Novel Naphthalene-*N*-sulfonyl-D-glutamic Acid Derivatives as Inhibitors of MurD, a Key Peptidoglycan Biosynthesis Enzyme[‡,§]

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Mur ligases have essential roles in the biosynthesis of peptidoglycan, and they represent attractive targets for the design of novel antibacterials. MurD (UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase) is the second enzyme in the series of Mur ligases, and it catalyzes the addition of D-glutamic acid (D-Glu) to the cytoplasmic intermediate UDP-*N*-acetylmuramoyl-L-alanine (UMA). Because of the high binding affinity of D-Glu toward MurD, we synthesized and biochemically evaluated a series of N-substituted D-Glu derivatives as potential inhibitors of MurD from *E. coli*, which allowed us to explore the structure−activity relationships. The substituted naphthalene-*N*-sulfonyl-D-Glu inhibitors, which were synthesized as potential transition-state analogues, displayed IC_{50} values ranging from 80 to 600 μM. In addition, the high-resolution crystal structures of MurD in complex with four novel inhibitors revealed details of the binding mode of the inhibitors within the active site of MurD. Structure−activity relationships and cocrystal structures constitute an excellent starting point for further development of novel MurD inhibitors of this structural class.

1. Introduction

Infectious diseases are one of the leading causes of death worldwide.[1] The emergence and dissemination of resistant bacterial strains have created an urgent need for the development of novel antibacterial agents.[2] The peptidoglycan, an essential cell-wall polymer that is unique to prokaryotic cells, is an important target for antibiotic research.[3] While the majority of approved drugs that target peptidoglycan biosynthesis act on the extracellular steps,[4] there has been increased interest in exploiting the early intracellular steps that are catalyzed by a series of Mur enzymes (MurA−MurF[a]).[5,6] Mur ligases (MurC−MurF) catalyze a series of reactions that start from UDP-*N*-acetylmuramic acid (UDP-Mur*N*Ac) to yield Park's nucleotide (UDP-Mur*N*Ac-pentapeptide) by sequentially adding L-Ala (MurC), D-Glu (MurD), *meso*-diaminopimelic acid or L-Lys (MurE), and the D-Ala-D-Ala dipeptide (MurF).[3] Recently, we have focused our attention on MurD, an ATP-dependent, amide-forming enzyme that acts through the catalytic mechanism illustrated in Figure 1. Initially, the carboxylic acid is phosphorylated, and the resulting acyl phosphate is attacked by the incoming amino acid (D-Glu) to form a high-energy tetrahedral intermediate, which subsequently collapses to the amide product and inorganic phosphate. All Mur ligases (MurC−MurF) act via similar mechanisms that have been confirmed by X-ray diffraction analysis,[7] by isotope transfer[8] and rapid quench[9] experiments, and by the chemical trapping method.[10]

The crystal structures of the Mur ligase family show that they have the same three-domain topology, with the N-terminal and central domains binding UDP precursor and ATP, respectively, while the incoming amino acid or dipeptide binds to the C-terminal part.[11] Interestingly, sequence alignments of Mur ligase orthologues and paralogues show relatively low overall homologies, with the exception of residues comprising the active sites.[12−15] Furthermore, the open and closed X-ray structures of free and complexed MurD[7,16,17] and MurF[18,19] show that the C-terminal domain undergoes substantial conformational changes upon substrate or inhibitor binding.[20]

The high stereospecificity of MurD for D-Glu, its ubiquitous nature among the bacteria, and its absence in mammals all make MurD a promising target for antibacterial therapy.[6] To date, several phosphinates of the general formula **1** (Figure 1) have been reported to be tetrahedral transition-state analogue inhibitors of MurD.[21−24] Macrocyclic inhibitors[25] and peptides[26] obtained from phage display screening have also been developed as MurD inhibitors. Furthermore, pulvinones[27] and pyrazole derivatives[28] have been reported as potential inhibitors of MurA−MurD and MurB−MurD, respectively. However, for all of these molecules, neither the MurD inhibition mechanisms nor the crystal structures of their complexes have been reported.

The search for new compounds with the potential to inhibit MurD led us to synthesize and biochemically evaluate N-substituted D-Glu derivatives. We postulated that they represent a promising starting point for the development of MurD inhibitors not only because of the highly stereospecific affinity of MurD for D-Glu and the conserved D-Glu-binding site across

‡ Dedicated to Professor Slavko Pečar on the occasion of his 60th birthday.

§ PDB accession codes: 2UUO, 2UUP, 2VTD, and 2VTE.

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[a] Abbreviations: UDP, uridine 5′-diphosphate; UMA, UDP-*N*-acetylmuramoyl-L-alanine; UMAG, UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate; MurA, UDP-*N*-acetylglucosamine enolpyruvyl transferase; MurB, UDP-*N*-acetylpyruvylglucosamine reductase; MurC, UDP-*N*-acetylmuramate:L-alanine ligase; MurD, UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase; MurE, UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate:*meso*-diaminopimelate ligase; MurF, UDP-*N*-acetylmuramoyl-L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate:D-alanyl-D-alanine ligase; GABA, γ-aminobutyric acid; PCC, pyridinium chlorochromate; DPPA, diphenylphosphoryl azide.

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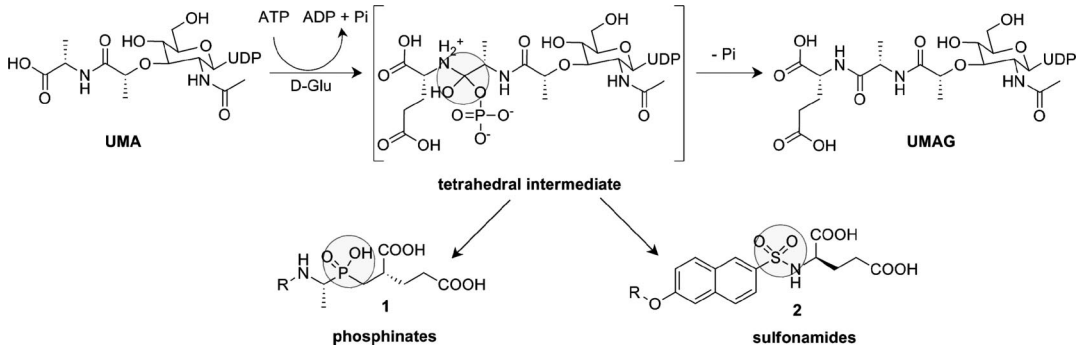
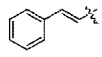
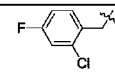
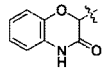
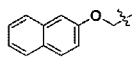
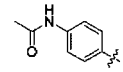
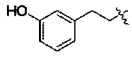
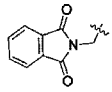
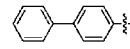
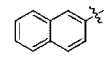
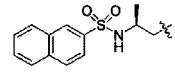


Figure 1. Reaction catalyzed by MurD and design of phosphinate and sulfonamide transition-state analogue inhibitors.

Table 1. Residual Activities of MurD in the Presence of 1 mM Inhibitors[b]

	R	RA (%) of MurD		R	RA (%) of MurD
5a		91	**8a**		86
5b		54	**8b**		84
5c		76	**11a**		79
5d		81	**11b**		66
5e		92	**11c**		43 (IC_{50} = 1720 μM)
			11d		35 (IC_{50} = 810 μM)
			12		75 (IC_{50} > 2,000 μM)[a]

[a] Published previously.[37] [b] Results represent the mean values of two independent experiments. Standard deviations were within ±10% of the mean values.

different bacterial species but also because of the 4-fold higher binding efficiency index[29] of D-Glu when compared to other substrates (UMA and ATP) of MurD. Additionally, it has been suggested recently that D-Glu represents an essential fragment of a potent inhibitor.[24,30,31]

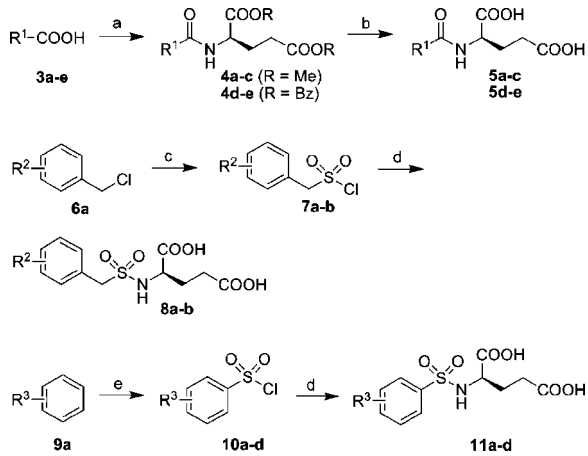
Among several crystal structures of Mur ligases[11,32] deposited in the Protein Data Bank, only two crystal structures of MurF in complex with inhibitors have been reported.[19] Recently, we published the first crystal structures of two isomers of an *N*-sulfonyl-Glu inhibitor in complex with MurD from *Escherichia coli*.[33] In the present study, we describe the synthesis and structure−activity relationships (SARs) of a series of N-substituted derivatives of D-glutamic acid, leading to naphthalene-*N*-sulfonyl-D-Glu derivatives of the general formula **2** (Figure 1), which most probably act as transition-state analogue inhibitors of MurD. In addition, we present four new crystal structures of small molecule inhibitors in complex with MurD.

2. Chemistry

Our investigations started with the synthesis of new carboxamides **5a**−**e** (Table 1) that were prepared by the coupling of C-protected D-Glu with proper carboxylic acids **3a**−**e** using DPPA, and final deprotection of **4a**−**c** and **4d**,**e** by alkaline hydrolysis or catalytic hydrogenation, to provide carboxamides **5a**−**c** and **5d**,**e**, respectively (Scheme 1). Carboxylic acid **3b** was prepared from the appropriate starting materials according to published procedures.[34] Sulfonamide compounds **8a**,**b** and **11a**−**d** (Table 1) were synthesized from sulfonyl chlorides **7a**,**b** and **10a**−**d**, respectively, and free D-Glu in the presence of aqueous 2 M NaOH (Scheme 1). Sulfonyl chlorides **7a**[35] and **10a**[36] were prepared from 2-chloro-1-(chloromethyl)-4-fluorobenzene (**6a**) and *N*-phenylacetamide (**9a**) according to literature procedures (Scheme 1).

With an IC_{50} of 810 μM, compound **11d** (Table 1) was selected for further optimization (see below). We proceeded with

Scheme 1. Synthesis of Carboxamide and Sulfonamide Derivatives[a]



[a] Reagents and conditions: (a) HCl·D-Glu(OR)$_2$, DPPA, Et_3N, DMF, room temp; (b) for compounds **5a**−**c**, 1 M NaOH, dioxane, room temp; for compounds **5e**,**d**, H_2, Pd/C, glacial acetic acid, room temp; (c) (i) $Na_2S_2O_3$, MeOH/H_2O, reflux; (ii) Cl_2, glacial acetic acid, 0 °C to room temp; (d) D-Glu, 2 M NaOH, pH 10−11, 70 °C; (e) $ClSO_3H$, 5−65 °C.

the synthesis of analogues of **11d** bearing substitutions on the naphthalene ring. Here, sodium hydroxynaphthalenesulfonic acids (**13**) were first alkylated with the corresponding alkyl halides to give alkyloxynaphthalenesulfonic acids **14a**−**o** and **32**−**34**, followed by chlorination using thionyl chloride to provide the desired sulfonyl chlorides **15a**−**o**. Treatment with C-protected D-Glu then provided sulfonamides **16a**−**o** and **35**−**37**, which were converted by alkaline hydrolysis, catalytic hydrogenation, or acidolysis into the target sulfonamide inhibitors **17a**−**o** and **38**−**40**, respectively (Scheme 2, Table 2). The same procedure was also used for the synthesis of the Gly derivative **19** (Table 3), using C-protected Gly instead of C-protected D-Glu, while the GABA and L-Glu derivatives **18** and **20** (Table 3), respectively, were synthesized from sulfonyl chloride **15c** and the appropriate free amino acid, using aqueous 2 M NaOH in acetone. For the production of compounds **38**−**40** (Table 5), a general synthetic approach for the synthesis of **17a**−**o** was used (Scheme 2). However, in the first step, Na/EtOH was replaced with powdered NaOH for the preparation of compounds **32**−**34**. Furthermore, in the synthesis of sulfonamides **16m**−**o** and **37**, the isolation of the corresponding sulfonyl chloride was omitted (Scheme 2) (see Experimental Section).

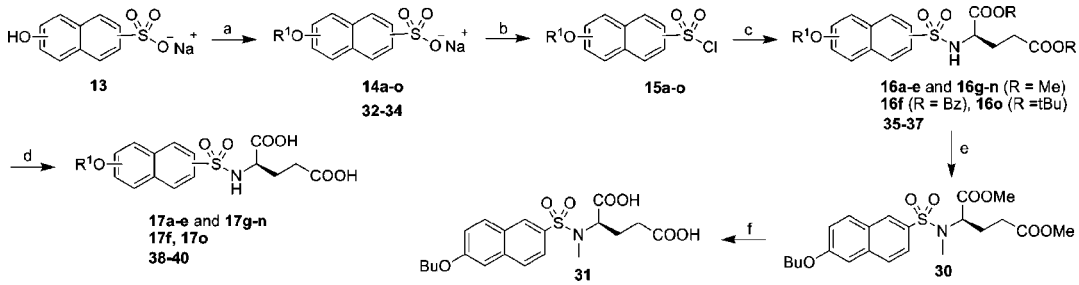
To determine the importance of the sulfonamide group, analogues of compound **17c** with carboxamide (**26**), a reduced amide bond (**29**) at the position of the sulfonamide bond, and the *N*-methyl derivative (**31**) were prepared (Table 4). Thus, 6-hydroxy-2-naphthoic acid (**21**) was first protected as the methyl ester to generate ester **22**, followed by O-alkylation with butyl bromide to give the corresponding ether **23** and alkaline hydrolysis for the carboxylic acid **24**. Subsequent DPPA-mediated coupling of carboxylic acid **24** with C-protected D-Glu gave **25**, followed by deprotection providing the desired carboxamide **26** (Scheme 3). Alternatively, treatment of intermediate **23** with $LiAlH_4$ gave alcohol **27**, which was converted to aldehyde **28** by oxidation with pyridinium chlorochromate (PCC). Treatment with D-Glu under reductive amination conditions gave the desired reduced amide **29** (Scheme 4). To probe the role of the sulfonamide proton of **17c**, the *N*-methyl derivative **31** was synthesized. Here, intermediate **16c** was first alkylated with MeI to give compound **30**, which was converted by alkaline hydrolysis into the target compound **31** (Scheme 2).

3. Results and Discussion

Target compounds **5a**−**e, 8a**,**b**, **11a**−**d, 16c**, **17a**−**o**, **18**−**20**, **26**, **29**, **31**, and **38**−**40** (Tables 1–5) were tested for their inhibitory activities on MurD from *E. coli.* The results are presented as residual activities (RAs) of the enzyme in the presence of 1 mM of each compound, and as IC_{50} values for active compounds. The first series of carboxamides **5a**−**e** and sulfonamides **8a**,**b** and **11a**,**b** proved to be practically inactive (Table 1). However, when the bulky biphenyl and naphthalene substituents were introduced, compounds **11c** and **11d** were obtained with IC_{50} values of 1720 and 810 μM, respectively (Table 1). It is interesting to compare the inhibitory activities of sulfonamides **11d** and **12** (Table 1). The latter compound, which contains a sulfonoalanine moiety between the naphthalene and D-Glu residues, was inactive,[37] in contrast to compound **11d**, in which the same naphthalene moiety is attached directly to D-Glu with a sulfonamide bond (Table 1). At this point, naphthalenesulfonamide **11d** was selected as the starting point for further development to obtain new transition-state analogue inhibitors of MurD. It is known that sulfonamides possess a geometry similar to that of the tetrahedral intermediate formed during the peptide bond cleavage and formation.[38] Further gradual improvements in inhibitor **11d** were achieved by substituting position 6 of the naphthalene ring with alkyloxy (compounds **17a**−**f**) or cyclopentyloxy (**17i**) substituents, giving compounds with IC_{50} values of between 170 and 590 μM (Table 2). Here, with the extension of the alkyl side chain length from methyl to pentyl, the inhibitory activity increased more than 3-fold (IC_{50} values for compound **17a** and **17d**: 590 and 170 μM, respectively). However, compounds **17g**,**h** bearing an acidic substituent on the naphthalene ring were less active (the IC_{50} value of **17g** was 630 μM, while **17h** was inactive). Inhibitory activity was further improved by introducing arylalkyloxy substituents (compounds **17j**−**o**), where the 3-phenylpropyl (**17k**, IC_{50} = 132 μM) and 4-cyanobenzyl (**17l**−**o**, $IC_{50} \approx$ 80−120 μM) derivatives were seen to be the most potent inhibitors of MurD.

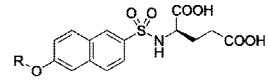
To further investigate the SARs, we prepared analogues of representative naphthylsulfonamide **17c**, incorporating changes that would help the elucidation of the structural features required for MurD inhibition (Tables 3−5). To determine the importance of the D-Glu α- and γ-carboxylate groups, we prepared compounds **18** and **19** as analogues of **17c** but lacking the α-carboxylate group and the glutamic acid side chain, respectively (Table 3). The importance of the γ-carboxylate group for the inhibition of MurD was recently demonstrated in the phosphinodipeptide series, where truncated analogues were seen to be devoid of inhibitory activity.[24] Our truncated sulfonamide analogues **18** and **19** did not inhibit MurD either, confirming that the presence of both α- and γ-carboxylate groups of the glutamate residue in this type of inhibitor is essential for good inhibition. In the complex of MurD with its product UMAG (MurD·UMAG, pdb entry 4UAG),[7] the α-carboxylate of D-Glu is hydrogen-bonded to Thr321 and forms a charge-based interaction with N^{ζ} of Lys348 and the γ-carboxylate is positioned through hydrogen bonds with Ser415 and Phe422. The absence of inhibition by analogues **18** and **19** is consistent with the loss of binding energy of the two hydrogen bonds provided by the α-carboxylate and γ-carboxylate group, respectively. Furthermore, the dimethyl ester derivative **16c** proved

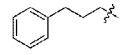
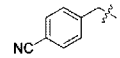
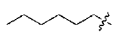
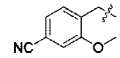
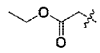
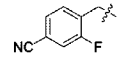
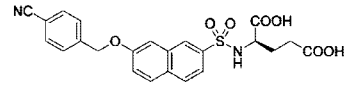
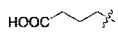
Scheme 2. Synthesis of Substituted Naphthalenesulfonamide Derivatives[a]



[a] Reagents and conditions: (a) for compounds **14a**−**o** (i) Na/EtOH, room temp, (ii) R^1-X, DMSO, room temp; for compounds **32**−**34**, R^1-Br, NaOH, DMSO, 60 °C; (b) $SOCl_2$, DMF, 0 °C to room temp; (c) HCl·D-Glu$(OR)_2$, Et_3N, CH_2Cl_2, room temp; (d) for compounds **17a–e**, **17g–n** and **38–40** 1 M NaOH, dioxane, room temp; for compound **17f**, H_2, Pd/C, MeOH, room temp; for compound **17o**, CF_3COOH, CH_2Cl_2, room temp; (e) MeI, K_2CO_3, DMF; (f) 1 M NaOH, dioxane, room temp.

Table 2. MurD Inhibitory Activity of Naphthalene-*N*-sulfonyl-D-Glu Derivatives[b]



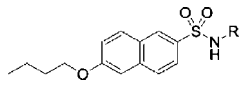
	R	IC_{50} (μM)		R	IC_{50} (μM)
17a		590	**17i**		400
17b		305	**17j**		239
17c		280[a]	**17k**		132
17d		170	**17l**		105
17e		176	**17m**		100
17f		192	**17n**		85
17g		630	**17o**		122
17h		> 1000			

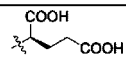
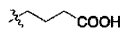
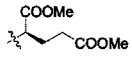
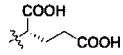
[a] Published previously.[33] [b] Results represent the mean values of two independent experiments. Standard deviations were within ±10% of the mean values.

to be inactive, further confirming the importance of the free carboxylate groups of D-Glu. On the other hand, we were surprised by the small difference in potency between the enantiomeric compounds **17c** (D-Glu derivative) and **20** (L-Glu derivative) (Table 3), which is somewhat contradictory to the high stereospecificity of MurD for the D-Glu substrate.[39] It was unexpected that a compound containing the L-Glu moiety would act as an inhibitor of this enzyme. Steady-state kinetics and X-ray diffraction studies unambiguously demonstrated that the L-Glu moiety of compound **20** binds into the D-Glu-binding site of MurD in a manner similar to that of D-Glu in UMAG or in compound **17c**.[33] Small differences in binding modes of the naphthalenesulfonamido group contributed to 3-fold better inhibition by compound **17c**.

In the next step, we wanted to investigate the importance of the intact sulfonamide group. As illustrated in Table 4, replacement of the sulfonamide in **17c** with carboxamide (**26**) led to diminished activity against MurD, demonstrating that the introduction of the planar carboxamide is not tolerated and that the tetrahedral geometry on the nitrogen substituent is preferred for inhibitory activity. To examine the importance of H-bond donor/acceptor properties of the sulfonamide group, the analogue with a reduced amide group **29** was synthesized. Compound **29** with a tetrahedral carbon atom and basic secondary amine group proved to be inactive (Table 4), indicating the importance of the hydrogen bond between the sulfonamide group oxygens and the enzyme. The acidic sulfonamide moiety is thus favored over the basic reduced amide. In further investigations, inhibitor **17c** was N-methylated to obtain compound **31**. The latter compound was devoid of inhibitory activity (Table 4), clearly demonstrating that proton donor properties on the sulfonamide nitrogen are also crucial for good inhibition.

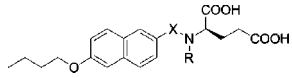
Table 3. Influence of Glutamate Moiety on Inhibitory Activity[b]



	R	IC_{50} (μM)
17c		280[a]
18		> 2,000
19		> 2,000
16c		> 2,000
20		710[a]

[a] Published previously.[33] [b] Results represent the mean values of two independent experiments. Standard deviations were within ±10% of the mean values.

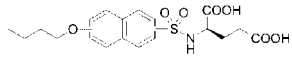
Table 4. Activities of Derivatives Obtained by Replacement or Methylation of the Sulfonamide Bond[b]



	X	R	IC_{50} (μM)
17c	$-SO_2-$	H	280[a]
26	$-CO-$	H	>1000
29	$-CH_2-$	H	>1000
31	$-SO_2-$	$-CH_3$	>1000

[a] Published previously.[33] [b] Results represent the mean values of two independent experiments. Standard deviations were within ±10% of the mean values.

Table 5. Influence of Substitution Patterns on Inhibitory Activity[b]



	substitution	IC_{50} (μM)
17c	2,6	280[a]
38	2,7	180
39	1,5	>1000
40	1,4	>1000

[a] Published previously.[33] [b] Results represent the mean values of two independent experiments. Standard deviations were within ±10% of the mean values.

Having identified that both the sulfonamido and D-Glu moieties are necessary for good inhibition, we next focused our attention on understanding the effects of naphthalene substitution patterns (Table 5). Here, 2,6- and 2,7-regioisomers (compounds **17c** and **38**, respectively) were preferred over 1,5- and 1,4-regioisomers (compounds **39** and **40**, respectively). In addition, compound **17l** and its 2,7-regioisomer **17o** were equipotent inhibitors of MurD (Table 2).

Finally, a common feature of all of the active compounds is *N*-(2-naphthylsulfonyl)-D-glutamic acid, which is substituted on position 6 or 7 by a lipophilic substituent. Because of the highly conserved active site of MurD among different bacteria,[15] it is expected that the identified inhibitors will also inhibit other bacterial MurD enzymes. To obtain additional information about the binding mode of the inhibitors, we also solved the high-resolution crystal structures of MurD in complex with four novel inhibitors (**17d**, **17l**, **17n**, **17o**).

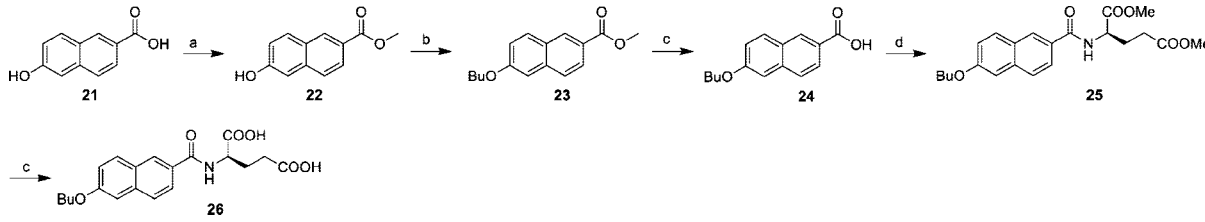
3.1. Crystal Structures of MurD in Complex with Compounds 17d, 17l, 17n, and 17o. The crystal structures of the MurD−inhibitor complexes were solved at a high resolution to determine the binding mode of the inhibitors within the active site of MurD.

In the structures of MurD complexed to **17d** and **17l** (Figures 2 and 3, respectively) the D-Glu moiety of both compounds occupies the same site as seen for related sulfonamide inhibitors (compounds **17c** and **20**)[33] and the product UMAG.[7] The α-carboxyl group of these inhibitors forms a charge based interaction with N^{ζ} of Lys348 and is additionally hydrogen-bonded with a conserved water molecule, W107 and W415 in the complexes with **17d** and **17l**, respectively. Within the active site, **17l** forms further hydrogen bonds to O^{γ} of Thr321 and the carboxyl group of Asp182. The carboxyl group of the D-Glu side chain is held in place by hydrogen bonds with Ser415 and Phe422. One of the carboxylic oxygen atoms forms hydrogen bonds with both the backbone nitrogen and the O^{γ} of Ser415, whereas the other oxygen atom interacts with the backbone nitrogen of Phe422 and, in the case of the MurD−**17l** complex, also with water molecule W413. The latter is further hydrogen-bonded to water molecule W412, which has a direct interaction with the nitrogen of the inhibitor, forming an extended ring-type system. The sulfonic group of both inhibitors also contributes to recognition within the active site by interacting with water molecule W185 (e.g., in complex with compound **17l**), which bridges between the oxygen atom of the sulfonic group and O^{γ} of Ser159, as well as O^{γ} of Asn138. The naphthalene ring of the inhibitors is positioned in the cleft between all three domains, making hydrophobic interactions with Leu416, Phe161, and C_{α} of Gly73. The functional groups of both compounds at position 6 of the naphthalene ring extend into the uracil-binding pocket. While the pentoxy group of compound **17d** probably makes hydrophobic interactions, the cyanobenzyloxy moiety of **17l** forms an additional hydrogen bond with the backbone nitrogen of Thr36 and makes $\pi-\pi$ interactions through its phenyl ring with the salt bridge Asp35−Arg37.

Both close analogues of **17l**, **17n**, and **17o** bind to MurD in a similar manner to **17I**. The additional fluorine atom at the ortho position of the *p*-cyanobenzyloxy group of **17n** faces the solvent area, similar to one of the carbonyl oxygen atoms of the uracil part of the product UMAG.[7] Not surprisingly, the naphthalene group of **17o**, the 2,7-substituted analogue of **17l**, occupies a slightly different position compared to its 2,6-substituted counterpart. Nevertheless, the cyanobenzyloxy group extends to the uracil-binding pocket in a similar manner, forming a hydrogen bond with the backbone nitrogen atom of Thr36.

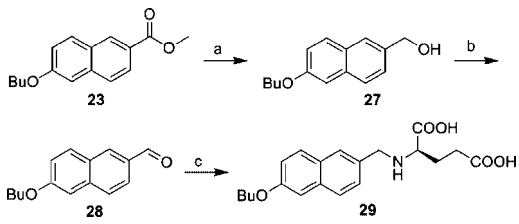
Experimentally determined positions and conformations of compounds **17d**, **17l**, **17n**, and **17o** in the MurD active site offer a solid foundation for optimization of inhibitors of this structural class. As suggested in Figure 4, the design of derivatives of inhibitor **17l** with improved interaction patterns within the active site of MurD could proceed in three directions. First, with the appropriate substitution of the N^{α} atom of **17l** we could gain additional interactions with Asn138 or with amino acid residues constituting the ATP-binding site (e.g., Lys115 or/and Lys319). The functional group added to N^{α} atom should be carefully selected, since the newly formed interactions between the introduced moiety and the protein have to energetically overcome a loss of a hydrogen bond that the sulfonamide hydrogen

Scheme 3. Synthesis of Carboxamide Analogue **26**[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) BuBr, K_2CO_3, acetone, reflux; (c) 1 M NaOH, dioxane, room temp; (d) HCl·D-Glu(OMe)$_2$, DPPA, Et_3N, DMF, room temp.

Scheme 4. Synthesis of Reduced Amide Analogue **29**[a]



[a] Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C to room temp; (b) PCC, $Na^+CH_3COO^-$, 4 Å molecular sieves, argon, CH_2Cl_2; (c) D-Glu, MeOH, NaOH, $NaBH_4$, 0 °C to room temp.

forms with a water molecule (W412, Figure 3b). Second, by substitution or bioisosteric replacement of the naphthalene ring, the strengthening of hydrophobic interactions with Leu416, Phe161, and C_α of Gly73 is expected (Figure 4b). Finally, the additional substitution or bioisosteric replacement of the *p*-cyanophenyl moiety of compound **17l** should result in the formation of additional hydrogen bonds with Ser71 and Thr36, both being part of the "uracil-binding pocket".

4. Conclusions

We have synthesized a series of naphthalene-*N*-sulfonyl-D-Glu derivatives as MurD inhibitors that show IC_{50} values from 80 to 600 μM. The SARs for these types of inhibitors were determined. Additionally, we solved four new crystal structures of MurD complexed with inhibitors to determine the binding mode of inhibitors within the enzyme active site. We have described the possible ways of optimizing the structures of reported inhibitors. Combinations of advantageous substitutions or/and bioisosteric replacements should considerably improve the interactions of these derivatives within the MurD active site and should have a beneficial impact on the inhibitory activities of these compounds against MurD. These compounds represent promising starting points for the development of MurD inhibitors with antibacterial activity.

5. Experimental Section

5.1. Inhibition Assay. The compounds were tested for their ability to inhibit the addition of D-[^{14}C]Glu to UMA in a mixture (final volume, 50 μL) containing 0.1 M Tris-HCl, pH 8.6, 5 mM $MgCl_2$, 25 μM UMA, 25 μM D-[^{14}C]Glu (50 000 cpm), 5% (v/v) DMSO, purified MurD from *E. coli*[40] (diluted with 20 mM potassium phosphate, pH 7.0, 1 mM DTT, 1 mg/mL BSA), and the test compound (compounds were soluble at all of the concentrations used in the enzyme assay mixture, containing 5% DMSO). The mixture was incubated for 30 min at 37 °C and the reaction stopped by adding 10 μL of glacial acetic acid. The mixture was lyophilized and taken up in HPLC elution buffer. The radioactive substrate and product were separated by reverse-phase HPLC with a Nucleosil 5C18 column (150 mm × 4.6 mm) as stationary phase and isocratic elution at a flow rate of 0.6 mL/min with 50 mM ammonium formate, pH 4.7. The compounds were detected and quantified with an LB 506 C-1 HPLC radioactivity monitor (Berthold France, Thoiry, France) using Quickszint Flow 2 scintillator (Zinsser Analytic, Maidenhead, U.K.) at 0.6 mL/min. The residual activities for each of the inhibitor concentrations were calculated with respect to a similar assay without inhibitor. The values are expressed as the mean of two independent experiments, and standard deviations were within ±10% of the mean values. The IC_{50} values were calculated from the fitted regression equation using the logit-log plot.

Control experiments were performed in the presence of Tween-20 (0.003%) to exclude nonspecific binding of inhibitors.[41] No significant differences were seen when compared with measurements without Tween-20.

5.2. Crystallization, Preparation of the Inhibitor Complexes, and Data Collection. Crystals were obtained from the 6×His-tagged enzyme as described previously[33] and were subsequently used for soaking with inhibitors by transfer to fresh reservoir solutions containing 2 mM inhibitor and 5% DMSO. After 5 h of soaking, the crystals were flash cooled in liquid nitrogen using Paratone oil as a cryoprotectant. All of the crystals tested belonged to space group $P4_1$ and had one molecule per asymmetric unit. Data sets were collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) BM30A, ID14-EH1, and ID29 beamlines.

5.3. Materials and Methods. Chemistry. Chemicals from Sigma-Aldrich, Acros Organics, and TCI-Europe 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). Compounds were visualized with ultraviolet light. Column chromatography was carried out on silica gel 60 (particle size 240−400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ or DMSO-d_6 solution, with TMS as the internal standard. IR spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer. Optical rotation was measured on a Perkin-Elmer 1241 MC polarimeter. Microanalyses were performed on a Perkin-Elmer C, H, N analyzer 240 C. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. LC−MS samples were analyzed with an ACQUITY UPLC separations module interfaced to a Waters Quattro Micro API mass spectrometer.

5.4. Experimental Procedures. Example for the Synthesis of Naphthalene-*N*-sulfonyl-D-Glu Analogues 17a−o. These compounds were prepared according to the five-step sequence outlined below.

Step 1. Anhydrous ethanol (80 mL) was put in a dry flask fitted with a reflux condenser and calcium chloride guard tube. Sodium (1.9 g, 82.6 mmol) was added portionwise to it to form sodium ethanolate. After the solution was cooled to room temperature, 6-hydroxy-2-naphthalenesulfonic acid sodium salt (**13**) (10.0 g, 40.6 mmol) was added and the reaction mixture was stirred for 2 h. The mixture was then filtered and the solid residue washed with MeOH to provide sodium 6-oxidonaphthalene-2-sulfonate (quantative), which was used in the next step without further purification.

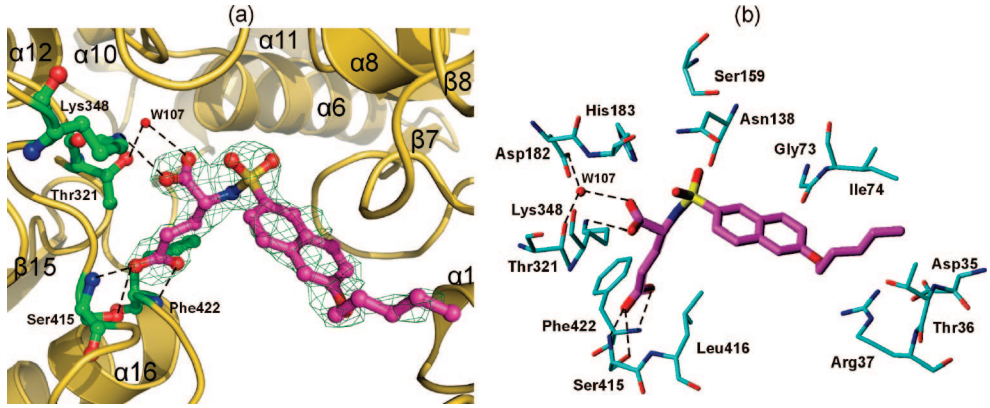


Figure 2. (a) Details of the ligand-binding site in the MurD complex with compound **17d**. The $F_o - F_c$ residual map is contoured at 3.0σ (green). (b) Binding mode of compound **17d** in the active site of *E. coli* MurD.

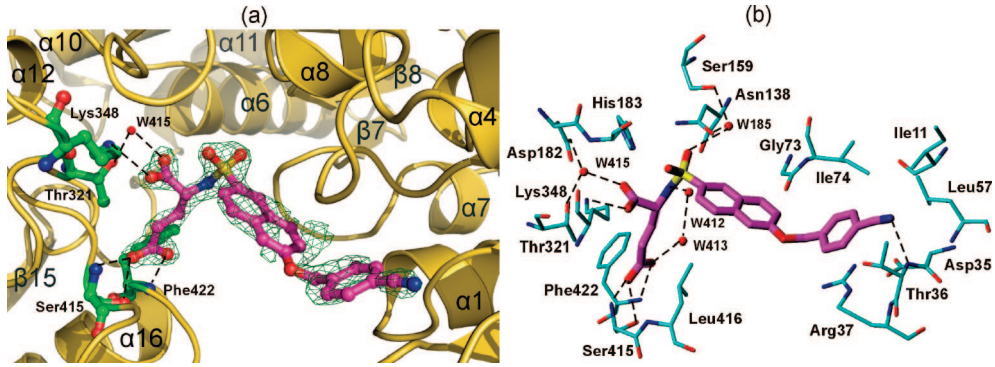


Figure 3. (a) Details of the ligand-binding site in the MurD complex with compound **17l**. The $F_o - F_c$ residual map is contoured at 3.0σ (green). (b) Binding mode of compound **17l** in the active site of *E. coli* MurD.

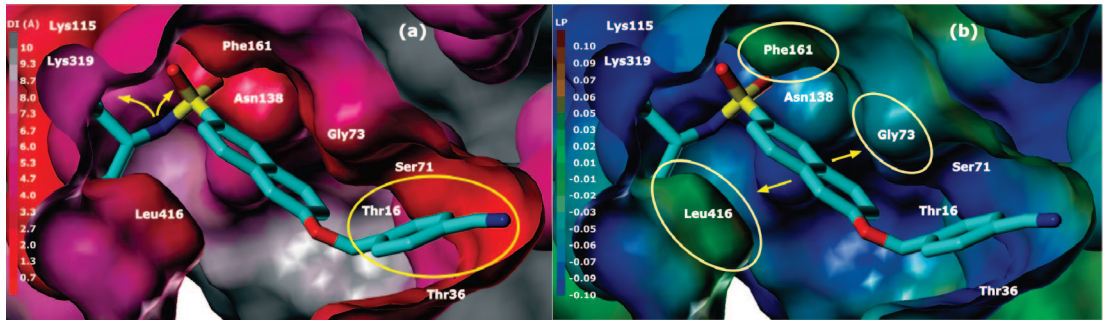


Figure 4. Possible directions of optimization of compound **17l**. The protein surface is colored in accordance with the protein−ligand distance (the legend in Å) (a) and by the calculated values of the lipophilicity potential (b).

Step 2. Sodium 6-(4-Cyanobenzyloxy)naphthalene-2-sulfonate (14l). To a solution of sodium 6-oxidonaphthalene-2-sulfonate (2.97 g, 11.10 mmol) generated in step 1 in DMSO (20 mL), 4-(bromomethyl)benzonitrile (4.35 g, 22.20 mmol) was added. The reaction mixture was stirred at room temperature overnight and then poured into ice-cold acetone (200 mL) with continuous stirring. The precipitated product was filtered off and washed with ice-cold acetone to provide compound **14l**, which was used without further purification. White solid (4.01 g, 98%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.35 (s, 2H, CH_2), 7.28 (dd, J = 9.0, 2.5 Hz, 1H, naph-H), 7.42 (d, J = 2.5 Hz, 1H, naph-H), 7.64−7.78 (m, 4H, naph-H and Ar−H), 7.83−7.95 (m, 3H, naph-H and Ar-H), 8.10 (s, 1H, naph-H); MS m/z 384 $(M + Na)^+$.

Step 3. 6-(4-Cyanobenzyloxy)naphthalene-2-sulfonyl Chloride (15l). To an ice-cooled suspension of the above sodium sulfonic acid **14l** (775 mg, 2.15 mmol) in DMF (10 mL), thionyl chloride was slowly added (0.32 mL, 4.40 mmol). The resulting mixture was stirred at room temperature for an additional 3 h. The reaction was then quenched by pouring onto crushed ice with continuous stirring, and the resulting mixture was filtered to provide the title compound **15l**, which was used in the next step without further purification.

Step 4. (*R*)-Dimethyl 2-(6-(4-cyanobenzyloxy)naphthalene-2-sulfonamido)pentanedioate (16l). Sulfonyl chloride **15l** (720 mg, 2.01 mmol) was dissolved in CH_2Cl_2 (10 mL), filtered to remove undissolved material if any, and added to a mixture of HCl·D-Glu(OMe)$_2$ (550 mg, 2.60 mmol) and Et_3N (0.6 mL, 4.30 mmol) in CH_2Cl_2 (10 mL). The resulting mixture was stirred overnight, quenched with ice-cold 2 M HCl (10 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting residue was purified by column chromatography to provide **16l**. White solid (224 mg, 21%, two steps); mp 163−165 °C; $[\alpha]_D^{23}$ −19.1 (c 0.287, CH_2Cl_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60−1.78 (m, 1H, CHC$\underline{H_2}$), 1.79−1.95 (m, 1H, CHC$\underline{H_2}$), 2.16−2.36 (m, 2H, CH_2COO), 3.30 (s, 3H, CH_3, overlaps with water), 3.40 (s, 3H, CH_3), 3.78−3.97 (m, 1H, CH), 5.40 (s, 2H, OCH_2), 7.41 (dd, J

= 9.0, 2.4 Hz, 1H, naph-H), 7.55 (d, J = 2.4 Hz, 1H, naph-H), 7.66−7.77 (m, 3H, 1 × naph-H and 2 × Ar-H), 7.86−7.93 (m, 2H, Ar-H), 7.97 (d, J = 8.8 Hz, 1H, naph-H), 8.10 (d, J = 9.0 Hz, 1H, naph-H), 8.30 (d, J = 1.6 Hz, 1H, naph-H), 8.37 (d, 1H, J = 7.5 Hz, NH); MS m/z 497 $(M + H)^+$. Anal. ($C_{25}H_{24}N_2O_7S$) C, H, N.

Step 5. *N*-(6-(4-Cyanobenzyloxy)naphthalene-2-sulfonamido)-D-glutamic Acid (17l). To a stirred solution of protected sulfonamide **16l** (124 mg, 0.25 mmol) in dioxane (2 mL), 1 M NaOH (2 mL) was added, and the reaction mixture was stirred for 3 h. The reaction mixture was diluted with H_2O (15 mL) and washed with EtOAc (2 × 15 mL). The aqueous phase was acidified to pH 1−2 using 2 M HCl and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (1 × 20 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure to provide the target compound **17l**. White solid (105 mg, 90%); mp 180−185 °C; $[\alpha]_D^{23}$ −16.0 (c 0.225, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.58−1.75 (m, 1H, CHC$\underline{H}_2$), 1.76−1.94 (m, 1H, CHC$\underline{H}_2$), 2.21 (t, J = 7.4 Hz, 2H, CH_2COO), 3.77−3.90 (m, 1H, CH), 5.40 (s, 1H, OCH_2), 7.40 (dd, J = 9.0, 2.4 Hz, 1H, naph-H), 7.54 (d, J = 2.4 Hz, 1H, naph-H), 7.57−7.79 (m, 3H, 1 × naph-H and 2 × Ar-H), 7.86−7.99 (m, 3H, 1 × naph-H and 2 × Ar-H), 8.08 (d, J = 9.0 Hz, 1H, naph-H), 8.31 (d, J = 1.6 Hz, 1H, naph-H); MS m/z 469 $(M + H)^+$. Anal. ($C_{23}H_{20}N_2O_7S$) C, H, N.

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Supporting Information Available: Description of crystal structure solution and model refinement, full synthetic experimental section, and microanalysis and HRMS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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