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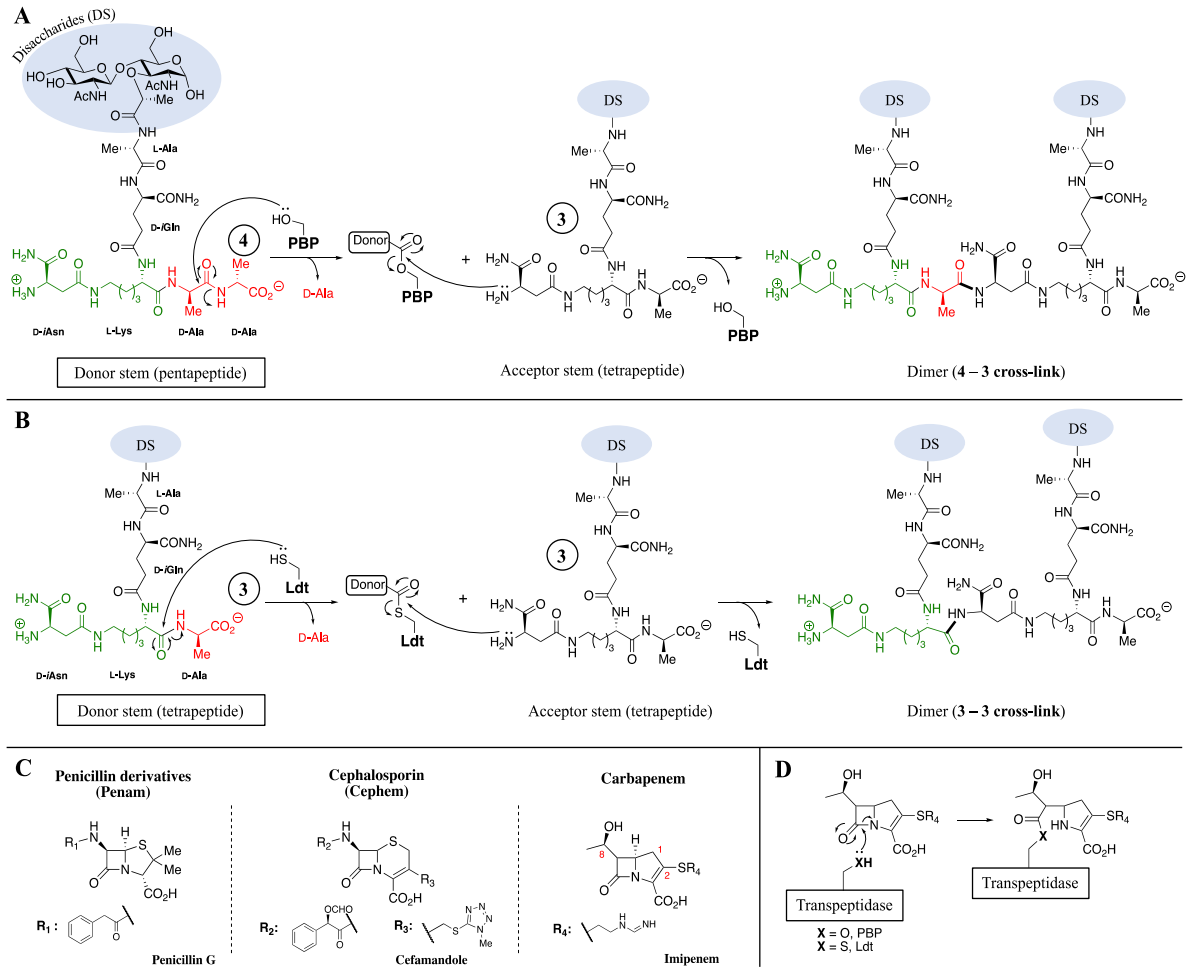
Abstract: The carbapenem class of β-lactams has been optimized against Gram-negative bacteria producing extended-spectrum β-lactamases by introducing substituents at position C_2. Carbapenems are currently investigated for the treatment of tuberculosis since these drugs are potent covalent inhibitors of L,D-transpeptidases involved in mycobacterial cell wall assembly. We sought to optimize carbapenems for inactivation of these unusual targets by exploiting the nucleophilicity of the C_8 hydroxyl group to introduce chemical diversity. Since β-lactams are structure analogues of peptidoglycan precursors, the substituents were chosen to increase similarity between the drug and the substrate. Fourteen peptido-carbapenems were efficiently synthesized. They were more effective than the reference drug, meropenem, due to the positive impact of a phenethylthio substituent introduced at position C_2 but the peptidomimetics added at position C_8 did not further improve activity. Thus, position C_8 can be modified to modulate the pharmacokinetic properties of highly efficient carbapenems.

Introduction

The biosynthesis of peptidoglycan (PG), the major constituent of the bacterial cell wall, has been intensively studied to identify targets for drug development. A number of inhibitors of the constitutive enzymes have been described,[1,2] the most successful as antibiotics being the β-lactams. These compounds inhibit the enzymes that catalyze the final transpeptidation step in which adjacent glycan chains made of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) are linked to each other by formation of an amide bond between

stem peptides carried by MurNAc residues (Scheme 1A). D,D-transpeptidases, commonly referred to as penicillin-binding proteins (PBPs), catalyze the formation of 4→3 cross-links connecting the fourth position of an acyl donor stem peptide to the side chain amino group of a di-amino acid located at the third position of an acyl acceptor stem peptide. Unusual 3→3 cross-links formed by unrelated L,D-transpeptidases[3] (Ldts) (Scheme 1B) are predominant in mycobacteria[4] and in β-lactam-resistant mutants of *Enterococcus faecium*[5] and *Escherichia coli*[6] selected *in vitro*.



Scheme 1. Transpeptidation reactions with the PG subunit of *E. faecium*, catalyzed by A) PBPs and B) Ldts. C) Examples of drugs belonging to the three main classes of β-lactams. D) Formation of the covalent adduct (acyl-enzyme) resulting from nucleophilic attack of a carbapenem by the active-site serine of PBPs or the active-site cysteine of Ldts.

In the β-lactam family, members of the carbapenem class (imipenem, meropenem, ertapenem, and doripenem) have been developed and optimized for the treatment of infections due to Gram-negative bacteria producing extended-spectrum β-lactamases active on third-generation

cephalosporins (Scheme 1C).[7] Optimization relied on introduction of various substituents at the C_2 position and of a methyl at position C_1. The latter modification increases the stability of the carbapenems with respect to hydrolysis by a host enzyme, albeit at the expense of a reduced reactivity of the drugs.[8] Carbapenems have been shown to be active against *Mycobacterium tuberculosis in vitro*[9,10] and *in vivo*[11] and are part of the recommended treatment of pulmonary infections due to *Mycobacterium abscessus* in cystic fibrosis patients.[12,13] The antibacterial activity of carbapenems against *M. tuberculosis* and *M. abscessus* involves inactivation of multiple targets including PBPs and Ldts.[14-16] The drugs irreversibly inactivate these enzymes by formation of a long-lasting covalent adduct resulting from nucleophilic attack of the β-lactam carbonyl by the active-site serine of PBPs or by the active-site cysteine of Ldts (Scheme 1D).[17-19]

β-Lactams are structure analogues of the PG precursors and act as suicide substrates of the transpeptidases. This conclusion was initially based on the pioneering work of Tipper and Strominger reported in 1965, who noticed that one of the possible conformations of the terminal D-Ala^4-D-Ala^5 dipeptide of stem pentapeptide is similar to that of penicillin (Figure 1A).[20] By analogy, we present a carbapenem with a conformation that simulates the D-Ala^4-D-Ala^5 (Figure 1A) or L-Lys^3-D-Ala^4 (Figure 1B) extremity of the pentapeptide or tetrapeptide stem peptides, which are used as acyl donor by transpeptidases of the D,D and L,D specificities, respectively.

Several groups have investigated the possibility of modifying the structure of the substituents linked to the bicyclic core of β-lactams to improve drug binding and antibacterial activity. This was investigated not only with D,D-transpeptidases but also with other members of the "D,D-peptidase" super family that share a common fold and catalytic mechanism but perform distinct functions in PG metabolism (Figure 2). This diversity is relevant to the current study since no general conclusion could be drawn for the various representatives of the D,D-peptidases subclasses. The premise of these investigations was that grafting groups resembling to PG precursors onto D-Ala-D-Ala and β-lactams should lead to parallel increases in the catalyzed acylation reaction rates with PG precursor analogues and β-lactams. The substituents of these peptidomimetics were designed to mimic the side or main chain of the PG precursors (in green and in blue in Figure 1C). Of note, the structure of the main chain is conserved whereas that of the side chain is variable in different bacterial species. Parallel increases in the acylation rates with peptidomimetic substrates and β-lactams were observed for the R61 D,D-peptidase isolated form a bacterial strain belonging to *Streptomyces genus* (L,L-DAP-Gly side chain). R61 is a class C low-molecular weight D,D-peptidase of uncertain function with respect to PG metabolism. *In*

vitro, purified R61 catalyzes formation of 4→3 cross-links (D,D-transpeptidase activity) and hydrolysis of D-Ala^5 (D,D-carboxypeptidase activity) by using as substrates synthetic peptides containing the terminal acyl-D-Ala-D-Ala moiety of PG precursors (Figure 1C).[22] The known structure of the PG of *Streptomyces spp*. implies that R61 functions *in vivo* with branched PG precursors composed of a linear stem peptide (L-Ala-γ-D-Glx-L,L-DAP-D-Ala-D-Ala) and a side chain composed of a single Gly residue linked to the ε amino group of L,L-DAP (Figure 1C).[23] Pratt *et al*. explored the impact of grafting the L,L-DAP(Gly) dipeptide onto D-Ala-D-Ala and representative β-lactams of the penam (penicillin) and cephem classes (Figure 1C). Introduction of the peptidoglycan-mimetic side chain specific of the *Streptomyces spp*. led to dramatic parallel increases in the activity of the R61 D,D-peptidase with PG precursor analogues and in the reactivity of the enzyme with β-lactams.[21]

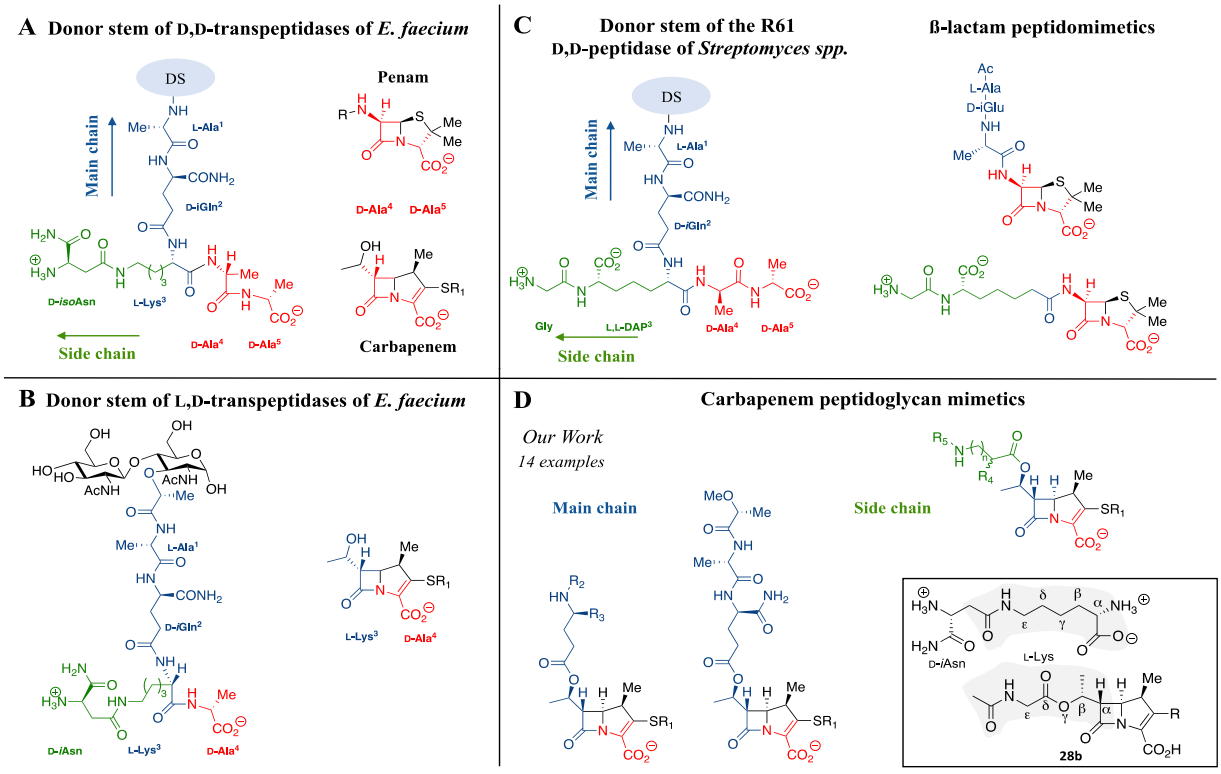


Figure 1. A) Structural similarity between β-lactams (penams and carbapenems) and the pentapeptide donor stem of PBPs. B) Structural similarity between carbapenems and the tetrapeptide donor stem of Ldts. C) Structure of the peptidoglycan subunit used by the R61 D,D-peptidase in *Streptomyces spp*. and peptidomimetic penams.[21] The third position of the stem pentapeptide is occupied by an L,L-diaminopimelyl (DAP) residue substituted by a Gly residue. D) Structure of peptidomimetic carbapenems synthesized in this study. On the left: the substituents of the carbapenem core mimic the main chain of the PG subunit of *E. faecium* (shown in blue). On the right: the substituents of the carbapenem core mimic the side chain of the PG subunit of *E. faecium* (shown in green). R_1 = -S$(CH_2)_2$Ph; R_2 = H, L-Ala or L-Ala-D-Lac; R_3 = H or $CONH_2$; R_4 = H, Me or NH_2; R_5 = H, Ac; n = 0-4. Inset: Structural analogy between carbapenem 28b and the L-Lys-D-iAsn moiety of peptidoglycan precursors.

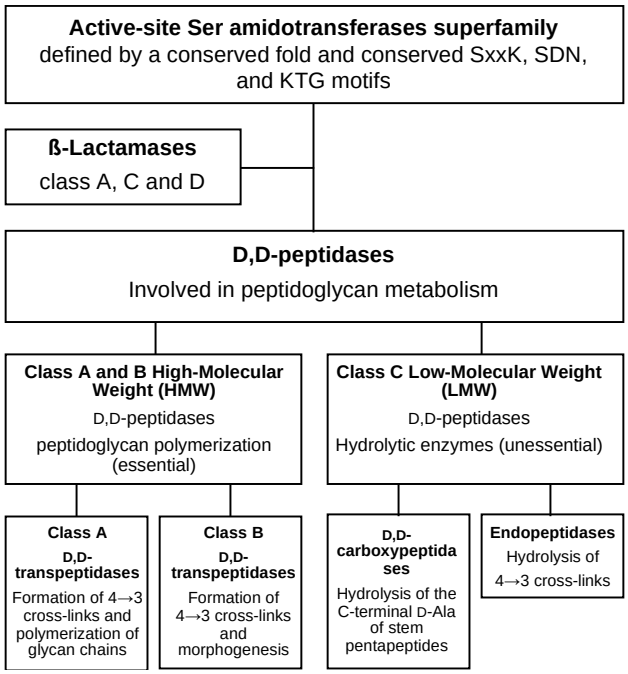


Figure 2. Classes of amidotransferases.

The inactivation rate constant of the R61 D,D-peptidase by the penicillin peptidomimetic containing the L,L-DAP(Gly) motif (1.5×10^7 $s^{-1}.M^{-1}$) was three orders of magnitude higher than that of penicillin (1.4×10^4 $s^{-1}.M^{-1}$), approached the diffusion limit, and was the largest rate constant yet reported for inactivation of a D,D-peptidase. The fact that the L,L-DAP(Gly) substituent of the L,L-DAP(Gly)-penicillin actually acted as a peptidomimetic was confirmed by the crystal structures of R61 in complex with L,L-DAP(Gly)-penicillin and L,L-DAP(Gly)-D-Ala-D-Ala.[24,25] These structures showed that the L,L-DAP(Gly) moieties of these molecules occupy nearly identical positions in the R61 catalytic cavity and form the same interactions with enzyme residues.

These observations suggest that the "perfect" penicillin with respect to inactivation of the R61 D,D-peptidase was obtained by the peptidomimetic approach.[21] Similar results, although quantitatively less impressive, were obtained with other peptidomimetic specific of the various PG chemotypes but this only concerned class C D,D-peptidases[26-29] and the side chain of peptidoglycan precursors.[26,30,31]

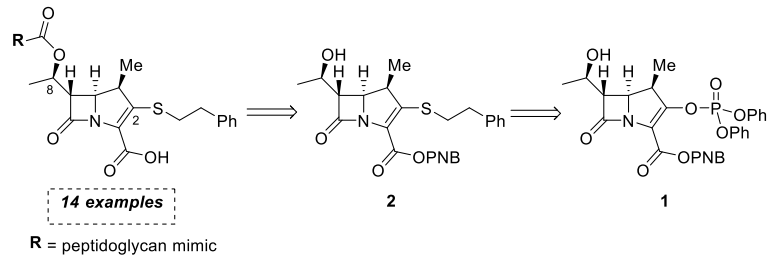
The group of Mobashery explored the bi-substrate approach by synthesizing a complex molecule consisting of a cephalosporin core substituted by mimics of the donor and acceptor stems of the transpeptidation reaction. Crystallization of the R61 D,D-peptidase acylated by this cephalosporin resulted in a model for the binding of the donor and acceptor substrates of the transpeptidation reaction.[32,33] The same approach adapted to the structure of the *E. coli* peptidoglycan structure led to a cephalosporin that behaved as a time-dependent and irreversible

inhibitor of *E. coli* PBP1b, a Class A D,D-transpeptidase (first-order inactivation rate constant k_{inact} of 0.0072 ± 0.0007 min^{-1} and inhibitor constant K_i of 2.5 ± 0.8 mM).[33]

The peptidoglycan mimetic approach has only been applied to D,D-transpeptidases of the PBP family and to β-lactams of the penam (penicillin) and cephalosporin classes. This prompted us to design and develop the synthesis of carbapenem-based peptidoglycan mimetics and to evaluate their efficacy in the inactivation of the prototypic L,D-transpeptidase from *E. faecium*.[3,18,19] In the literature, the two main synthetic strategies providing access to functionalized carbapenems are either based on (i) the formation of the 4,5-fused nuclei from acyclic precursors (*i.e.* the carbapenem core including the ß-lactam ring)[34] or (ii) a direct addition of functionalities at the C_2 and C_8 positions on the preformed skeleton.[35] In this study, we took advantage of the nucleophilicity of the hydroxyl group at position C_8 to connect various PG fragments *via* the formation of an ester bond. The resulting carbapenem-based peptidoglycan mimetics were divided into two categories containing portions of either the main chain or the side chain of the *E. faecium* PG subunits (Figure 1D, in blue and green, respectively). A functionalized carbapenem with a phenethylthio group at the C_2 position was chosen as the model building block for modifications at C_8 since previous work indicated that the latter led to effective inactivation of Ldts.[36]

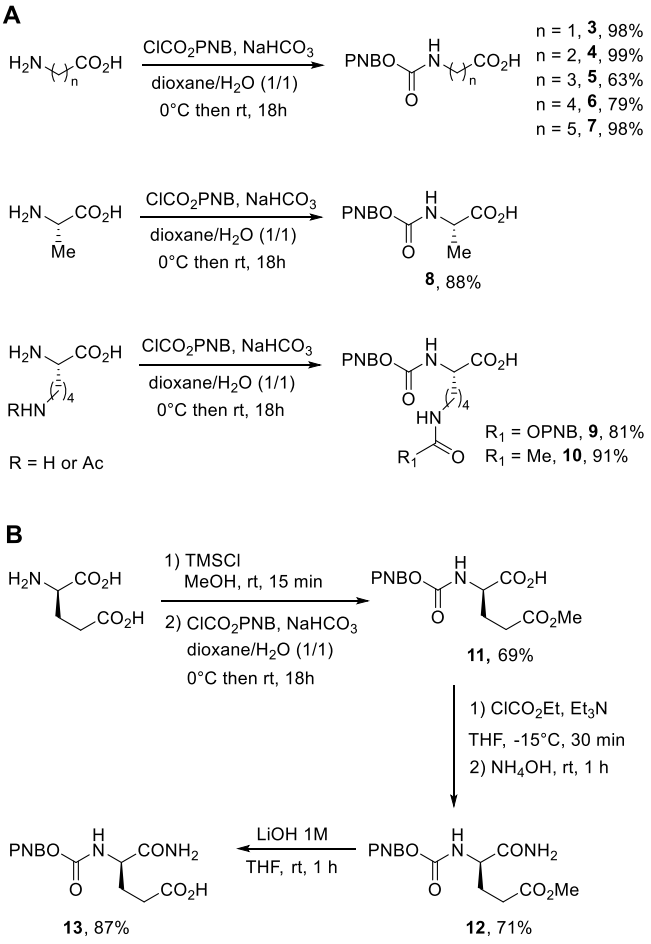
Results and Discussion

Synthesis of carbapenem-based peptidoglycan mimetics (peptido-carbapenems). The functionalized carbapenems were synthesized according to the retrosynthesis outlined in Scheme 2 starting from the commercially available β-methyl-vinyl-phosphate **1**. The phenethyl side chain was introduced at the C_2 position by nucleophilic substitution of the phenyl-phosphate.[36] The protected amino acids or peptides mimicking various moieties of the stem peptide of PG precursors were incorporated *via* esterification of the C_8 hydroxyl group. In this strategy, the *p*-nitrobenzyl (PNB) carbamate group was chosen as the main protecting group for the synthesis of peptidoglycan mimetics, enabling a single final deprotection step. Of note, the initial syntheses of the former derivatives conducted with benzyl and benzyloxycarbonyl protecting groups were unsuccessful since these groups could not be efficiently removed during the final deprotection step.



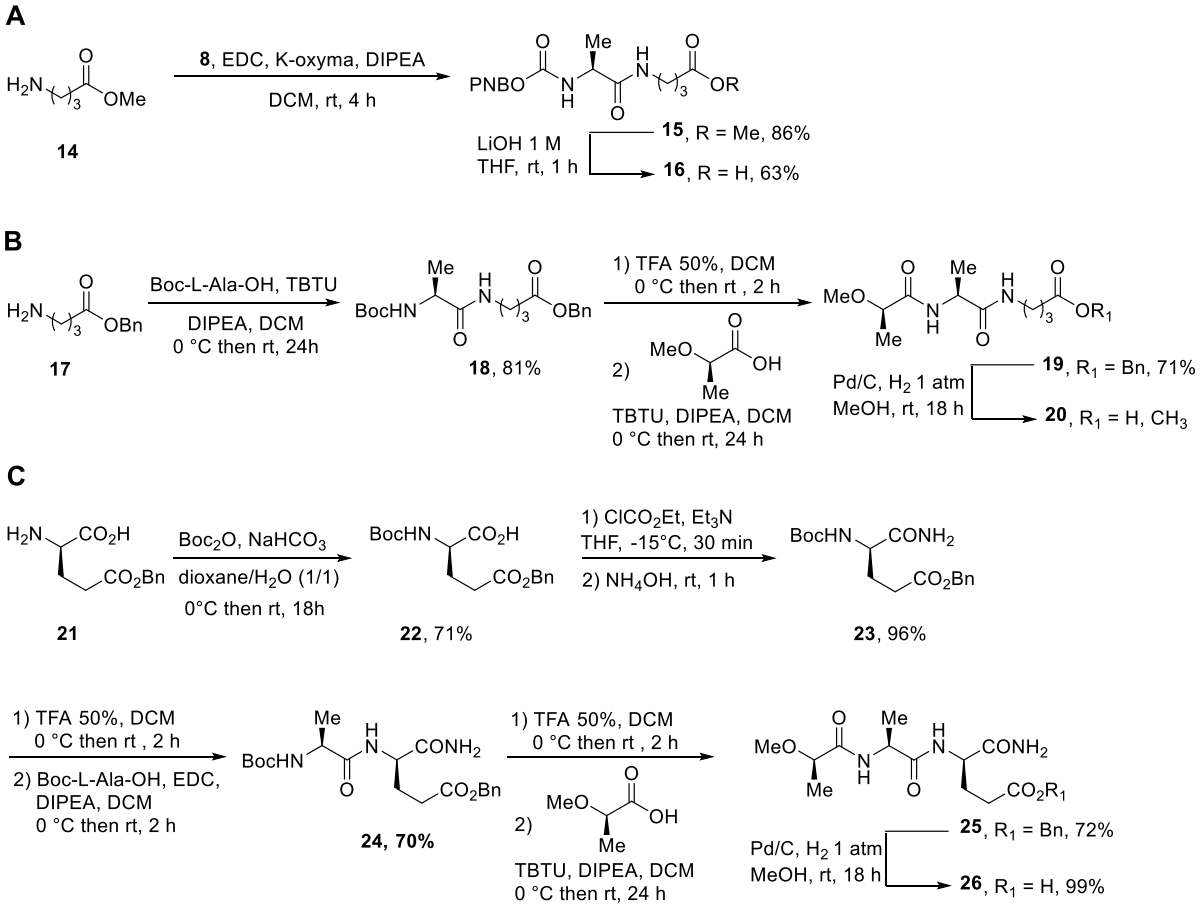
Scheme 2. Retrosynthesis of peptido-carbapenems from **1**.

The general procedure for *N*-CO_2PNB protection were found to be highly efficient and versatile providing the protected amino acids and analogues **3** to **10** in 63% to 99% yield (Scheme 3A). D-iso-glutamine derivative **13** was prepared in a three-step procedure (Scheme 3B). The γ-carboxylic acid of D-glutamic acid was selectively protected using trimethylsilyl chloride and methanol[37] followed by the introduction of the PNB group onto the amine affording amino acid **11** in 69% yield. The carboxylic acid was then activated with ethyl chloroformate and the reaction with aqueous ammonia provided compound **12** in 71% yield. In the last step, deprotection of the carboxylic acid was performed with LiOH affording the D-iso-glutamine derivative **13** in 87% yield.



Scheme 3**.** A) Synthesis of *N*-CO_2PNB carbamates protected compounds **3-10**. B) Synthesis of compound **13**.

Peptidoglycan mimetic **16** was obtained by using as the starting material 4-aminobutyric acid, which was first converted into the corresponding methyl ester **14**.[38] Coupling of **14** with alanine **8** assisted by K-oxyma® and EDC as coupling reagents afforded **15** in 86% yield. Deprotection of the methyl ester was performed with LiOH leading to the corresponding acid **16** in 63% yield (Scheme 4A). The synthesis of dipeptide derivative **20** started with the formation of the amino benzyl ester **17** as previously described.[39] Coupling with Boc-L-Ala-OH was achieved using TBTU as the coupling agent. Compound **18** was treated with TFA to remove the Boc group and reacted with (*2R*)-2-methoxypropanoic acid affording compound **19** in 71% yield. The final deprotection step was achieved by hydrogenolysis in methanol providing acid **20** in 76% yield (calculated by NMR) together with the corresponding methyl ester (Scheme 4B).



Scheme 4. Synthesis of peptidomimetics **16** (A), 20 (B) and 26 (C).

To obtain peptide **26** (Scheme 4C), the γ-benzylated D-glutamic acid **21** was protected with a Boc group affording **22** in 71% yield. Subsequent amidation afforded **23** in 96% yield. The *N*-Boc group of **23** was cleaved and coupling with Boc-L-Ala-OH afforded **24** in 70% yield. Compound **24** was treated with TFA and coupling with (*2R*)-2-methoxypropanoic acid afforded

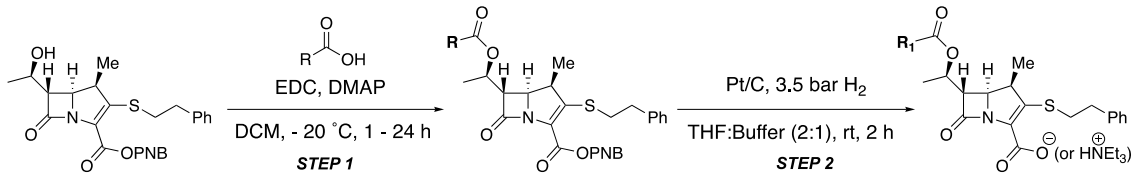
25 in 72% yield. Final deprotection of the benzyl ester was achieved by hydrogenolysis leading to peptide **26** in almost quantitative yield.

The synthesis of peptido-carbapenems **28a**-**n** was achieved in two steps (Table 1). Esterification of carbapenem **2** was first conducted with the PG analogues using a published procedure[40] affording carbapenems **27a-n** in 35 to 99% yield. Finally, the peptido-carbapenems **28a**-**n** were obtained by hydrogenolysis of **27a**-**n** after HPLC purification. Using this approach, a full mimetic of the main chain was obtained (**28n**). In contrast, the best mimic of the side chain was **28b** (inset in Figure 2D) since incorporation of the D-iAsn extremity of peptidoglycan precursor in the carbapenem could not be obtained due to cyclisation of D-iAsn during the synthesis of the L-Lys-D-iAsn dipeptide.

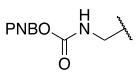
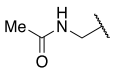
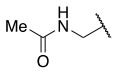
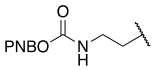
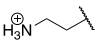
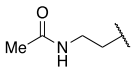
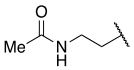
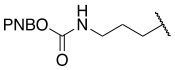
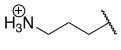
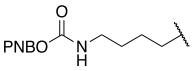
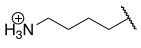
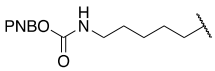
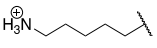
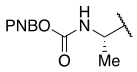
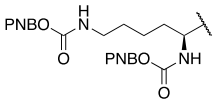
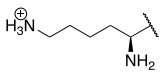
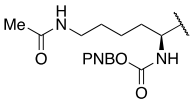
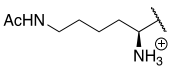
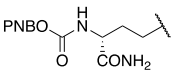
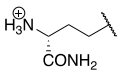
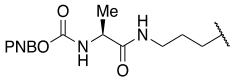
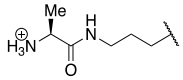
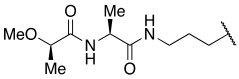
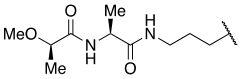
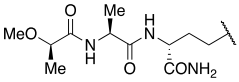
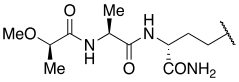
Efficacy of acylation of the Ldt_{fm} L,D-transpeptidase by the peptidomimetic carbapenems. Inactivation of L,D-transpeptidases was previously shown to be a two-step reaction[8] starting with nucleophilic attack of the β-lactam carbonyl by the sulfur of the catalytic cysteine (Scheme 5). This first step was proposed to lead to reversible formation of an amine anion.[41] The second step is irreversible, except for the cephalosporin nitrocefin, due to rupture of the β-lactam C-N bond followed by protonation of the nitrogen in the case of carbapenems.[42,43] Mass spectrometry analyses showed that all carbapenems synthesized in this study formed the expected acylenzymes without any secondary modification of the compound following the acylation step, as is the case for other classes of β-lactams (Table 2).[10,17] These results also indicated that the ester link at the C_8 position was not hydrolyzed in the Ldt_{fm} active site. This was further confirmed by incubating peptido-carbapenem **28g** in the presence or absence of Ldt_{fm} for 0, 90, and 240 minutes. Loss of 114,2 Da expected for the hydrolysis of the ester at C_8 was observed in neither cases.

The efficacy of Ldt_{fm} inactivation by peptidomimetic carbapenems was evaluated by determining the k_1 and k_2 kinetic constants for the formation of the amine anion and the acylenzyme (Scheme 5), respectively.[8,17,41,44] These kinetic constants were determined by stopped-flow fluorimetry as the intrinsic fluorescence of the Ldt_{fm} tryptophan residues varies for the free enzyme, the amine anion, and the acylenzyme.[41] Fluorescence kinetics were determined with a minimum of three concentrations for each carbapenem. The rate constants k_1, k_{-1} and k_2 were determined by fitting differential equations to these kinetics using the DynaFit software,[45] as described in section 5 of the Supplementary Information. Peptido-carbapenems **28a** to **28n** harbored a phenethylthio substituent at position C_2. In comparison to the reference carbapenem, meropenem, the phenethyl side chain improved both the k_1 and k_2

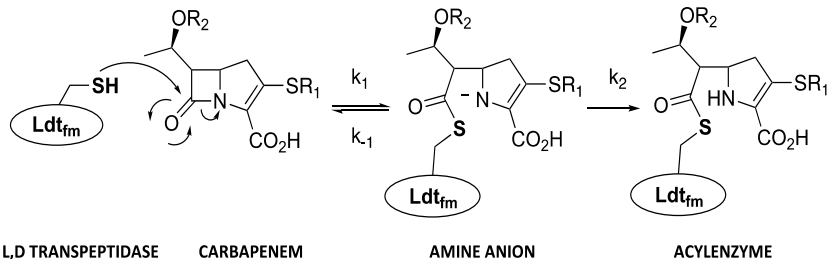
Table 1. Synthesis of peptido-carbapenems **28a-n**.



2 **27a-n** **28a-n**

	STEP 1 - Coupling			*STEP 2* - Deprotection		
	**Cpd**	**R**	**Yield[a] (%)**	**Cpd**	**R_1**	**Yield[d] (%)**
3	**27a**		89	**28a**		21
Ac-Gly-OH[b]	**27b**		93	**28b**		26
4	**27c**		96	**28c**		6
Ac-ß-Ala-OH[b]	**27d**		87	**28d**		40
5	**27e**		99	**28e**		40
6	**27f**		97	**28f**		6
7	**27g**		68	**28g**		17
8	**27h**		98	**28h**		8
9	**27i**		90	**28i**		9
10	**27j**		84	**28j**		32
13	**27k**		82	**28k**		14
16	**27l**		86	**28l**		15
20	**27m**		35[c]	**28m**		31
26	**27n**		87	**28n**		21

[a] Isolated yield. [b] Commercially available. [c] NMR calculated yield. [d] Isolated yield after HPLC purification.



Scheme 5. Two-step acylation of L,D-transpeptidase Ldt_{fm} by carbapenems. The evidence for the formation of amine anion in the first step of the acylation reaction is supported by previous studies.[8,43,44]

Table 2. Mass of adducts formed between Ldt_{fm} and carbapenems.

Cpd	Mass of carbapenem (Da)	Mass of adduct (Da)	
		Calculated	Observed
None	Not Applicable	16,639.3	16,640.2
Side chain mimetics			
28a	404.5	17 043.8	17 045.2
28b	446.5	17 085.8	17 087.9
28c	418.5	17 057.8	17 059.6
28d	460.5	17 099.9	17 100.7
28e	432.5	17 071.9	17 073.6
28f	446.6	17 085.9	17 088.2
28g	460.6	17 099.9	17 098.0
28h	418.5	17 057.8	17 059.1
28i	476.6	17 115.9	17 116.2
28j	517.6	17 156.9	17 158.7
Main chain mimetics			
28k	475.6	17 114.9	17 116.3
28l	503.6	17 142.9	17 143.6
28m	589.7	17 259.0	17 259.5
28n	632.7	17 272.1	17 273.4

kinetic parameters (compare meropenem and compound **2'** in Table 3). In comparison to **2'**, introduction of peptidomimetics at the C_8 position had minor negative impacts on the k_2 kinetic constant for all compounds (**28a** to **28n**). Thus, the peptidomimetics reduced the efficacy of the chemical step of the reaction involving the rupture of the β-lactam ring and protonation of its nitrogen atom. The peptidomimetics had minor positive and negative impacts on kinetic constant k_1, which is the critical constant determining the efficacy of Ldt_{fm} inactivation.[41]

These results show that substitutions at the C_8 position marginally modulate the efficacy of inactivation of Ldt_{fm}, as previously found for high-molecular weight PBPs.

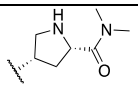
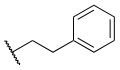
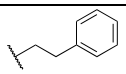
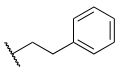
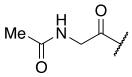
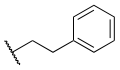
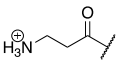
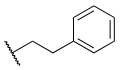
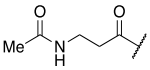
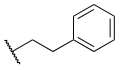
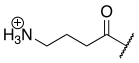
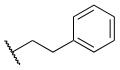
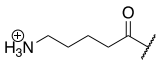
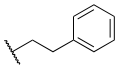
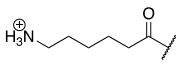
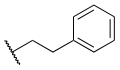
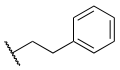
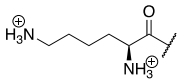
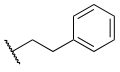
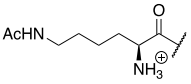
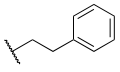
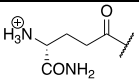
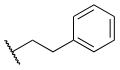
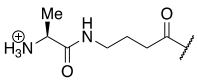
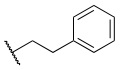
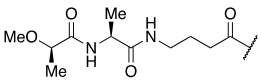
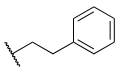
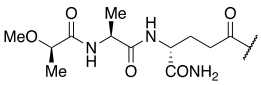
Antibacterial activity. Peptido-carbapenems were tested by the microdilution technique in 96-well plates against *E. faecium* strain M5 in the presence of ampicillin (32 µg/ml), as previously described.[46] Under such conditions, Ldt_{fm} is the only functional transpeptidase due to inhibition of PBPs by ampicillin.[47] The minimal inhibitory concentrations (MICs) of peptido-carbapenems **28a** to **28n** were equal to or higher than that of meropenem indicating that the peptidomimetic did not improve antibacterial activity.

Conclusion

We show that the C_8 position of carbapenems can be efficiently functionalized by esterification to introduce chemical diversity in carbapenems. We added to this position mimics of the side chain (L-Lys-D-iAsn) and main chain (D-Lac-L-Ala-D-iGln-L-Lys) of peptidoglycan precursors from *E. faecium*. Biological evaluation of the resulting series of 14 peptido-carbapenems indicated that increasing similarity between the drug and the stem peptide of PG precursors does not improve the efficacy of inactivation of the L,D-transpeptidase produced by this species. Similar negative results were previously reported for other species-specific β-lactam peptidomimetics designed to inhibit the transpeptidase activity of HMW class A and B PBPs (see introduction section). As proposed by Josephine *et al*.,[27] this could be accounted for by the mode of substrate recognition by class A and B D,D-transpeptidases that may involve regions of the enzyme other than the transpeptidase active site and the glycan chains rather than the stem peptides. Such interactions were identified by solid-state NMR analysis of an L,D-transpeptidase from *Bacillus subtilis*.[48]

The carbapenems reported in this study were more effective than the reference drug of this class, meropenem, due to the positive impact of the phenethylthio substituent at position C_2 combined to the mostly neutral impact of the substituents at position C_8. Thus, the latter position can be modified to modulate the pharmacokinetic or other properties of carbapenems without any deleterious effect on the efficacy of $_{L,D}$-transpeptidase inactivation or antibacterial activity. This strategy could be used to optimize carbapenems for the treatment of lung infections due to *Mycobacterium tuberculosis* and *Mycobacterium abscessus* since $_{L,D}$-transpeptidases are major contributors to peptidoglycan polymerization in these bacteria.[4,10,11,49,50]

Table 3. Efficacy of acylation of the Ldt_{fm} L,D-transpeptidase by meropenem and peptido-carbapenems and antibacterial activity against *E. faecium* M512.

Cpd	R_1	R_2	k_1 ($\mu M^{-1}min^{-1}$)[a]	k_2 (min^{-1})[a]	MIC ($\mu g\ ml^{-1}$)[b]
Meropenem		H	0.0819 ± 0.0004	1.59 ± 0.01	1
2'[c]		H	0.292 ± 0.004	14.8 ± 0.18	2
Side chain Mimetics					
28a			0.248 ± 0.004	5.10 ± 0.05	4
28b			0.347 ± 0.010	4.68 ± 0.08	16
28c			0.572 ± 0.007	7.40 ± 0.06	2
28d			0.343 ± 0.009	3.92 ± 0.06	16
28e			0.268 ± 0.003	7.49 ± 0.07	4
28f			0.109 ± 0.001	3.16 ± 0.04	8
28g			0.138 ± 0.002	3.43 ± 0.03	8
28h			0.379 ± 0.005	5.20 ± 0.05	1
28i			0.278 ± 0.005	3.80 ± 0.04	4
28j			0.168 ± 0.002	2.70 ± 0.02	8
Main chain Mimetics					
28k			0.239 ± 0.010	4.60 ± 0.11	4
28l			0.192 ± 0.001	4.79 ± 0.03	4
28m			0.355 ± 0.006	7.88 ± 0.11	8
28n			0.121 ± 0.002	9.07 ± 0.15	> 16

[a] The methods used to determine kinetics parameters k_1 and k_2 are described in *Section 4* of the *Supplementary Information*. [b] Median from three independent experiments. [c] Synthesis of carbapenem **2'** was previously described.[36]

Experimental Section

General procedure for the synthesis of peptido-carbapenems **27a-n** (Step 1). Carbapenem **2** (1 equiv.) in presence of peptidoglycan mimetic (4 equiv. up to 8 equiv.) was treated with DMAP (0.9 equiv.) and then with small portions of EDC (8 equiv. up to 16 equiv.) at – 20 ˚C. The reaction mixture was stirred for 1 – 24 hours while maintaining a temperature between – 30 to – 15 ˚C. After the completion of the reaction, EtOAc was added to dissolve the crude. The organic layer was washed with a sat. solution of $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by silica gel chromatography (cyclohexane:EtOAc or DCM:MeOH as eluent systems) to afford the desired peptido-carbapenem derivatives.

General procedure for the synthesis of peptido-carbapenems **28a-n** (Step 2). The corresponding carbapenem derivative (1 equiv.) was treated with Pt/C 10% wt. (1 equiv. mass.) in a mixture of THF:triethylammonium bicarbonate buffer (1 M, pH = 8.5) (2:1) and the reaction mixture was hydrogenated under 3.5 bars for 2 hours at room temperature using the PARR apparatus. The crude mixture was filtered through a celite pack to remove the catalyst. THF was removed under reduced pressure, and H_2O was added. The crude mixture was purified by HPLC using a solvent system consisting of H_2O and acetonitrile (linear gradient, 0 to 100% over 45 minutes) and the selected fractions were collected and lyophilized to afford the final peptido-carbapenem derivatives.

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