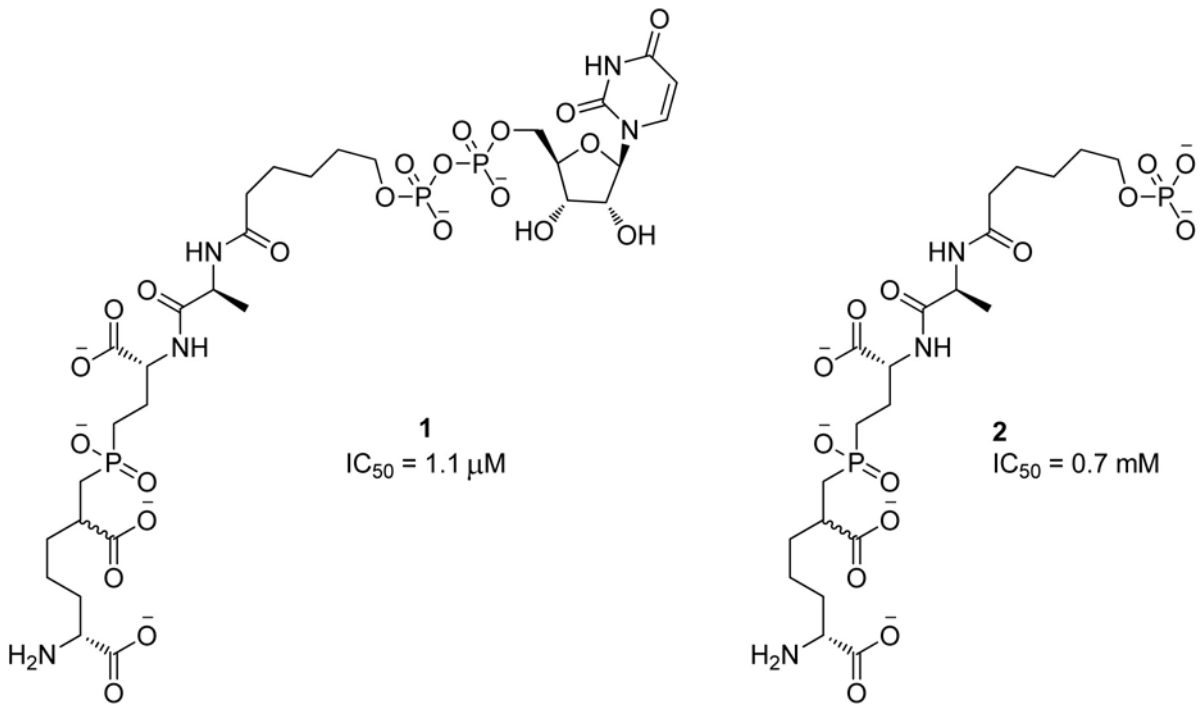


Figure 1: Phosphinate inhibitors of MurE (from *E. coli*).

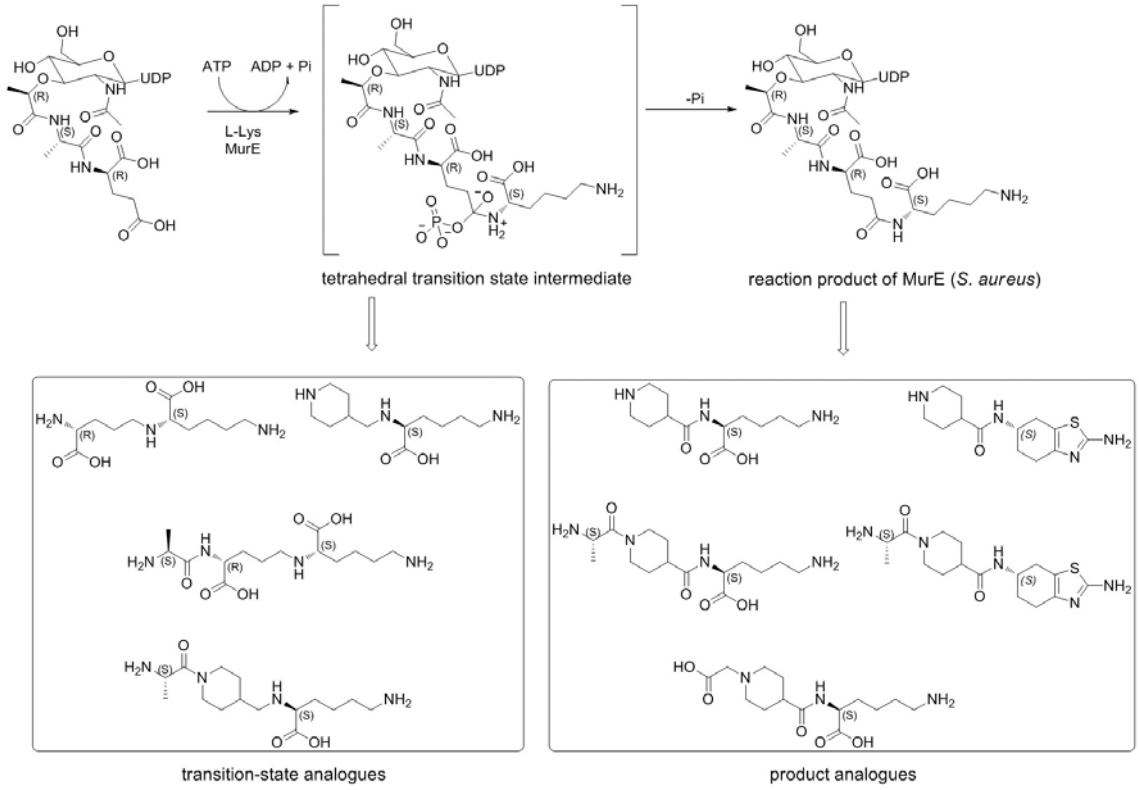
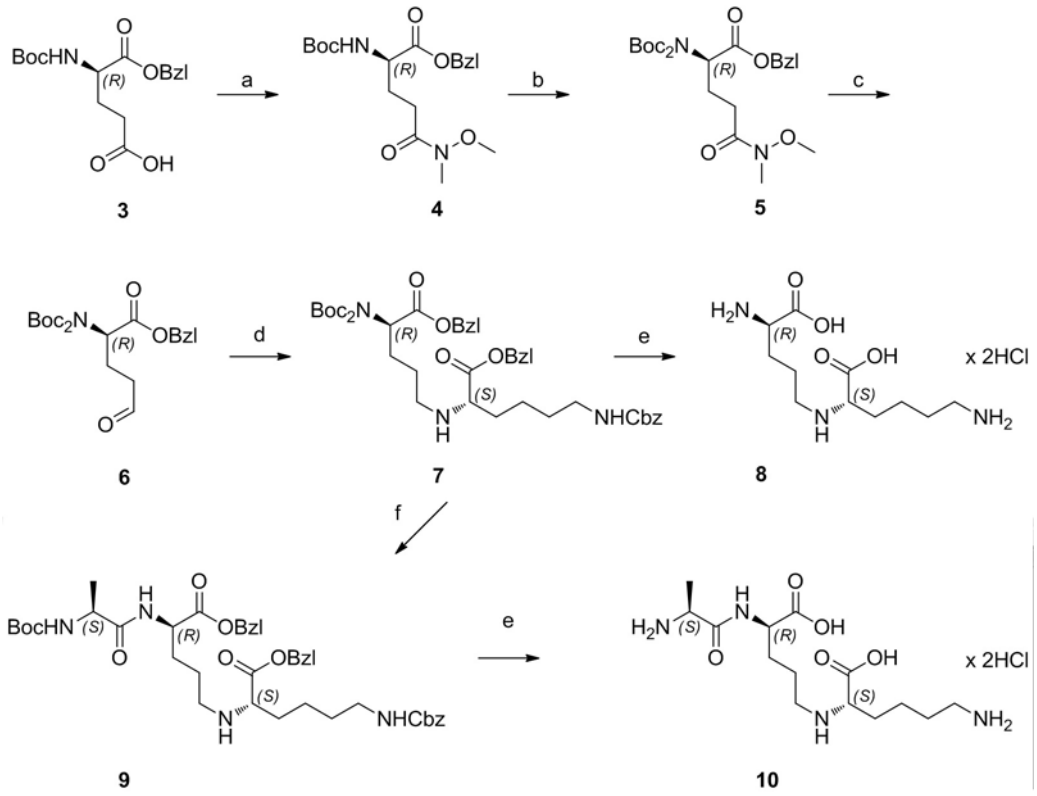


Figure 2: Reaction mechanism of MurE, showing the putative tetrahedral transition state intermediate, the product of the reaction, and the designed transition-state analogues and product analogues.

2. Results and Discussion

Compounds **8** and **10** were prepared using standard peptide chemistry approaches (Scheme 1). Here, 5-(benzyloxy)-4-[(Boc)amino]-5-oxopentanoic acid **3** (Boc representing *tert*-butoxycarbonyl) was first converted to benzyl 2-[(Boc)amino]-5-[methoxy(methyl)amino]-5-oxopentanoate **4**. A second Boc protective group was introduced to the α-amino group of **4**, to obtain the di-Boc-protected Weinreb amide **5**. This second protecting group was needed to avoid the reaction of the monoprotected α-amino group with the γ-aldehyde that is formed in the next reaction step, which would potentially lead to unwanted side products and a lower reaction yield.[23] Weinreb amide **5** was then converted to benzyl *N*,*N*-di-Boc-glutamate γ-semialdehyde **6** by reduction with tris-*tert*-butoxy lithium aluminium hydride ($LiAl(^tBuO)_3H$) in diethyl ether at room temperature. The transition-state analogue, methyleneamino pseudodipeptide D-Glu-ψ[CH_2NH]-L-Lys **8**, was prepared from aldehyde **6** in two steps. Reductive amination of **6** with H-L-Lys(Z)-OBzl using sodium triacetoxyborohydride in 1,2-dichloroethane (DCE) gave di-Boc-D-Glu-ψ[CH_2NH]-L-Lys(Z)-OBzl **7**, which was deprotected to give the target reduced amide **8**. Alternatively, compound **7** was Boc deprotected with CF_3CO_2H in CH_2Cl_2, followed by coupling with Boc-L-Ala using the benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent to obtain the protected pseudotripeptide Boc-L-Ala-D-Glu-ψ[CH_2NH]-L-Lys(Z)-OBzl **9**. This was finally deprotected using standard procedures, to give the desired reduced methyleneamino pseudotripeptide (L-Ala-D-Glu-ψ[CH_2NH]-L-Lys) **10** (Scheme 1).

Methyleneamino pseudopeptides are usually prepared by reductive amination reactions between an *N*-protected α-amino aldehyde and an amino acid.[24] Although several options are usually available for the preparation of *N*-protected α-amino aldehydes,[25] dicarboxylic amino acids are a special case, due to the presence of a second carboxylic group.[26] Several options for the preparation of *N*,*N*-di-Boc-glutamate γ-semialdehydes were presented in a review by Constantinou-Kokotou and Magrioti.[26] Accordingly, *N*,*N*-di-Boc-glutamate γ-semialdehydes were prepared mainly by two methods: (i) selective reduction of a γ-methyl ester to the corresponding *N*,*N*-di-Boc-glutamate γ-semialdehyde, using DIBAL at –78 °C; or (ii) reduction of a Weinreb amide with diisobutylaluminium hydride (DIBAL) at –78 °C.[23,27] All of the described methods were applied to the synthesis of either α-methyl or α-*tert*-butyl *N*,*N*-di-Boc-glutamate γ-semialdehydes with good yields. Recently, the successful application of a method using DIBAL for the selective reduction of a γ-methyl ester for the synthesis of benzyl *N*,*N*-di-Boc-glutamate γ-semialdehyde **6** was reported.[28] All of these methods require



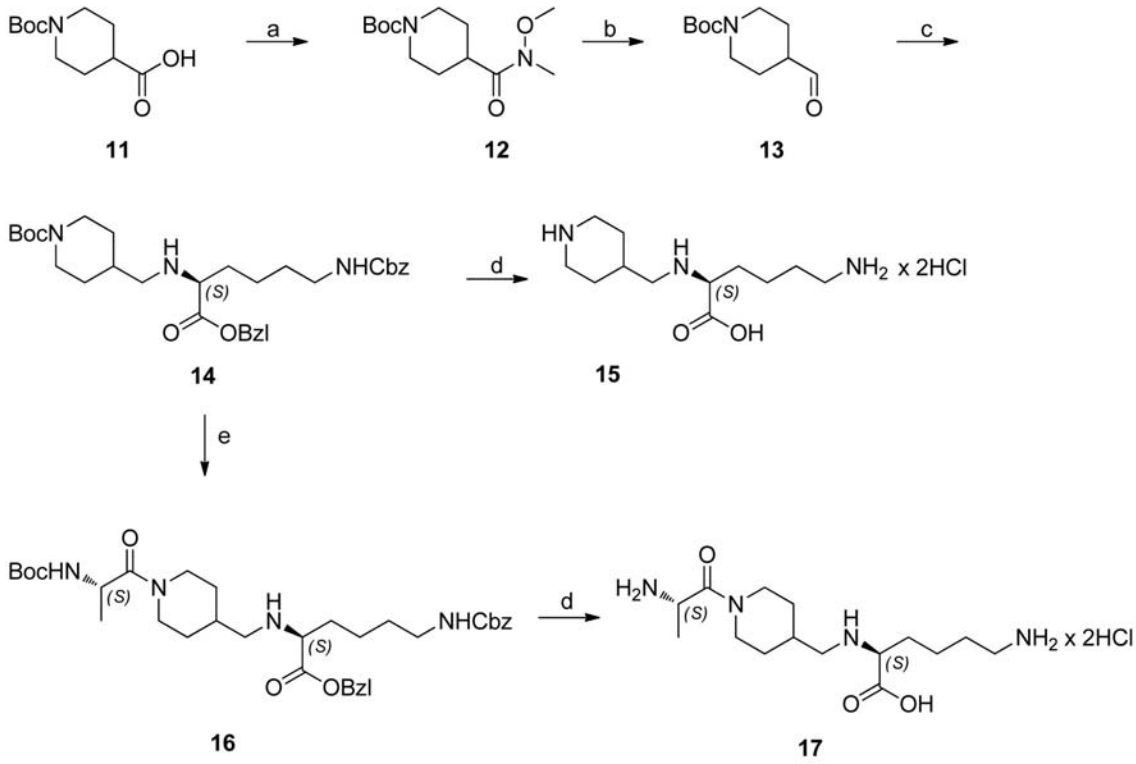
Scheme 1: Synthesis of methyleneamino-containig pseudopeptides. Reagents and conditions: a) HCl × $HN(OCH_3)CH_3$, BOP, Et_3N, CH_2Cl_2, 0 °C; b) Boc_2O, DMAP, AcCN; c) $LiAl(^tBu)_3H$, diethyl ether, r.t.; d) H-L-Lys(Z)-OBzl, DCE, $NaBH(OAc)_3$, r.t.; e) first H_2, Pd/C, glacial acetic acid, then $HCl_{(g)}$, glacial acetic acid; f) first CH_2Cl_2/CF_3CO_2H = 9:1 for 1h, then Et_3N, BOP, Boc-L-Ala-OH, CH_2Cl_2, 0 °C.

the synthesis to be performed at –78 °C. In a study where different reagents were assessed for their potential as reducing agents for the selective reduction of Weinreb amides to aldehydes, Paris et al. proposed the use of $LiAl(^tBuO)_3H$ as a mild reducing agent that allows for the reduction of Weinreb amides at room temperature.[29] A comparison of the stabilities of the different ester functionalities (but not the benzyl ester) to various reducing agents at room temperature showed that ester functionalities were not affected by $LiAl(^tBuO)_3H$, while the appearance of polar compounds was detected for both $LiAlH_4$ and DIBAL.[29] We explored the potential of $LiAl(^tBuO)_3H$ for the reduction of Weinreb amide **5** to the desired benzyl *N*,*N*-di-Boc-glutamate γ-semialdehyde **6**. Our data show that $LiAl(^tBuO)_3H$ can be used for the reduction of the Weinreb amide **5** to the semialdehyde **6** with good yield. Moreover, the reaction can be carried out at room temperature, as compared to other methods that need to be performed at –78 °C.

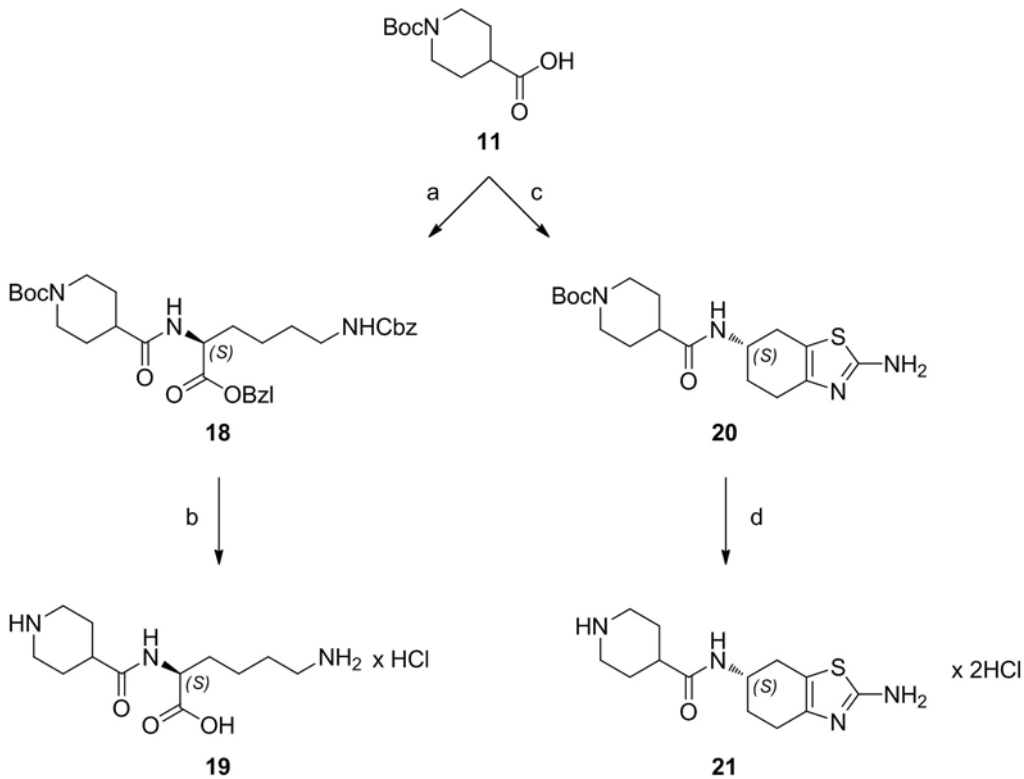
Transition-state analogues containing 4-piperidinecarboxylic acid (i.e. **15** and **17**) (Scheme 2) were prepared in a similar fashion. 1-(Boc)piperidine-4-carboxylic acid **11** was converted to the Weinreb amide **12** by coupling with *N*,*O*-dimethylhydroxylamine. Reduction of the latter with $LiAlH_4$ gave aldehyde **13**, which was used in the subsequent reductive amination reaction with L-Lys(Z)-OBzl, to give the reduced dipeptide analogue **14**. Deprotection of the latter gave the target compound **15** as a constrained mimetic of the methyleneamino pseudodipeptide (D-Glu-ψ[CH_2NH]-L-Lys) **8**. The reduced tripeptide analogue **17** was prepared by Boc deprotection of the reduced dipeptide analogue **14**, followed by coupling with Boc-L-Ala to give the protected tripeptide analogue **16**. Deprotection of **16** by standard procedures gave compound **17** as a constrained mimetic of the methyleneamino pseudotripeptide (L-Ala-D-Glu-ψ[CH_2NH]-L-Lys) **10**.

The MurE product analogues of compounds **19** and **21** were prepared from 1-(Boc)piperidine-4-carboxylic acid **11** in two reaction steps (Scheme 3). Dipeptide analogue **18** was prepared by coupling of **11** with H-L-Lys(Z)-OBzl, using the diphenylphosphoryl azide (DPPA) reagent. Compound **18** was then deprotected to give the target dipeptide analogue **19**. To further increase the rigidity of compound **19**, we replaced the Lys moiety with (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole, to give the target dipeptide analogue **21**. This was prepared first by coupling of carboxylic acid **11** with (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole to give dipeptide analogue **20**, followed by its deprotection to give the desired compound **21**.

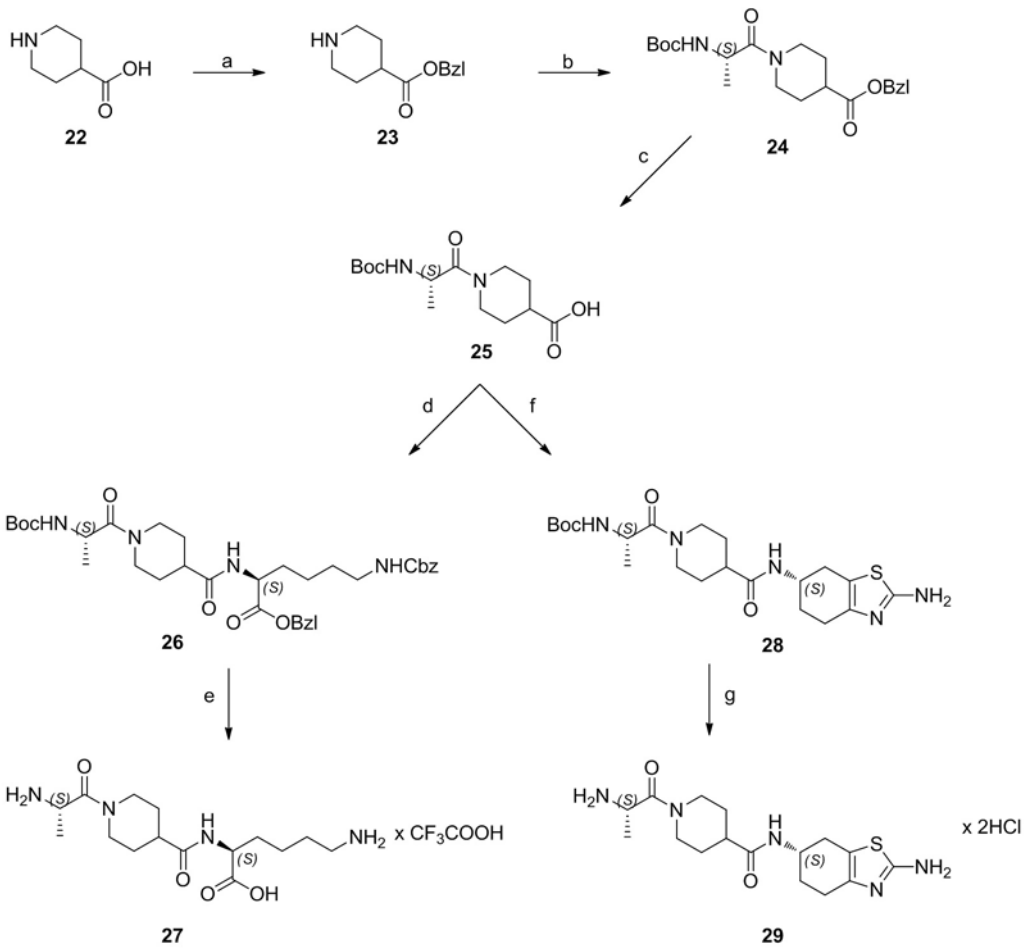
Product analogues containing an L-Ala moiety (**27** and **29**) were synthesized in five reaction steps from 4-pi-



Scheme 2: Synthesis of methyleneamino-containing pseudopeptides containing 4-piperidinecarboxylic acid. Reagents and conditions: a) HCl × $HN(OCH_3)CH_3$, BOP, Et_3N, CH_2Cl_2, 0 °C; b) $LiAlH_4$, ether, 0 °C; c) H-L-Lys(Z)-OBzl, DCE, $NaBH(OAc)_3$, r.t.; d) first H_2, Pd/C, glacial acetic acid, then $HCl_{(g)}$, glacial acetic acid; e) first CH_2Cl_2/CF_3CO_2H = 9:1 for 1 h, then Et_3N, BOP, Boc-L-Ala-OH, CH_2Cl_2, 0 °C.



Scheme 3: Syntheses of product-like pseudodipeptides. Reagents and conditions: a) H-L-Lys(Z)-OBzl, Et_3N, DPPA, DMF, 0 °C; b) first H_2, Pd/C, glacial acetic acid, then $HCl_{(g)}$, glacial acetic acid; c) (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole, DPPA, Et_3N, DMF, 0 °C; d) $HCl_{(g)}$, EtOH.



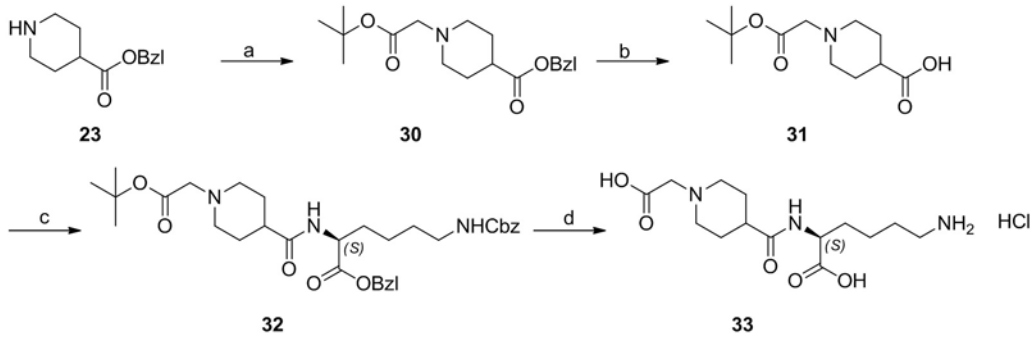
Scheme 4: Synthesis of product analogues based on the 4-piperidinecarboxylic acid scaffold. Reagents and conditions: a) first *p*-$TolSO_3H$, benzene, benzyl alcohol, then $NaHCO_3$; b) Boc-L-Ala-OH, Et_3N, DPPA, DMF, 0 °C; c) H_2, Pd/C, MeOH, r.t.; d) H-L-Lys(Z)-OBzl, Et_3N, DPPA, DMF, 0 °C; e) first CH_2Cl_2/CF_3CO_2H = 9:1, then H_2, Pd/C, glacial acetic acid; f) (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole, BOP, CH_2Cl_2, 0 °C; g) $HCl_{(g)}$, THF.

peridinecarboxylic acid **22** (Scheme 4). Compound **22** was first transformed to its benzyl ester **23**, which was next coupled with Boc-L-Ala to give L-Ala-D-Glu analogue **24**. Catalytic hydrogenation of the latter yielded a carboxylic acid **25**, which was coupled to either H-L-Lys(Z)-OBzl or (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole to give compounds **26** and **28**, respectively. These were finally deprotected to give the target L-Ala-D-Glu-L-Lys tripeptide analogues **27** and **29**.

In compounds **15**, **17**, **19**, **21**, **27** and **29**, D-glutamic acid was replaced by 4-piperidinecarboxylic acid, which confers a higher rigidity to these molecules and maintains the correct distance between the Nα-atoms of the D-Glu and L-Lys fragments; however, this loses the α-Glu carboxylic functionality. To assess the possibility of compensating for this loss by adding a carboxylic functionality through a methylene spacer on the amino group of the piperidine moiety, we synthesised MurE product analogue **33** (Scheme 5). Here, benzyl 4-piperidinecarboxylate **23** was alkylated with *tert*-butyl bromoacetate, to give benzyl 1-(2-*tert*-butoxy-2-oxoethyl)piperidine-4-carboxylate **30**. After deprotection of the benzyl ester by catalytic hydrogenation, the free *N*-substituted 4-piperidinecarboxylic carboxylic acid **31** was coupled with L-Lys(Z)OBzl to give pseudodipeptide **32**, which was deprotected to give the desired product analogue **33**.

Specific rotations as well as NMR spectra, where no multiplications of signals could be observed, suggest that the chirality of the starting reagents was preserved during all reactions described in this paper.

All of these compounds were tested for inhibition of MurE from *S. aureus*. Among these, only compounds **8** (63% inhibition at 2 mM) and **15** (73% inhibition at 2 mM; IC_{50}, 1.12 mM) showed MurE inhibitory activity. Both of these compounds are pseudodipeptides that are based on the methyleneamino core, and in both cases the inhibitory activity was lost upon extension of the molecule by an L-Ala moiety (i.e., compounds **10** and **17** showed no MurE inhibition). The similar inhibitory activities of compounds **8** and **15** indicated that 4-piperidine



Scheme 5: Synthesis of product analogue **33**. Reagents and conditions: a) $BrCH_2CO_2{}^tBu$, Et_3N, CH_2Cl_2, 0 °C; b) H_2, Pd/C, MeOH, r.t.; c) H-L-Lys(Z)-OBzl, Et_3N, DPPA, DMF, 0 °C; d) first H_2, Pd/C, glacial acetic acid, then $HCl_{(g)}$.

carboxylic acid is a plausible replacement for D-Glu. Also, the lack of activity of compound **19** indicated that the methyleneamino linker is needed between the piperidine and L-Lys moieties for the inhibitory activity of compound **15**.

However, none of these active compounds had potent enough MurE inhibitory activity to be selected as potential lead compounds. Nevertheless, these results still offered insight into the future design of MurE inhibitors. Thus, if we compare the activities of compounds **8** and **15** with the phosphinate inhibitors of MurE from *E. coli* presented by Tanner et al. (their compounds **1** and **2**),[18] we can see that although our compounds have a 1,000-fold lower inhibitory activity than their compound **1**, they were approximately only 2-fold less active than the truncated compound **2**. As most of the reported inhibitors of MurE are most likely substrate analogues, we can conclude that design and synthesis of substrate analogues appears to be the best method to obtain inhibitors of this MurE.

3. Conclusions

We have designed and synthesised a focused library of peptidomimetic compounds as potential inhibitors of MurE from *S. aureus*. The compounds were designed as transition-state analogues based on the methyleneamino core, or as product analogues. Biological evaluation of these potential inhibitors identified two methyleneamino-based pseudodipeptides **8** and **15** that inhibited MurE in the mM range, and thus they represent initial hit compounds for further development.

4. Experimental

4. 1. Chemistry

Chemicals were from Sigma-Aldrich, Acros Organics and Bachem, and were used without further purification. Solvents were used without purification or drying, unless otherwise stated. Analytical TLC was performed on Merck silica gel ($60F_{254}$) plates (0.25 mm), and the compounds were visualised under 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 spectrometer at 300 MHz in $CDCl_3$, DMSO-d_6, MeOH-d_4 and D_2O solution, with TMS as the internal standard. IR spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer. Microanalyses were performed on a 240 C Perkin-Elmer C, H, N analyser. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer.

(*R*)-Benzyl 2-[(*tert*-Butoxycarbonyl)amino]-5-[methoxy(methyl)amino]-5-oxopentanoate (4)[29]

Boc-D-Glu-OBzl (1.67 g, 5 mmol) was dissolved in 30 mL CH_2Cl_2 and cooled in an ice-bath. Then BOP (2.30 g, 5.20 mmol) and Et_3N (0.55 g, 5.50 mmol) were added to the solution and left to react for 10 min, followed by addition of Et_3N (0.55 g, 5.50 mmol) and *N*,O-dimethylhydroxylamine (0.54 g, 5.50 mmol). The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with 100 mL CH_2Cl_2. The organic layer was washed successively with 1 M aqueous HCl (3 × 30 mL), a saturated aqueous solution of $NaHCO_3$ (3 × 30 mL), and brine (40 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give an oily residue. The product was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **4** as a colourless oil (1.62 g, 85%). $[\alpha]^{20}_D = 6.95$ ($c = 0.285$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3337, 2976, 1743, 1711, 1658, 1512, 1366, 1250, 1170, 999. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37–1.50 (m, 9H, 3 × CH_3), 1.91–2.30 (m, 2H, CH_2CO), 2.42–2.56 (m, 2H, $CHCH_2CH_2$), 3.15 (s, 3H, NCH_3), 3.61 (s, 3H, OCH_3), 4.36 (d, J = 4.7 Hz, 1H, CH), 5.10–5.23 (m, 2H, OCH_2), 5.32 (d, J = 6.8 Hz, 1H, NHCO), 7.29–7.39 (m, 5H, Ar-H). MS (ESI) *m/z*: 381 (M+H, 10), 281 (100). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{29}N_2O_6$, 381.2026; found:

381.2024. Anal. Calcd for $C_{19}H_{28}N_2O_6$: C 59.98, H 7.42, N 7.36. Found: C 59.94, H 7.65, N 7.44.

(*R*)-Benzyl 2-[Bis(*tert*-butoxycarbonyl)amino]-5-[methoxy(methyl)amino]-5-oxopentanoate (5)

Di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) and 4-dimethylaminopyridine (DMAP; 0.31 g, 2.50 mmol) were added to a solution of Weinreb amide **4** (1.90 g, 5.00 mmol) in acetonitrile (30 mL) and left to react at room temperature for 24 h. Di-*tert*-butyl dicarbonate (1.09 g, 5.00 mmol) and DMAP (0.15 g, 1.25 mmol) were again added, and the solution was left stirring for 18 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the residue dissolved in diethyl ether (150 mL). The organic phase was washed with 1 M aqueous HCl (3 × 30 mL) and brine (40 mL), and dried over Na_2SO_4. The solvent was evaporated and the product purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **5** as a colourless oil that solidified upon standing (1.89 g, 79%), mp 55–57 °C. $[\alpha]^{20}_D = 26.7$ ($c = 0.220$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 2979, 2361, 1735, 1697, 1667, 1455, 1367, 1245, 1144, 995. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 18H, 6 × CH_3), 2.14–2.19 (m, 1H, 1H of CH_2), 2.45–2.60 (m, 3H, 1H of CH_2, CH_2), 3.16 (s, 3H, NCH_3), 3.65 (s, 3H, OCH_3), 4.98–5.03 (m, 1H, CH), 5.16 (s, 2H, OCH_2), 7.30–7.40 (m, 5H, Ar-H). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{37}N_2O_8$, 481.2550; found: 481.2548. Anal. Calcd for $C_{24}H_{36}N_2O_8$: C 59.98, H 7.55, N 5.83. Found: C 59.99, H 7.78, N 5.56.

(*R*)-2-[Bis(*tert*-butoxycarbonyl)amino]-5-oxopentanoate (6)

Compound **5** (1.44 g, 3.00 mmol) was dissolved in diethyl ether (30 mL) and $LiAl(O^tBu)_3H$ (1.52 g, 6.00 mmol) was added at room temperature. After 2 h, the solution was hydrolysed with a 5% aqueous solution of $KHSO_4$ (20 mL).The layers were separated, and the aqueous layer was extracted with diethyl ether (4 × 20 mL). The combined organic layers were washed successively with a saturated solution of $NaHCO_3$ (3 × 20 mL) and brine (30 mL), and dried over Na_2SO_4. The solution was concentrated *in vacuo* and the oily residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **6** as a pale yellow oil (0.74 g, 59%). $[\alpha]^{20}_D = 41.7$ ($c = 0.225$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3483, 2980, 2367, 1747, 1456, 1368, 1250, 1143, 994, 853. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 18H, 6 × CH_3), 2.14–2.27 (m, 1H, 1H of CH_2), 2.46–2.64 (m, 3H, 1H of CH_2, CH_2), 4.89–4.94 (m, 1H, CH), 5.16 (dd, J_1 = 12.5 Hz, J_2 = 1.7 Hz, 2H, OCH_2), 7.30–7.40 (m, 5H, Ar-H), 9.77 (s, 1H, CHO). ^{13}C NMR (75.5 MHz, $CDCl_3$) × δ 22.16, 27.88, 40.46, 57.46, 66.87, 83.38, 127.97, 128.14, 128.43, 135.50, 152.07, 170.08, 200.83. HRMS-ESI (*m/z*): $[M-H]^-$ calcd for $C_{22}H_{31}NO_7$, 420.2022; found: 420.2026.

(*S*)-Benzyl 2-({(*R*)-5-(Benzyloxy)-4-[bis(*tert*-butoxycarbonyl)amino]-5-oxopentyl}amino)-6-benzyloxycarbonylaminohexanoate (7)

Compound **6** (1.49 g, 3.5 mmol) was dissolved in DCE (30 mL), and HCl × H-L-Lys(Z)-OBzl (1.57 g, 3.80 mmol) and Et_3N (0.38 g, 3.80 mmol) were added. Then, $NaBH(OAc)_3$ (1.06 g, 5.00 mmol) was added, and the mixture was left to react overnight at room temperature under an Ar atmosphere. The reaction mixture was quenched by adding 10% aqueous solution of $NaHCO_3$ (40 mL), and the product was extracted with diethyl ether (4 × 30 mL). The solution was dried over Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **7** as a colourless oil (1.49 g, 55%). $[\alpha]^{20}_D = 10.47$ ($c = 0.235$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3386, 2978, 2361, 1731, 1524, 1368, 1247, 1130, 1028, 854. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.73 (m, 27H, 6 × CH_3, 4 × CH_2, NH), 1.84–2.01 (m, 1H, 1H of $CH_{2\beta}$-Glu), 2.10–2.26 (m, 1H, 1H of $CH_{2\beta}$-Glu), 2.35–2.52 (m, 1H, 1H of $CH_{2\delta}$-Glu), 2.55–2.70 (m, 1H, 1H of $CH_{2\delta}$-Glu), 3.13 (dd, J_1 = 12.8 Hz, J_2 = 6.4 Hz, 2H, $CH_{2\varepsilon}$-Lys), 3.24 (t, J = 6.6 Hz, 1H, CH_{α}-Lys), 4.78 (bs, 1H, $NHCO_2$), 4.89 (dd, J_1 = 9.6 Hz, J_2 = 5.2 Hz, 1H, CH_{α}-Glu), 5.06–5.25 (m, 6H, 3 × OCH_2), 7.29–7.44 (m, 15H, Ar-H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.95, 26.93, 27.13, 27.92, 33.07, 47.62, 58.07, 61.35, 66.39, 66.70, 83.00, 126.95, 127.93, 128.02, 128.06, 128.35, 128.40, 128.47, 128.57, 135.72, 135.81, 136.67, 152.26, 156.32, 170.69, 175.27. MS (ESI) *m/z*: 776 (M+, 57), 676 (100). Anal. Calcd for $C_{43}H_{57}N_3O_{10}$: C 66.56, H 7.40, N 5.42. Found: C 66.61, H 7.67, N 5.38.

(*S*)-6-Amino-2-((*R*)-4-amino-4-carboxybutylamino)hexanoic Acid Dihydrochloride (8)

Compound **7** (1.00 g, 1.29 mmol) was dissolved in acetic acid (20 mL) and Ar was passed through the solution. Pd/C (0.10 g) was added, and the reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo* and the residue was freeze-dried. This gave title compound **8** as a very hygroscopic brown foam (0.38 g, 88%). $[\alpha]^{20}_D$ = 0.91 ($c = 0.286$, H_2O). IR (nujol) ν/cm^{-1}: 3394, 2962, 2364, 1993, 1735, 1604, 1500, 1219. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.39–1.78 (m, 4H, 2 × CH_2), 1.80–2.12 (m, 6H, 3 × CH_2), 2.84–3.01 (m, 2H, $CH_{2\varepsilon}$-Lys), 3.02–3.21 (m, 2H, $CH_{2\delta}$-Glu), 3.88–4.07 (m, 2H, 2 × CH). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 23.08, 23.45, 28.04, 28.70, 30.50, 40.32, 47.38, 53.50, 61.27, 171.24, 171.55. HRMS-ESI (*m/z*): $[M-H]^-$ calcd for $C_{11}H_{22}N_3O_4$, 260.1610; found: 260.1603. Anal. Calcd for $C_{11}H_{23}N_3O_4$ × 2 HCl × 3.5 H_2O: C 33.25, H 8.12, N 10.58. Found: C 33.49, H 8.18, N 10.42.

(6*S*,9*R*,14*S*)-Benzyl 9-Benzyloxycarbonyl-14-(4-benzyloxycarbonylaminobutyl)-2,2,6-trimethyl-4,7-dioxo-3-oxa-5,8,13-triazapentadecan-15-oate (9)

Compound **7** (1.49 g, 1.92 mmol) was dissolved in a mixture of CH_2Cl_2/CF_3CO_2H (9:1; 20 mL) and stirred at room temperature until disappearance of the starting material, as determined by TLC. The solvent was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 mL) and Et_3N was added to pH 8. Boc-L-Ala (0.38 g, 2.00 mmol) was dissolved in CH_2Cl_2 (10 mL), and BOP (0.93 g, 2.10 mmol) and Et_3N (0.22 g, 2.10 mmol) were added at 0 °C. The two solutions were mixed together and the reaction mixture was left to react for 2 h. After completion of the reaction, CH_2Cl_2 (80 mL) was added. The organic layer was washed successively with a saturated aqueous solution of $NaHCO_3$ (3 × 20 mL) and brine (20 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* to give an oily residue. The residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **9** as a colourless oil (1.00 g, 70%). $[\alpha]^{20}_D = -16.58$ ($c = 0.205$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3347, 2937, 1718, 1522, 1456, 1366, 1250, 1167, 1026, 751. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10–1.80 (m, 23H, 4 × CH_3, 5 × CH_2, NH), 2.27–2.50 (m, 2H, CH_2), 2.93 (m, 2H, CH_2), 3.10–3.14 (m, 1H, CH), 3.98–4.02 (m, 1H, CH), 4.22–4.29 (m, 1H, CH), 5.00 (s, 2H, OCH_2), 5.12 (s, 4H, 2 × OCH_2), 6.79–6.87 (m, 1H, CONH), 7.18 (t, J = 7.5 Hz, 1H, NHCO), 7.30–7.40 (m, 15H, Ar-H), 8.11 (d, J = 7.5 Hz, 1H, CONH). MS (ESI) *m/z*: 747 (M+H, 100). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{41}H_{55}N_4O_9$, 747.3969; found: 747.3974. Anal. Calcd for $C_{41}H_{54}N_4O_9 \times H_2O$: C 64.38, H 7.38, N 7.32. Found: C 64.02, H 7.56, N 7.61.

(*S*)-6-Amino-2-{[(*R*)-4-((*S*)-2-aminopropanamido)-4-carboxybutyl]amino}hexanoic Acid Dihydrochloride (10)

Compound **9** (0.75 g, 1.00 mmol) was dissolved in acetic acid (15 mL), and Ar was passed through the solution. Pd/C (0.07 g) was added and the reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo*, and the residue was freeze-dried. This gave title compound **10** as a very hygroscopic colourless foam (0.34 g, 84%). $[\alpha]^{20}_D$ = 11.23 (c = 0.285, H_2O). IR (nujol) ν/cm^{-1}: 3430, 2968, 1993, 1735, 1684, 1560, 1498, 1397, 1210, 1149, 1004, 854. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.42–2.15 (m, 13H, 5 × CH_2, CH_3), 2.98 (t, J = 7.3 Hz, 2H, $CH_{2\varepsilon}$-Lys), 3.07–3.20 (m, 2H, $CH_{2\delta}$-Glu), 3.96–4.17 (m, 2H, 2 × CH), 4.38–4.50 (m, 1H, CH). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 18.05, 23.03, 24.05, 27.99, 29.53, 29.95, 40.30, 47.64, 50.38, 53.33, 60.99, 170.97, 171.35, 174.27. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{29}N_4O_5$, 333.2138; found: 333.2130. Anal. Calcd for $C_{14}H_{28}N_4O_5 \times 2$ HCl × 2 H_2O × 0.5 CH_3CO_2H: C 38.22, H 7.70, N 11.89. Found: C 38.25, H 8.07, N 11.79.

***tert*-Butyl 4-[Methoxy(methyl)carbamoyl]piperidine-1-carboxylate (12)**[31]

1-(Boc)piperidine-4-carboxylic acid (2.29 g, 10 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled in an ice-bath. Then BOP (4.86 g, 11 mmol) and Et_3N (1.11 g, 11 mmol) were added to the solution, and left to react for 10 min, followed by addition of Et_3N (1.11 g, 11 mmol) and *N*,O-dimethylhydroxylamine (1.07 g, 11 mmol). The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 (150 mL). The organic phase was washed successively with 1 M aqueous HCl (3 × 40 mL), a saturated aqueous solution of $NaHCO_3$ (3 × 40 mL) and brine (50 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give an oily residue. The product was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **12** as a colourless oil that solidifies on air (2.30 g, 85%), mp 51–54 °C (lit. 54 °C). IR (nujol) ν/cm^{-1}: 3568, 2972, 1692, 1420, 1365, 1234, 1170, 984, 870. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H, 3 × CH_3), 1.59–1.79 (m, 4H, 2 × CH_2), 2.73–2.87 (m, 3H, CH_2, CH), 3.19 (s, 3H, NCH_3), 3.71 (s, 3H, OCH_3), 4.29–4.00 (m, 2H, CH_2). MS (ESI) *m/z*: 273 (M+H, 50), 217 (100). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{25}N_2O_4$, 273.1814; found: 273.1818.

***tert*-Butyl 4-Formylpiperidine-1-carboxylate (13)**[32]

Compound **12** (1.36 g, 5.00 mmol) was dissolved in diethyl ether (30 mL) and $LiAlH_4$ (0.23 g, 6 mmol) was added at –10 °C. After 1 h, the solution was hydrolysed with a 5% aqueous solution of $KHSO_4$ (20 mL) and the product was extracted with diethyl ether (4 × 30 mL). The organic phase was washed successively with a saturated solution of $NaHCO_3$ (3 × 40 mL) and brine (40 mL), and dried over Na_2SO_4. The solution was concentrated *in vacuo* and the oily residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **13** as a colourless oil (0.92, 86%). IR (nujol) ν/cm^{-1}: 3422, 2974, 2361, 1691, 1424, 1366, 1237, 1167, 865. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H, 3 × CH_3), 1.47–1.62 (m, 2H, CH_2), 1.80–1.97 (m, 2H, CH_2), 2.35–2.43 (m, 1H, CH), 2.92 (m, 2H, CH_2), 3.97 (m, 2H, CH_2), 9.65 (s, 1H, CHO).

(*S*)-*tert*-Butyl 4-{[(1-Benzyloxy-6-benzyloxycarbonylamino-1-oxohexan-2-yl)amino]methyl}piperidine-1-carboxylate (14)

Compound **13** (0.75 g, 3.50 mmol) was dissolved in DCE (30 mL), and HCl × H-L-Lys(Z)-OBzl (1.51 g, 3.70 mmol) and Et_3N (0.37 g, 3.70 mmol) were added. Then, $NaBH(OAc)_3$ (1.00 g, 4.7 mmol) was added, and the mixture was left to react overnight at room temperature under

an Ar atmosphere. The reaction mixture was quenched by adding 1 M NaOH (30 mL) and the product was extracted with diethyl ether (3 × 40 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **14** as a colourless oil (1.40 g, 71%). $[\alpha]^{20}_D = -7.36$ (c = 0.315, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3337, 2931, 2365, 1726, 1691, 1533, 1425, 1365, 1247, 1170, 725. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (m, 2H, CH_2), 1.28–1.75 (m, 19H, 3 × CH_3, 4 × CH_2, CH, NH), 2.28 (dd, J_1 = 11.4 Hz, J_2 = 6.4 Hz, 1H, 1H of CH_2), 2.44 (dd, J_1 = 11.4 Hz, J_2 = 6.9 Hz, 1H, 1H of CH_2), 2.65 (m, 2H, CH_2), 3.23–3.07 (m, 3H, CH_2, CH_α), 4.09–3.99 (m, 2H, CH_2), 4.74 (s, 1H, NHCO), 5.08 (s, 2H, OCH_2), 5.15 (dd, J_1 = 12.3 Hz, J_2 = 6.1 Hz, 2H, OCH_2), 7.40–7.27 (m, 10H, Ar-H). MS (ESI) *m/z*: 568 (M+H, 100). Anal. Calcd for $C_{32}H_{45}N_3O_6$: C 67.70, H 7.99, N 7.40. Found: C 67.82, H 8.28, N 7.55.

(*S*)-6-Amino-2-[(piperidin-4-ylmethyl)amino]hexanoic Acid Dihydrochloride (15)

Compound **14** (0.62 g, 1.1 mmol) was dissolved in acetic acid, and Ar was passed through the solution. Pd/C (0.06 g) was added, and reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo*, and the residue was freeze-dried. This gave title compound **15** as a very hygroscopic foam (0.28 g, 89%). $[\alpha]^{20}_D = 6.74$ (c = 0.270, H_2O). IR (nujol) ν/cm^{-1}: 3400, 2963, 2043, 1735, 1618, 1458, 1397, 1211, 1005, 958. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.85–1.48 (m, 6H, 3 × CH_2), 2.31–2.01 (m, 5H, 2 × CH_2, CH), 3.19–2.93 (m, 6H, 3 × CH_2), 3.45 (m, 2H, CH_2), 4.06 (t, J = 6.12 Hz, 1H, CH_α). ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 23.29, 27.54, 27.58, 27.99, 30.00, 32.69, 40.29, 44.47, 44.49, 52.65, 61.61, 170.96. MS (ESI) *m/z*: 244 (M+H, 100). Anal. Calcd for $C_{12}H_{25}N_3O_2$ × 2 HCl × 3.3 H_2O × 0.3 CH_3CO_2H: C 38.44, H 8.91, N 10.67. Found: C 38.64, H 9.14, N 10.42.

(*S*)-Benzyl 6-Benzyloxycarbonylamino-2-[({1-[(*S*)-2-(*tert*-butoxycarbonylamino)propanoyl]piperidin-4-yl}methyl)amino]hexanoate (16)

Compound **14** (1.00 g, 1.76 mmol) was dissolved in a mixture of CH_2Cl_2/CF_3CO_2H (9:1; 10 mL) and stirred at room temperature until disappearance of the starting material, as determined by TLC. The solvent was evaporated *in vacuo* and the residue dissolved in CH_2Cl_2 (10 mL). Et_3N was added to pH 8. Boc-L-Ala (0.34 g, 1.80 mmol) was dissolved in CH_2Cl_2 (10 mL) and BOC (0.84 g, 1.90 mmol) and Et_3N (0.20 g, 2.00 mmol) were added at 0 °C. The two solutions were mixed together and the reaction mixture was left to react for 2 h at room temperature. After completion of the reaction, CH_2Cl_2 (100 mL) was added. The organic layer was washed successively with a saturated aqueous solution of $NaHCO_3$ (3 × 20 mL) and brine (40 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, to give an oily residue that was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **16** as a colourless oil (0.88 g, 78%). $[\alpha]^{20}_D = -10.57$ (c = 0.210, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3328, 2933, 2364, 1712, 1637, 1528, 1455, 1367, 1249, 1168, 1054, 1026. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78–1.06 (m, 2H, CH_2), 1.11 (d, J = 6.2 Hz, 3H, CH_3), 1.22–1.77 (m, 18H, 3 × CH_3, 4 × CH_2, CH), 2.16–2.41 (m, 2H, CH_2NH), 2.43–2.50 (m, 1H, 1H of CH_2), 2.94 (dd, J_1 = 12.5 Hz, J_2 = 6.4 Hz, 3H, $CH_{2\varepsilon}$, 1H of CH_2), 3.08–3.22 (m, 1H, CH), 3.74–3.94 (m, 1H, 1H of CH_2), 4.21–4.53 (m, 2H, CH_α-Ala, 1H of CH_2), 5.00 (s, 2H, CH_2O), 5.13 (s, 2H, CH_2O), 6.76–6.90 (m, 1H, CONH), 7.19 (t, J = 5.4 Hz, 1H, CONH), 7.26–7.46 (m, 10H, Ar-H). MS (ESI) *m/z*: 639 (M+H, 48), 180 (100). Anal. Calcd for $C_{35}H_{50}N_4O_7$ × 0.3 H_2O: C 65.20, H 7.92, N 8.70. Found: C 65.26, H 8.31, N 8.50.

(*S*)-6-Amino-2-({[1-((*S*)-2-aminopropanoyl)piperidin-4-yl]methyl}amino)hexanoic Acid Dihydrochloride (17)

Compound **16** (0.70 g, 1.10 mmol) was dissolved in acetic acid, and Ar was passed through the solution. Pd/C (0.07 g) was added, and reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo*, and the residue was freeze-dried. This gave title compound **17** as a very hygroscopic colourless foam (0.38 g, 89%). $[\alpha]^{20}_D = 11.23$ (c = 0.285, H_2O). IR (nujol) ν/cm^{-1}: 3429, 2946, 1993, 1735, 1640, 1490, 1388, 1270, 1107, 1002, 729. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.14–1.83 (m, 9H, 3 × CH_2, CH_3), 1.86–2.24 (m, 5H, 3 × CH_2, CHCH_2NH), 2.70–3.27 (m, 6H, 2 × CH_2, 2 × 1H of CH_2), 3.86–3.96 (m, 1H, 1H of CH_2), 3.98–4.05 (m, 1H, CH_α-Lys), 4.35–4.60 (m, 2H, 1H of CH_2, CH_α-Ala). ^{13}C NMR (300 MHz, MeOH-d_4) δ 17.36, 23.35, 28.03, 30.06, 30.27, 31.03, 34.82, 40.31, 43.08, 45.80, 48.23, 53.19, 61.65, 169.15, 171.03. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{31}N_4O_3$, 315.2396; found: 315.2403. Anal. Calcd for $C_{15}H_{30}N_4O_3$ × 2 HCl × 3 H_2O: C 40.82, H 8.68, N 12.69. Found: C 40.90, H 8.97, N 12.43.

(*S*)-*tert*-Butyl 4-[(1-Benzyloxy-6-benzyloxycarbonylamino-1-oxohexan-2-yl)carbamoyl]piperidine-1-carboxylate (18)

1-(Boc)piperidine-4-carboxylic acid (0.92 g, 4.00 mmol) and HCl × H-L-Lys(Z)-OBzl (1.67 g, 4.1 mmol) were dissolved in dimethylformamide (DMF). Et_3N (0.81 g, 8 mmol) and DPPA (1.13 g, 4.1 mmol) were added at 0 °C, and the reaction was left to react for 18 h. The solution was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed successively with 10% citric acid (3 × 30 mL), a saturated solu-

tion of $NaHCO_3$ (3 × 30 mL) and brine (40 mL), and dried over Na_2SO_4. The product was precipitated from ethyl acetate. This gave title compound **18** as a white solid (1.25 g, 54%), mp 91–94 °C. $[\alpha]^{20}_D$ = –3.52 (*c* = 0.210, $CHCl_3$). IR (nujol) *ν*/cm^{-1}: 3350, 2948, 1748, 1689, 1643, 1541, 1425, 1263, 1175, 952, 752. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21–1.49 (m, 15H, 3 × CH_2, 3 × CH_3), 1.49–1.80 (m, 4H, 2 × CH_2), 2.37 (ddd, J_1 = 14.3 Hz, J_2 = 6.9 Hz, J_3 = 2.9 Hz, 1H, CHCO), 2.72 (t, *J* = 9.5 Hz, 2H, $CH_{2\varepsilon}$), 2.96 (m, 2H, CH_2), 3.90 (d, *J* = 11.3 Hz, 2H, CH_2), 4.23 (dd, J_1 = 13.4 Hz, J_2 = 8.2 Hz, 1H, CH_α), 5.00 (s, 2H, OCH_2), 5.10 (dd, J_1 = 12.6 Hz, J_2 = 3.6 Hz, 2H, OCH_2), 7.21 (t, *J* = 5.0 Hz, 1H, NHCO), 7.26–7.46 (m, 10H, Ar-H), 8.17 (d, *J* = 7.4 Hz, 1H, NHCO). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{32}H_{44}N_5O_7$, 582.3179; found 582.3196. Anal. Calcd for $C_{32}H_{43}N_3O_7$: C 66.07, H 7.45, N 7.22. Found: C 66.32, H 7.45, N 7.22.

6-Amino-2-(piperidine-4-carboxamido)hexanoic Acid Hydrochloride (19)

Compound **18** (1.16 g, 2.00 mmol) was dissolved in acetic acid (20 mL), and Ar was passed through the solution. Pd/C (0.1 g) was added, and the reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo*, and the residue was freeze-dried. This gave title compound **19** as a very hygroscopic colourless foam (0.53 g, 89%). $[\alpha]^{20}_D$ = –13.46 (*c* = 0.260, D_2O). IR (nujol) *ν*/cm^{-1}: 3403, 2962, 2044, 1725, 1649, 1546, 1455, 1396, 1221, 959. ^{1}H NMR (300 MHz, D_2O) δ 1.35–1.50 (m, 2H, CH_2), 1.58–1.95 (m, 6H, 3 × CH_2), 1.97–2.10 (m, 2H, CH_2), 2.69 (tt, J_1 = 11.4 Hz, J_2 = 3.8 Hz, 1H, CHCO), 2.93–3.00 (m, 2H, CH_2), 3.06 (dt, J_1 = 12.8 Hz, J_2 = 3.0 Hz, 2H, CH_2), 3.46 (td, J_1 = 13.0 Hz, J_2 = 3.3 Hz, 2H, CH_2), 4.32 (m, 1H, CH_α). HRMS-ESI (*m/z*): $[M–H]^-$ calcd for $C_{12}H_{22}N_3O_3$, 256.1661; found 256.1663. Anal. Calcd for $C_{12}H_{23}N_3O_3$ × HCl × 3 H_2O: C 41.44, H 8.69, N 12.08. Found: C 41,57, H 8,78, N 12,04.

tert-Butyl 4-[(2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)carbamoyl]piperidine-1-carboxylate (20)

1-(Boc)piperidine-4-carboxylic acid (1.15 g, 5.00 mmol) and (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (0.93 g, 5.50 mmol)[33] were dissolved in DMF (15 mL). Et_3N (1.01 g, 10.00 mmol) and DPPA (1.51 g, 5.50 mmol) were added at 0 °C, and the reaction was left to react for 18 h. The solution was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed successively with 10% citric acid (3 × 30 mL), a saturated solution of $NaHCO_3$ (3 30 mL) and brine (30 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* to give an oily residue. Title compound **20** was crystallized from ethyl acetate to give a white solid (1.18 g, 62%), mp 153–155 °C. $[\alpha]^{20}_D$ = –27.82 (*c* = 0.220, MeOH). IR (nujol) *ν*/cm^{-1}: 3355, 3143, 2930, 2368, 1677, 1537, 1418, 1365, 1168, 1121, 948. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32–1.47 (m, 11H, 3 × CH_3, CH_2), 1.54–1.90 (m, 4H, 2 × CH_2), 2.21–2.48 (m, 4H, 2 × CH_2), 2.60–2.80 (m, 3H, CHO, CH_2), 3.94 (d, *J* = 10.8 Hz, 3H, C*H*NH, CH_2), 6.63 (s, 2H, NH_2), 7.86 (d, *J* = 7.6 Hz, 1H, NHCO). MS (ESI) *m/z*: 403 (M+Na), 381 (M+H, 79), 325 (100). Anal. Calcd for $C_{18}H_{28}N_4O_3S$ × 0.5 H_2O: C 55.50, H 7.50, N 14.38. Found: C 55.50, H 7.53, N 14.29.

(*S*)-*N*-(2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)piperidine-4-carboxamide Dihydrochloride (21)

Compound **20** (0.51, 1.33 mmol) was dissolved in absolute EtOH (20 mL) and treated with gaseous HCl. The solvent was evaporated *in vacuo*, and the product triturated with diethyl ether. This gave title compound **21** as a hygroscopic white solid (0.45 g, 95%), mp 235–240 °C. $[\alpha]^{20}_D$ = –19.61 (*c* = 0.205, H_2O). IR (nujol) *ν*/cm^{-1}: 3420, 3246, 3093, 2948, 2802, 2495, 1618, 1438, 1311, 1244, 953. ^{1}H NMR (300 MHz, D_2O) δ 1.73–2.07 (m, 6H, 3 × CH_2), 2.39–2.70 (m, 4H, CH_2CS, CH_2CN), 2.77–2.89 (m, 1H, CHCO), 2.94–3.09 (m, 2H, CH_2NH), 3.40–3.50 (m, 2H, CH_2NH), 4.07–4.22 (m, 1H, CHNH). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{21}N_4OS$, 281.1436; found: 281.1429. Anal. Calcd for $C_{13}H_{20}N_4OS$ × 2 HCl × 3.3 H_2O: C 37.83, H 6.98, N 13.57. Found: C 38.00, H 6.89, N 13.21.

4-Benzyloxycarbonylpiperidin-1-ium 4-methylbenzenesulfonate (23)

Piperidine-4-carboxylic acid (6.52 g, 50 mmol), p-TolSO_3H (9.70 g, 0.051 mmol), benzyl alcohol (25 mL) and benzene (70 mL) were refluxed in a Dean–Stark apparatus overnight. The solvent was removed *in vacuo*, and the residue was triturated with diethyl ether. This gave title compound **23** as a white solid (15.18 g, 78%), mp 68–72 °C, which was used in the next step of the reaction without further purification. IR (nujol) *ν*/cm^{-1}: 3448, 3032, 2980, 1724, 1453, 1191, 1034, 1010, 817. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64–1.81 (m, 2H, CH_2CH_2CH), 2.00 (dd, J_1 = 14.3 Hz, J_2 = 3.2 Hz, 2H, CH_2CH_2CH), 2.29 (s, 3H, Ar-CH_3), 2.75 (tt, J_1 = 11.0 Hz, J_2 = 3.8 Hz, 1H, CHCO), 2.94 (dt, J_1 = 12.5 Hz, J_2 = 2.9 Hz, 2H, CH_2NH), 3.25 (td, J_1 = 12.6 Hz, J_2 = 3.5 Hz, 2H, CH_2NH), 5.12 (s, 2H, $ArCH_2O$), 7.12 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.43–7.29 (m, 5H, Ar-H), 8.42 (bs, 2H, NH_2). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{18}NO_2$, 220.1338; found: 220.1333.

(*S*)-Benzyl 1-(2-*tert*-Butoxycarbonylaminopropanoyl) piperidine-4-carboxylate (24)

4-[(Benzyloxy)carbonyl]piperidin-1-ium 4-methylbenzenesulfonate (1.96 g, 5 mmol) was partitioned between ethyl acetate and aqueous $NaHCO_3$. The organic layer was separated, and evaporated to dryness. The residue was dissolved in DMF, and Boc-L-Ala (0.94 g, 5 mmol),

Et_3N (1.01 g, 10 mmol) and DPPA (1.51 g, 5.5 mmol) were added at 0 °C. The reaction was left to react for 18 h. The solution was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed successively with 10% citric acid (3 × 30 mL), a saturated solution of $NaHCO_3$ (3 × 30 mL) and brine (40 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, to give an oily residue. The residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **24** as a colourless oil (1.17 g, 59%). $[\alpha]^{20}_D$ = 10.75 (*c* = 0.225, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3422, 3310, 2977, 2362, 1731, 1644, 1454, 1366, 1250, 1168, 1025, 752. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (d, *J* = 6.0 Hz, 3H, CH_3), 1.36 (s, 9H, 3 × CH_3), 1.38–1.60 (m, 2H, CH_2), 1.86 (d, *J* = 12.1 Hz, 2H, CH_2), 2.60–2.88 (m, 2H, CHCO, 1H of CH_2), 3.00–3.20 (m, 1H, 1H of CH_2), 3.74–3.96 (m, 1H, 1H of CH_2), 4.09–4.33 (m, 1H, 1H of CH_2), 4.36–4.48 (m, 1H, CH_α), 5.11 (s, 2H, OCH_2), 6.79–7.04 (m, 1H, NHCO), 7.45–7.26 (m, 5H, Ar-H). HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{21}H_{30}N_2O_5Na$, 413.2052; found 413.2051. Anal. Calcd for $C_{21}H_{30}N_2O_5$: C 64.59, H 7.74, N 7.17. Found: C 64.48, H 8.08, N 7.17.

(*S*)-1-(2-*tert*-Butoxycarbonylaminopropanoyl)piperidine-4-carboxylic acid (25)

Compound **24** (1.80 g, 4.60 mmol) was dissolved in MeOH, and Ar was passed through the solution. Pd/C (0.18 g) was added, and the reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was concentrated *in vacuo*. This gave title compound **25** as a white solid (1.27 g, 92%), mp 85–90 °C. $[\alpha]^{20}_D$ = –27.41 (*c* = 0.205, MeOH). IR (nujol) ν/cm^{-1}: 3452, 1700, 1542, 1250, 1168, 1115, 1025, 924, 861. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (d, *J* = 6.0 Hz, 3H, CH_3), 1.36 (s, 9H, 3 × CH_3), 1.39–1.58 (m, 2H, CH_2), 1.71–1.90 (m, 2H, CH_2), 2.41–2.55 (m, 1H, CHCO), 2.73 (m, 1H, 1H of CH_2), 3.08 (m, 1H, 1H of CH_2), 3.82 (s, 1H, 1H of CH_2), 4.00–4.31 (m, 1H, 1H of CH_2), 4.33–4.48 (m, 1H, CH_α), 6.74–7.07 (m, 1H, NHCO), 12.32 (bs, 1H, COOH). HRMS-ESI (*m/z*): $[M–H]^-$ calcd for $C_{14}H_{23}N_2O_5$, 299.1607; found: 299.1605. Anal. Calcd for $C_{14}H_{24}N_2O_5$: C 55.98, H 8.05, N 9.33. Found: C 55.83, H 8.09, N 9.32.

(*S*)-Benzyl 6-Benzyloxycarbonylamino-2-[1-((*S*)-2-*tert*-butoxycarbonylaminopropanoyl)piperidine-4-carboxamido]hexanoate (26)

Compound **25** (1.20 g, 4.00 mmol) and HCl × L-Lys(Z)OBzl (1.62 g, 4 mmol) were dissolved in DMF. Et_3N (0.81 g, 8 mmol) and DPPA (1.21 g, 4.4 mmol) were added at 0 °C, and the reaction was left to react for 18 h. The solution was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed successively with 10% citric acid (3 × 30 mL), a saturated solution of $NaHCO_3$ (3 × 30 mL) and brine (30 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, to give an oily residue, which was purified by column chromatography using a CH_2Cl_2/MeOH elution system. This gave title compound **26** as a colourless oil (2.08 g, 80%). $[\alpha]^{20}_D$ = –10.30 (*c* = 0.20, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3315, 2933, 2362, 1702, 1638, 1534, 1456, 1366, 1251, 1169, 1024, 954. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21–1.32 (m, 5H, CH_3, CH_2), 1.42 (s, 9H, 3 × CH_3), 1.44–1.51 (m, 2H, CH_2), 1.57–1.94 (m, 6H, 3 × CH_2), 2.36 (tt, J_1 = 10.8 Hz, J_2 = 3.9 Hz, 1H, CH_2CHCH_2), 2.61–2.81 (m, 1H, CHCO), 2.94–3.18 (m, 3H, 1H of CH_2, $CH_{2\varepsilon}$), 3.77–3.91 (m, 1H, 1H of CH_2), 4.38–4.63 (m, 3H, 1H of CH_2, 2 × CH_α), 4.74–4.85 (m, 1H, NHCO), 5.07 (s, 2H, OCH_2), 5.15 (m, 2H, OCH_2), 5.48–5.58 (m, 1H, NHCO), 6.10–6.24 (m, 1H, NHCO),7.30–7.40 (m, 10H, Ar-H). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{35}H_{49}N_4O_8$, 653.3564; found: 653.3569. Anal. Calcd for $C_{35}H_{48}N_4O_8$: C 64.40, H 7.41, N 8.58. Found: C 64.16, H 7.64, N 8.42.

(*S*)-6-Amino-2-[1-((*S*)-2-aminopropanoyl)piperidine-4-carboxamido]hexanoic Acid 2,2,2-Trifluoroacetate (27)

Compound **26** (1.49 g, 2.28 mmol) was dissolved in a mixture of CH_2Cl_2/CF_3CO_2H (9:1; 10 mL) and stirred at room temperature until disappearance of the starting material, as determined by TLC. The solvent was evaporated *in vacuo*, and the residue dissolved in glacial acetic acid. Ar was passed through the solution. Pd/C was added, and reaction mixture was stirred under H_2 over night. After the reaction, the Pd/C was filtered off and the acetic acid was evaporated *in vacuo*. The wet product was then freeze-dried. This gave title compound **27** as a very hygroscopic colourless foam (0.89 g, 88%). $[\alpha]^{20}_D$ = 2.48 (*c* = 0.355, H_2O). IR (nujol) ν/cm^{-1}: 3430, 2948, 1668, 1538, 1452, 1392, 1201, 1134, 1024, 957. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.95–1.26 (m, 13H, 5 × CH_2, CH_3), 2.48–2.61 (m, 1H, CHCO), 2.66–2.81 (m, 1H, 1H of CH_2), 2.85 (t, *J* = 7.5 Hz, 2H, $CH_{2\varepsilon}$), 3.13 (m, 1H, 1H of CH_2), 3.82 (m, 1H, 1H of CH_2), 4.47–4.20 (m, 3H, 1H of CH_2, 2 × CH_α). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{29}N_4O_4$, 329.2189; found: 329.2190. Anal. Calcd for $C_{15}H_{28}N_4O_4$ × 1.5 CF_3CO_2H × 1.5 H_2O: C 41.06, H 6.22, N 10.64. Found: C 41.31, H 6.13, N 10.28.

***tert*-Butyl {(*S*)-1-[4-((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-ylcarbamoyl)piperidin-1-yl]-1-oxopropan-2-yl}carbamate (28)**

Compound **25** (0.90 g, 3.00 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled in an ice-bath. Then BOP (1.39 g, 3.1 mmol) and Et_3N (0.31 g, 3.10 mmol) were added to the solution and left to react for 10 min, followed by addition of Et_3N (0.31 g, 3.10 mmol) and (*S*)-2,6-diamino-4,5,6,7-tetrahydrobenzothiazole (0.59 g, 3.5 mmol). The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 (100 mL). The organic phase was washed successively with 1 M

aqueous HCl (3 × 30 mL), a saturated aqueous solution of $NaHCO_3$ (3 × 30 mL) and brine (30 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give an oily residue. The product was purified by column chromatography using a CH_2Cl_2/MeOH elution system. This gave title compound **28** as a pale yellow solid (0.78 g, 58%), mp 140–143 °C. $[\alpha]^{20}_D = -31.74$ ($c = 0.235$, MeOH). IR (nujol) ν/cm^{-1}: 3315, 2977, 2932, 1698, 1637, 1526, 1453, 1367, 1165, 1062, 948. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.28 (dd, $J_1 = 12.7$ Hz, $J_2 = 7.0$ Hz, 3H, CH_3), 1.46 (s, 9H, 3 × CH_3), 1.54–2.05 (m, 6H, 3 × CH_2), 2.36–2.98 (m, 6H, 2 × CH_2, CHCO, 1H of CH_2), 3.26–3.06 (m, 1H, 1H of CH_2), 3.97–4.26 (m, 2H, C*H*NH, 1H of CH_2), 4.41–4.73 (m, 2H, CH_α, 1H of CH_2). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{34}N_5O_4S$, 452.2332; found 452.2321. Anal. Calcd for $C_{21}H_{33}N_5O_4S \times 1.3\ H_2O$: C 53.10, H 7.55, N 14.74. Found: C 53.18, H 7.78, N 14.67.

N-[(*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl]-1-[(*S*)-2-aminopropanoyl]piperidine-4-carboxamide Hydrochloride (29)

Compound **28** (0.55 g, 1.22 mmol) was dissolved in anhydrous THF (20 mL) and treated with gaseous HCl. The solvent was evaporated *in vacuo*, and the product triturated with diethyl ether. The wet solid was freez-dried to give title compound **29** as a very hygroscopic colourless solid (0.46 g, 90%). $[\alpha]^{20}_D = -28.49$ ($c = 0.308$, H_2O). IR (nujol) ν/cm^{-1}: 3409, 2949, 1640, 1543, 1448, 1370, 1267, 1209, 1095, 948. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.49 (dd, $J_1 = 10.1$ Hz, $J_2 = 7.0$ Hz, 3H, CH_3), 1.55–2.13 (m, 6H, 3 × CH_2), 2.45–2.98 (m, 6H, 2 × CH_2, CHCO, 1H of CH_2), 3.17–3.30 (m, 1H, 1H of CH_2), 3.86–4.03 (m, 1H, 1H of CH_2), 4.15–4.26 (m, 1H, C*H*NH), 4.38–4.60 (m, 2H, CH_α, 1H of CH_2). HRMS-ESI (*m/z*): $[M+H]^+$ $C_{16}H_{26}N_5O_2S$, 352.1807; found: 352.1823. Anal. Calcd for $C_{16}H_{25}N_5O_2S \times 1.5\ HCl \times 3.3\ H_2O$: C 41.27, H 7.17, N 15.04. Found: C 41.57, H 7.49, N 14.69.

Benzyl 1-(2-*tert*-Butoxy-2-oxoethyl)piperidine-4-carboxylate (30)

4-[(Benzyloxy)carbonyl]piperidin-1-ium 4-methylbenzenesulfonate (1.96 g, 5 mmol) was partitioned between a 10% aqueous solution of $NaHCO_3$ (50 mL) and ethyl acetate (3 × 30 mL). The combined organic layers were evaporated to dryness to give an oily residue. This was dissolved in THF and cooled in an ice bath, and Et_3N (1.21 g, 12 mmol) was added. Then $BrCH_2CO_2{}^tBu$ (1.95, 10 mmol) was added dropwise over 5 min at 0 °C. The reaction was left to proceed at room temperature overnight. Triethylammonium bromide was filtered off by suction filtration, and the filtrate was concentrated *in vacuo* to give an orange oily residue. The residue was purified by column chromatography using an ethyl acetate/hexane elution system. This gave title compound **30** as an orange oil (1.23 g, 74%). IR (nujol) ν/cm^{-1}: 3448, 2944, 2362, 1735, 1454, 1368, 1260, 1158, 1046, 1015, 751. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H, 3 × CH_3), 1.77–1.99 (m, 4H, 2 × CH_2CH), 2.18–2.41 (m, 3H, CH_2N, CHCO), 2.86–2.94 (m, 2H, CH_2N), 3.10 (s, 2H, NCH_2CO), 5.12 (s, 2H, OCH_2), 7.30–7.38 (m, 5H, Ar-H). MS (ESI) *m/z*: 334, 278 (100). Anal. Calcd for $C_{19}H_{27}NO_4 \times 0.5\ H_2O$: C 66.64, H 8.24, N 4.09. Found: C 66.55, H 8.42, N 3.89.

1-(2-*tert*-Butoxy-2-oxoethyl)piperidine-4-carboxylic Acid (31)

Compound **30** (1.00 g, 3.00 mmol) was dissolved in MeOH, and Ar was passed through the solution. Pd/C (0.18 g) was added, and reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was concentrated *in vacuo*. This gave title compound **31** as a yellow solid (0.672 g, 92%), mp 148–152 °C. IR (nujol) ν/cm^{-1}: 3447, 2976, 1718, 1458, 1373, 1282, 1157, 942, 847. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H, 3 × CH_3), 1.74–2.05 (m, 4H, 2 × CH_2CH), 2.30 (tt, $J_1 = 10.8$ Hz, $J_2 = 4.1$ Hz, 1H, CHCO), 2.36–2.48 (m, 2H, CH_2N), 2.98–3.10 (m, 2H, CH_2N), 3.23 (s, 2H, NCH_2CO), 10.73 (bs, 1H, CO_2H). MS (ESI) *m/z*: 266 (M+Na, 4), 244 (M+H, 21), 188 (100). Anal. Calcd for $C_{12}H_{21}NO_4$: C 59.24, H 8.70, N 5.76. Found: C 59.34, H 8.95, N 5.74.

(*S*)-Benzyl 6-Benzyloxycarbonylamino-2-[1-(2-*tert*-butoxy-2-oxoethyl)piperidine-4-carboxamido]hexanoate (32)

Compound **31** (0.66 g, 2.72 mmol) and HCl × L-Lys(Z)-OBzl (1.11 g, 2.72 mmol) were dissolved in DMF. Et_3N (0.61 g, 6 mmol) and DPPA (0.82 g, 3.00 mmol) were added at 0 °C, and the reaction was left to react for 18 h. The solution was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed successively with 10% citric acid (3 × 30 mL), a saturated solution of $NaHCO_3$ (3 × 30 mL) and brine (40 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the product was purified by column chromatography using a CH_2Cl_2/MeOH elution system. This gave title compound **32** as a white solid (1.19 g, 74%), mp 70–74 °C. $[\alpha]^{20}_D = -6.87$ ($c = 0.230$, $CHCl_3$). IR (nujol) ν/cm^{-1}: 3344, 2941, 1747, 1691, 1642, 1542, 1264, 1151, 956. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12–1.92 (m, 19H, 3 × CH_3, 5 × CH_2), 2.06–2.30 (m, 3H, CH_2N, CHCO), 2.89–2.99 (m, 2H, CH_2N), 3.07–3.16 (m, 4H, NCH_2CO, $CH_{2\varepsilon}$), 4.63 (dt, $J_1 = 7.7$ Hz, $J_2 = 5.1$ Hz, 1H, CH_α), 4.80 (s, 1H, NHCO), 5.03–5.25 (m, 4H, 2 × OCH_2), 6.10 (d, $J = 7.6$ Hz, 1H, NHCO), 7.27–7.44 (m, 10H, Ar-H). HRMS-ESI (*m/z*): $[M+H]^+$ $C_{33}H_{46}N_3O_7$, 596.3336; found: 596.3333. Anal. Calcd for $C_{33}H_{45}N_3O_7$: C 66.53, H 7.61, N 7.05. Found: C 66.31, H 7.83, N 7.05.

(*S*)-6-Amino-2-[1-(carboxymethyl)piperidine-4-carboxamido]hexanoic Acid Hydrochloride (33)

Compound **32** (1.00 g, 1.68 mmol) was dissolved in acetic acid, and Ar was passed through the solution. Pd/C

was added, and the reaction mixture was stirred under H_2 until no starting material was detected by TLC. After the reaction, the Pd/C was filtered off and the solution was treated with gaseous HCl for 0.5 h. Acetic acid was removed *in vacuo*, and the residue was freeze-dried. This gave the title compound **33** as a very hygroscopic colourless foam (0.55 g, 95%). $[\alpha]^{20}_D = -8.16$ (c = 0.245, H_2O). IR (nujol) ν/cm^{-1}: 3422, 2954, 1994, 1736, 1654, 1543, 1401, 1232, 1160, 953. ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.79–0.96 (m, 2H, $CH_{2\gamma}$), 1.01–1.33 (m, 4H, $CH_{2\beta}$, $CH_{2\delta}$), 1.36–1.62 (m, 4H, CH_2CH), 2.07–2.22 (m, 1H, CHCO), 2.27–2.39 (m, 2H, $CH_{2\varepsilon}$), 2.57–2.79 (m, 2H, CH_2NH), 2.97–3.17 (m, 2H, CH_2NH), 3.48 (s, 2H, CH_2CO_2H), 3.63–3.72 (m, 1H, CH_α). ^{13}C NMR (75.5 MHz, MeOH-*d*4) δ 23.80, 27.75, 27.78, 31.45, 40.35, 40.43, 53.49, 168.02, 175.03, 175.79. HRMS-ESI (*m/z*): $[M+H]^+$ $C_{14}H_{26}N_3O_5$, 316.1872; found: 316.1868. Anal. Calcd for $C_{14}H_{25}N_3O_5 \times HCl \times 2.9\ H_2O$: C 41.61, H 7.93, N 10.40. Found: C 41.88, H 7.93, N 10.40.

4. 2. Biochemical Evaluation of the Compounds

The compounds were tested for inhibition of addition of L-[^{14}C]Lys to UMAG in a mixture (final volume, 50 µl) containing 0.1 M Tris-HCl, pH 8.6, 15 mM $MgCl_2$, 5 mM ATP, 0.1 mM UMA, 198.4 µM L-Lys, 1.6 µM L-[^{14}C]Lys (50,000 cpm), 30 µM Tween-20, 5% (v/v) DMSO, purified MurE from *S. aureus* (diluted in 20 mM potassium phosphate (pH 7.2), 1 mM DTT), and the test compound (compounds were soluble in the enzyme assay mixture containing 5% DMSO at all of the concentrations used). The samples were incubated for 30 min at 37 °C, and the reaction was stopped by addition of 10 µL glacial acetic acid. The mixture was lyophilised and dissolved in water. This solution was then analysed by TLC. The plates were run in a mobile phase of *n*-propanol:NH_3:water (6:3:1) for 8 h. The radioactive substrate and product were detected and quantified with a radioactivity monitor (Berthold France, Thoiry, France). The residual activity for each inhibitor concentration was calculated with respect to a similar assay without inhibitor, and the IC_{50} values were calculated from the fitted regression equation using the logit-log plot.

5. Acknowledgements

This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project No. LSHM-CT-2004-512138) under the thematic priority of Life Sciences, Genomics and Biotechnology for Health. Support from the Ministry of Higher Education, Science and Technology of the Republic of Slovenia and the Slovenian Research Agency are also acknowledged.

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Povzetek

Zaradi pojavljanja in širjenja bakterijskih sevov, ki so hkrati odporni na več vrst protimikrobnih učinkovin, obstaja velika potreba po razvoju novih zdravilnih učinkovin. Eden izmed pomembnih virov novih tarč za razvoj protimikrobnih učinkovin je biosinteza bakterijskega peptidoglikana. Biosintezo peptidne verige v peptidoglikanu katalizirajo encimi ligaze Mur (C, D, E in F), ki so nujni za bakterijsko preživetje. Načrtovali in sintetizirali smo serijo spojin kot potencialnih inhibitorjev UDP-*N*-acetilmuramoil-L-alanil-D-glutamat:L-lizin ligaze (MurE) iz *Staphylococcus aureus*. Pri tem smo uporabili dva pristopa: (i) sintezo metilenaminskih derivatov kot mimetikov prehodnega stanja in (ii) sintezo produktnih ananlogov reakcije MurE. Dve spojini z metilenaminskim ogrodjem sta primerni za nadaljni razvoj inhibitorjev MurE.