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Stereoselective synthesis of lanthionine derivatives in aqueous solution and their incorporation into the peptidoglycan of *Escherichia coli*

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ABSTRACT

The three diastereoisomers—(*R*,*R*), (*S*,*S*) and *meso*—of lanthionine were synthesized in aqueous solution with high diastereoselectivity (>99%). The (*S*) and (*R*) enantiomers of two differently protected sulfamidates were opened by nucleophilic attack of (*R*) or (*S*)-cysteine. Acidification and controlled heating liberated the free lanthionines. Using the same chemistry, an α-benzyl lanthionine was also prepared. The proposed method, which avoids the need of enrichment by recrystallization, opens the way to the labelling of these compounds with ^{35}S. Furthermore, in vivo bioincorporation into *Escherichia coli* W7 was studied. No incorporation of α-benzyl lanthionine was observed. In contrast, *meso*-lanthionine can effectively replace *meso*-diaminopimelic acid in vivo, while in the presence of (*R*,*R*)-lanthionine the initial increase of bacterial growth was followed by cell lysis. In the future, *meso*-[^{35}S]lanthionine could be used to study the biosynthesis of peptidoglycan and its turnover in relation to cell growth and division.

1. Introduction

meso-Lanthionine (*meso*-Lan, **1**, Fig. 1), the monosulfur analogue of *meso*-diaminopimelic acid (*meso*-A_2pm, **2**), is a non-proteinogenic diamino diacid. Since its discovery by Horn[1] and its synthesis by Du Vignaud,[2] Lan has been known to play the role of crosslinker in the peptidoglycan (PG) of several *Fusobacterium* species.[3] It is also an important constituent in a family of polypeptidic antibiotics known as lantibiotics.[4]

The PG surrounding bacterial cells is essential for their survival. It provides the cells with osmotic stability and has also a vital function in bacterial morphogenesis. The biosynthesis of PG starts in the cytoplasm by the formation of UDP-MurNAc from UDP-GlcNAc catalyzed by the enzymes MurA and MurB (Fig. 2).

After the successive addition of different amino acids, catalyzed by different Mur ligases ((*S*)-Ala/MurC, (*R*)-Glu/MurD; **2**/MurE, (*R*)-Ala-(*R*)-Ala/MurF), the MurNAc-moiety of UDP-MurNAc pentapeptide (Park's nucleotide) is transferred onto membrane-bound bactoprenyl phosphate by MraY. After reaction with UDP-GlcNAc under catalysis by MurG, the resulting product lipid II is translocated to the periplasmic side of the plasma membrane. Once there, it is used as a substrate for the polymerisation reactions catalyzed by the transglycosylase and transpeptidase activities of penicillin-binding proteins (PBPs) (Fig. 2).

In the biosynthesis of PG, MurE catalyzes the formation of UDP-MurNAc-tripeptide from UDP-MurNAc-dipeptide and **2** (Fig. 2).[5] Although the specificity of *Escherichia coli* MurE for **2** is very high, certain A_2pm analogues are in vivo and in vitro substrates, whereas others have an inhibitory effect.[6–9] Experiments have demonstrated that under certain circumstances, *meso*-Lan can replace *meso*-A_2pm in vivo (Fig. 1).[10–13]

Several syntheses of Lan or its analogues, performed in aqueous solution, are reported in the literature.[14–20] However, side reactions (racemisation,[17,21] elimination and Michael addition,[18] formation of isomeric products[16]) lead to low yields or tedious isolation. A more convenient way for a stereoselective synthesis of the Lan diastereomers is thus mandatory. The approach selected in our lab to reach this goal, starting from sulfamidates, is presented in Scheme 1.

Click chemistry is an important field of research in organic chemistry. As a model for a compound bearing a substituted aromatic, the preparation in aqueous solution of the α-benzyl

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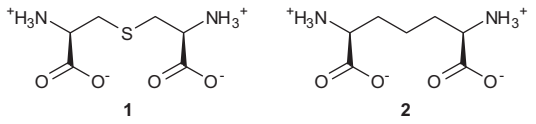


Figure 1. *meso*-Lan **1** and *meso*-A_2pm **2**.

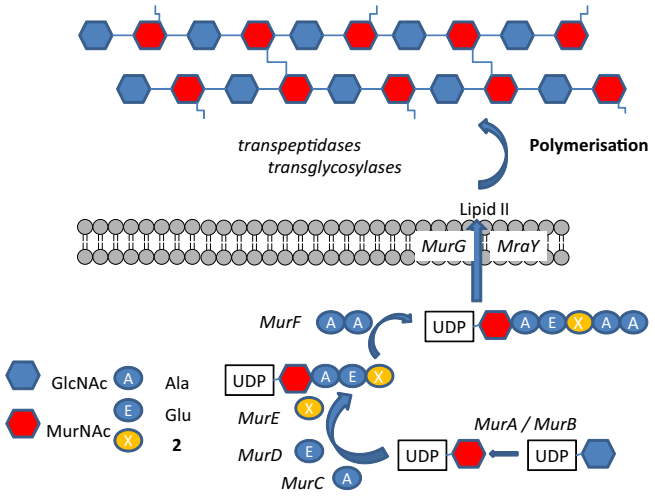


Figure 2. Metabolism of PG in *E. coli*. The biosynthesis of PG starts with the synthesis of UDP-MurNAc-pentapeptide from UDP-GlcNAc in the cytoplasm (enzymes: MurA, MurB, MurC, MurD, MurE and MurF). The second part, the synthesis of lipid II catalyzed by MraY and MurG, takes place at the level of the cytoplasmic membrane. Finally, the polymerisation reactions (transglycosylation and transpeptidation) takes place in the periplasm.

analogue Bn-Lan **3** (Scheme 1) is described. Results of attempts of incorporation of these molecules into *E. coli* PG are also presented.

2. Results and discussion

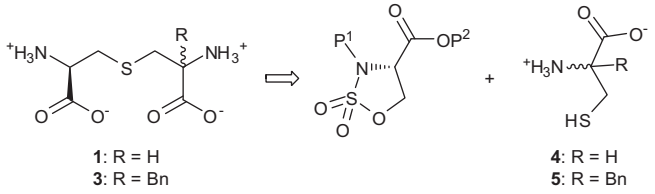
2.1. Chemical synthesis

2.1.1. Retrosynthetic analysis

Recent results published by Cobb and Vederas[22] describe the synthesis of protected lanthionines by opening of suitably protected sulfamidates under anhydrous conditions. Another research group has prepared *S*-linked glycosyl amino acids in aqueous solution from serine- and threonine-derived cyclic sulfamidates (such as **15**, Scheme 2).[23] According to these results, we envisioned that Lan **1** could result from the S_N2 opening of a sulfamidate precursor with cysteine **4** in water. By using α-benzyl cysteine **5**, the corresponding Bn-Lan **3** should be obtained (Scheme 1).

2.1.2. Synthesis of sulfamidates 10 and 14

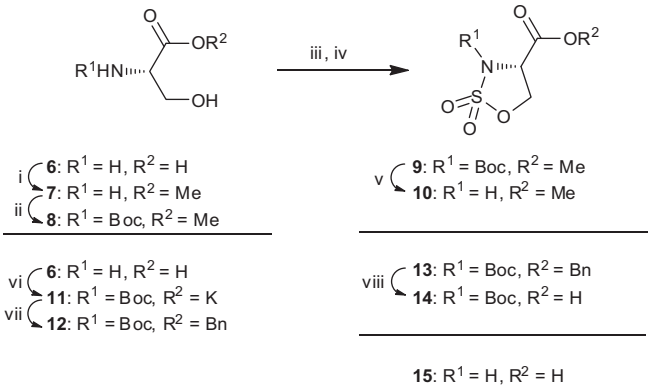
The main drawback of sulfamidate synthesis is the need to protect the nitrogen atom during cyclization and oxidation (steps iii and iv, Scheme 2).[24] Most of the time, an *N*-benzyl protection



1: R = H
3: R = Bn

4: R = H
5: R = Bn

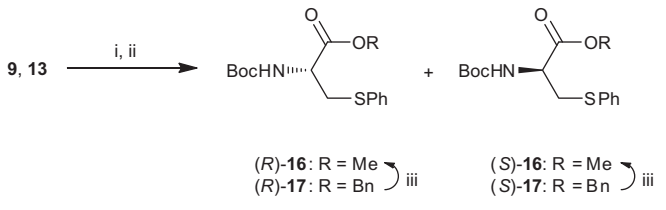
Scheme 1. Retrosynthetic approach to Lan **1** (R = H) and Bn-Lan **3** (R = Bn). P^1 = H, Boc; P^2 = Me, H (respectively).



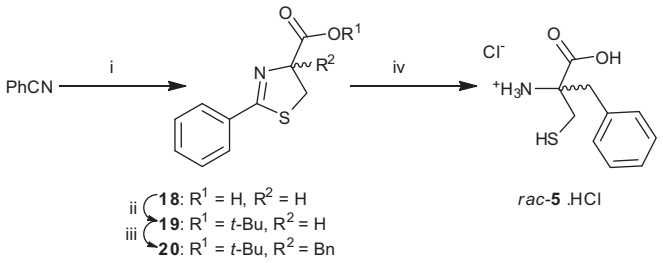
Scheme 2. Synthesis of cyclic sulfamidates derived from serine. Reagents and conditions: (i) AcCl, MeOH (99%); (ii) Boc_2O, Na_2CO_3 (pH 10), THF/H_2O (70%); (iii) $SOCl_2$, CH_3CN, pyridine; (iv) $NaIO_4$, cat. $RuCl_3$, CH_3CN/H_2O (33–68% two steps); (v) TFA, CH_2Cl_2 (81%); (vi) K_2CO_3, Boc_2O, THF/H_2O; (vii) BnBr, DMF (93% for two steps); (viii) H_2, cat. Pd/C (97%).

strategy is used.[25] However, Boc-sulfamidate **9** has been synthesized[26] from Boc-serine methyl ester **8** using the method of Posakony et al.[27] Likewise, Boc-threonine benzyl ester was prepared following the said procedure.[28] As the Boc protection is particularly convenient to remove (TFA or HCl), this protective group was selected. To protect the carboxylic acid, a methyl ester was chosen. Thus, (*S*)-serine **6** was esterified in methanol using acetyl chloride (Scheme 2, steps i).[29] Thereafter, the amino group of **7** was protected with Boc_2O and Na_2CO_3 (pH 10) in THF/H_2O (steps ii). The cyclization of **8** with thionyl chloride and pyridine was done according to the general method for Boc-protected amino alkanol (step iii).[27] The crude oil was used in the subsequent ruthenium-catalyzed oxidation (step iv) without purification.[30] The sulfamidate **9** was then purified by recrystallization to give a homogeneous crystalline compound in 48% global yield (steps i–iv, Scheme 2). The product **9** can be kept at 2 °C for several years without decomposition. After removal of the Boc-protecting group with TFA, the water soluble sulfamidate **10** was obtained in 81% yield after crystallization (Et_2O) (Scheme 2, step v).

Because of the enhanced reactivity of Boc-protected sulfamidates,[31,32] compound **14** was also synthesized. Transformation of (*S*)-serine **6** into the potassium salt of Boc-serine **11** using Boc_2O and K_2CO_3 followed by a '*one pot*' alkylation with benzyl bromide in DMF, gave **12** in 93% yield (steps vi and vii). By using the same reaction conditions that were used for the methyl ester, a crude product was obtained (step iii). Ru-catalyzed oxidation and recrystallization afforded the cyclic sulfamidate **13** in 33% yield (step iv). Hydrogenation[23,28] gave the free acid **14** after recrystallization from toluene in quantitative yield (step viii). By using the same approach, the (*R*) enantiomers of **10** and **14** were synthesized from (*R*)-serine.



Scheme 3. Derivatization of (*S*)-**9** and (*S*)-**13**: (i) PhSNa (1 equiv), DMF, rt, 2 h (80%); (ii) 10% aq NaH_2PO_4, EtOAc, 50 °C, 2 h; (iii) (a) HCl (1 M) in MeOH, reflux, 5 h; (b) Boc_2O, NEt_3, semi-preparative HPLC purification. Chiral HPLC conditions: Chiralcel OD-H 9:1 (Hexanes/*i*-PrOH), 1.0 mL/min: t_R (*S*)-**16** = 6.6 min, t_R (*R*)-**16** = 7.6 min, *R*/*S*:>99/1. ee of **17** was determined after transformation of **17** into **16** (step iii), *R*/*S*:>99/1.



Scheme 4. Preparation of racemic α-benzyl cysteine. Reagents and conditions: (i) (a) (*R*)-Cys, NaOH, MeOH, N_2, rt, 16 h; (b) HCOOH, water, 0 °C (80%); (ii) a) Boc_2O (2 equiv), DMAP (10 mol %), 1:1 CH_2Cl_2/*t*-BuOH, reflux 1 h; b) imidazole, $NaHSO_4$ (97%); (iii) BnBr (5 equiv), KOH (5 equiv), TBAB (10 mol %), toluene, 0 °C, 4 h (99%); (iv) 6 M HCl, reflux 36 h (85%).

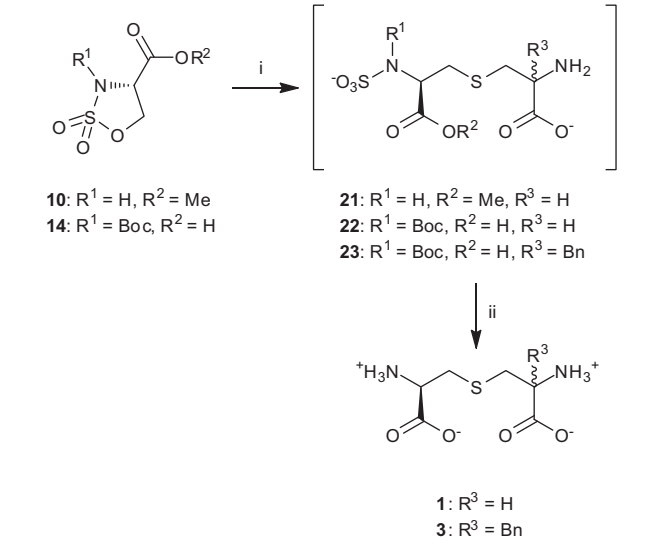
2.1.3. Enantiomeric excess of 9 and 13

For enantiomeric purity determination, a chromophore group was introduced on (*R*) and (*S*) sulfamidates **9** and **13**. The reaction of **9** with sodium thiophenolate in DMF, followed by selective hydrolysis of the *N*-sulfamate in the presence of the Boc-group with aqueous NaH_2PO_4[33], gave the protected *S*-phenyl-cysteine derivative **16** in good yield (80%) (Scheme 3, steps i-ii). The specific rotation of (*R*)-**16** ($[\alpha]_D^{20}$) was in accordance to the literature data.[34] In the same way, **13** gave the compound **17**. The enantiomers of **16** were separated on a Chiracel OD-H column and detected at 254 nm. By integration, an enantiomeric excess (ee) >99% was determined. Unfortunately, enantiomers of compound **17** could not be resolved on the OD-H column. Thus, the benzyl ester **17** was converted into methyl ester **16** by reflux with methanolic HCl followed by reprotection of the amino group by Boc_2O/NEt_3 (step iii). After semi-preparative HPLC purification, the ee of the corresponding compound **16** was >99%. In this way, the ee of (*R*)-**9**, (*S*)-**9**, (*R*)-**13** and (*S*)-**13** were determined to be >99%.

2.1.4. Synthesis of α-benzyl cysteine 5

As an expedient way to prepare thiazoline **18**, we first reacted benzonitrile and cysteine in aqueous methanol in the presence of NaOH, followed by HCOOH precipitation of the acid (Scheme 4, step i).[35,36] The *tert*-butyl ester **19** was then synthesized using the procedure of Takeda for protection of carboxylic acids with DMAP and Boc_2O (step ii).[37] Due to the low solubility of the acid **18** in *t*-BuOH we used CH_2Cl_2 at reflux as a co-solvent. Excess of Boc_2O was removed by using imidazole followed by acidification.[38] Using this synthetic procedure, **19** was obtained as a yellow oil in two steps (77% yield).

To obtain α-benzyl cysteine **5**, the route described by Kim et al. was used.[39] This involved alkylation of the thiazoline precursor **19** using tetrabutylammonium bromide (TBAB) as a phase transfer catalyst (PTC) (step iii). The racemic product **20** was hydrolyzed with 6 M HCl at reflux to afford the racemic α-benzyl cysteine *rac*-**5** as the hydrochloride salt (step iv). Extensive contamination by the corresponding cystine was observed when the zwitterion of *rac*-**5** was generated on Dowex 50WX8 according to the procedure of Kim et al.[39] Thus, the hydrochloride of *rac*-**5** was used for lanthionine synthesis.



Scheme 5. Preparation of Lan **1** and (*R*,*R*/*S*)-α-Bn-Lan **3**. Reagents and conditions: (i) (a) (*R* or *S*)-Cys **4** or *rac*-**5** (1 equiv), $NaHCO_3$ or $CsHCO_3$ (3.15 equiv), water, N_2, rt, 16 h; (b) HCl_{aq}, heat, isolation by Dowex 50WX8 (eluting with ammonia) or crystallization (pH 6).

2.1.5. Synthesis of lanthionines 1 and 3

The three diastereomers of Lan **1** ((*R*,*R*), (*S*,*S*) and *meso*) were synthesized in water at room temperature from **10** or **14** and cysteine (Scheme 5).[23] In the same way, (*R*,*R*/*S*)-α-Bn-Lan **3** was obtained from (*S*)-**14** and *rac*-**5**. The lanthionines **21–23** were deprotected via aqueous HCl hydrolysis. Because of the described epimerization of **1**[17,40] by aqueous HCl at *T* >95 °C, we conducted the hydrolysis at low temperature for Boc, methyl ester and sulfamate (2.5 M HCl at 50 °C for 30 min or 5 M HCl at 70 °C for 6 h[41]) (Scheme 5).

Next, the diastereomeric excess (de) of crude **1** was determined by HPLC, after *o*-phthaldehyde/*N*-Ac-(*R*)-cysteine (OPA/NAC) derivatization of an aliquot of the solution (Table 1).[42] The OPA/NAC derivatives were analyzed on a C_{18} HPLC column with a phosphate buffer (pH 7.5)/CH_3CN eluent. These conditions allowed us to separate (*R*,*R*) and (*S*,*S*)-Lan from *meso*-Lan but not the optical antipodes from each other.

According to results presented in Table 1, 7% of *meso*-Lan were observed when sulfamidate (*S*)-**10** (0.25 M) was reacted with (*R*)-cysteine **4** in 0.8 M aqueous $NaHCO_3$ to yield (*R*,*R*)-Lan **1** (86% de, entry 1, Table 1). We concluded that elimination to a dehydroalanine and subsequent Michael addition did occur.[18,43] In contrast to Cohen who observed, by ^1H NMR, no elimination for the sulfamidate **15**, we detected with **10**, in the same conditions[23] (0.2 M in D_2O containing 0.5 M $NaHCO_3$ at pH 8 and 23 °C), within 6 h the formation of alkene protons at 5.3 and 5.6 ppm. After elution on a Dowex 50WX8, Lan **1** was isolated with yields >95% and chemical purity >95%. Trace amounts of serine and cystine present in product **1** (as shown by ^{13}C NMR) were removed by sparingly washing with cold water. This step enables an increase both in the chemical purity (99%) and in the de (99%) but with a small decrease in yield (70%).

In order to increase the de, $CsHCO_3$ was substituted[23] for $NaHCO_3$ and the concentration of the sulfamidate **10** in the

Table 1
Preparation of lanthionine **1** and α-benzyl lanthionine **3**

Entry	Sulfamidate	Cys	Base[a]	H_3O^+[b]	de[c]	Product
1	(*S*)-**10**	(*R*)-Cys	$NaHCO_3$	A	86%	(*R*,*R*)-Lan
2	(*R*)-**10**	(*R*)-Cys	$CsHCO_3$	A	94%	*meso*-Lan
3	(*R*)-**14**	(*S*)-Cys	$NaHCO_3$	B	88%	(*S*,*S*)-Lan
4	(*R*)-**14**	(*S*)-Cys	$CsHCO_3$	B	>99%	(*S*,*S*)-Lan
5	(*S*)-**14**	(*R*)-Cys	$CsHCO_3$	B	>99%	(*R*,*R*)-Lan
6	(*R*)-**14**	(*R*)-Cys	$CsHCO_3$	B	>99%	*meso*-Lan
7	(*S*)-**14**	rac-**5**	$CsHCO_3$	B	N.D.	**3** (60%)[d]

[a] $NaHCO_3$ (0.8 M, 3.15 equiv) or $CsHCO_3$ (1.6 M, 3.15 equiv) was used.
[b] A: 5 M HCl_{aq}, 70 °C, 5 h; B: 2.5 M HCl_{aq}, 50 °C, 30 min.
[c] de of the crude product in an aliquot of hydrolyzed solution.
[d] Yield of the crystallized product.

aqueous solution was doubled (0.5 M). By doing so, the de increased to 94% (entry 2, Table 1).

When sulfamidate **14** was used instead of **10**, with $NaHCO_3$, the de of the (*S*,*S*)-Lan measured was found to be slightly higher (88%) (entries 1 and 3). Finally, the use of **14** and $CsHCO_3$ led to a de >99% for the three diastereomers of Lan **1** (entries 4–6). This can be explained by the increased reactivity of carbamate-substituted sulfamidates such as **14**.[31,32]

This highly diastereoselective method to Lan **1** (de >99%), without any additional enrichment step such as recrystallization, opens the way to the synthesis of lanthionines labelled with ^{35}S.

For biological investigation, a new compound, the (*R*,*R*/*S*)-α-Bn-Lan **3** was also prepared from **14** and the previously synthesized *rac*-**5** in place of cysteine. As an expedient purification process, **3** was easily precipitated at pH 6 in 60% yield (entry 7, Table 1).

2.2. Biological experiments

For biological incorporation experiments, *meso*-A_2pm **2**, *meso*-Lan **1**, (*R*,*R*)-Lan and Bn-Lan **3** have been used. It has been shown[11] that A_2pm auxotrophy in *E. coli* W7 can be complemented by exogenous lanthionine in the presence of lysine (50 μg/mL). However, certain growth conditions must be fulfilled. Minimal medium is preferred and preliminary growth with A_2pm is essential before inoculation.[11,44]

Our first experiments showed that a minimal concentration of 1 μg/mL A_2pm (commercially available mixture of diastereomers) is sufficient for the growth of bacterial cells (Fig. 3). By contrast, no growth of *E. coli* cells W7 was observed in minimal medium in the presence of 50 μg/mL *meso*-Lan, (*R*,*R*)-Lan or **3** in the absence of 1 μg/mL A_2pm (data not shown).

Then, cultures were grown in the presence of 1 μg/mL A_2pm and *meso*-Lan, (*R*,*R*)-Lan or **3** (50 μg/mL) was added when the stationary phase of bacterial growth was reached (t = 7 h) (Fig. 4).

After 9 h, the increase of optical density in the control experiment was small; indicating that maximal bacteria growth in the presence of 1 μg/mL A_2pm was achieved after 7 h and all supplementary growth was induced by addition of higher concentration of A_2pm or lanthionines. Thus, an increase of optical density in the presence of *meso*-Lan or (*R*,*R*)-Lan, as with additional A_2pm, was observed after a further two hours of incubation (t = 9 h) (Fig. 4), indicating that these molecules can replace *meso*-A_2pm. With α-benzyl derivative **3** a small but insignificant (p >0.05) decrease of bacterial growth was observed at t = 9 h (Fig. 4).

In a second experiment, bacteria were grown in the presence of 1 μg/mL A_2pm and 50 μg/mL of *meso*-Lan, (*R*,*R*)-Lan or **3**. After 7 h and 24 h incubation time, the bacterial growth was measured (Fig. 5).

After 24 h, in the presence of *meso*-Lan, an increase of the bacterial growth was still observed, indicating its incorporation into PG. After 24 h, an important decrease of optical density was found

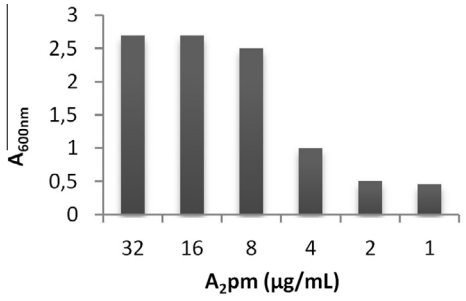


Figure 3. Optical density (600 nm) after 18 h of incubation of *E. coli* cells W7 at 37 °C in minimal medium containing 50 μg/mL (*S*)-Lys.

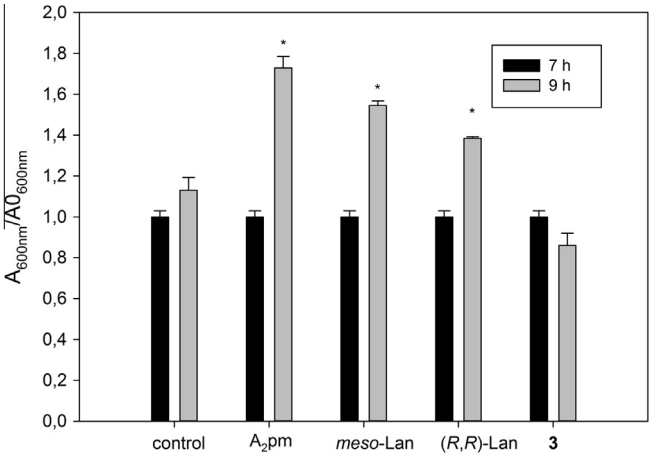


Figure 4. Bacteria were grown in the presence of 1 μg/mL A_2pm and 50 μg/mL of A_2pm, *meso*-Lan, (*R*,*R*)-Lan or **3** were added after 7 h when the stationary phase of bacterial growth was reached. A_{600nm}: optical density of the different cultures. $A0_{600nm}$: optical density of culture at t = 7 h in the presence of 1 μg/mL A_2pm. Control: no addition at t = 7 h. $^*p < 0.05$.

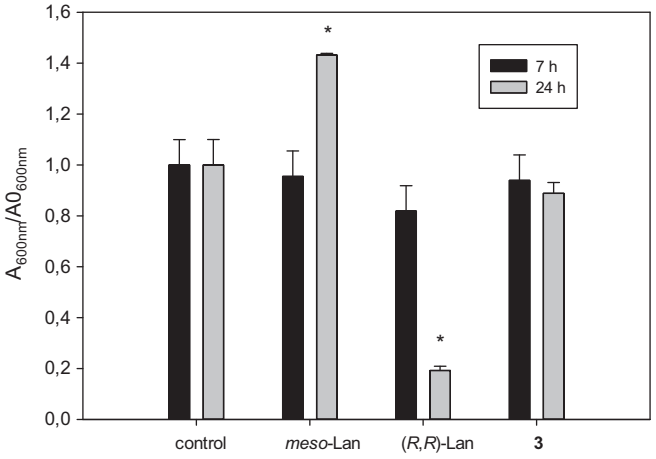


Figure 5. Bacteria were grown in the presence of 1 μg/mL A_2pm and 50 μg/mL of *meso*-Lan, (*R*,*R*)-Lan or **3**. Control: bacteria were grown with 1 μg/mL A_2pm. A_{600nm}: optical density of the different cultures. $A0_{600nm}$: optical density of culture in the presence of 1 μg/mL A_2pm. $^*p$ <0.05.

Table 2
Incorporation of *meso*-A_2pm **2** and lanthionines *meso*-Lan and **3** into PG[a]

	PG1[b]	PG2[c]	PG3[d]
GlcNAc[e]	1	1	1
MurNAc[f]	0.82	0.87	0.85
Ala	2.04	1.35	1.32
Glu	1.18	0.83	0.82
2	0.80	0.25	0.85
epi-Lan[g]	<0.01	0.03	<0.01
meso-Lan	<0.01	0.53	<0.01
3	<0.01	<0.01	<0.01

[a] Amino acid and hexosamine ratios are expressed with respect to glucosamine.
[b] PG1: culture with 50 μg/mL A_2pm.
[c] PG2: culture with 1 μg/mL A_2pm and 50 μg/mL *meso*-Lan.
[d] PG3: culture with 1 μg/mL A_2pm and 50 μg/mL **3**.
[e] Detected as glucosamine.
[f] Detected as muramic acid.
[g] A mixture of (*R*,*R*) and (*S*,*S*)-Lan not separated on analyzer.

with (*R*,*R*)-Lan. The decrease of optical density in the presence of **3** at t = 9 h and 24 h was very small and is probably due to a coincidence arising from random sampling (p >0.05). This indicates that

3 was probably not incorporated and its presence had no effect on cells. However, the sensitivity of the presented in vivo assays may not be sufficient to study the incorporation of **3**.

Thus, one-liter cultures have been grown in the presence of A_2pm (1 μg/mL) and A_2pm, *meso*-Lan or **3** (50 μg/mL). PGs (with A_2pm: PG1; with *meso-Lan*: PG2; with **3**: PG3) from the different cultures have been purified, hydrolyzed and analyzed (Table 2). Results indicate an important incorporation of *meso*-Lan into PG2. Of note, a small amount of a mixture of (*R*,*R*)-Lan and (*S*,*S*)-Lan was also found in PG2. This can be explained by the partial epimerization of Lan that occurred during HCl hydrolysis of the PG at T >95 °C.[17,38] No incorporation of **3** was detected in PG3.

2.3. Discussion

In the literature, the replacement of A_2pm by several sulfur-containing compounds, lanthionine,[10–13] cystathionine,[10] and djenkolic acid[11] was described. Experiments with A_2pm derivatives containing different substituents at position 4 (4-methyl-A_2pm,[11] 4-methylene-A_2pm,[45] 4-hydroxy-A_2pm,[13] 4-fluoro-A_2pm,[13] 4-oxo-A_2pm[13]) or at position 3 (3-hydroxy-A_2pm[11,46]) showed that it is possible to introduce A_2pm derivatives with different groups at position 3 or 4 in vivo. Furthermore, growth inhibition in the presence of α,α′-dimethyl-A_2pm and 3-methyl-lanthionine was described.[11] In *E. coli*, the addition of *meso*-A_2pm **2** to UDP-MurNAc-(*S*)-Ala-(*R*)-Glu is catalyzed by MurE. *meso*-Lan **1** is a good substrate of this enzyme (52% relative specific activity compared with *meso*-A_2pm).[10] (*R*,*R*)-Lan and (*S*,*S*)-A_2pm are poor substrates of MurE with 1.5% and 2.6% relative specific activity, respectively, compared with *meso*-A_2pm.[10] Incorporation of (*S*,*S*)-A_2pm into *E. coli* mutants lacking diaminopimelate epimerase *dap*F is possible, but limiting steps for this incorporation are its addition to UDP-MurNAc-(*S*)-Ala-(*R*)-Glu and the impossibility to form (*R*)-alanyl-(*S*,*S*)-A_2pm cross-bridges.[7,10] In the present work, we confirmed that *meso*-Lan can effectively replace *meso*-A_2pm in vivo if the culture medium contains a minimal concentration of 1 μg/mL A_2pm. Concerning (*R*,*R*)-Lan an initial increase of bacterial growth was observed (Fig. 4) followed by cell lysis (Fig. 5) also in the presence of a minimal concentration of A_2pm. (*R*,*R*)-Lan could be incorporated into PG but when its proportion becomes important, cells are probably destabilized owing to the impossibility to form (*R*)-alanyl-(*R*,*R*)-Lan cross bridges, as already observed for (*S*,*S*)-A_2pm[7] and (*S*)-lysine.[47] No incorporation of **3** was observed. Obviously, substitution at the α-position is detrimental for the incorporation of **3**, as already shown with α,α′-dimethyl-A_2pm.[12]

3. Conclusion

A new stereoselective synthesis of the three lanthionine diastereomers (*meso*-Lan **1**, (*R*,*R*)-Lan and (*S*,*S*)-Lan) and of the α-benzylated analogue **3** was developed. This procedure, conducted in aqueous solution, affords good yields and excellent de (>99%) without any recrystallization step. Thus, starting from commercially available (*R*)-[^{35}S]cysteine, this straightforward approach could be applicable for the preparation of diastereomerically pure [^{35}S]lanthionine. Moreover, the coupling of the protected sulfamidates **9** and **13** in aprotic solvent could also provide access to orthogonally protected lanthionines with good de.[22]

Biological experiments showed the incorporation of *meso*-Lan and (*R*,*R*)-Lan into PG. With (*R*,*R*)-Lan an important lysis was observed. Thus, [^{35}S]lanthionine diastereomers could be used to study the biosynthesis of PG and its turnover in relation to cell growth and division. Unfortunately, the α-benzylated lanthionine **3** was not incorporated. This result indicates that the introduction of substituted aromatic groups at this position is not feasible.

4. Experimental section

4.1. General

All solvents and chemicals were of analytical grade and used without further purification. DMF, EtOAc and CH_3CN were dried over 3 Å molecular sieves. TLC: Macherey–Nagel Polygram SIL G/UV_{254} using UV light or an anisaldehyde/sulfuric acid/AcOH/EtOH:1/1/0.04/18:v/v/v/v stain. Column chromatography (CC): silica gel Acros, 0.060–0.200 mm, 60 Å. HPLC: Waters system (600 pump, 717 autosampler, 996 PDA detector) with a XTerra RP18 column (4.6 × 150 mm; 3.5 μm) or for enantiomeric excess a Chiralcel OD-H column (250 × 4.6 mm, 5 μm, Daicel Chemical Industries LTD). Mp: Büchi Melting Point B-545 calibrated on three points (83, 136 and 237 °C). ^{1}H and ^{13}C NMR: Bruker Avance DRX 400 (^{1}H at 400 MHz and ^{13}C at 101 MHz) and Bruker AM 250 (^{1}H at 250 MHz and ^{13}C at 63 MHz) δ in parts per million relative to ^{13}C or the residual proton signal of deuterated solvent, T = 298 K. MS: Thermoquest Finnigan TSQ 7000 mass spectrometer operating in full scan MS mode with an ESI source. HRMS: ESI-FT-ICR mass spectrometer (SolariX, Bruker) in positive ion mode. For some samples 1 mM LiI was used for adducts. External calibration was done over the range of m/z 150 to 700 and mean residual error obtained was <1 ppm. Elemental analysis: Flash EA 1112 Series (Thermo Electron Corporation), the maximum deviation from theoretical results was 0.4%.

4.2. (*R*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxy propanoate (12)

A solution of Boc_2O (46.3 g, 212 mmol, 1.2 equiv) in dioxane (100 mL) was added to a solution of (*R*)-Serine (18.6 g, 177 mmol) and K_2CO_3 (24.4 g, 177 mmol) in water (100 mL). The solution was stirred for 16 h at room temperature (rt). The dioxane was evaporated and the aqueous solution was washed with 3 × 50 mL Et_2O. Water was evaporated in vacuo and remaining traces were azeotropically removed with EtOH. The resulting white powder was suspended in DMF (200 mL) and BnBr (24 mL, 200 mmol) was added. The mixture was stirred at rt for 16 h. The DMF was evaporated in vacuo and the residue was extracted with toluene (2 × 200 mL). The pooled fractions were washed twice with water and brine and dried over $MgSO_4$. After filtration, the solvent was evaporated at 90 °C in vacuo. The *title compound* was obtained as golden oil that crystallized upon standing (48.8 g, 93% for two steps). Mp 62–64 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.43 (s, 5H, Ar-H), 5.70 (b, 1H, NH), 5.28 (s, 2H, CH_2Ph), 4.49 (b, 1H, H-α), 4.07 (dd, J = 11.1, 3.2 Hz, 1H, Ser-CH_2), 3.97 (dd, J = 11.1, 3.2 Hz, 1H, Ser-CH_2), 3.09 (b, 1H, OH), 1.52 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 170.9 (CO), 155.9 (Boc-CO), 135.3 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 128.2 (Ar-C), 80.3 (*C*(CH_3)$_3$), 67.4 (CH_2Ph), 63.4 (C-β), 55.9 (C-α), 28.4 (C(*C*H_3)$_3$); m/z (ESI) 296 [MH]$^+$.

4.3. General procedure for the preparation of sulfamidites

Sulfamidites were synthesized according to a protocol described in literature.[27] In a dry round bottom flask under nitrogen, equipped with a guard tube and a pressure equalized addition funnel, was added $SOCl_2$ (15 mL, 200 mmol) in 100 mL of CH_3CN. The solution was cooled to −42 °C. (*R*)-Boc-serine ester **8** or **12** (80 mmol) in 100 mL of degassed CH_3CN was placed in the funnel and this solution was added dropwise in 1 h. Next, pyridine (34 mL, 420 mmol) was introduced in the funnel and added dropwise during 30 min to the flask. The yellow mixture was stirred for 2 h more at −42 °C. The reaction was then quenched on crushed ice and acidified with aqueous 10% $NaHSO_4$. The aqueous layer was

extracted with 3 × 100 mL CH_2Cl_2 and the combined organic fractions were washed with water, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated in vacuo to yield the crude product as a yellow oil which was used without purification for the next step.

4.4. General procedure for the preparation of sulfamidates

This is a modification of the protocol described in literature.[30] The crude sulfamidite was dissolved in CH_3CN (160 mL) and cooled in an ice bath. $RuCl_3.xH_2O$ (90 mg, 0.5 mol %) was added followed by $NaIO_4$ (18.8 g, 88 mmol) and water (160 mL). The green–brown solution with a white precipitate was stirred for 15 min at 0 °C and allowed to return to rt. After 4 h, the mixture was diluted with Et_2O (200 mL) and brine (200 mL). The aqueous layer was extracted with Et_2O (3 × 200 mL) and the pooled fractions were washed with saturated $NaHCO_3$ (2 × 300 mL) and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated in vacuo to yield a beige powder. The crude product was dissolved in the minimum amount of CH_2Cl_2 and then an equal volume of Et_2O was added to induce crystallization. After 16 h at −18 °C the crystals were filtered and dried to constant weight.

4.4.1. Methyl (4*R*)-3-(*tert*-butyloxycarbonyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate (9)

Yield for two steps 68% (15.3 g); colorless crystals; mp 100–101 °C (dec) (lit.[26] 71 °C); Anal (C,H,N,S) $C_9H_{15}NO_7S$; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 4.86–4.59 (m, 3H), 3.84 (s, 3H, OCH_3), 1.53 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.6 (CO), 148.2 (Boc-CO), 86.3 ($C(CH_3)_3$), 67.6 (C-β), 57.6 (C-α), 53.7 (OCH_3), 27.9 ($C(CH_3)_3$); HRMS *m*/*z* (ES+) Calcd for $C_9H_{15}LiNO_7S$ 288.0724, found 288.0722 $[MLi]^+$.

4.4.2. Benzyl (4*R*)-3-(*tert*-butyloxycarbonyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate (13)

Yield for two steps 33% (9.4 g); colorless crystals; mp 121–122 °C (dec); R_f (1:1 EtOAc/Hexanes, anisaldehyde) 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36 (s, 5H, Ar-H), 5.32 (d, *J* = 12.2 Hz, 1H, CH_2-Ph), 5.23 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.83 (br d, *J* = 6.5 Hz, 1H, H-α), 4.76 (dd, *J* = 9.2, 6.8 Hz, 1H, Ser−CH_2), 4.67 (dd, *J* = 9.3, 1.7 Hz, 1H, Ser−CH_2), 1.49 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167.0 (CO), 148.1 (Boc-CO), 134.5 (Ar-C), 128.8 (Ar-C), 128.8 (Ar-C), 128.4 (Ar-C), 86.2 ($C(CH_3)_3$), 68.5 (CH_2Ph), 67.5 (C-β), 57.7 (C-α), 27.8 ($C(CH_3)_3$); HRMS *m*/*z* (ES+) Calcd for $C_{15}H_{19}LiNO_7S$ 364.1037, found 364.1035 $[MLi]^+$.

4.5. Methyl (4*R*)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate (10)

A solution of **4** (1.405 g, 5 mmol) in 5 mL of CH_2Cl_2 and 5 mL of TFA was refluxed for 30 min. The volatiles were removed in vacuo and the residue was triturated in boiling Et_2O (20 mL). The suspension was cooled to −18 °C for 16 h. The crystals were filtered, washed with Et_2O and dried to give the *title compound* as colourless needles 735 mg (81%). Mp 102–103 °C; ^{1}H NMR (250 MHz, D_2O) δ_H 5.20 (b, 1H), 4.77 (dd, *J* = 8.9, 7.8 Hz, 1H, H-α), 4.61 (dd, *J* = 8.9, 5.3 Hz, 1H, CH_2), 4.55–4.43 (m, 1H, CH_2), 3.90 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 168.8 (CO), 69.9 (C-β), 56.2 (C-α), 54.1 (OCH_3); HRMS *m*/*z* (ES+) Calcd for $C_4H_7LiNO_5S$ 188.0199, found 188.0198 $[MLi]^+$.

4.6. (4*R*)-3-(*tert*-Butyloxycarbonyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylic Acid (14)

The literature protocol was modified as follows.[23] To a solution of **13** (1.785 g, 5 mmol) in EtOAc (25 mL) was added Pd/C (10%, 159 mg, 3 mol %). The suspension was stirred for 8 h under H_2 (70 psi). TLC analysis indicated total conversion to a less mobile product. The mixture was diluted with EtOAc, filtered on Celite and washed with EtOAc. The solvent was evaporated in vacuo to give a golden oil. This was dissolved in toluene (50 mL) and evaporated in vacuo until the start of crystallization. After 10 min, an equal volume of hexanes was added and the crystallization was completed at 0 °C. The crystals were filtered, washed with hexanes and dried to constant weight in vacuo to give the *title compound* as colorless needles (2.6 g, 97%). Mp 71–72 °C (dec); R_f (1:1 EtOAc/hexanes, anisaldehyde stain) 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.04 (b, 1H, OH), 4.86 (dd, *J* = 6.4, 1.4 Hz, 1H, H-α), 4.82 (dd, *J* = 9.2, 6.6 Hz, 1H, CH_2), 4.76 (dd, *J* = 9.2, 1.7 Hz, 1H, CH_2), 1.56 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 170.6 (CO), 148.4 (Boc-CO), 87.0 ($C(CH_3)_3$), 67.8 (C-β), 57.5 (C-α), 27.9 ($C(CH_3)_3$); HRMS *m*/*z* (ES+) Calcd for $C_8H_{13}LiNO_7S$ 274.0567, found 274.0568 $[MLi]^+$.

4.7. Enantiomeric purity: General procedure

To sulfamidate **9** or **13** (1 mmol) in degassed DMF (3 mL) was added sodium thiophenolate (132 mg, 1 mmol). The reaction mixture was stirred at rt for 2 h under nitrogen. The solution was poured in EtOAc (20 mL) and 10% aq NaH_2PO_4 (20 mL). After 2 h of stirring at 50 °C, the organic layer was decanted and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with water (2 × 50 mL) and brine. After drying over $MgSO_4$ and filtration, the solvent was evaporated in vacuo to give an oil. The purification conducted by CC on silica with 1:1 (EtOAc/hexanes) afforded a colorless oil. Chiral HPLC conditions: Chiralcel OD-H 9:1 (Hexanes/*i*-PrOH), 1.0 mL/min: t_R (*S*)-**16** = 6.6 min, t_R (*R*)-**16** = 7.6 min, ee:>99%. The enantiomers of **17** were not separated on the Chiralcel OD-H column. Thus, the benzyl ester **17** was converted into methyl ester **16** by refluxing with excess 1 M methanolic HCl, followed by evaporation and reprotection of the amino group using a one-pot Boc_2O/NEt_3 procedure. Compound **16** was then purified by semi-preparative HPLC and injected on the Chiralcel OD-H column.

4.7.1. (*S*)-Methyl 2-(*tert*-butoxycarbonylamino)-3-(phenylthio)propanoate ((*S*)-16)

Yield 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.50–7.14 (m, 5H, Ar-H), 5.37 (s, 1H, NH), 4.58 (s, 1H, H-α), 3.54 (s, 3H, OCH_3), 3.39 (s, 2H, CH_2S), 1.43 (s, 9H, $C(CH_3)_3$); *m*/*z* (ESI) 312 $[MH]^+$; Chiralcel OD-H 9:1 (Hexanes/*i*-PrOH), 1,0 mL/min: t_R (S)-**16** = 6,6 min, t_R (*R*)-**16** = 7,6 min, *S*/*R*:>99/1. (*S*)-**16** $[\alpha]_D^{20}$ +22° (*c* 1, MeOH) (lit.[32] $[\alpha]_D^{20}$ −19° (*c* 1, MeOH) for (*R*)-**16**).

4.8. 2-Phenyl-thiazoline-4-carboxylic acid (18)

The literature protocol was modified as follows.[35,36] A solution of cysteine (12.1 g, 100 mmol) in degassed aqueous NaOH (0.5 M, 100 mL) was treated under nitrogen with a degassed solution of benzonitrile (10.3 g, 100 mmol) in MeOH (200 mL). The solution was stirred at rt for 16 h. MeOH was evaporated and **18** was precipitated at 0 °C by the addition of HCOOH (5.7 mL, 150 mmol). The crystals were filtered, washed with cold water and dried in vacuo to constant weight. The *title compound* (16.6 g, 80%) was obtained as a yellow powder. Mp 128–129 °C; ^{1}H NMR (250 MHz, D_2O + NaOH) δ_H 8.04–7.74 (m, 2H, Ar-H), 7.74–7.21 (m, 3H, Ar-H), 4.34 (dd, *J* = 7.2, 4.0 Hz, 1H, H-α), 3.07 (dd, *J* = 13.4, 3.1 Hz, 1H, Cys−CH_2), 2.92 (dd, *J* = 13.1, 8.2 Hz, 1H, Cys−CH_2); ^{13}C NMR (63 MHz, D_2O + NaOH) δ_C 178.1 (CO), 170.1 (CN), 133.4 (Ar-C), 132.2 (Ar-C), 128.8 (Ar-C), 127.2 (Ar-C), 59.3 (C-α), 27.1 (C-β); *m*/*z* (ESI) 208 $[MH]^+$.

4.9. *tert*-Butyl 2-phenyl-thiazoline-4-carboxylate (19)

The general method of Takeda[37] was followed, with a special emphasis on the poor solubility of the acid **18**. Under nitrogen, **18** (5.2 g, 25 mmol), DMAP (610 mg, 20 mol %), CH_2Cl_2 (30 mL) and *t*-BuOH (30 mL) were introduced in a 250 mL round bottom flask equipped with an efficient condenser. The solution was heated at reflux and Boc_2O (10.9 g, 50 mmol) was added via a syringe in two portions. *Caution:* this step produces substantial gas evolution. The mixture was heated for 1 h then imidazole (3.4 g, 50 mmol) was added and reflux was maintained for 30 min. The mixture was cooled to rt. Aqueous $NaHSO_4$ (10%, 60 mL) was added and stirring was continued for 30 min. The organic layer was decanted and the aqueous solution was extracted once with CH_2Cl_2. The combined organic layers were washed twice with water then with saturated $NaHCO_3$ and brine. The CH_2Cl_2 layer was dried over $MgSO_4$, filtered and the solvent evaporated in vacuo to yield the *title compound* (6.4 g, 97%) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.90–7.80 (m, 2H, Ar-H), 7.48–7.33 (m, 3H, Ar-H), 5.18 (t, J = 8.9 Hz, 1H, H-α), 3.61 (d, J = 9.1 Hz, 2H, Cys−CH_2), 1.50 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 170.5 (CN), 170.0 (CO), 132.9 (Ar-C), 131.6 (Ar-C), 128.7 (Ar-C), 128.5 (Ar-C), 82.2 (*C*(CH_3)$_3$), 79.4 (C-α), 35.8 (C-β), 28.1 (C(*C*H_3)$_3$); *m*/*z* (ESI) 264 $[MH]^+$.

4.10. *tert*-Butyl 4-benzyl-2-phenyl-4,5-dihydrothiazole-4-carboxylate (rac-20)

The *title compound* was obtained from **19** (25 mmol) as described in literature [19] but by using TBAB (10 mol %) as PTC. Yield quantitative. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.06–7.93 (m, 2H, Ar-H), 7.63–7.50 (m, 3H, Ar-H), 7.50–7.29 (m, 5H, Ar-H), 3.88 (d, J = 11.5 Hz, 1H, Cys−CH_2), 3.50 (d, J = 11.5 Hz, 1H, Cys−CH_2), 3.47 (d, J = 13.7 Hz, 1H, CH_2Ph), 3.40 (d, J = 13.7 Hz, 1H, CH_2Ph), 1.56 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (63 MHz,$CDCl_3$) δ_C 171.2 (CO), 168.4 (CN), 136.2 (Ar-C), 133.2 (Ar-C), 131.5 (Ar-C), 130.6 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 128.2 (Ar-C), 126.9 (Ar-C), 89.3 (C-α), 82.2 (*C*(CH_3)$_3$), 43.2 (CH_2Ph), 39.4 (Cys−CH_2), 28.0 (C(*C*H_3)$_3$); *m*/*z* (ESI) 354 $[MH]^+$.

4.11. 2-Amino-3-mercapto-2-phenylpropanoic acid hydrochloride (rac-5)

The *title compound* was obtained from **20** (10 mmol) as described in literature[39] but with a longer reflux time and a different work-up. A suspension of **20** (3.53 g, 10 mmol) in 6 M HCl (30 mL) was heated at reflux for 36 h under nitrogen. After evaporation of the solvent in vacuo, the residue was dissolved in water (20 mL) and washed with EtOAc (3 × 10 mL). The aqueous solution was evaporated to dryness and the powder was triturated with acetone. The product was filtered and dried in vacuo to constant weight to give the *title compound* (2.1 g, 85%) as a pale yellow solid. Attempt to purify via Dowex 50WX8[39] resulted in a contamination by the corresponding cystine. Mp 114–118 °C; ^{1}H NMR (250 MHz, D_2O) δ_H 7.21–7.01 (m, 3H, Ar-H), 7.01–6.87 (m, 2H, Ar-H), 3.18–2.78 (m, 2H, CH_2Ph), 3.01 (d, J = 14.9 Hz, 1H, Cys−CH_2), 2.67 (d, J = 15.2 Hz, 1H, Cys−CH_2); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.1 (CO), 132.0 (Ar-C), 129.9 (Ar-C), 129.0 (Ar-C), 128.2 (Ar-C), 65.5 (C-α), 40.4 (CH_2Ph), 28.7 (Cys−CH_2); *m*/*z* (ESI) 212 $[MH]^+$.

4.12. General procedure for the preparation of lanthionines

To a degassed solution of $CsHCO_3$ (611 mg, 3.15 mmol) in water (2 mL), cysteine or α-benzyl cysteine **5** (1 mmol) was added followed by the sulfamidate **10** or **14** (1 mmol). This solution was stirred for 16 h at rt under nitrogen. Concentrated HCl (10 M, 10 mmol if **14** was used, 25 mmol if **10**) was added. The solution was heated under nitrogen at 50 °C for 30 min or at 70 °C for 5 h with compound **14** or **10**, respectively.

For diastereomeric excess determination, an aliquot of the crude reaction mixture (100 μL) was diluted with 20 mL (for **10**) or 30 mL (for **14**) of water. To this solution, $K_2B_4O_7.4H_2O$ (100 mg) was added and the pH of the solution was adjusted to 9.3 with NaOH (1 M). Meanwhile, a solution of *N*-Ac-cysteine (66 mg) and *o*-phthaldehyde (27 mg) in MeOH (0.5 mL) was added to $K_2B_4O_7{\cdot}4H_2O$ (122 mg) in water (4.5 mL). Then, the lanthionine solution (300 μL) and the NAC-OPA solution (300 μL) were mixed in a small vial. After heating to 37 °C for 3 min, the resulting solution was analyzed immediately by HPLC. Eluent was CH_3CN/aqueous NaH_2PO_4 (0.05 M, pH adjusted to 7.2 with NaOH) (12/88, v/v). The isoindoles were quantified at 355 nm.

Purification of the lanthionine from the reaction mixture was performed as follows. The aqueous HCl was evaporated in vacuo and the product was purified by DOWEX 50WX8 (eluting with ammonia). After lyophilization, the yield was 95% and the chemical purity was >95% (containing some serine and cystine). Alternatively, the product can be dissolved in 2 mL of water and the pH adjusted to 6 with ammonia to crystallize the zwitterion. After 16 h at 2 °C, the lanthionine was filtered, washed sparingly with cold water and dried in vacuo to constant weight. In this case, the yield was 70% and chemical purity was >99%.

4.12.1. (*R*)-2-Amino-3-((*S*)-2-amino-2-carboxyethylthio)propanoic acid (*meso*-Lan, 1)

Colorless powder; mp 274–276 °C (dec) (lit.[2] 270 (dec)); ^{1}H NMR (400 MHz, D_2O) δ_H 4.22 (dd, J = 7.3, 4.4 Hz, 2H, H-α), 3.15 (dd, J = 15.1, 4.4 Hz, 2H, CH_2), 3.03 (dd, J = 15.0, 7.4 Hz, 2H, CH_2); ^{13}C NMR (63 MHz, D_2O) δ_C 170.0 (CO), 52.2 (C-α), 31.5 (CH_2); *m*/*z* (ESI) 209 $[MH]^+$.

4.12.2. (*R*/*S*)-2-Amino-3-((*R*)-2-amino-2-carboxyethylthio)-2-benzylpropanoic acid (3)

Yield 60% after crystallization from water; mixture of two diastereomers; colorless powder; mp 218–220 °C (dec); ^{1}H NMR (250 MHz, D_2O) δ 7.19–7.02 (m, 3H, Ar-H), 7.02–6.85 (m, 2H, Ar-H), 4.15–3.99 (m, 1H, H-α), 3.20 (d, J = 14.8, 0.5H, CH_2), 3.16 (d, J = 14.8, 0.5H, CH_2), 3.16–2.76 (m, 5H, CH_2); ^{13}C NMR (63 MHz, D_2O) δ 171.1 (0.5C, CO), 171.0 (0.5C, CO), 169.57 (0.5C, CO), 169.55 (0.5C, CO), 131.73 (0.5C, Ar-C), 131.69 (0.5C, Ar-C), 129.98 (2C, Ar-C), 129.02 (2C, Ar-C), 128.3 (Ar-C), 64.46 (0.5C, C-α), 64.24 (0.5C, C-α), 52.27 (0.5C, CH-α), 51.92 (0.5C, CH-α), 40.88 (0.5C, CH_2), 40.75 (0.5C, CH_2), 37.27 (0.5C, CH_2), 36.55 (0.5C, CH_2), 32.86 (0.5C, CH_2), 32.44 (0.5C, CH_2); *m*/*z* (ESI) 299 $[MH]^+$; HRMS *m*/*z* (ES+) Calcd for $C_{13}H_{19}N_2O_4S$ 299.1060, found 299.1057 $[MH]^+$.

5. Biological experiments

5.1. Strains and media

W7 (*dap lysA*)[48] was obtained from A. Derouaux (Newcastle University, Newcastle, United Kingdom). The cells were grown at 37 °C in a minimal salts medium[44] containing (per liter) 6.33 g of $K_2HPO_4{\cdot}3H_2O$, 2.95 g of KH_2PO_4, 1.05 g of $(NH_4)_2SO_4$, 1.0 g of $MgSO_4{\cdot}7H_2O$, 0.1 mg of $FeSO_4{\cdot}7H_2O$, 2.8 mg of $Ca(NO_3)_2{\cdot}4H_2O$, 4 mg of thiamine, 5 g (0.5%) or 2 g (0.2%) of glucose, 50 mg of (*S*)-lysine and various concentrations of A_2pm (commercially available mixture of diastereomers) and/or lanthionines.

5.2. Purification of PG

The preparation of highly purified PG has been done from one-liter culture medium by modifying a protocol described by

Girardin.[49] Briefly, bacteria were harvested in the exponential growth phase at an optical density (600 nm) of 0.4–0.6. After centrifugation (10 min, 6000 g) the pellets were suspended in ice-cold water (20 mL) and the suspension was added dropwise to 8% boiling SDS (20 mL). Samples were boiled for 45 min. SDS treatment removed contaminating proteins, non-covalently bound lipoproteins and LPS. Polymeric PG, which remained insoluble, was recovered by ultracentrifugation (45 min, Beckmann L2-65B, rotor Type 60Ti, 50,000 rpm) and washed five times with water in order to remove SDS. PGs were further treated with α-amylase (200 μg/mL, 20 mM sodium phosphate, 7 mM NaCl, pH 6.9, 3 h at 37 °C) to remove oligosaccharides, with trypsin (200 μg/mL, 20 mM sodium phosphate, 7 mM NaCl, pH 8, 3 h at 37 °C) and pronase (500 μg/mL, 100 mM Tris, 10 mM $CaCl_2$, pH 8, 3 h at 40 °C) to remove proteins. After all treatments, PG was recovered by ultracentrifugation (60 min, 50,000 rpm). Finally, it was washed with water, with 8 M LiCl to remove any polypeptidic contamination, again with water, and lyophilized.

5.3. Analysis of purified PG

Aliquots were hydrolyzed in 6 M HCl containing 0.05% (v/v) 2-mercaptoethanol at 95 °C for 16 h. The reducing agent prevented the oxidation of lanthionine into sulfoxide. After evaporation, the pellet was dissolved with 67 mM sodium citrate-HCl buffer (pH 2.2) and injected into a Hitachi L-8800 amino acid analyzer equipped with a 2620MSC-PS column (ScienceTec). Amino acids and hexosamines were detected after post-column reaction with ninhydrin. Lanthionine derivatives did not co-elute with any other amino acid or hexosamine; retention times were: (*R*,*R*)-lanthionine, 19.9 min; (*S*,*S*)-lanthionine, 19.9 min; *meso*-lanthionine, 21.7 min; (*R*,*R*/*S*)-α-Bn-Lan 3, 30.7 and 31.3 min.

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Supplementary data

Supplementary data (NMR spectra of the newly synthesized molecules are reproduced) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.023.

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