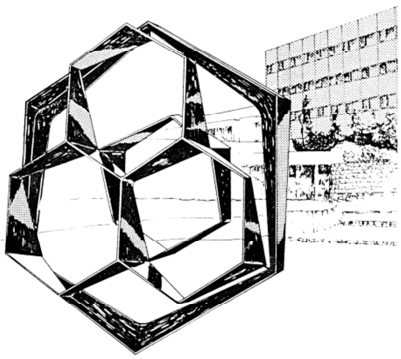
FACULTÉ DES SCIENCES – DÉPARTEMENT DE CHIMIE

CENTRE DE RECHERCHES DU CYCLOTRON

SYNTHESIS AND BIOLOGICAL STUDIES OF LANTHIONINE DERIVATIVES

Promoteur: **Professeur André LUXEN**



Dissertation présentée par

Thibaut DENOËL

pour l'obtention du grade de

Docteur en Sciences

Année académique 2013 - 2014

Membres du Jury :

Promoteur :	Professeur André Luxen (Université de Liège)
Président :	Professeur Albert Demonceau (Université de Liège)
Autres membres :	Docteur Didier Blanot (Université Paris-Sud)
	Docteur David Thonon (Uteron Pharma)
	Professeur Bernard Joris (Université de Liège)
	Docteur Christian Lemaire (Université de Liège)

Remerciements

Les travaux présentés dans cette thèse ont été réalisés sous la direction du Professeur André Luxen dans les laboratoires du Centre de Recherches du Cyclotron de l'Université de Liège.

Je voudrais tout d'abord remercier le Professeur André Luxen pour son accueil au sein du Cyclotron, sa grande patience et son exigence. Il a aussi inspiré nombre de manipulations et m'a guidé dans mes recherches. En outre, il a mis à ma disposition tout le matériel et la quantité de produits chimiques nécessaires à la réalisation de ce travail.

Je tiens également à remercier Messieurs les membres du Jury pour m'avoir fait l'honneur d'accepter d'examiner ce travail, en l'occurrence le Docteur Didier Blanot (Université Paris-Sud), le Docteur David Thonon (Uteron Pharma), le Professeur Bernard Joris, le Docteur Christian Lemaire et le Professeur Albert Demonceau dans le rôle de Président.

Je remercie aussi les Docteurs Didier Blanot et Mireille Hervé pour la réalisation des essais in vitro *avec l'enzyme Mpl et l'aide dans les publications. Je remercie grandement le Docteur Astrid Zervosen pour la réalisation des manipulations biochimiques, ses conseils, sa disponibilité et la correction lors de la rédaction. Tous mes remerciements vont aussi au Docteur Christian Lemaire pour la relecture fastidieuse des manuscrits et les moments partagés. Enfin, je suis très reconnaissant au Professeur Albert Demonceau pour les nombreuses discussions et conseils judicieux prodigués tout au long de mon cursus.*

Je remercie également Thomas Gerards pour les centrifugations, le Docteur Gabriel Mazzucchelli et ses collègues du GIGA MSL pour les mesures HRMS, Christian Damblon et les membres du CREMAN pour la RMN, Guillermo Zaragozza pour la diffraction des rayons X, Eric Zigler pour la relecture de l'anglais de l'article Tetrahedron et Vedran Hasimbegovic pour l'aide apportée à la correction de l'anglais des publications.

Je n'oublie pas mes collègues et amis : Alain, André B., Annick, Audrey, Brigitte, Caro, Cécile, Corentin, David G., David T., Eve, Fabrice, Fred Me., Fred Mi., Geoffroy, Jean, Jérôme, Jessica, Julien, Justine, Lionel, Louis, Ludo, Marlies, Mathias, Mathurin, Muhammad, Nathalie, Nico, Steve, Sylvestre, Vincent et tous les membres du Cyclotron.

Enfin, pour leur soutien et leurs encouragements durant toutes ces années, je tiens à exprimer ma reconnaissance aux membres de ma famille, maman, papa, Clément, Louis, Mélanie, Philippe, Roseline.

List of abbreviations

$[\alpha]_D^{20}$	specific rotation
Abu	2-aminobutyric acid
A_2pm	diaminopimelic acid
Ac	acetyl
Acm	acetamidomethyl
ADDP	1,1′-(azodicarbonyl)dipiperidine
AD-mix α	reagent for asymmetric dihydroxylation (Sharpless)
AG50-X8	strongly acidic cation exchange resin
Ala	alanine
Alloc	allyloxycarbonyl
allo-Thr	*allo*-threonine
ATP	adenosine triphosphate
β-Me-Lan	β-methyl-lanthionine
BDP	benzotriazol-1-yl diethyl phosphate
Bn	benzyl
Boc	*tert*-butoxycarbonyl
$(Boc)_2O$	di-*tert*-butyl dicarbonate
Burgess reagent	methyl *N*-(triethylammoniumsulfonyl)carbamate
br	broad
C_{18} column	reversed phase column with octadecyl carbon chain bonded silica
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CC	column chromatography
Chiralcel OD-H	chiral stationary phase for liquid chromatography
Chirasil-Val	chiral stationary phase for gas chromatography
COSY	correlation spectroscopy
Cys	cysteine
dansyl	5-(dimethylamino)naphthalene-1-sulfonyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DCE	dichloroethane
de	diastereomeric excess
DEAD	diethylazodicarboxylate

Dha	dehydroalanine
Dhb	dehydrobutyrine
DiPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMEDA	*N*,*N*'-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNs	dinitrobenzenesulfonyl
EA	elemental analysis
E. coli W7	*Escherichia coli* strain auxotrophic for A_2pm and for Lys
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
eq	equivalents
ES+	positive ion electrospray ionisation
ES-	negative ion electrospray ionisation
ESI	electrospray ionization
Fmoc	9-fluorenylmethoxycarbonyl
FT-ICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
GlcNAc	*N*-acetylglucosamine
Glu	glutamic acid
HMBC	heteronuclear multiple bond correlation spectroscopy
HOBt	1-hydroxybenzotriazole
HSQC	heteronuclear single quantum correlation spectroscopy
HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HP-20	polyaromatic adsorbent resin for hydrophobic compounds
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
i-Pr	isopropyl
ivDde	2-isovaleryldimedone
J	coupling constant
KO*t*-Bu	potassium *tert*-butoxide
Lan	lanthionine
Lipid I	undecaprenyl diphosphate-MurNAc-pentapeptide
Lipid II	undecaprenyl diphosphate-(MurNAc-pentapeptide)-GlcNAc
lit.	literature reference
LPS	lipopolysaccharides

Lys	lysine
m	multiplet
MeLan	β-methyllanthionine
mesylate	methanesulfonate
mp	melting point
Mpl	murein peptide ligase
MPLC	medium pressure liquid chromatography
MppA	murein peptide permease A
MS	mass spectrometry
Ms	methanesulfonyl
MurNAc	*N*-acetylmuramic acid
NBD	7-nitro-2,1,3-benzoxadiazol-4-yl
NMM	*N*-methylmorpholine
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
nor-Lan	norlanthionine
OPA/NAC	*o*-phthaldehyde/*N*-Ac-(*R*)-cysteine
Opp	oligopeptide permease
OSu	oxysuccinimide
PBP	penicillin-binding protein
PDA	photodiode array
Pd/C	palladium on carbon
PG	peptidoglycan
PMB	*para*-methoxybenzyl
pNB	*para*-nitrobenzyl
pNZ	*para*-nitrobenzyloxycarbonyl
PTC	phase transfer catalys(is/t)
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	quartet
R_f	retention factor
RP18 column	C_{18} column
rt	room temperature
s	singlet
SDS	sodium dodecyl sulfate
Ser	serine
SIL G/UV254	standard silica TLC plates with UV indicator

S_N2	bimolecular nucleophilic substitution
SPPS	solid phase peptide synthesis
t	triplet
TBAB	tetra-*n*-butylammonium bromide
TBAF	tetra-*n*-butylammonium fluoride
TBAHS	tetra-*n*-butylammonium hydrogensulfate
t-Bu	*tert*-butyl
Tce	2,2,2-trichloroethyl
Teoc	β-(trimethylsilyl)ethoxycarbonyl
TES	triethylsilane
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
TFFH	fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate
THF	tetrahydrofuran
TIS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSE	trimethylsilylethyl
tosyl	*p*-toluene sulfonyl
Tr	trityl
t_R	retention time
Tris	trishydroxymethylaminomethane
Triton B	benzyltrimethylammonium hydroxide
Trityl	triphenylmethyl
Troc	2,2,2-trichloroethoxycarbonyl
UDP	uridine diphosphate
UMP	uridine monophosphate
UV	ultraviolet
Weinreb amide	*N*,*O*-dimethylhydroxyamide
Z	Cbz

Contents

Chapter 1

Introduction

1.1 General introduction

Bacteria are prokaryotic microorganisms of typical length between 0.5 and 5 µm displaying different shapes. The bacterial cells are protected from osmotic lysis by the cell envelope (Figure 1). Peptidoglycan (PG) is unique to bacteria and essential for cell survival. It lies outside of the cytoplasmic membrane. A classification of bacteria into two groups was done based on their ability to retain crystal violet when treated with the Gram stain. The thickness of PG differs in Gram+ (20 - 80 nm) from that of Gram- (1 - 3 nm) bacteria. In contrast to Gram+ bacteria, Gram- bacteria also possess an outer lipid-based membrane (Figure 1).

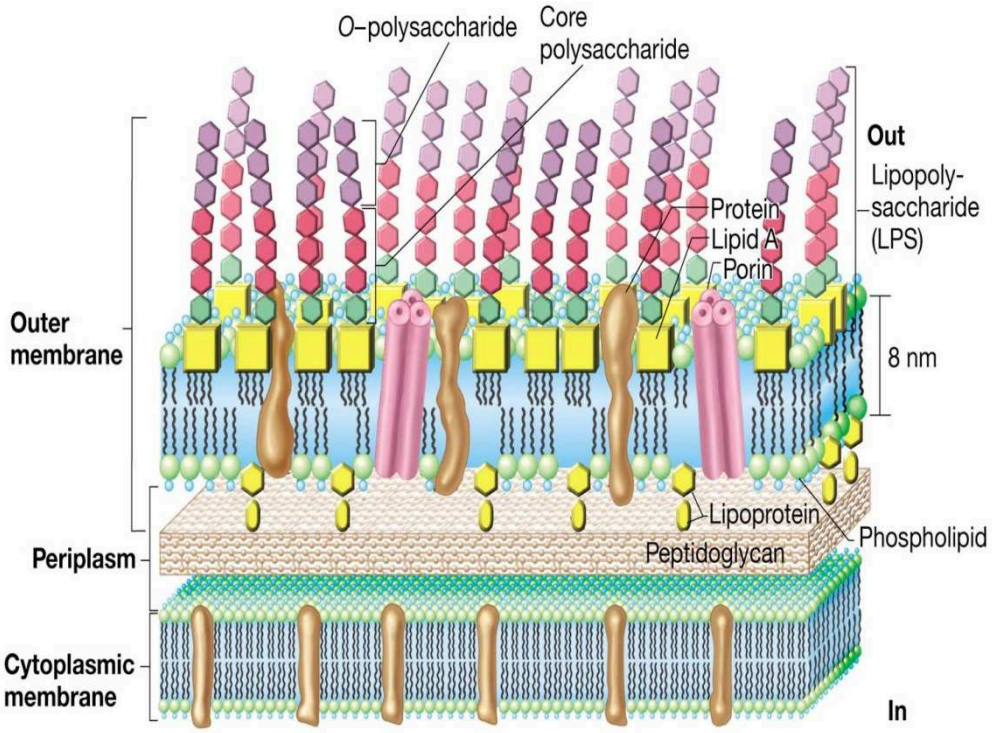


Figure 1. Cell envelope of a Gram- bacteria. Figure reproduced from Martinko.[1]

PG provides the cells with osmotic stability and has also a vital function in bacterial morphogenesis. Enzymes catalysing the biosynthesis of PG are attractive targets for the development of new antibiotics. Indeed, PG is only found in bacteria thus inhibitors of its biosynthesis will probably not interfere with eukaryote enzymes.[2]

PG is a polymeric macromolecule consisting of glycan strands cross-linked by small peptide chains. The sugar component is made up of alternating *N*-acetyl-glucosamine (GlcNAc) and *N*-acetyl-muramic acid (MurNAc) fragments. In Gram- bacteria, the peptide moiety consists of pentapeptide chains that can be cross-linked due to the presence of a diamino diacid, namely *meso*-diaminopimelic acid (*meso*-A_2pm, Figure 2). The amino acids are attached to the MurNAc lactyl group.

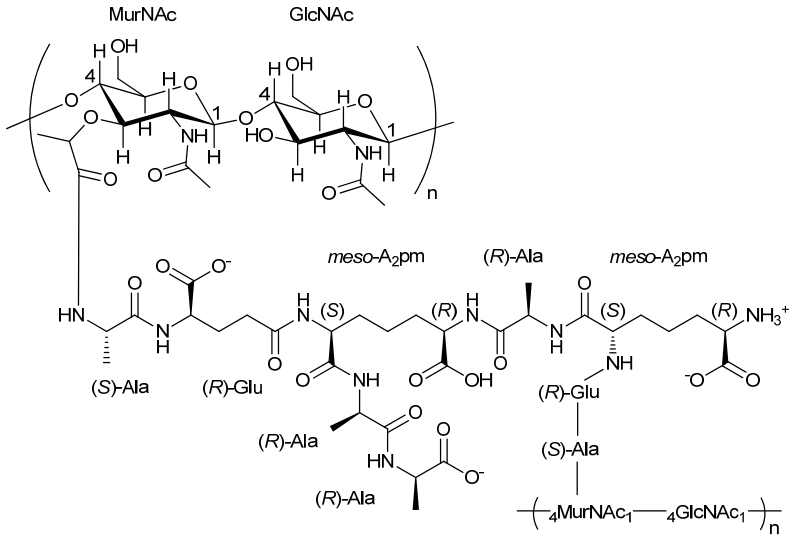


Figure 2. General structure of peptidoglycan in Gram- bacteria.

1.2 Peptidoglycan biosynthesis in *Escherichia coli*

PG biosynthesis starts in the cytoplasm by formation of UDP-MurNAc from UDP-GlcNAc through the involvement of the enzymes MurA and MurB which catalyse the addition of enolpyruvate and its reduction into a lactyl group (Figure 3). The successive addition of different amino acids is catalysed by different Mur ligases ((*S*)-Ala/MurC, (*R*)-Glu/MurD, *meso*-A_2pm/MurE, (*R*)-Ala-(*R*)-Ala/MurF). The synthesis of the bacteria specific (*R*)-Ala-(*R*)-Ala was catalysed in a previous step by Ala racemase (Alr) and (*R*)-Ala-(*R*)-Ala ligase (DdlB). Then, the MurNAc-peptide monophosphate moiety of UDP-MurNAc-pentapeptide is transferred on membrane-bound undecaprenyl phosphate by MraY yielding lipid I and UMP. The next step is the formation of lipid II by reaction with UDP-GlcNAc under catalysis

of the membrane-bound glycosyltransferase MurG. Lipid II is translocated to the periplasmic side of the plasma membrane. Probably an enzyme "flippase" is involved in this translocation. There, it is used as a substrate for the polymerisation reactions, transglycosylation followed by transpeptidation, both catalysed by penicillin-binding proteins (PBPs, Figure 3).

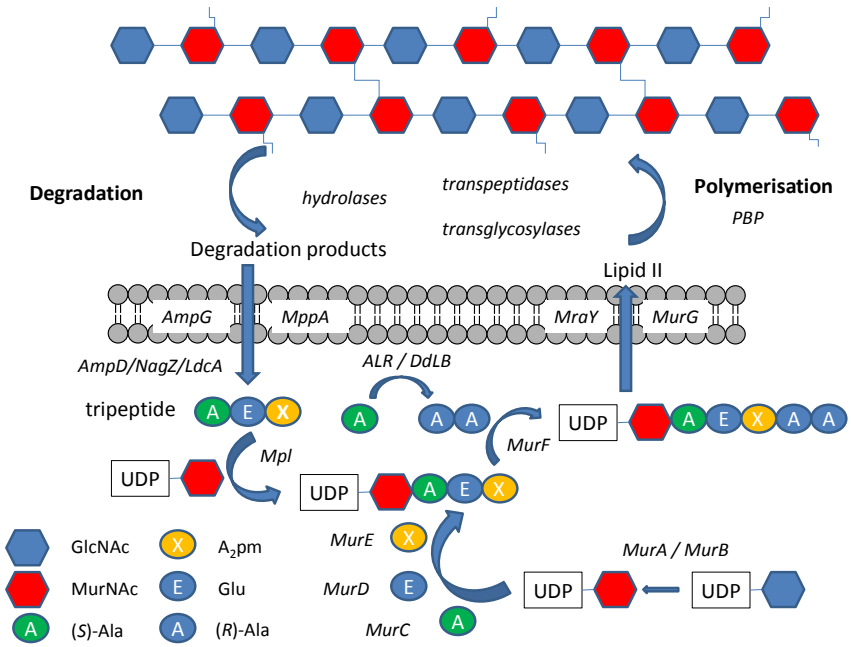


Figure 3. Metabolism of PG in *E. coli*. The *de novo* biosynthesis of PG starts with the synthesis of UDP-MurNAc-pentapeptide from UDP-GlcNAc in the cytoplasm (enzymes: MurA, MurB, MurC, MurD, MurE and MurF). The (*R*)-Ala-(*R*)-Ala is previously synthesized under catalysis of Alr and DdlB. The second part, the synthesis of Lipid II catalysed by MraY and MurG, takes place at the level of the cytoplasmic membrane. Finally, the polymerisation reactions (transglycosylation and transpeptidation) catalysed by PBPs, take place in the periplasm. During the cell growth and cell division the PG is partly degraded by numerous hydrolases. The degradation products are re-imported by permeases (AmpG, MppA) and further degraded (AmpD, NagZ, LdcA). The end-product, the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm, is re-introduced in the biosynthesis of PG by murein peptide ligase (Mpl).

In *E. coli*, during growth and cell division, a high fraction of PG is broken down by the action of lytic transglycosylases, amidases and endopeptidases. Besides *de novo* biosynthesis of PG, recycling of PG fragments generated during the growth and division processes is observed. Thus, one end-product of the degradation process,

the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm, is transported by the MppA (murein peptide permease A) into the cytoplasm where it is directly bound to UDP-MurNAc under catalysis by Mpl (murein peptide ligase). The resulting product is the natural substrate of MurF and thus can again enter the biosynthetic pathway of PG (Figure 3).

1.3 *meso*-Diaminopimelic acid replacement

In the biosynthesis of PG, MurE catalyses the formation of UDP-MurNAc-tripeptide from UDP-MurNAc-dipeptide and *meso*-A_2pm (Figure 3).[3] Although the specificity of *E. coli* MurE for *meso*-A_2pm is very high, certain A_2pm analogues are *in vivo* and *in vitro* substrates, whereas others have an inhibitory effect.[4-7] The replacement of A_2pm by several sulfur-containing compounds, lanthionine,[8-11] cystathionine[8] and djenkolic acid[9] has been described in the literature (Figure 4).

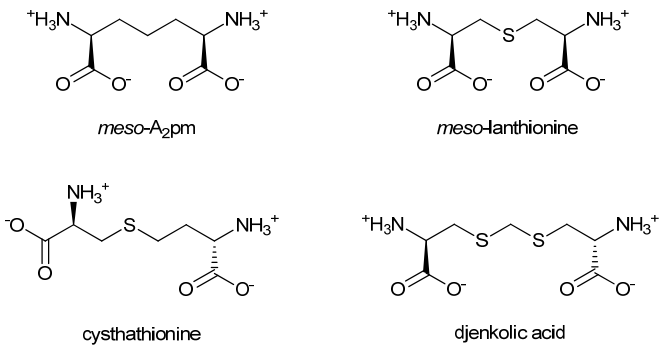


Figure 4. *meso*-A_2pm and some sulfur analogues.

Experiments with A_2pm derivatives containing different substituents at position 4 (4-methyl-A_2pm,[9] 4-methylene-A_2pm,[12] 4-hydroxy-A_2pm,[11] 4-fluoro-A_2pm,[11] 4-oxo-A_2pm[11]) or at position 3 (3-hydroxy-A_2pm[9,13]) 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.[9] In *E. coli*, *meso*-lanthionine (Figure 4) is a good substrate of MurE (52% relative specific activity compared with *meso*-A_2pm).[8] (*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.[8] Incorporation of (*S*,*S*)-A_2pm into *E. coli* mutants lacking diaminopimelate epimerase dapF 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 (Figure 2).[5,8]

Modified amino acids (A_2pm and Lys derivatives) in the form of tripeptide analogs of (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm can be introduced *in vivo* in *E. coli* through the PG recycling pathway.[14,15] The permeases and Mpl activity of *E. coli* thus allow the biosynthesis of modified UDP-MurNAc-tripeptides from exogenous tripeptides.[16]

1.4 Inhibitors of peptidoglycan biosynthesis

The PG biosynthesis can be inhibited at different steps. Here, the most common inhibitors are briefly presented. The compounds are classed by following the chronological order of the enzymes involved in PG biosynthesis (Figure 3).

1.4.1 Inhibitor of MurA

Fosfomycin is a naturally occuring broad-spectrum antibiotic that inhibit the first step of PG biosynthesis, namely the ligation of phosphoenolpyruvate to UDP-GlcNAc catalysed by MurA (Figure 5).[17]

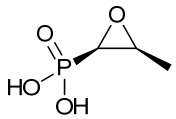


Figure 5. Fosfomycin, a MurA inhibitor.

1.4.2 Inhibitor of Alr and DdlB

(*R*)-Cycloserine is a structural analogue of (*R*)-Ala and inhibits both Ala racemase (Alr), which converts (*S*)-Ala to (*R*)-Ala, and (*R*)-Ala-(*R*)-Ala ligase (DdlB) (Figure 6).[18]

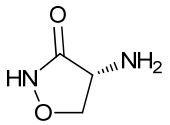


Figure 6. (*R*)-Cycloserine, an Alr and DdlB inhibitor.

1.4.3 Inhibitor of MraY

Tunicamycin prevents the synthesis of Lipid I by inhibiting MraY, the enzyme responsible for the transfer of the monophosphate of MurNac-pentapeptide onto the membrane-bound undecaprenyl phosphate (Figure 7).[19]

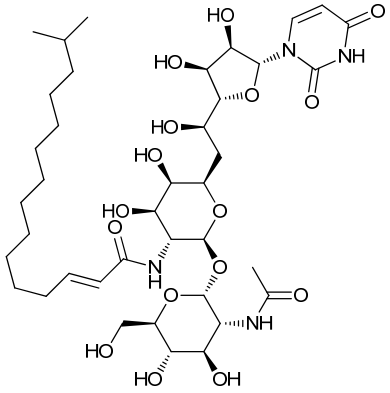


Figure 7. Tunicamycin, a MraY inhibitor.

1.4.4 Inhibitor of MurG

Ramoplanin inhibits the formation of Lipid II from Lipid I by hampering the addition of UDP-GlcNac catalysed by MurG (Figure 8).[20]

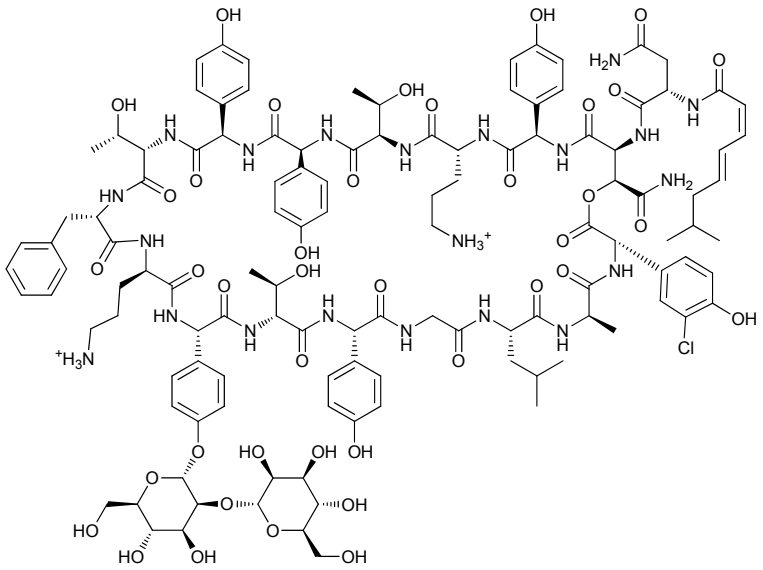


Figure 8. Ramoplanin, a MurG inhibitor.

1.4.5 Interaction with Lipid II

Vancomycin is a glycopeptide antibiotic that target Lipid II at the (*R*)-Ala-(*R*)-Ala moiety of the pentapeptide chain and thus inhibits the PG biosynthesis at the level of transpeptidation. It exerts its action outside of the cytoplasmic membrane (Figure 9).[21]

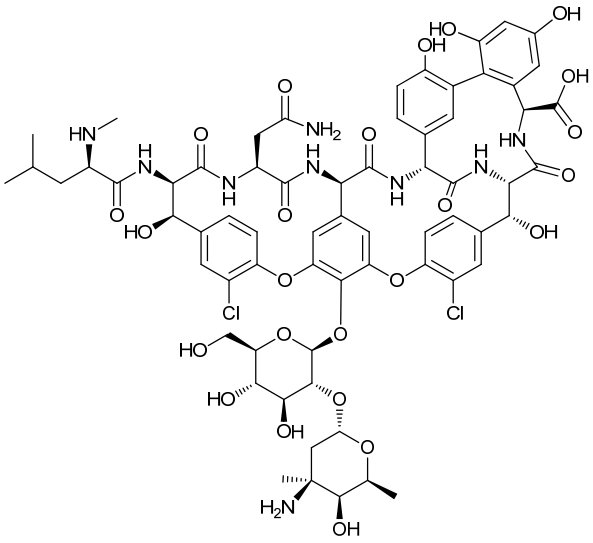


Figure 9. Vancomycin, a Lipid II inhibitor.

Mersacidin is a biologically active polypeptide containing a lanthionine (ie a lantibiotic) that interacts with a different part of Lipid II (the terminal GlcNac) than vancomycin and thus inhibits the PG biosynthesis at the level of transglycosylation (Figure 10).[21,22]

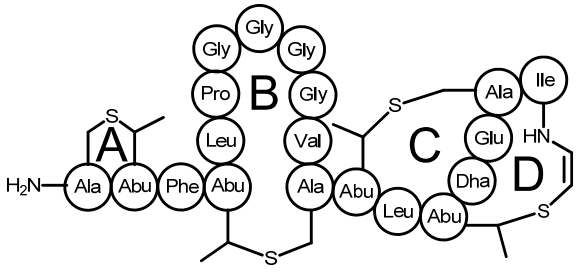


Figure 10. Mersacidin, a Lipid II inhibitor.

1.4.6 Interaction with the cytoplasmic membrane

Lantibiotics such as nisin interract with Lipid II and with the cytoplasmic membrane. Indeed, in the presence of Lipid II, nisin is able to form pores in the plasma membrane, leading to a potent (nmol) antibiotic effect. A second mechanism (sequestration of Lipid II) that does not involve pore formation is currently investigated (Figure 11).[21,23]

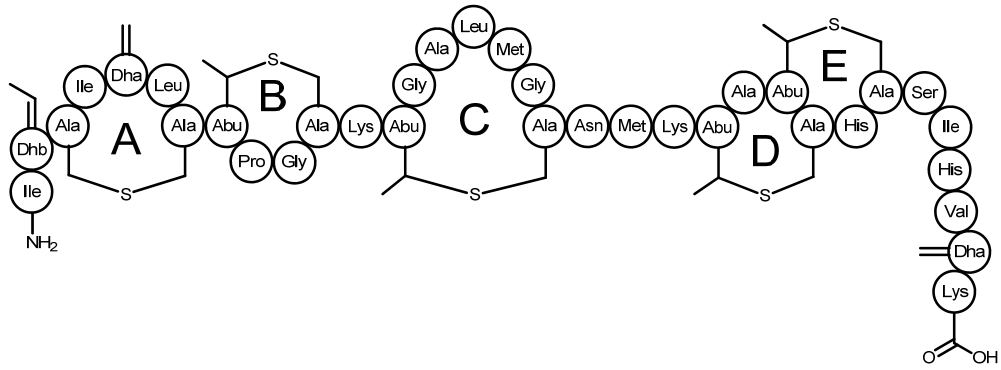


Figure 11. Nisin, a cytoplasmic membrane-targeting lantibiotic.

1.4.7 Inhibitor of PBP (tranglycosylation)

Moenomycin is a phosphoglycolipid that inhibits the transglycosylation stage of PG polymerisation by mainly targeting PBP1b, a bifunctionnal transpeptidase/glycosyl transferase (Figure 12).[24]

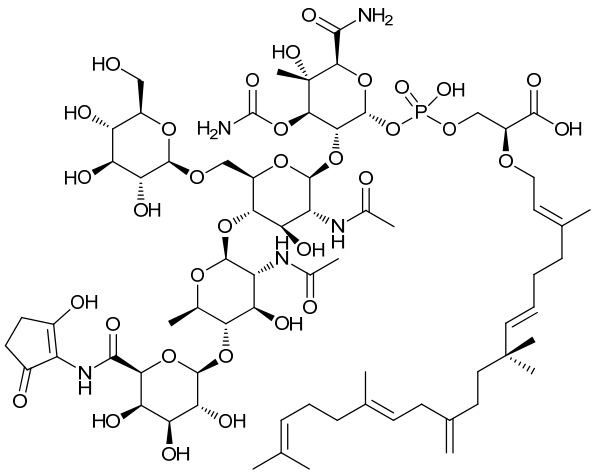


Figure 12. Moenomycin, a PBP1b-transglycosylase inhibitor.

1.4.8 Inactivator of PBP (transpeptidation)

The β-lactam family of antibiotic with its well-known member penicillin G, inactivates the DD-transpeptidase step of PG reticulation by substituting for (*R*)-Ala-(*R*)-Ala and forming a covalent bond with the active Ser of the active site of PBPs (Figure 13).

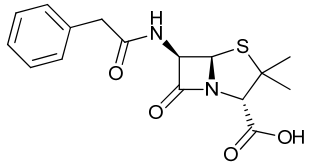


Figure 13. Penicillin G, a PBP DD-transpeptidase inactivator.

1.5 Labeling biomolecules

The widespread use of antibiotics has caused bacteria to become drug resistant. Efforts are done by many research groups to identify new molecules as inhibitors or inactivators of resistant proteins like PBPs or to find new targets which allow putting PG biosynthesis under control.

To attain these objectives, labeled molecules can be useful in four ways:

i) studies of biosynthesis with labeled substrates *in vivo* and *in vitro* (for example synthetic tripeptides as substrates of Mpl,[16] 1,6-AnhMurNAc derivatives for assay of AmiD,[25] MurG assay using a *N*-dansyl-Lipid I analogue,[26]…)

ii) studies of inhibitors of different enzymes using labeled substrates (for instance screening of inhibitors of PG glycosyltransferase using a displacement assay with a fluorescent moenomycin analogue,[27] continuous fluorescence assay for PBP1b inhibitors using *N*-dansyl-Lipid II,[28] [^{14}C]glutamic acid for evaluation of MurD inhibitors,[29]…)

iii) imaging PG biosynthesis *in vitro* and *in vivo* using fluorescent probes (for example fluorescent vancomycin and ramoplanin for imaging

PG,[30] investigation of the mechanism of Lipid II interaction with nisin by fluorescence,[23] NBD-tripeptides for cell wall labeling *via* the PG recycling pathway,[31] imaging of live bacteria using taged (*R*)-Ala analogues,[32,33]…)

iv) studies of enzyme interactions by introduction of a photoactivatable crosslinker (for instance an [^{125}I]4-azidosalicylic-labeled UDP-MurNAc-pentapeptide to identify proteins in contact with the resulting Lipid II,[34] photoaffinity-probe and biotin tagged moenomycin;[35,36] a mini-review on the topic of photoaffinity labeling as well as a recent book describe bioconjugate techniques[37,38]).

1.6 Objective

In this work, we will try to develop a novel labeling method of the PG of *E. coli* through the replacement of natural A_2pm by derivatives of lanthionine (Figure 4 and Figure 14). In a first approach, we will investigate the synthesis of derivatives **1**-**2** and the feasibility of their utilisation in PG biosynthesis *via* MurE enzymatic addition. Another part of this work will concern the replacement of tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm by analogues **3**-**4** and their incorporation *via* the recycling pathway. Those labeling strategies may open ways to the study of PG synthesis and turnover in relation with cell growth and division.

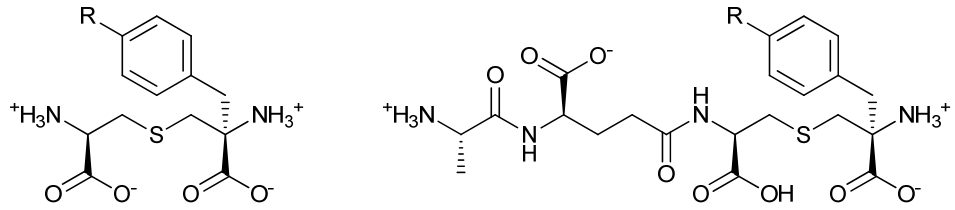


Figure 14. Structural formulas of natural and labelled tripeptides.

In particular, studies of PG biosynthesis using compounds **1**-**4** can shed light on enzyme-substrate specificities. In addition, by using an azido group, analogues such as **2** and **4** can be useful for click-chemistry ligation with a fluorescent probe and imaging of bacteria. Furthermore, **2** and **4** containing [^{35}S]lanthionine and a photoactivatable moiety such as phenylazido could potentially be used to study enzymes interacting with PG. *In vivo*, upon UV irradiation, identification of proteins involved in the biosynthesis or biodegradation of the PG or in the cell morphogenesis and division should be feasible.

During the course of the present work, a literature survey of known lanthionine syntheses was performed. The main routes are summarized in the bibliographic part. The results of our research have been published in three papers, all of which are presented in the following sections.

References

1. Martinko, J.; Madigan, M. *Brock Biology Of Microorganisms*. 11th ed.; 2005.
2. Zervosen, A.; Sauvage, E.; Frère, J.M.; Charlier, P.; Luxen, A. Development of new drugs for an old target: the penicillin binding proteins. *Molecules* **2012**, *17*, 12478-12505.
3. van Heijenoort, J. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Natural Product Reports* **2001**, *18*, 503-519.
4. Auger, G.; van Heijenoort, J.; Vederas, J.C.; Blanot, D. Effect of analogues of diaminopimelic acid on the *meso*-diaminopimelate-adding enzyme from *Escherichia coli*. *FEBS Lett.* **1996**, *391*, 171-174.
5. Mengin-Lecreulx, D.; Michaud, C.; Richaud, C.; Blanot, D.; van Heijenoort, J. Incorporation of LL-diaminopimelic acid into peptidoglycan of *Escherichia coli* mutants lacking diaminopimelate epimerase encoded by dapF. *J. Bacteriol.* **1988**, *170*, 2031-2039.
6. Michaud, C.; Mengin-Lecreulx, D.; van Heijenoort, J.; Blanot, D. Over-production, purification and properties of the uridine-diphosphate-*N*-acetylmuramoyl-L-alanyl-D-glutamate: *meso*-2,6-diaminopimelate ligase from *Escherichia coli*. *Eur. J. Biochem.* **1990**, *194*, 853-861.
7. van Assche, I.; Soroka, M.; Haemers, A.; Hooper, M.; Blanot, D.; van Heijenoort, J. Synthesis and antibacterial evaluation of phosphonic acid analogues of diaminopimelic acid. *European Journal of Medicinal Chemistry* **1991**, *26*, 505-515.
8. Mengin-Lecreulx, D.; Blanot, D.; van Heijenoort, J. Replacement of diaminopimelic acid by cystathionine or lanthionine in the peptidoglycan of *Escherichia coli*. *J. Bacteriol.* **1994**, *176*, 4321-4327.
9. Rhuland, L.E. Role of α,ε-diaminopimelic acid in the cellular integrity of *Escherichia coli*. *J. Bacteriol.* **1957**, *73*, 778-783.
10. Richaud, C.; Mengin-Lecreulx, D.; Pochet, S.; Johnson, E.J.; Cohen, G.N.; Marliere, P. Directed evolution of biosynthetic pathways. Recruitment of cysteine thioethers for constructing the cell wall of *Escherichia coli*. *J. Biol. Chem.* **1993**, *268*, 26827-26835.
11. Chaloupka, J.M.; Strnadová, M.; Caslavská, J.; Veres, K. Growth and cell division of *Escherichia coli* 173-25 in the presence of some analogues of diaminopimelic acid. *Z. All. Mikrobiol.* **1974**, *14*, 283-296.
12. Girodeau, J.M.; Agouridas, C.; Masson, M.; Pineau, R.; Le Goffic, F. The lysine pathway as a target for a new genera of synthetic antibacterial antibiotics? *J. Med. Chem.* **1986**, *29*, 1023-1030.
13. Sundharadas, G.; Gilvarg, C. A new synthesis and some biological properties of β-hydroxy-α,ε-diaminopimelic acid. *J. Biol. Chem.* **1966**, *241*, 3276-3282.
14. Goodell, E.W. Recycling of murein by *Escherichia coli*. *J. Bacteriol.* **1985**, *163*, 305-310.
15. Hervé, M.; Boniface, A.; Gobec, S.; Blanot, D.; Mengin-Lecreulx, D.; Hervé, M.; Kovač, A.; Cardoso, C.; Patin, D.; Brus, B.*, et al.* Biochemical characterization and physiological properties of *Escherichia coli* UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate ligase. *J. Bacteriol.* **2007**, *189*, 3987-3995.
16. Hervé, M.; Kovač, A.; Cardoso, C.; Patin, D.; Brus, B.; Barreteau, H.; Mengin-Lecreulx, D.; Gobec, S.; Blanot, D. Synthetic tripeptides as alternate substrates of murein peptide ligase (Mpl). *Biochimie* **2013**, *95*, 1120-1126.
17. Eschenburg, S.; Priestman, M.; Schönbrunn, E. Evidence That the Fosfomycin Target Cys115 in UDP-N-acetylglucosamine Enolpyruvyl Transferase (MurA) Is Essential for Product Release. *J. Biol. Chem.* **2005**, *280*, 3757-3763.
18. Noda, M.; Kawahara, Y.; Ichikawa, A.; Matoba, Y.; Matsuo, H.; Lee, D.-G.; Kumagai, T.; Sugiyama, M. Self-protection Mechanism in D-Cycloserine-producing Streptomyces lavendulae: Gene cloning, characterization, and kinetics of its alanine racemase and D-alanyl-D-alanine ligase, which are target enzymes of D-cycloserine. *J. Biol. Chem.* **2004**, *279*, 46143-46152.
19. Price, N.P.J.; Tsvetanova, B. Biosynthesis of the Tunicamycins: A Review. *J. Antibiot.* **2007**, *60*, 485-491.

20. McCafferty, D.G.; Cudic, P.; Frankel, B.A.; Barkallah, S.; Kruger, R.G.; Li, W. Chemistry and biology of the ramoplanin family of peptide antibiotics. *Peptide Science* **2002**, *66*, 261-284.
21. Breukink, E.; de Kruijff, B. Lipid II as a target for antibiotics. *Nat. Rev. Drug Discov.* **2006**, *5*, 321-323.
22. Ross, A.C.; Vederas, J.C. Fundamental functionality: recent developments in understanding the structure-activity relationships of lantibiotic peptides. *J. Antibiot.* **2011**, *64*, 27-34.
23. Hasper, H.E.; Kramer, N.E.; Smith, J.L.; Hillman, J.D.; Zachariah, C.; Kuipers, O.P.; de Kruijff, B.; Breukink, E. An Alternative Bactericidal Mechanism of Action for Lantibiotic Peptides That Target Lipid II. *Science* **2006**, *313*, 1636-1637.
24. Baizman, E.R.; Branstrom, A.A.; Longley, C.B.; Allanson, N.; Sofia, M.J.; Gange, D.; Goldman, R.C. Antibacterial activity of synthetic analogues based on the disaccharide structure of moenomycin, an inhibitor of bacterial transglycosylase. *Microbiology* **2000**, *146*, 3129-3140.
25. Mercier, F.; Zervosen, A.; Teller, N.; Frère, J.-M.; Herman, R.; Pennartz, A.; Joris, B.; Luxen, A. 1,6-AnhMurNAc derivatives for assay development of amidase AmiD. *Biorg. Med. Chem.* **2010**, *18*, 7422-7431.
26. Auger, G.; van Heijenoort, J.; Mengin-Lecreulx, D.; Blanot, D. A MurG assay which utilises a synthetic analogue of lipid I. *FEMS Microbiol. Lett.* **2003**, *219*, 115-119.
27. Gampe, C.M.; Tsukamoto, H.; Doud, E.H.; Walker, S.; Kahne, D. Tuning the Moenomycin Pharmacophore To Enable Discovery of Bacterial Cell Wall Synthesis Inhibitors. *J. Am. Chem. Soc.* **2013**, *135*, 3776-3779.
28. Schwartz, B.; Markwalder, J.A.; Seitz, S.P.; Wang, Y.; Stein, R.L. A Kinetic Characterization of the Glycosyltransferase Activity of *Eschericia coli* PBP1b and Development of a Continuous Fluorescence Assay. *Biochemistry* **2002**, *41*, 12552-12561.
29. Barreteau, H.; Sosič, I.; Turk, S.; Humljan, J.; Tomašić, T.; Zidar, N.; Hervé, M.; Boniface, A.; Peterlin-Mašič, L.; Kikelj, D.*, et al.* MurD enzymes from different bacteria: Evaluation of inhibitors. *Biochem. Pharmacol.* **2012**, *84*, 625-632.
30. Tiyanont, K.; Doan, T.; Lazarus, M.B.; Fang, X.; Rudner, D.Z.; Walker, S. Imaging peptidoglycan biosynthesis in Bacillus subtilis with fluorescent antibiotics. *Proceedings of the National Academy of Sciences* **2006**, *103*, 11033-11038.
31. Olrichs, N.K.; Aarsman, M.E.; Verheul, J.; Arnusch, C.J.; Martin, N.I.; Hervé, M.; Vollmer, W.; de Kruijff, B.; Breukink, E.; den Blaauwen, T. A novel in vivo cell-wall labeling approach sheds new light on peptidoglycan synthesis in *Escherichia coli*. *ChemBioChem* **2011**, *12*, 1124-1133.
32. Siegrist, M.S.; Whiteside, S.; Jewett, J.C.; Aditham, A.; Cava, F.; Bertozzi, C.R. D-Amino Acid Chemical Reporters Reveal Peptidoglycan Dynamics of an Intracellular Pathogen. *ACS Chemical Biology* **2012**, *8*, 500-505.
33. Kuru, E.; Hughes, H.V.; Brown, P.J.; Hall, E.; Tekkam, S.; Cava, F.; de Pedro, M.A.; Brun, Y.V.; VanNieuwenhze, M.S. In Situ Probing of Newly Synthesized Peptidoglycan in Live Bacteria with Fluorescent D-Amino Acids. *Angew. Chem. Int. Ed.* **2012**, *51*, 12519-12523.
34. van Dam, V.; Olrichs, N.; Breukink, E. Specific labeling of peptidoglycan precursors as a tool for bacterial cell wall studies. *ChemBioChem* **2009**, *10*, 617-624.
35. Volke, D.; Daghish, M.; Hennig, L.; Findeisen, M.; Giesa, S.; Oehme, R.; Welzel, P. On Penicillin-Binding Protein 1b Affinity-Labeling Reagents. *Helv. Chim. Acta* **2003**, *86*, 4214-4232.
36. Rühl, T.; Hennig, L.; Hatanaka, Y.; Burger, K.; Welzel, P. A trifunctional reagent for photoaffinity labeling. *Tetrahedron Lett.* **2000**, *41*, 4555-4558.
37. Hashimoto, M.; Hatanaka, Y. Recent Progress in Diazirine-Based Photoaffinity Labeling. *Eur. J. Org. Chem.* **2008**, *2008*, 2513-2523.
38. Hermanson, G.T. *Bioconjugate Techniques*. Third ed.; 2013.

Chapter 2

Bibliography

2.1 Background

Around 1925, human hair was the main material used to prepare (*R,R*)-cystine (**1**, Figure 1).[1] The process involved a preliminary wash of the hair with soap to remove oil and waxes. A cold aqueous Na_2CO_3 (1-2%) solution was also often used for this purpose. A 5% (w/w) yield of **1** was thus obtained after heating at 100 °C with aqueous HCl (6 M) for 120 h, followed by neutralization and precipitation.[1] Other keratinous materials such as wool and feather were also used. Nevertheless, Hoffman discovered that this procedure did not lead to any cystine isolation if the preliminary wash was done with *hot* (100 °C) aqueous Na_2CO_3.[2] In that case, after 2 h of stirring with hot aqueous Na_2CO_3 (1%), a loss of 23.8% of the sulfur content of the hair was recorded. As crystallization of cystine was prevented, Hoffman assumed that its structure had been altered.

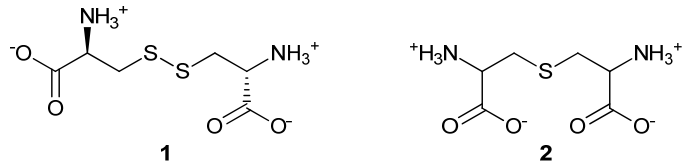


Figure 1. (*R,R*)-cystine **1** and β-amino-β-carboxy-ethylsulfide (lanthionine) **2.**

In 1941, Horn et al. reported the isolation of a new amino acid after boiling wool with aqueous Na_2CO_3 (2%) for 1 h.[3] This amino acid was optically inactive and was identified as the symmetrical β-amino-β-carboxy-ethylsulfide **2** based on the evidence of elemental analysis, general properties and chemical behaviour. This compound **2** crystallized in triangle-like hexagonal plate in a yield of 0.5% (w/w) (Figure 2).

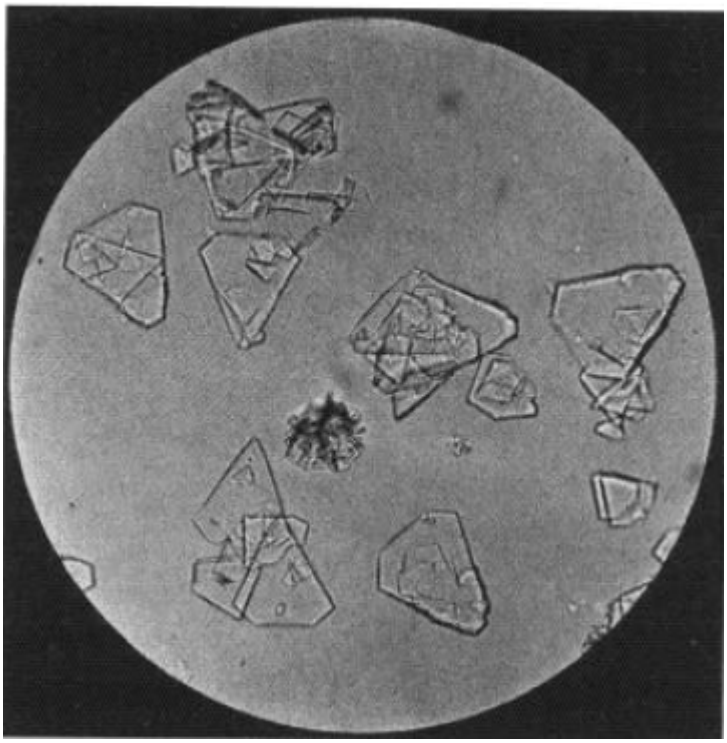
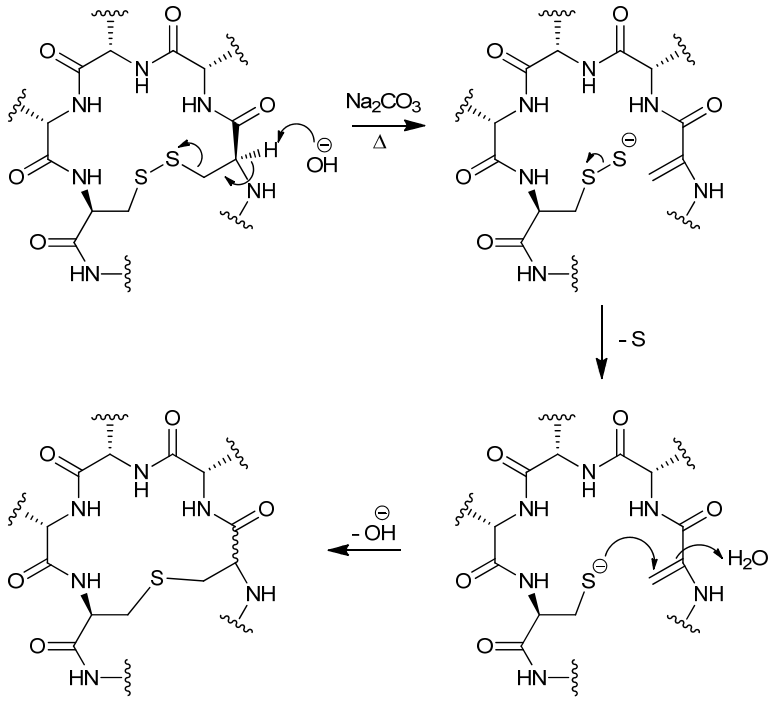


Figure 2. Triangle-like hexagonal plate of the more insoluble isomer of **2**.[3]

As compound **2** was isolated from wool and contains sulfur, it was named lanthionine (Lan). Its structure was confirmed by Brown and Du Vigneaud after synthesis (yield 4.2%) and comparison of optical-crystallographic data.[3,4] The optical inactivity of **2**, crystallizing in triangle-like shape, suggested that it was the *meso*-isomer. During the process, a second compound, more soluble and differing in crystalline form, was also recovered (yield 0.5%). Those latter crystals presented the same composition as the *meso*-form and, except solubility, mp and crystalline form, displayed the same chemical properties. This new derivative, crystallizing in various forms depending of the solvent used (dimorphism), was also optically inactive. It was concluded this last compound was a mixture (1:1) of both enantiomers of Lan (ie *dl*-Lan).[5] *meso*-Lan was also isolated from chicken feather (0.25%, w/w), human hair (2.5%), lactalbumin (0.25%) and insulin (1%).[6-8] A recent review by Galande et al. describes the mechanism of the base-induced desulfurization of the cystine residue to Lan inside small peptides (Scheme 1).[9]



Scheme 1. Probable mechanism for the formation of Lan in peptides upon base treatment.[10-12]

The transformation involves a β-elimination from cystine giving a dehydroalanine (Dha) and an unstable disulfide anion.[11] Subsequent loss of sulfur and Michael addition of the liberated cysteine (Cys) residue on the Dha provides Lan bridged peptide (Scheme 1).[9,10]

The main chemical and enzymatic syntheses of Lan were reviewed by Paul and Van der Donk in 2005.[13] Another review on the principal chemical syntheses of Lan and a discussion on the preparation of biologically active peptides containing a Lan (lantibiotics) was published in 2011 by Tabor.[14] In the following sections the most important routes to unprotected Lan and to protected Lan as well as two examples of applications in the lantibiotic field are presented.

2.2 Chemical synthesis of unprotected lanthionines

2.2.1 Generality

As described in the previous section, Lan is a diamino diacid with a central monosulfur linked to two alanine residues each of *R* or *S* configuration. Thereby, different stereomeric forms of Lan are possible. Those are summarized in Figure 3.

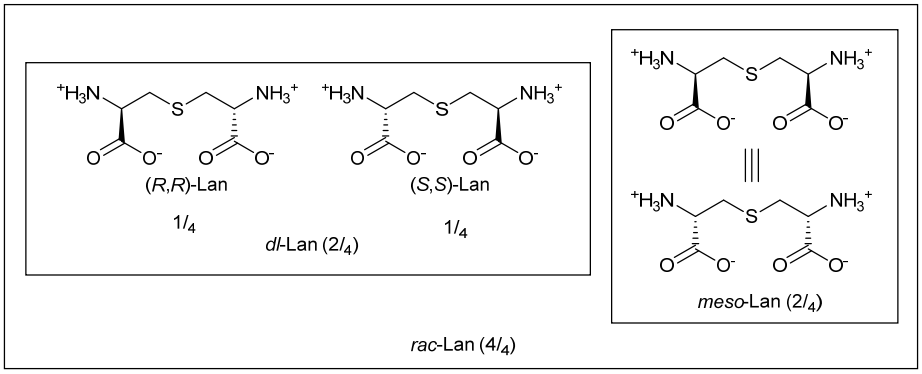


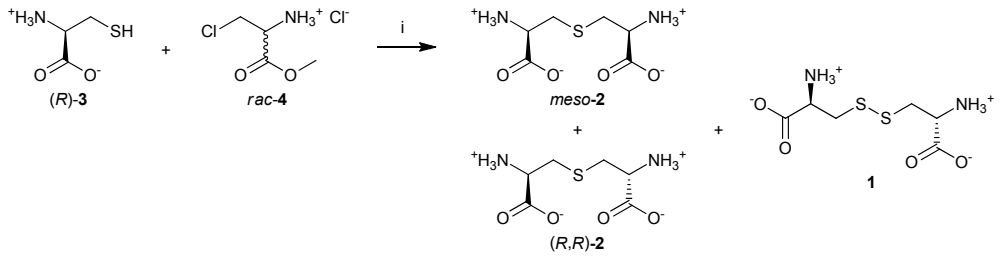
Figure 3. Stereochemical forms of Lan **2**.

Assuming a Lan synthesis from racemic starting materials, the ratio of each diastereomer would be as follows: the two optically active forms [(*R*,*R*)-Lan and (*S*,*S*)-Lan] contribute each for 25% and the *meso*-Lan for 50%. The nomenclature presented in Figure 3 will be used throughout this chapter.

In the following paragraphs different syntheses of unprotected Lan are presented using various precursors.

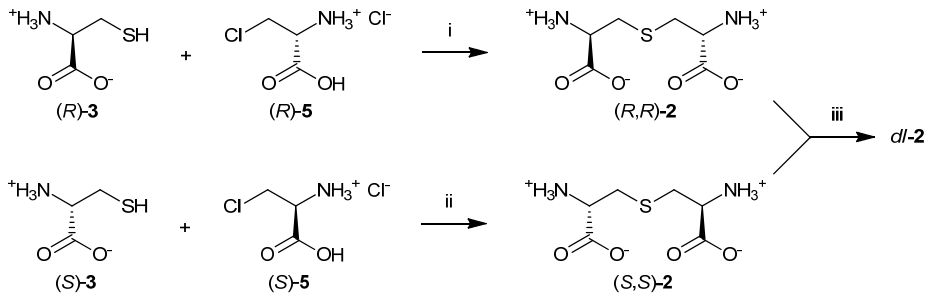
2.2.2 Synthesis from chloroalanine

The first chemical synthesis of Lan **2** and the structural proof were reported, in 1941, by Brown and Du Vigneaud.[4] Lan **2** was prepared through the reaction of (*R*)-Cys (*R*)-**3** with *rac*-chloroalanine methyl ester hydrochloride *rac*-**4** (Scheme 2).



Scheme 2. The first route to Lan **2**. i) (*R*)-**3** (250 mmol, 2.17 eq), *rac*-**4** (115 mmol, 1 eq), KOH (33% aq, 648 mmol), 45 °C, 3 h, fractional crystallization, 4.2% (*meso*-**2**, 1 g).

The reaction, occurring in a basic solution of KOH, led to a mixture of products. Indeed, Lan was contaminated with (*R*,*R*)-cystine **1** (from the dimerization of **3**) and another compound. After sequential fractional crystallizations and microscopic examination, pure triangle-like crystals of Lan **2** were obtained (yield 4.2%). The physical and chemical properties of this compound were identical to the first form of Lan isolated from wool by Horn et al..[3] If reaction between *rac*-**4** and (*R*)-**3** proceeded without racemization, only (*R*,*R*)-Lan and *meso*-Lan could have been formed (Scheme 2). Since the triangle-like crystals (cfr. 2.1 Generality) were optically inactive, the *meso*-Lan (*meso*-**2**) was synthesized.[4]



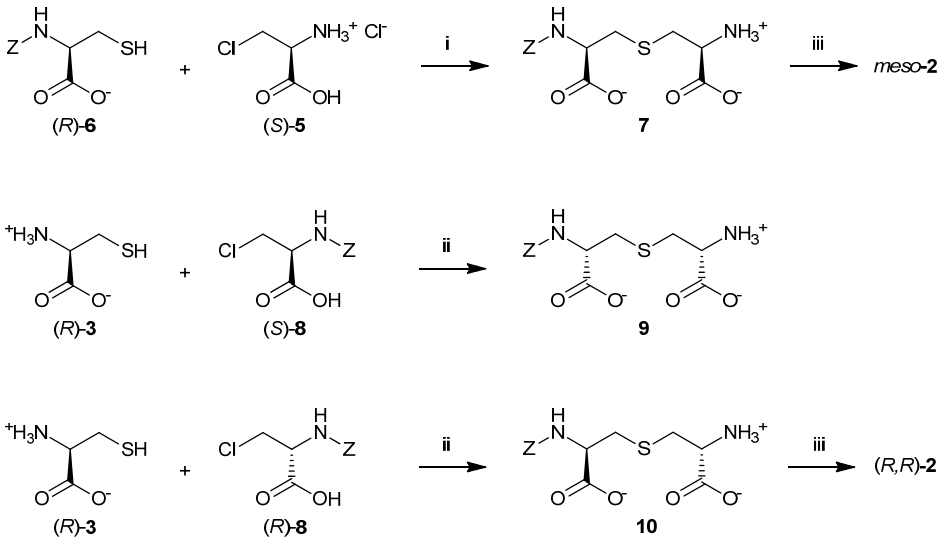
Scheme 3. Diastereoselective synthesis of Lan **2**. i) (*R*)-**3** (117 mmol, 1.34 eq), (*R*)-**5** (87 mmol, 1 eq), KOH (33% aq, 250 mmol), 45 °C to rt, 4 h, recrystallization (NaCN), 34%; ii) (*R*)-**3** (71 mmol, 1 eq), (*R*)-**5** (81 mmol, 1.15 eq), KOH (33% aq), 45 °C to rt, 4 h, recrystallization, 21%; iii) equimolar (*R*,*R*)-**2** and (*S*,*S*)-**2**, NH_4OH, AcOH.

In order to get the (*R*,*R*)-Lan diastereoisomer, Brown and Du Vigneaud reacted (*R*)-chloroalanine hydrochloride (*R*)-**5** and Cys (*R*)-**3** (prepared *in situ* from (*R*,*R*)-cystine) with aqueous KOH for 4 h (Scheme 3).[15] Following neutralization, precipitation, cystine removal (NaCN) and recrystallization, (*R*,*R*)-Lan was obtained

in 34% yield. Using a similar procedure, the (*S*,*S*)-Lan diastereoisomer was obtained from (*S*)-**5** (previously prepared *in situ* from (*S*)-BnS-Cys) and (*S*)-**3** in 21% yield. An equal amount of (*R*,*R*)-Lan and (*S*,*S*)-Lan were then mixed in aqueous ammonia and acetic acid was added to crystallize *dl*-Lan (*dl*-**2**, Figure 3 and Scheme 3).[15] This method was also used to prepare a homologue of Lan, namely cystathionine.[16,17]

In 1979, the same procedure was used by Photaki et al. to synthesize (*R*,*R*)-Lan, in a higher yield (75%), from commercially available Cys (*R*)-**3**. Likewise, using (*S*)-**5** and (*R*)-**3**, *meso*-Lan was obtained in 55% yield.[18]

Moreover, the same paper also described a synthesis of cyclolanthionine from *N*-CBz protected Lan **7** (Scheme 4).[18] This latter compound was obtained from *N*-CBz-(*R*)-Cys (*R*)-**6** and chloroalanine (*S*)-**5**. After reaction with 1 M KOH at rt for 3 h, crystallization of **7** occurred at pH 3.1. Subsequent recrystallization afforded pure **7** in 37% yield.



Scheme 4. Diastereoselective synthesis of CBz-Lan. i) (*R*)-**6** (9.2 mmol, 1.47 eq), (*S*)-**5** (6.25 mmol, 1 eq), KOH (1.8 M, 45 mmol), 50 °C to rt, 4 h, Et_2O (to remove excess (*R*)-**6**), pH 3.1, recrystallization, 37%; ii) (*R*)-**3** (6 mmol, 2 eq), (*S*)-**8** (3 mmol, 1 eq), KOH (33% aq., 16 mmol), THF, 70 °C, Triton B, 5 h, Et_2O, pH 2.7, recrystallization, 46-68%; iii) 10 M HCl in AcOH (1/1), 60 °C, 5-15 h, de 97.9-99.9%.

Furthermore, compound **9** was obtained by reacting (*R*)-**3** and (*S*)-**8** in a two-phase system, using phase transfer catalysis (PTC), in 68% yield. The same reaction was also realized from (*R*)-**3** and (*R*)-**8** to give, after recrystallization, **10** (yield 46%).

The CBz-protecting group of **7** and **10** was then removed by hydrolysis with concentrated HCl in AcOH at 60 °C for 5 - 15 h to yield the corresponding Lan **2** in high diastereomeric excess (de) (97.9-99.9%; yield unknown) (Scheme 4).

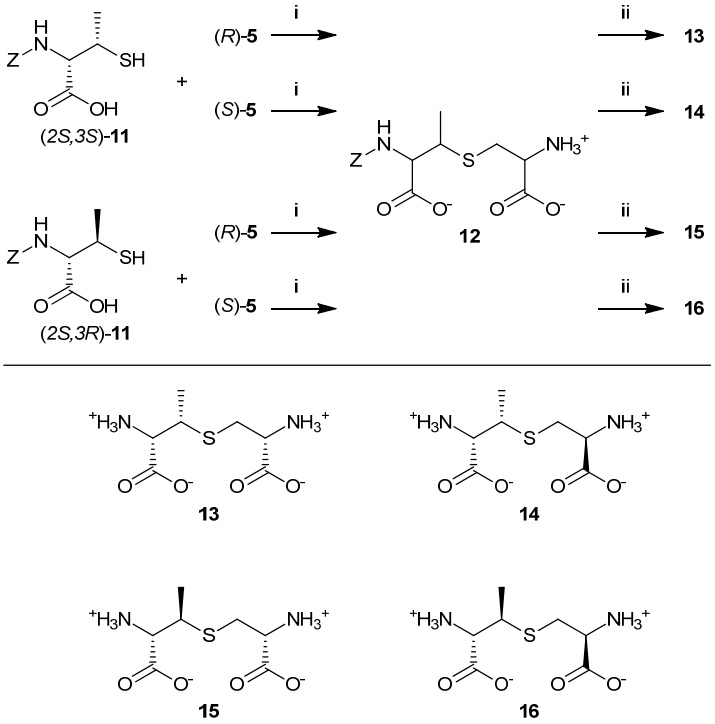
The racemization study of **2** in HCl (6 M) showed that at temperature > 96 °C, the de was decreasing (Table 1). Fortunately, no racemization occurred at 60 °C.[18,19]

Table 1. Moore and Stein amino-acid analyses after heating acidic solutions of (*R*,*R*)-Lan

T (°C)	T (h)	Acid	(*R*,*R*)-Lan	*meso*-Lan
20	-	-	100	0
96	5	a	97.8	2.2
96	15	a	95	5
110	6	a	92.1	7.9
110	24	a	75.8	24.2
110	48	a	63.2	36.8
110	72	a	56.7	43.3
110	168	a	50.4	49.6
60	5	b	100	0
96	5	b	98	2
96	15	b	93.3	6.7
96	25	b	90.4	9.6
96	45	b	85.3	14.7

[a] 6 M HCl; [b] acetic acid-concentrated HCl (1:1). Table reproduced from Photaki.[18]

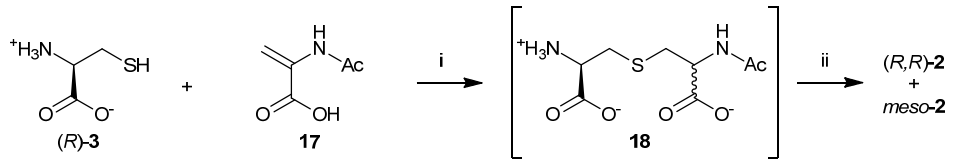
In order to identify the natural β-methyl-lanthionine (β-Me-Lan) presents in nisin, Wakamiya et al., in 1985, synthesized four β-Me-Lan diastereomers.[20] The procedure of Photaki et al., starting from (2*S*,3*S*)-**11** or (2*S*,3*R*)-**11** and (*R*)-**5** or (*S*)-**5** was used (Scheme 5). The resulting compounds **12** were chromatographed, crystallized and treated with HBr in AcOH at rt to remove the CBz protecting group. Four diastereomeric β-Me-Lan **13-16** were thus obtained. The natural compound isolated from nisin was thereby identified as isomer [2*S*,3*S*,6*R*] (**13**, Scheme 5).



Scheme 5. Diastereoselective synthesis of four diastereomers of β-Me-Lan. i) **11** (2.5 mmol, 1 eq), **5** (2.5 mmol, 1 eq), KOH (3.4 M, 11.5 mmol), 50 °C (30 min) to rt, 44 h, Et_2O (to remove unreacted **11**), HP-20 chromatography (50% aq. MeOH), crystallization, 35-53%; ii) 25% HBr in AcOH, rt, 2 h, Et_2O, pyridine, recrystallization, 87-99%.

2.2.3 Synthesis from dehydroalanine

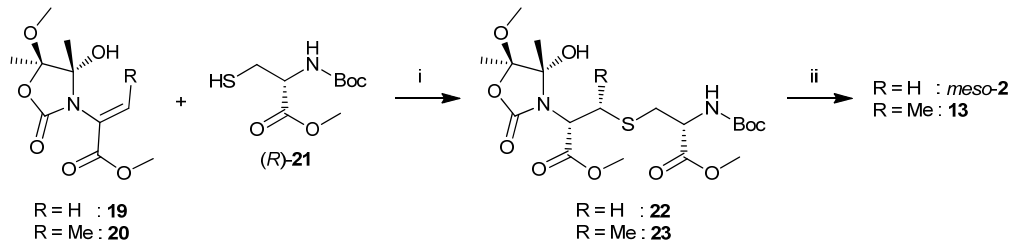
In 1947, Schöberl and Wagner reported that the reaction of **17**, a Dha derivative, with (*R*)-**3** led to a mixture of (*R*,*R*)-Lan and *meso*-Lan (Scheme 6).[21] The Michael addition occurred readily at 100 °C. Although the reaction proceeded in good yield (83%), it suffered from the lack of diastereospecificity due to the planarity of **17**.



Scheme 6. Synthesis of Lan from a Dha derivative. i) (*R*)-**3** ·HCl (10 mmol, 1 eq), **17** (20 mmol, 2 eq), NaOH (1 M, 28 mmol, pH 7-8), 50 °C to 100 °C, 70 min; ii) HCl (20% aq, 300 mmol), reflux, 4h30, AcONa, recrystallization, 83%.

In 1966, *rac*-[^{35}S]Cys, diluted eleven times with enantiomerically pure (*R*)-**3**, was used by Gansser for the synthesis of radioactive [^{35}S]Lan.[22] Recrystallization of the crude product gave mainly radioactive *meso*-**2** in 70% yield.[22,23] In 1975, the same procedure was used by Hanus et al. with undiluted *rac*-[^{35}S]Cys.[24] A mixture of all three diastereomers of **2** was obtained using this latter method (73% yield).

In 2011, Aydillo et al. described a diastereoselective Michael addition of protected Cys **21** on chiral dehydroamino acid precursors **19**-**20** as a route to Lan and β-Me-Lan (Scheme 7).[25]

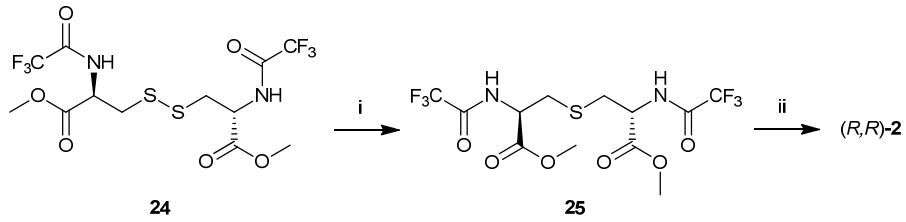


Scheme 7. Diastereoselective synthesis of *meso*-Lan and β-Me-Lan **13**. i) **19** (0.13 mmol, 1 eq) or **20** (0.35 mmol, 1 eq), (*R*)-**21** (1.1 eq), DBU (1.1 eq), THF, rt, 30 min (for **19**) or 7 h (for **20**). Yields: 90% (**22**), 57% (**23**), de >90%; ii) HCl (6 M), reflux, 12 h, 96-97%.

In particular, addition of (*R*)-**21** to **19** with DBU in THF provided **22** in de > 90% and in 90% yield. Hydrolysis in refluxing HCl for 12 h afforded *meso*-**2**. The same reaction using **20** led to **23** (57% yield) which was in turn hydrolysed to β-Me-Lan **13.**

2.2.4 Synthesis from cystine

In 1971, Gleason and Harpp prepared (*R*,*R*)-Lan through desulfurization of a protected cystine **24**.[26] An aminophosphine, $P(NEt_2)_3$ in benzene, was used for the disulfide contraction of **24**. Formation of **25** proceeded in 96% yield (Scheme 8).

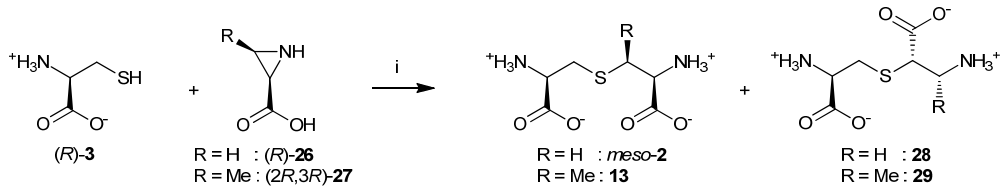


Scheme 8. Synthesis of (*R*,*R*)-Lan by desulfurization of a protected cystine. i) **24** (5 mmol, 1 eq), $(Et_2N)_3P$ (5.5 mmol, 1.1 eq), benzene, rt, 10 min, 96%; ii) **25** (3 mmol), dioxane, NaOH (1 M, 27 mmol), 5 °C, 30 min, pH 6, 64%.

The trifluoroacetyl protecting group was chosen because of its easy removal under mildly basic conditions. Deprotection was achieved with 1 M aq. NaOH in dioxane (final NaOH concentration 0.7 M). Indeed, using a stronger concentration (2.4 M NaOH), Lan racemized completely in 4 h. The proposed procedure was not suitable for preparation of unsymmetrical disulfide due to the ease of disulfide exchange in the reaction conditions.

2.2.5 Synthesis from aziridine

In 1987, Hata and Watanabe investigated the reaction of Cys, in water, on the unprotected aziridinecarboxylic acid **26** and its 3-Me derivative **27** (Scheme 9).[27]

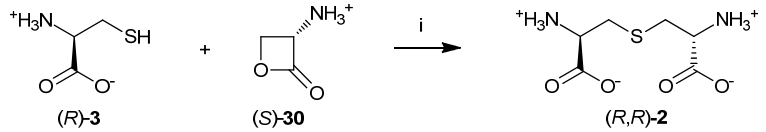


Scheme 9. Synthesis of *meso*-Lan and β-Me-Lan **13** from an aziridine derivative. i) (*R*)-**3** (1 mmol, 1 eq), (*R*)-**26** or (2*R*,3*R*)-**27** (40 mmol, 40 eq), pH 8, 37 °C, 20 h, preparative HPLC. Yields: 28% (*meso*-**2**) + 67% (**28**); 18% (**13**) + 62% (**29**).

In contrast to reaction of aziridinecarboxylates bearing an electron withdrawing group on the nitrogen, which form α-amino acids on opening, compound **26** and **27** afforded predominantly β-amino acid derivatives. Thus, reaction of a large excess of (*R*)-**3** with (*R*)-**26**, in pH 8 water, provided 28% of *meso*-**2** contaminated with 67% of norlanthionine (*nor*-Lan) **28**. In the same manner, starting from (*R*)-**3** and (*R*)-**27**, β-Me-Lan **13** (18% yield) was obtained with **29** (62%). Preparative HPLC was required to recover the pure Lan **2** and β-Me-Lan **13**.

2.2.6 Synthesis from serine β-lactone

In 1988, the synthesis of (*R*,*R*)-**2** through the opening of a β-lactone derivative was described by Arnold et al. (Scheme 10).[28]



Scheme 10. Synthesis of (*R*,*R*)-Lan from Ser β-lactone. i) (*R*)-**3** (3.9 mmol, 3 eq), (*S*)-**30** (1.3 mmol, 1 eq), NaOH (1 M, pH 5-5.5), rt, 40 min, AG50-X8 (80 mL), gradient HCl (0 to 5M, 1.5 L), recrystallization (pH 6), 93%.

A high yield (93%) of (*R*,*R*)-**2** was obtained by reaction of Ser β-lactone (*S*)-**30** with 3 eq of (*R*)-**3** and slow NaOH addition to maintain pH at 5.5. However, this efficient method required the removal of excess (*R*)-**3** by a gradient chromatography on an AG50-X8 cation-exchange resin.

2.3 Chemical synthesis of protected lanthionines

2.3.1 Generality

Peptide synthesis often requires protected synthons (Fmoc, Boc, etc…). In order to achieve chemoselectivity between the two carboxylic functions and the two amino functions of Lan, different synthetic approaches were described in the literature.

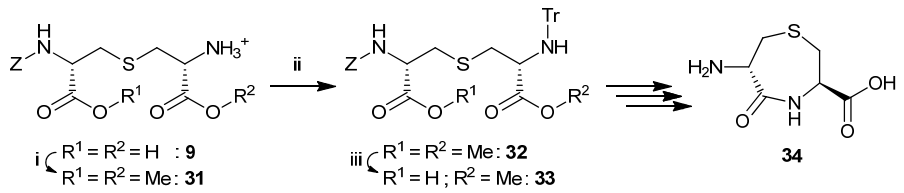
Three types of protected Lan are described in the following section:

- Protected Lan: no restriction on the nature of the protecting group used.
- Orthogonally protected Lan: the protecting groups can each be removed independently from each other.
- Mutually orthogonally protected Lan: the protecting groups are removable independently from the protecting groups used on another Lan. This is useful in preparation of peptides containing several Lan.

In the paragraphs that follow, different syntheses of protected Lan using various precursors are presented.

2.3.2 Synthesis from chloroalanine

An early synthesis of a protected Lan **33** was attempted by Photaki et al. toward the synthesis of cyclolanthionine **34** (Scheme 11).[18]



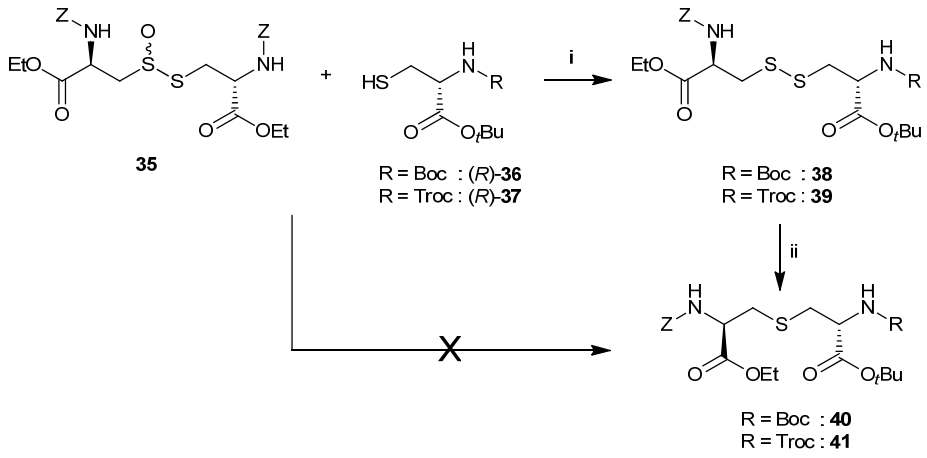
Scheme 11. Synthesis of a protected Lan derivative. i) **9** (1 eq), $SOCl_2$ (2.6 eq), rt, 12 h, 92%; ii) **31** (1 eq), trityl chloride (1.1 eq), NEt_3 (1.1 eq), $CHCl_3$, rt, 12 h, 88%; iii) **32** (1 eq), KOH (1 M, 1.05 eq), acetone, rt, 50 min, 81%.

Starting from previously described compound **9** (Scheme 4), diester **31** was synthesized using thionyl chloride. The amino group of **31** was protected with a bulky trityl (Tr) group leading to **32**. Partial saponification of **32** with KOH

(1.05 eq) gave **33**. However, the author did not provide satisfactory evidence regarding purity of **33** (no NMR, no elemental analysis, broad mp (60 - 75°C)).

2.3.3 Synthesis from cystine

In 1985, in order to synthesize quinomycin, Olsen et al. prepared an orthogonally protected Lan.[29] Because of the lack of selectivity in the saponification step, the Photaki's method to (*R*,*R*)-**33** (Scheme 11, step iii) could not be reproduced.[18] Harpp's disulfide contraction was then considered (Scheme 8).[26] However, this approach was not suitable for asymmetric Lan synthesis due to the fact that disulfide exchange gives multiple products. Synthesis of Lan **40-41** *via* a disulfide contraction route using thiosulfinate **35** in presence of Cys **36**-**37** was therefore developed (Scheme 12).[29]

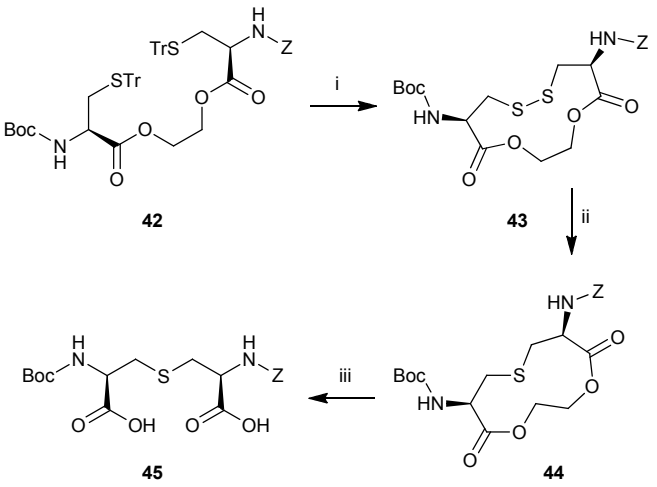


Scheme 12. Synthesis of orthogonally protected Lan by desulfurization of unsymmetrical cystines. i) (*R*)-**36** (4 mmol, 1 eq) or (*R*)-**37** (1.78 mmol, 1 eq), **35** (1 eq), $P(NEt_2)_3$ (1 eq), benzene, rt, 5 h, MPLC, 55-65%; ii) **38** or **39** (1 eq), $P(NEt_2)_3$ (1.1-2.5 eq), benzene, rt, 5-12 h, MPLC, 52-58%.

Accordingly, a solution of thiosulfinate derivative **35** in benzene was reacted with (*R*)-**36** in the presence of $P(NEt_2)_3$. Unfortunately, none of the desired Lan **40** was obtained. Instead, even in the presence of an excess of phosphine, unsymmetrical cystine **38** was formed. Isolated **38** readily performed the disulfide contraction when treated again with $P(NEt_2)_3$. After purification by MPLC, the target Lan **40** was

obtained in 52% yield. Using the same approach, **41** was prepared from **35** and (*R*)-**37** in two steps (Scheme 12).

In another publication, in order to avoid the disulfide exchange giving a mixture of products, Cavelier-Frontin et al. realized the cyclization of the protected cystine before sulfur contraction (Scheme 13).[30]



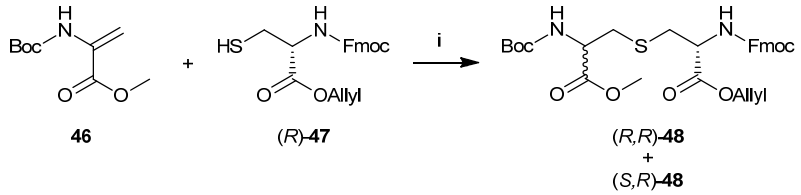
Scheme 13. Synthesis of protected Lan by desulfurization of a cyclic cystine. i) **42** (1 eq), $PhI(OAc)_2$ (1.1 eq), CH_2Cl_2, rt, 1 h, 70%; ii) **43** (1 eq), $P(NEt_2)_3$ (25 eq), DMF, rt, 12 h, 22%; iii) **44** (5 mmol, 1 eq), NaOH, *i*-PrOH.

Two differently protected Cys were coupled into **42** *via* an ethylene diester bridge. The disulfide linkage was formed by treatment of **42** with (diacetoxyiodo)benzene. Concomitant deprotection of the trityl groups occurred. Sulfur contraction, without racemization, was done with $P(NEt_2)_3$. The large excess of phosphine used resulted in tedious isolation of **44** (22%). After basic hydrolysis of the diester, Lan **45** was obtained.

2.3.4 Synthesis from dehydroalanine

An orthogonally protected Lan suitable for solid phase peptide synthesis (SPPS) was synthesized by Probert et al. to prepare small fragments of nisin.[31] Multiple attempts at $P(NEt_2)_3$-induced desulfurization, on solid phase, by adapting the conditions of Harpp and Wakamiya, gave poor yields (32-44%).[26,32] Therefore, a

route using a Michael addition of **47** to the protected Dha **46** was envisaged (Scheme 14).

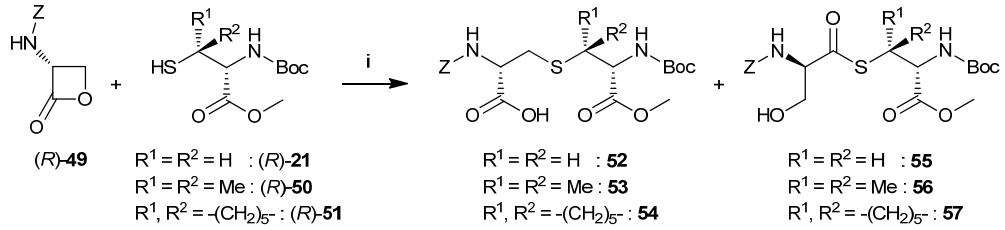


Scheme 14. Synthesis of an orthogonally protected Lan by Michael addition. i) **46** (2.14 mmol, 1 eq), (*R*)-**47** (2.15 mmol, 1 eq), Cs_2CO_3 (0.06 mmol, cat.), CH_3CN, rt, 2 h, 72% (before C_{18} column).

Treatment of **46** with (*R*)-**47** in CH_3CN and a catalytic amount of Cs_2CO_3 gave the orthogonally protected Lan **48** as a mixture of diastereomers (72% yield). A preparative HPLC purification on a C_{18} column was necessary to separate both diastereomers. Deprotection of the allyl or methyl ester of **48** (in the presence of the Fmoc group) was then accomplished to afford Lan suitable for SPPS.

2.3.5 Synthesis from serine β-lactone

In 1995, Arnold's method (Scheme 10) was modified by Shao et al. to synthesize protected Lan from Cbz-Ser β-lactone (*R*)-**49** (Scheme 15).[28,33] The reaction proceeded in organic solvents and basic conditions rather than in water at pH 5.5. However, in preliminary experiments, the main product of the reaction was the thioester **55**.

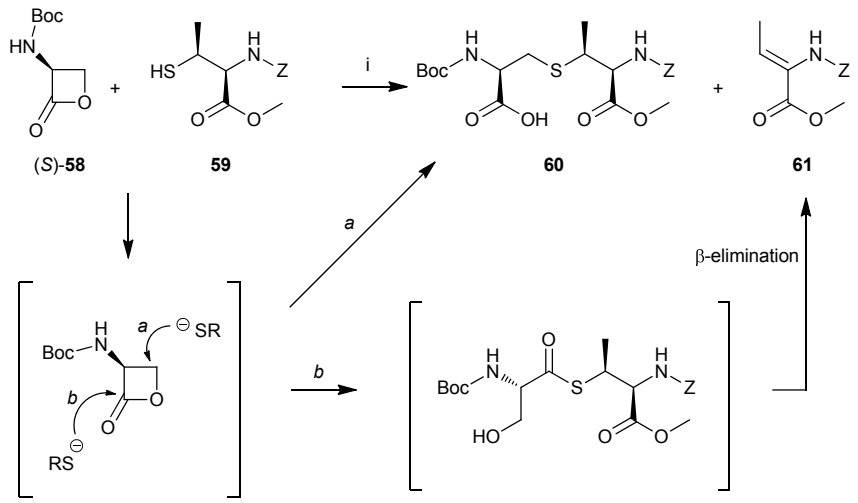


Scheme 15. Synthesis of orthogonally protected Lan using Cbz-Ser β-lactone. i) (*R*)-**49** (4.5 mmol, 1.05 eq), **21, 50** or **51** (4.3 mmol, 1 eq), Cs_2CO_3 (2.2 mmol), DMF, rt, 4 h. Yields: 50% (**52**) + 30-40% (**55**); 78% (**53**) + **56** (traces); 92% (**54**) + **57** (traces).

After optimisation of the reaction conditions, Lan **52** (50%) and thioester **55** (30-40%) were obtained from (*R*)-**49** and (*R*)-**21** in presence of Cs_2CO_3 or $CsHCO_3$

in DMF. Further trials with more hindered nucleophiles such as (*R*)-**50** or (*R*)-**51** gave higher yields of Lan **53** or **54** and only traces of thioesters **56**-**57**. According to Shao, this increase of selectivity happened due to steric factors, namely restricted access to the carbonyl carbon but not to the β-methylene of (*R*)-**49**.[33] However, this route was unsuccessful when Fmoc-(*R*)-Cys-allyl ester (*R*)-**47** was used instead of (*R*)-**21**.[31]

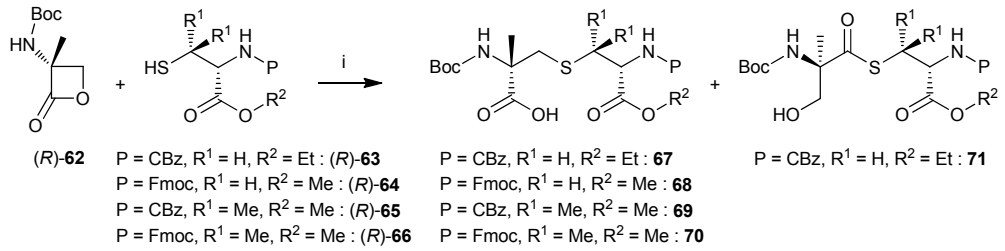
In 2005, a similar method was followed by Narayan and VanNieuwenhze to prepare the orthogonally protected β-Me-Lan **60** from β-Me-Cys **59** and Boc-Ser β-lactone (*S*)-**58** (Scheme 16).[34]



Scheme 16. Synthesis of orthogonally protected β-Me-Lan using Boc-Ser β-lactone. i) (*S*)-**58** (0.71 mmol, 1 eq), **59** (0.71 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 4 h, 52% (**60**) + 20% (**61**).

This approach afforded, in 52% yield, a single isomer of **60** as shown by NMR (pathway a). A side reaction led to 20% of dehydrobutyrine (Dhb) **61**, presumably through the competing opening of the lactone at the carbonyl group followed by β-elimination of the resulting thioester (pathway b).

In 2003, Goodman and Smith reported the preparation of orthogonally protected α-Me-Lan **67**-**70**, by reaction of Cys **63**-**66** with Boc α-Me-Ser β-lactone (*R*)-**62** (Scheme 17).[35]

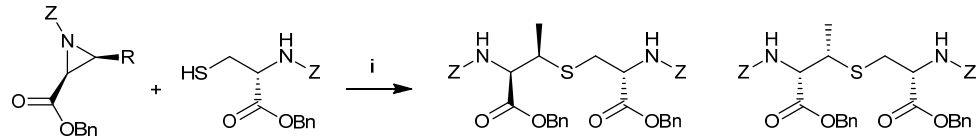


Scheme 17. Synthesis of orthogonally protected α-Me-Lan using Boc α-Me-Ser β-lactone. i) (*R*)-**62** (0.12 mmol, 1 eq), **63**-**66** (0.15 mmol, 1.25 eq), Cs_2CO_3 (1.25 eq), CH_3CN, rt. Yields: 76-83% (**67**) + 17-24% (**71**); 78% (**68**); 99% (**69**); 80% (**70**).

The Boc α-Me-Ser β-lactone (*R*)-**62** was synthesized because of a lack of reactivity with thiols of a mesylate derived from Boc α-Me-Ser. In contrast, stirring (*R*)-**62** with protected Cys (*R*)-**63** in CH_3CN with Cs_2CO_3 gave the orthogonally protected α-Me-Lan **67** (76% yield) contaminated with the thioester by-product **71** (24%) (Scheme 17). The increased bulkiness of Fmoc-protected (*R*)-**64** led to **68** (78%) without any thioester formation. In that case, some Fmoc cleavage occurred, slightly decreasing the yield. More hindered nucleophiles such as **65**-**66** gave Lan **69-70** as a sole product in good yield (80-99%).

2.3.6 Synthesis from aziridine

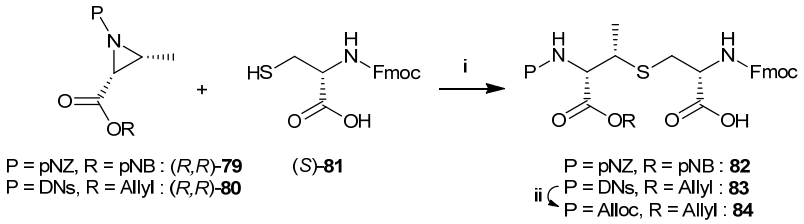
In 1983, Nakajima et al. prepared protected Lan **75** and **77** and β-Me-Lan **76** and **78** through aziridine intermediates (Scheme 18).[36]



Scheme 18. Synthesis of protected Lan and β-Me-Lan using aziridine. i) (*S*)-**72** or (*S,S*)-**73** (1 mmol, 1 eq), (*R*)-**74** (1 mmol, 1 eq), $BF_3.Et_2O$ (3 drops), CH_2Cl_2, rt, 120 h. Yields: 37% (**75**); 22% (**76**); using (*R*)-**72** or (*R,R*)-**73**: 21% (**77**); 12% (**78**).

This approach involved treatment of a mixture of (*S*)-**72** or (*S*,*S*)-**73** with (*R*)-**74** in CH_2Cl_2 containing $BF_3.Et_2O$. After five days at rt, **75** (37%) or **76** (22%) were obtained. Likewise, **77** and **78** were synthesized from (*R*)-**72** or (*R*,*R*)-**73** with (*R*)-**74**, in yield of 21% and 12%, respectively. The low yields were the major drawback of this method.

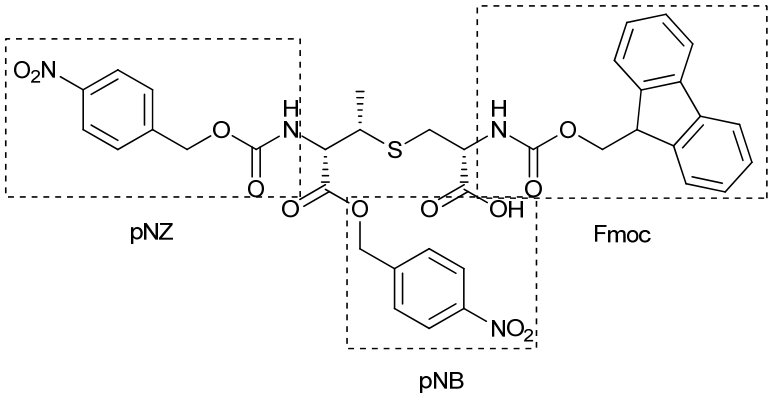
In 2011, both components of lacticin 3147 were prepared by Liu et al. using two mutually orthogonally protected β-Me-Lan **82** and **84** (Scheme 19).[37]



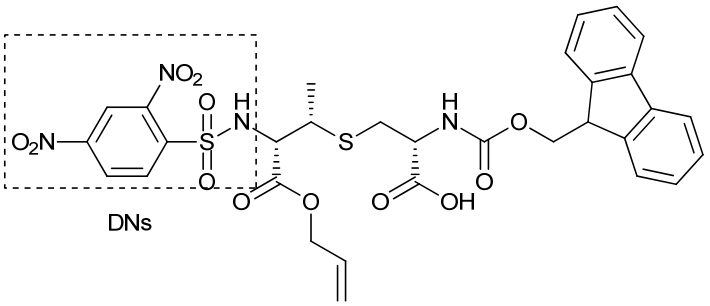
Scheme 19. Synthesis of mutually orthogonally protected β-Me-Lan using aziridine. i) (*R,R*)-**79** (24 mmol, 1 eq), (*S*)-**81** (24 mmol, 1 eq), $BF_3.Et_2O$ (4 eq), CH_2Cl_2, 4 °C, 72 h, 46% (**82**); or (*R,R*)-**80** (2.8 mmol, 1 eq), (*S*)-**81** (11 mmol, 4 eq), $BF_3.Et_2O$ (8 eq), CH_2Cl_2, rt, 48 h, 40% (**83**); ii) **83** (0.12 mmol, 1 eq), thioglycolic acid (0.14 mmol, 1.2 eq), DiPEA (6 eq), Alloc-Cl (1.5 eq), 0 °C → rt, 2+24 h, 47%.

The aziridine route was selected because of its good stereochemical control. A *p*-nitrobenzyloxycarbonyl (pNZ) and a *p*-nitrobenzyl (pNB) groups were chosen to protect the aziridine and ester function of (*R*,*R*)-**79**. Reaction of this compound with (*S*)-**81** in CH_2Cl_2 and $BF_3.Et_2O$ gave the β-Me-Lan **82** in 46% yield. The two other mutually orthogonal protecting groups chosen for the second β-Me-Lan were Alloc and allyl. For this β-Me-Lan **84** synthesis, the starting aziridine (*R*,*R*)-**80** was protected with a dinitrobenzenesulfonyl (DNs) and an allyl groups. Coupling with (*S*)-**81** afforded **83** in 40% yield. The DNs group was then removed in basic media with thioglycolic acid and the amino group was reprotected in one pot with Alloc-Cl to give **84** (47%). The expanded structures of the β-Me-Lan **82**-**84** are illustrated in Figure 4.

82:



83:



84:

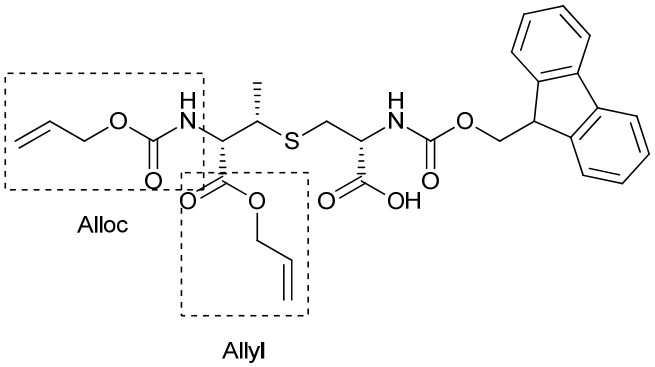
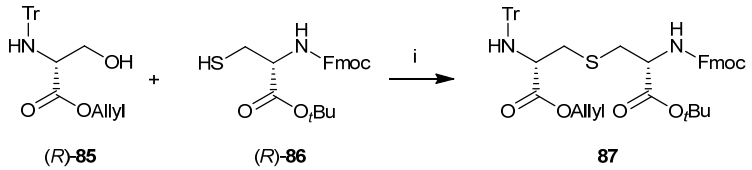


Figure 4. Orthogonally protected β-Me-Lan **83** and mutually orthogonally protected β-Me-Lan **82** and **84**.

2.3.7 Synthesis from serine

In 2002, Swali et al. tried to synthesize, without success, the protected Lan **87** through a Mitsunobu reaction.[38] In 2003, Mohd Mustapa et al. investigated the same approach (Scheme 20).[39]



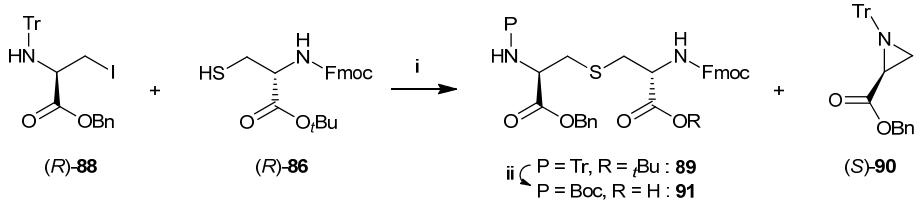
(*R*)-**85** (*R*)-**86** **87**

Scheme 20. Synthesis of a protected Lan by a modified Mitsunobu reaction. i) (*R*)-**85** (0.25 mmol, 1 eq), (*R*)-**86** (0.25 mmol, 1 eq), ADDP (2 eq), PMe_3 (2 eq), Zn tartrate (0.2 eq), $CHCl_3$, rt, 168 h, 52%.

In the Mitsunobu reaction, the relatively non-acidic thiols are usually unreactive. So, more powerful reagents such as 1,1′-(Azodicarbonyl)dipiperidine (ADDP) and PMe_3 were used. Unfortunately, as observed by Swali et al., no reaction occurred with (*R*)-**86**. The reaction conditions were further modified by Mohd Mustapa et al.. The addition of a catalytic amount of zinc tartrate allowed to increase the kinetic and the yield of this sluggish reaction. When (*R*)-**85**, pre-treated with ADDP and PMe_3, was added to a mixture of (*R*)-**86** and zinc tartrate in $CHCl_3$, protected Lan **87** was obtained in 52% yield after seven days at rt.

2.3.8 Synthesis from iodoalanine

In 1997, Dugave and Ménez described the preparation of orthogonally protected Lan **91** using a trityl protected iodoalanine derivative (*R*)-**88** (Scheme 21).[40]



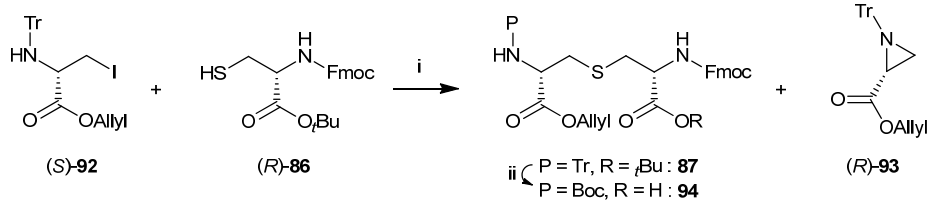
(*R*)-**88** (*R*)-**86** (*S*)-**90**

Scheme 21. Synthesis of orthogonally protected Lan using trityl iodoalanine. i) (*R*)-**88** (1 mmol, 1 eq), (*R*)-**86** (1 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 4 h, 88% (**89**) + 8% (**90**); ii) **89** (0.5 mmol, 1 eq), TFA (50% in CH_2Cl_2, 20 mL), rt, 30 min, evaporation, NEt_3, Boc_2O (1 eq), rt, 12 h, 94%.

Reaction of (*R*)-**86** with (*R*)-**88** and Cs_2CO_3 in DMF at rt yielded Lan **89** (88%) and 8% of aziridine (*S*)-**90**. Deprotection of the *tert*-butyl ester and trityl groups of **89** with TFA followed by Boc reprotection of the free amine gave orthogonally protected Lan **91** (94%). For this synthesis, iodoalanine (*R*)-**88** was previously synthesized by reaction of the corresponding mesylate with a 10 molar excess of NaI in acetone.[41] The trityl protecting group was chosen to prevent C_α-proton abstraction and subsequent elimination to Dha.[41,42] Compound (*R*)-**88** was reported as a 2:1 mixture of rotamers that did not coalesce on heating but was instead converted to the aziridine (*S*)-**90**. The optical activity of this particular (*R*)-**88** was $[\alpha]_D^{20}$ = +5° (c 1, $CHCl_3$). Furthermore, Lan **89** was also described as a mixture of rotamers.

In 2002, the route shown in Scheme 21 was adapted to SPPS by Rew et al..[43] In this case, (*R*)-**88** was prepared in 93% yield by the Garegg iodination (PPh_3/I_2/imidazole) of (*S*)-Tr-Ser-OBn. The optical rotation of this batch of (*R*)-**88** was $[\alpha]_D^{20}$ = +14.7° (c 0.37, $CHCl_3$), which is considerably higher than the value reported by Dugave and Ménez.

In 2002, Swali et al. prepared an orthogonally protected Lan for use in SPPS by a modification of the method of Dugave et al. (Scheme 22).[38]

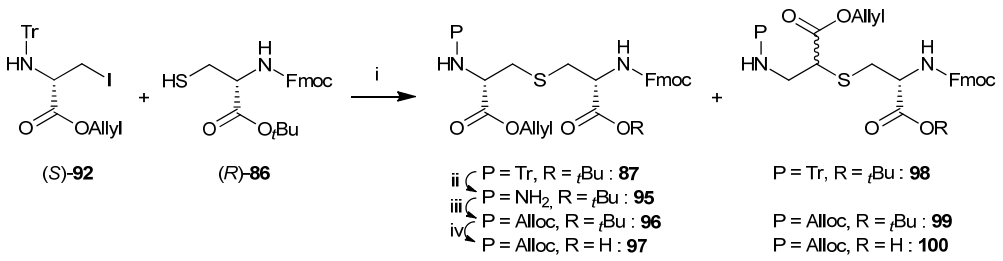


Scheme 22. Synthesis of orthogonally protected Lan using trityl iodoalanine. i) (*S*)-**92** (1 mmol, 1 eq), (*R*)-**86** (1 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 4 h, 74% (**87**) + 16% (**93**); ii) **87** (2.7 mmol, 1 eq), TFA (50% in CH_2Cl_2 with 2% TIS, 10 mL), rt, 1 h, evaporation, DiPEA, Boc_2O (1 eq), rt, 12 h, 62%.

For this application, a Fmoc strategy was chosen and an allyl ester was used as an orthogonal protecting group. After reaction of iodoalanine (*S*)-**92** with (*R*)-**86** and Cs_2CO_3 in DMF, Lan **87** (74%) contaminated with aziridine (*R*)-**93** (16%) was

obtained. The iodoalanine (*S*)-**92** was previously prepared by the Garegg iodination of (*R*)-**85** in 84% yield. The authors reported (*S*)-**92** to be a 2:1 mixture of rotamers that degraded to aziridine (*R*)-**93** upon heating. Furthermore, the ^{13}C NMR of Lan **87** showed dedoubled peaks for the three methyl of the *tert*-butyl, both β-methylene carbons, both α-carbons and the three allylic carbons. Acidolysis of trityl and *tert*-butyl groups was followed by Boc protection of the free amine to provide **94** (62%). The SPPS step was conducted with the orthogonally protected Lan **94** and a protected valine to give a dipeptide in 16% yield.

In 2002 and 2003, Mohd Mustapa et al. demonstrated that the interpretation made by Dugave and Ménez and by Swali et al. for the product of the reaction between (*R*)-**86** and (*R*)-**88** or (*S*)-**92** was not totally correct.[39,44] When Mohd Mustapa synthesized iodoalanine (*S*)-**92** from the mesylate according to the Dugave's method, two distinct isomeric forms were again obtained as shown by ^{1}H NMR. From the reaction between (*S*)-**92** and (*R*)-**86**, Lan **87** was prepared (Scheme 23).

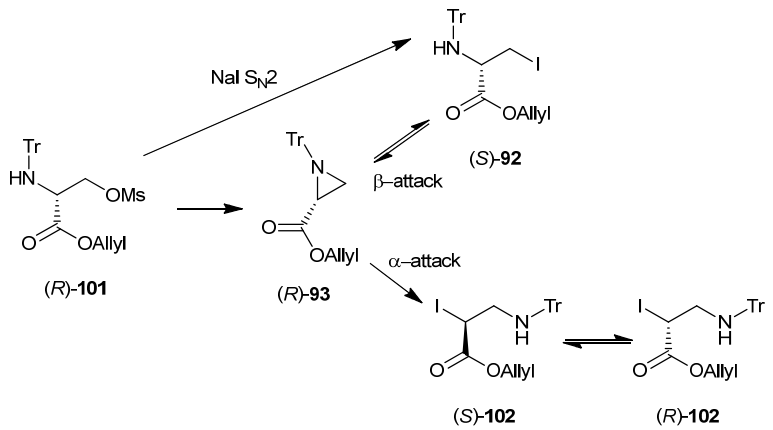


Scheme 23. Synthesis of orthogonally protected Lan and *nor*–Lan using trityl iodoalanine. i) (*S*)-**92** (16 mmol, 1.14 eq), (*R*)-**86** (14 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 4 h. Yields: 15% (**87**) + 75% (**98**); ii) **87**+**98** (4.6 mmol, 1 eq), TFA (5% in $CHCl_3$, 27 mL, 4 eq), rt, 4 h; iii) Alloc-OSu (9.2 mmol, 2 eq), $NaHCO_3$ (5%, 30 mL), dioxane, 5 °C, 18 h. Yields: 14% (**96**), 73% (**99**); iii) **96**+**99** (3.4 mmol, 1 eq), TFA (50% in $CHCl_3$, 10 mL), 4 h, rt. Yields: 0% (**97**), 76% (**100**).

The ^{1}H NMR analysis showed that the synthesis product consisted of two sets of peaks in a 4:1 ratio. Moreover, the HMBC-NMR analysis showed correlations between the methylene protons of the Fmoc part of Lan and the CH next to the allyl carboxylate (trityl part). Based on these results, Mohd Mustapa concluded that the reaction between (*R*)-**86** and (*S*)-**92** gave a mixture of Lan **87** (minor product) and

nor-Lan **98** (major product), rather than the two previously described rotamers of **87**.[38,40] It was also shown that Lan **87** is a pure diastereomer while *nor*-Lan **98** is a mixture of diastereomers.[39,44] The absence of rotamers was confirmed after complete NMR characterization of the two HPLC purified isomers **96** and **99** obtained after substitution of Alloc for trityl on the mixture of **87** and **98**.[39,44]

Furthermore, it was shown by HSQC that the iodoalanine, prepared from mesylate (*S*)-**101**, was in fact mainly a mixture of enantiomers of α-iodo-β-alanine (*R*/*S*)-**102** (Scheme 24). The desired β-iodo-α-alanine (*S*)-**92** was the minor product.[39,44]



Scheme 24. Mechanism for transformation of Ser mesylate to trityl-iodoalanine using Dugave's method. Conditions: (*R*)-**101** (46 mmol, 1 eq), NaI (10 eq), acetone, rt, 72 h. Yields: 15% [(*S*)-**92**]; 72% [(*R*/*S*)-**102**].

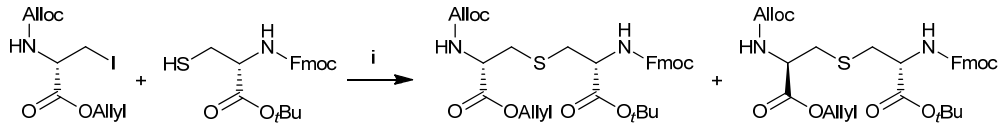
In 2003, Mohd Mustapa et al. published an additional study on the mechanism of conversion of mesylate (*R*)-**101** to a mixture of (*S*)-**92**, (*R*)-**93** and (*R*/*S*)-**102**.[39] Briefly, during the reaction of mesylate (*R*)-**101** with NaI (10 eq) in acetone at rt, aziridine (*R*)-**93** was formed. Subsequent ring-opening with NaI occurred in either the α-position (major) or the β-position (minor) giving (*S*)-**102** or (*S*)-**92**, respectively. By direct S_N2 reaction of NaI on the mesylate, (*S*)-**92** can also be obtained. Attack of excess iodide on α-iodo-β-alanine (*S*)-**102** led to (*R*)-**102** through Walden inversion.

When aziridine (*R*)-**93** was subjected to the same conditions as described for the formation of Lan **87**, namely cysteine (*R*)-**86** and Cs_2CO_3 in DMF, no reaction

occurred. Therefore, *nor*-Lan **98** was formed by S_N2 on the major iodoalanine regioisomer (*R*/*S*)-**102**, while Lan **87** was the product from the minor isomer (*S*)-**92** (Scheme 23 and Scheme 24).

The *tert*-butyl esters of the 1:6 mixture of the two isomers **96** and **99** were removed to give, after reverse phase chromatography, pure *nor*-Lan **100** (76%, Scheme 23).[39] A cyclic peptide containing *nor*-Lan was prepared in Fmoc-based SPPS from **100**.[45]

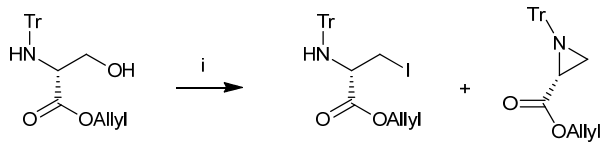
In 2005, Bregant and Tabor investigated the use of carbamate-protected iodoalanine in the preparation of protected Lan (Scheme 25).[46]



Scheme 25. Synthesis of protected Lan using carbamate-protected iodoalanine. i) (*S*)-**103** (0.12 mmol, 1.1 eq), (*R*)-**86** (0.11 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 3 h. Yields: 74% (**96**) + 13% (**104**), de: 70%.

As the trityl group was electron donating and promoted aziridine formation, a carbamate group was selected to circumvent this problem. Indeed, when Alloc iodoalanine (*S*)-**103** was reacted with (*R*)-**86**, Lan **96** was obtained in good yield (74%). However, the diastereomer **104** (13%) was also present as a side product. Iodoalanine (*S*)-**103** was previously generated from the mesylate with NaI and the corresponding Dha derivative was also recovered. The latter was readily removed from the iodoalanine (*S*)-**103** by chromatography. Freshly prepared iodoalanine (*S*)-**103** was then used directly because of its instability. Nevertheless, under the reaction conditions depicted in Scheme 25, some β-elimination occurred affording more Dha derivative. Secondary reaction with this Dha, gave rise to a 1:1 mixture of **96** and **104**. *In fine* as much as 13% of **104** was present in the final product (de 70%). This Michael addition was already described in Scheme 14 for the similar Dha **56**. The formation of the diastereomer **104** could not be avoided despite attempts using other reaction solvents or bases.

These failures prompted Bregant and Tabor to reinvestigate the trityl protected iodoalanine (*S*)-**92**.[46] A new synthesis was developed in order to minimize aziridine (*R*)-**93** formation as well as its conversion into α-iodo-β-alanine (*R*/*S*)-**102** (Scheme 24). In this case, a Mitsunobu reaction between protected Ser (*R*)-**85** and iodomethane was done at -2 °C in the presence of PPh_3 and diethylazodicarboxylate (DEAD) (Scheme 26).

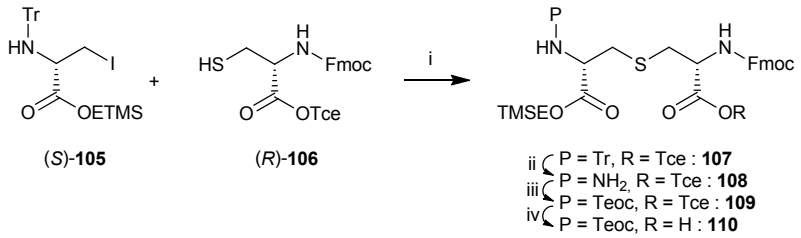


(*S*)-**85** (*S*)-**92** (*R*)-**93**

Scheme 26. Regioselective preparation of trityl iodoalanine. i) (*S*)-**85** (4 mmol, 1 eq), PPh_3 (1.5 eq), DEAD (1.5 eq), CH_3I (1.5 eq), CH_2Cl_2, -2 °C, 3 h. Yields: 72% (**92**); (*S*)-**93** (traces).

By using this method, (*S*)-**92** was obtained with only traces of aziridine. Moreover, formation of α-iodo-β-alanine (*R*/*S*)-**102** was not observed. Orthogonally protected Lan **97** was then synthesized from iodoalanine (*S*)-**92** and (*R*)-**86** by following the route previously reported (Scheme 23). Using this regio- and stereo-selective way to prepare (*S*)-**92**, only Lan **97** was recovered and *nor*-Lan **100** was not detected.[46] This result contrasts with the one of Mohd Mustapa et al. who obtained only *nor*-Lan **100**.[39] Lan **97** was then incorporated into an analogue of nisin ring C by SPPS synthesis.[46]

In 2011, Mothia et al. prepared an analogue of nisin rings D and E by SPPS.[47] Those two rings being constituted of overlapping thioether bridges, a new approach was considered. This route required the synthesis of the mutually orthogonally protected Lan **97** and **110** (Scheme 27 and Figure 5).

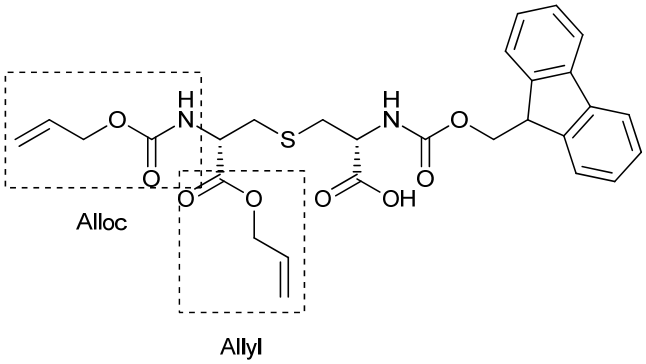


Scheme 27. Synthesis of protected Lan using trityl iodoalanine. i) (*S*)-**105** (0.16 mmol, 1 eq), (*R*)-**106** (0.16 mmol, 1 eq), Cs_2CO_3 (1 eq), DMF, rt, 4 h, 58%; ii) **107** (0.11 mmol, 1 eq), TFA (10% in CH_2Cl_2 with 5% TES, 4 mL), rt, 24 h; iii) **108** (0.11 mmol, 1 eq), Teoc-OSu (5 eq), DMAP (1 eq), rt, 24 h, 66%; iii) **109** (0.075 mmol, 1 eq), Zn (2.7 mmol, 36 eq), NH_4OAc (1 M, 0.56 mL), THF, rt, 24 h, 40%.

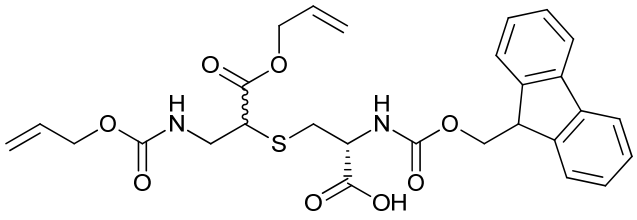
For **110**, the trimethylsilylethyl (TMSE) and the β-(trimethylsilyl)ethoxycarbonyl (Teoc) protections were chosen. Indeed, their removal by tetra-*n*-butylammonium fluoride (TBAF) is orthogonal to Alloc/allyl as well as the transient Fmoc and permanent Boc/*t*-Bu groups used in SPPS. Iodoalanine (*S*)-**105** was prepared by the Mitsunobu reaction with iodomethane as described above (Scheme 26). Coupling in DMF of (*S*)-**105** with (*R*)-**106** in the presence of Cs_2CO_3 gave the orthogonally protected Lan **107** (58%). After trityl removal and subsequent protection of the amine of **108** with a Teoc, **109** was obtained in 66% yield (two steps). The trichloroethyl ester (Tce) was removed in neutral conditions with Zn and ammonium acetate to provide Lan **110**. Synthesis of the bicyclic peptide analogue of nisin rings D and E was then done successfully.

The expanded structures of Lan **97**, *nor*-Lan **100** and Lan **110** are illustrated in Figure 5.

97:



100:



110:

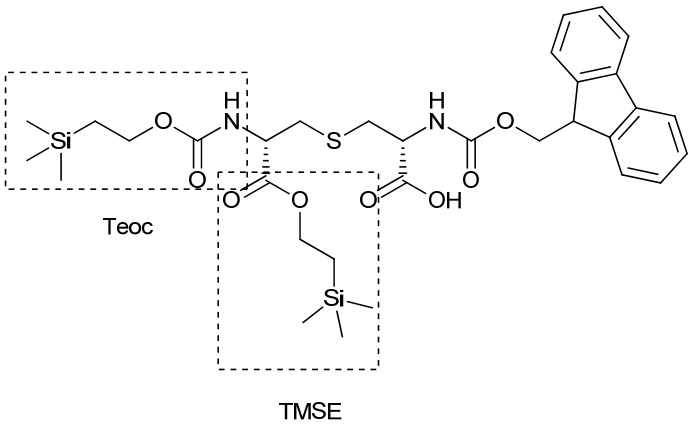
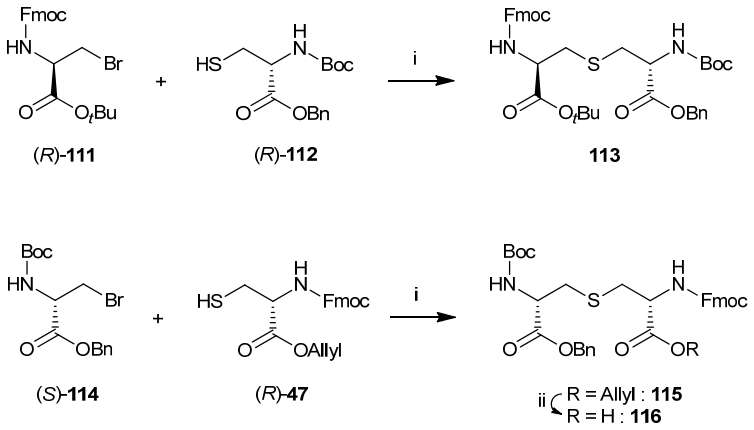


Figure 5. Representation of mutually orthogonally protected Lan **97** and **110** as well as protected *nor*-Lan **100**.

2.3.9 Synthesis from bromoalanine

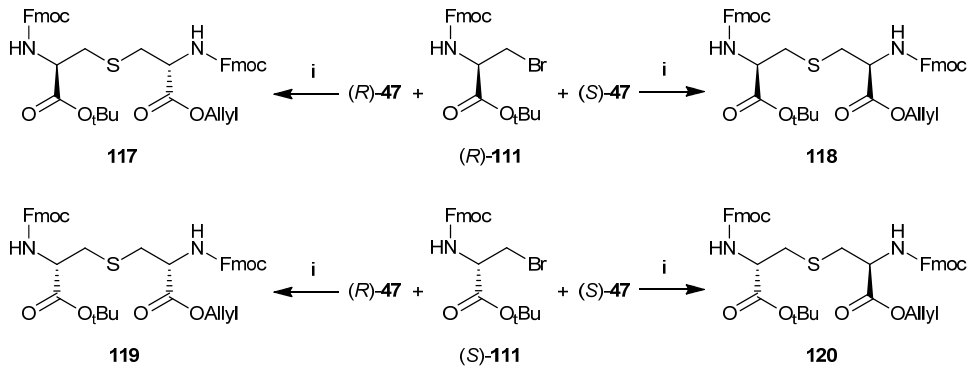
In 2003, Schmidt and Zhu pioneered the use of β-bromoalanine derivatives in the preparation of Lan (Scheme 28).[48]



Scheme 28. Synthesis of orthogonally protected Lan using bromoalanine. i) (*R*)-**111** or (*S*)-**114** (0.21 mmol, 1.05 eq), (*R*)-**112** or (*R*)-**47** (0.2 mmol, 1 eq), $NaHCO_3$ (3 mL, pH 8.5), TBAHS (4 eq), EtOAc, rt, 12 h. Yields: 92% (**113**), 84% (**115**); ii) **115** (0.095 mmol, 1 eq), *N*-methylaniline (3 eq), $Pd(PPh_3)_4$ (1.05 eq), THF, rt, 1 h, 94%.

They postulated that brominated compounds such as (*R*)-**111** or (*S*)-**114**, under mildly basic conditions ($NaHCO_3$ at pH 8.5), would avoid elimination to Dha. The reaction of (*R*)-**111** with (*R*)-**112** was performed in a biphasic mixture of aqueous $NaHCO_3$ and EtOAc with tetra-*n*-butylammonium hydrogensulfate (TBAHS) as a PTC. Orthogonally protected Lan **113** was obtained in 92% yield. The same reaction with (*S*)-**114** and (*R*)-**47** led to **115** (84%). The Lan **113** and **115** were diastereoselectively pure and did not contain any *nor*-Lan. Treatment of **115** with $Pd(PPh_3)_4$ and *N*-methylaniline gave the free acid **116** (94%).

In 2006, Seyberth prepared the four diastereomers (**117**-**120**) of a protected Lan (Scheme 29) from cysteine **47** and bromoalanine **111**.[49]



Scheme 29. Synthesis of protected Lan using bromoalanine. i) (*R*)-**111** or (*S*)-**111** (1 mmol, 1.1 eq), (*R*)-**47** or (*S*)-**47** (0.91 mmol, 1 eq), DiPEA (5% in DMF, 8 mL), rt, 4 h. Yields: 65% (**117**), 62% (**118**), 58% (**119**), 56% (**120**).

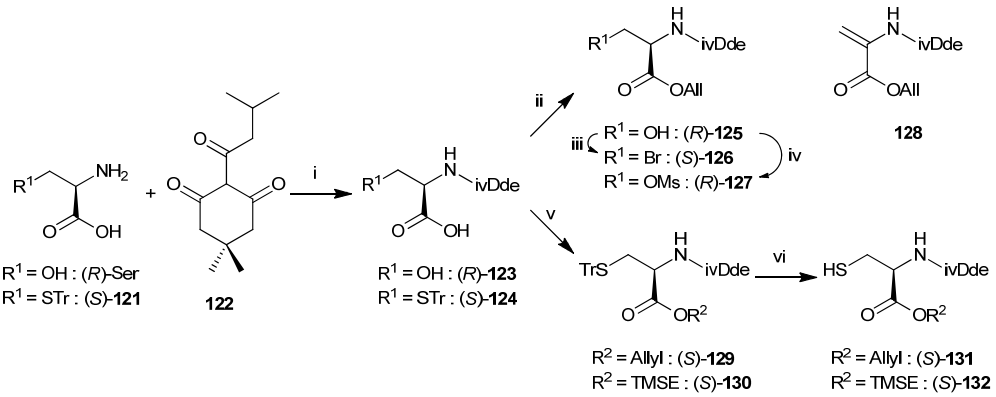
First experiments were attempted with an allyl ester bromoalanine synthon. Its preparation (Fmoc-Ser allyl ester with PPh_3/CBr_4) was unsuccessful as a 4:1 mixture of the Dha and bromoalanine derivatives was obtained. Following the same procedure and the more hindered Fmoc-Ser *tert*-butyl ester, bromoalanine **111** was obtained in good yield (85%). No contamination by Dha was reported. The four Lan diastereoisomers **117**-**120** were prepared from (*R*)-**111** or (*S*)-**111** with (*R*)-**47** or (*S*)-**47**. Reactions were conducted with DiPEA (5%) in DMF rather than by PTC (Zhu conditions). As shown by 2D-NMR, the carbamate protection avoided the formation of *nor*-Lan.[49] The same result was previously reported by Bregant and Tabor (Scheme 25).[46] The diastereomeric purity of **117**-**120** was determined by chiral GC-MS on a Chirasil-Val column after derivatization. The diastereomeric excesses were >91% (Table 2).

Table 2. Diastereomeric purities of Lan 117-120 [a]

Lan	(2*R*, 6*R*)	(2*R*, 6*S*), (2*S*, 6*R*)	(2*S*, 6*S*)
117 (2*R*, 6*R*)	97.3	2.7	0
118 (2*R*, 6*S*)	1.3	97.1	1.6
119 (2*S*, 6*R*)	2.6	96.7	0.7
120 (2*S*, 6*S*)	0.1	4.1	95.8

[a] Chiral GC-MS according to Küsters.[50] Analysis on a Chirasil-Val column after derivatization to the *N,N'*-bis-(trifluoroacetyl)-Lan-α-allyl-ε-methyl diester. Table reproduced from Seyberth.[49]

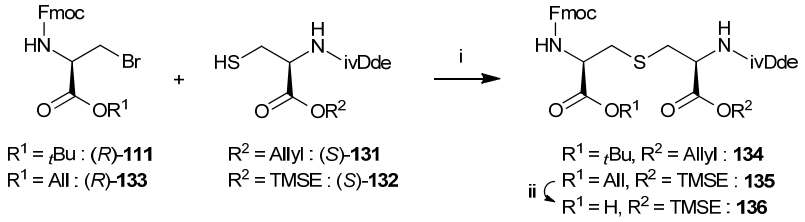
In 2008, Martin developed a synthesis for alternative Lan building blocks suitable for SPPS.[51] The amine was protected with an isovaleryldimedone (ivDde) group. Even if ivDde is orthogonal to allyl ester, *tert*-butyl ester and Fmoc deprotection, its removal by dilute hydrazine also cleaves Fmoc. Ser was reacted with **122** in MeOH containing DiPEA to give (*R*)-**123** in 86% yield (Scheme 30).



Scheme 30. Synthesis of 2-isovaleryldimedone (ivDde) protected synthons. i) (*R*)-Ser (40 mmol, 1.2 eq) or (*S*)-**121** (16.3 mmol, 1.1 eq), **122** (33 mmol or 14.8 mmol resp., 1 eq), DiPEA (4 eq), MeOH, reflux, 14 h. Yields: 86% (**123**), 95% (**124**); ii) (*R*)-**123** (28.7 mmol, 1 eq), $NaHCO_3$ (1.33 eq), allyl-Br (1.2 eq), DMF, rt, 14 h, 91%; iii) (*R*)-**125**, CBr_4, PPh_3, CH_2Cl_2, inseparable mixture of (*S*)-**126** and **128**; iv) (*R*)-**125** (2.85 mmol, 1 eq), MsCl (1 eq), NEt_3 (1 eq), CH_2Cl_2, 30 min, 99%; v) (*S*)-**124** (7 mmol, 1 eq), $NaHCO_3$ (1.33 eq), allyl-Br (1.2 eq), DMF, rt, 14 h, 92% or (*S*)-**124** (17.6 mmol, 1 eq), trimethylsilylethanol (2 eq), DCC (1.5 eq), DMAP (0.2 eq), CH_2Cl_2, 0 °C →rt, 14 h, 92%; vi) (*S*)-**129** (2.0 mmol, 1 eq), TFA (8% in CH_2Cl_2 with 2 eq TIS, 43 mL), rt, 1 h, 70% or (*S*)-**130** (7.5 mmol, 1 eq), TFA (12% in CH_2Cl_2 with 2 eq TIS, 110 mL), rt, 1 h, 87%.

The protection of (*R*)-**123** with an allyl ester gave (*R*)-**125**. The bromoalanine (*S*)-**126**, synthesized from PPh_3/CBr_4, was contaminated with the Dha derivative **128**. Moreover, the instability of (*S*)-**126** precluded its purification by chromatography. The mesylate (*R*)-**127** was thus prepared from (*R*)-**125**. After reaction with MsCl and NEt_3, (*R*)-**127** was obtained without Dha **128**. The protected Cys (*R*)-**86** and the freshly synthesized (*R*)-**127** were then coupled by using the Zhu conditions ($NaHCO_3$/EtOAc). The corresponding protected Lan was obtained with a good yield but as a mixture of diastereomers resulting from elimination to Dha **128** during the synthesis.

Therefore, the author decided to interchange the protecting groups between the bromoalanine and the Cys. Starting from trityl-protected Cys (*S*)-**121** and **122**, compound (*S*)-**124** was obtained in 95% yield (Scheme 30). Protection of the carboxylic acid with allyl bromide led to allyl ester (*S*)-**129**. After *S*-trityl deprotection with TFA, (*S*)-**131** was recovered. Lan **134** was synthesized from (*R*)-**111** and (*S*)-**131** according to the Zhu protocol (52%, Scheme 31). The fair yield resulted from a competing degradation of (*S*)-**131**, presumably due to the allyl group. Thus, an alternative Cys protecting group was selected. Compound (*S*)-**124** was reacted with trimethylsilylethanol and 1,3-dicyclohexylcarbodiimide (DCC) in presence of catalytic DMAP to provide TMSE ester (*S*)-**130**. Trityl deprotection with TFA gave the free thiol (*S*)-**132** (Scheme 30). Lan **135** was then prepared from (*S*)-**132** and (*R*)-**133** in 88% yield as a single diastereomer (Scheme 31). Removal of the allyl ester of **135** was done using $Pd(PPh_3)_4$ (5 mol%) and *N*-methylaniline in 87% yield. Protected Lan **136** is illustrated in Figure 6 (page 55).

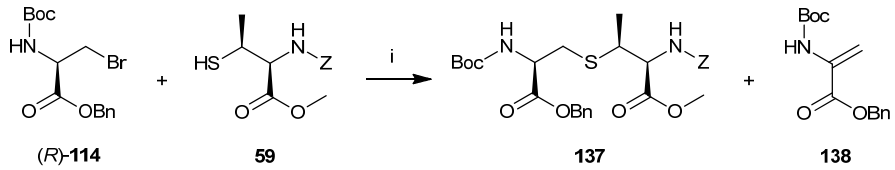


Scheme 31. Synthesis of protected Lan using bromoalanine. i) (*R*)-**111** (1.54 mmol, 1.1 eq) or (*R*)-**133** (7.2 mmol, 1.1 eq), (*S*)-**131** (1.4 mmol, 1 eq) or (*S*)-**132** (6.5 mmol, 1 eq), $NaHCO_3$ (saturated, pH 8.5), TBAB (4 eq), EtOAc, rt, 14 h. Yields: 52% (**134**), 88% (**135**); ii) **135** (0.39 mmol, 1 eq), *N*-methylaniline (3 eq), $Pd(PPh_3)_4$ (5 mol%), THF, rt, 1 h, 87%.

In 2005, Narayan and VanNieuwenhze prepared the orthogonally protected β-Me-Lan **137** from β-Me-Cys **59** and bromoalanine (*R*)-**114** (Scheme 32).[34]

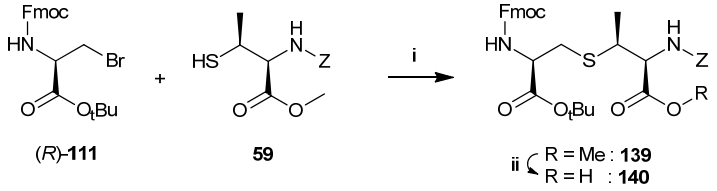
The method of Zhu gave β-Me-Lan **137** in 60% yield and the by-product Dha **138** in 21% yield. By substituting Cs_2CO_3 (pH 12) for $NaHCO_3$ (pH 8.5) the yield of **137** increased to 72% while **138** was formed in 13% yield. The isomeric purity was established on basis of ^1H and ^{13}C NMR. Small peaks that did not disappear on

heating seem to indicate that diastereomers are presents. In the paper, no de value of **137** is reported.



Scheme 32. Synthesis of orthogonally protected β-Me-Lan using bromoalanine. i) *Zhu conditions*: (*R*)-**114** (0.34 mmol, 1 eq), **59** (0.34 mmol, 1 eq), $NaHCO_3$ (saturated, pH 8.5), TBAHS (4 eq), EtOAc, rt, 12 h. Yields: 60% (**137**) + 21% (**138**); *Narayan conditions*: (*R*)-**114** (0.34 mmol, 1 eq), **59** (0.34 mmol, 1 eq), Cs_2CO_3 (saturated, pH 12), TBAHS (4 eq), EtOAc, rt, 12 h. Yields: 72% (**137**) + 13% (**138**).

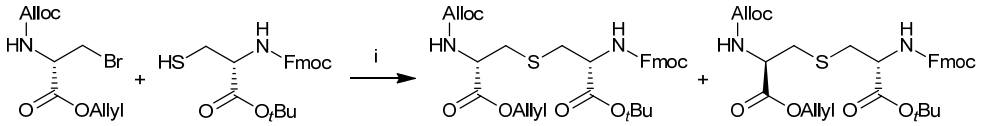
In 2012, Carillo and VanNieuwenhze synthesized the D-ring of Mersacidin in liquid phase synthesis. An orthogonally protected β-Me-Lan was prepared from **59** and bromoalanine (*R*)-**111** (Scheme 33).[52]



Scheme 33. Synthesis of orthogonally protected β-Me-Lan using bromoalanine. i) (*R*)-**111** (22 mmol, 1 eq), **59** (22 mmol, 1 eq), Cs_2CO_3 (220 mL, pH 13), TBAHS (4 eq), EtOAc, rt, 12 h, 65%; ii) **139** (0.77 mmol, 1 eq), Me_3SnOH (6 eq), $ClCH_2CH_2Cl$, 78 °C, 4 h, 70%.

The protocol of Zhu was used with the alkaline modifications introduced by Narayan. The reaction of **59** with (*R*)-**111** in a biphasic mixture of aqueous Cs_2CO_3 (pH 12) and EtOAc with TBAHS as PTC gave β-Me-Lan **139** in 60% yield. The methyl ester of this β-Me-Lan was selectively deprotected by reflux in dichloroethane with Me_3SnOH. Orthogonally protected β-Me-Lan **140** was obtained in 70% yield.

In order to synthesize bis(desmethyl) lacticin 3147 A2, an analogue with two Lan substituted for β-Me-Lan, the protected Lan **96** was prepared, in 2008, by Pattabiraman et al. (Scheme 34).[53]



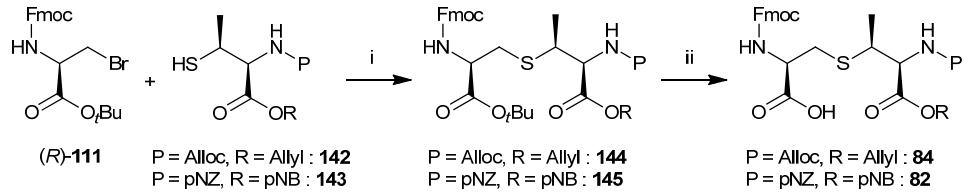
Scheme 34. Synthesis of protected Lan using bromoalanine. i) (*S*)-**141** (6.9 mmol, 1 eq), (*R*)-**86** (6.9 mmol, 1 eq), $NaHCO_3$ (0.5 M, pH 8.5), TBAB (4 eq), EtOAc, rt, 24 h, 73%. Yields: 66% (**96**) + 7% (**104**), de: 81%.

Reaction of bromoalanine (*R*)-**114** with Cys (*R*)-**86** and TBAB in a biphasic medium of EtOAc and $NaHCO_3$ (0.5 M, pH 8.5) afforded protected Lan **96**. However, ^{13}C NMR analysis indicated the presence of diastereomer **104** in the product and a de of 81% was extrapolated.[53] The same compounds **96** and **104** were previously made by Bregant and Tabor from the identically protected iodoalanine (*S*)-**103** with a de of 70% (cfr. Scheme 25).[46] From these results, it appears that the synthesis of Lan from carbamate protected bromo or iodoalanine, lead to diastereoisomers formation. This probably happens due to a Dha by-product generated during the coupling followed by Michael addition of the thiol. A Fmoc-based SPPS strategy to the lacticin 3147 A2 analogue was used by Pattabiraman et al. using Lan **96**.[53] To avoid dimerized peptide, a low resin loading was required. The lacticin 3147 A2 analogue was obtained in 1.3% global yield after 22 coupling reactions and three cyclizations.

In 2012, Knerr and van der Donk wanted to synthesize epilancin 15X analogues.[54] This lantibiotic contains three rings, one with Lan and two with β-Me-Lan. Two of those rings are constituted of overlapping thioether bridges. Therefore, two mutually orthogonally protected β-Me-Lan **82** and **84** were synthesized (Scheme 35).

Preparation of **144** from a β-Me-bromoalanine according to the protocol of Pattabiram depicted in Scheme 34 gave low yields and partial epimerization. So, the authors synthesized β-Me-Cys **142**. Lan **144** was then formed by reacting **142** and (*R*)-**111** in a mixture of EtOAc and $NaHCO_3$ (0.5 M, pH 8.5) with TBAB and PBu_3 (64%, Scheme 35). TFA acidolysis of **144** in the presence of $PhSiH_3$ removed the *tert*-butyl ester to give **84** (95%). To prepare Lan **145**, Knerr and van der Donk

reacted β-Me-Cys **143** with (*R*)-**111** using the same reaction conditions (85% yield). TFA acidolysis of **145** provided Lan **82** in 95% yield.



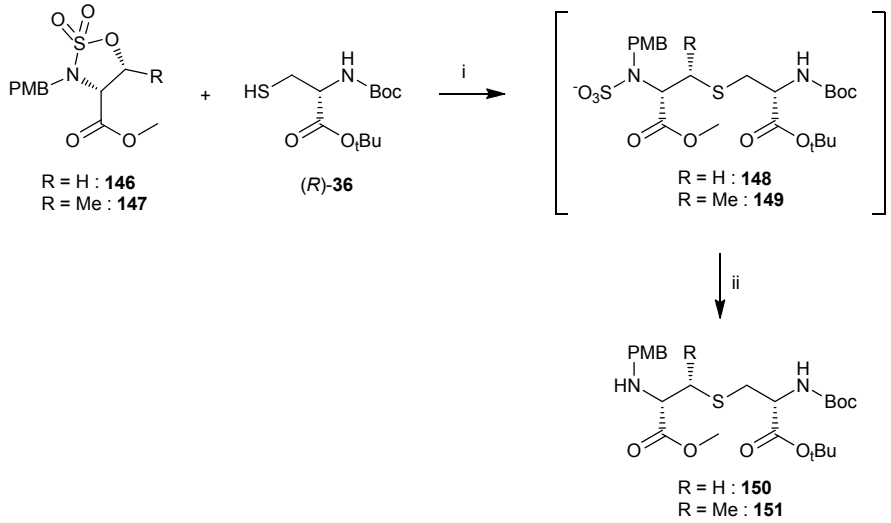
Scheme 35. Synthesis of mutually orthogonally protected β-Me-Lan using bromoalanine. i) (*R*)-**111** (1 mmol, 1 eq), **142** or **143** (1.5 mmol, 1.5 eq), $NaHCO_3$ (0.5 M, pH 8.5), TBAB (4 eq), EtOAc, PBu_3 (0.5 eq), rt, 24 h. Yields: 64% (**144**), 85% (**145**); ii) **144** or **145** (0.8 mmol, 1 eq), TFA (50% in CH_2Cl_2 with 1.05 eq $PhSiH_3$, 6 mL), rt, 2 h, 95%.

After SPPS, the epilancin 15X analogues were obtained in 1.6-1.9% yield (92-93% average per step). The diastereomeric purity of Lan and of β-Me-Lan in the peptide was verified by chiral GC-MS on a Chirasil-Val column. The hydrolysate (HCl 6 M, 100 °C, 20 h) of the synthetic epilancin analogues was derivatised and injected. The *meso*-Lan contained small amounts of (*R*,*R*)-Lan and (*S*,*S*)-Lan. This probably resulted from epimerization during the hydrolysis step. The incorporated β-Me-Lan was of an excellent diastereomeric purity.

2.3.10 Synthesis from sulfamidate

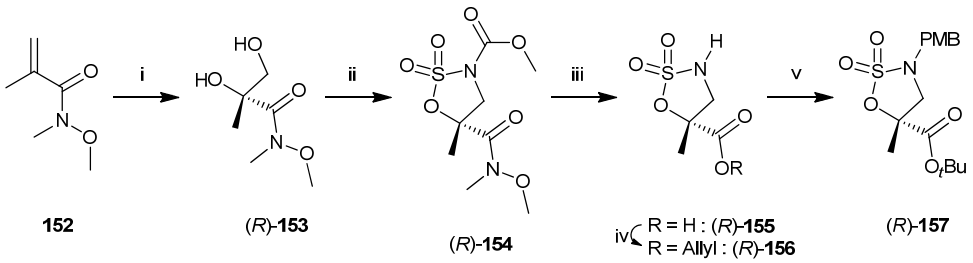
In 2007, the preparation of Lan and β-Me-Lan derivatives from cyclic sulfamidates was investigated by Cobb and Vederas (Scheme 36).[55] Allyl and Alloc groups are not appropriate with the oxidative conditions required for sulfamidate preparation. Hence, sulfamidates **146** and **147** were prepared from *para*-methoxybenzyl (PMB) protected Ser and *allo*-Thr methyl ester, respectively. After reaction of **146** with (*R*)-**36** in the presence of Cs_2CO_3 in DMF, the intermediate sulfamate **148** was obtained. Hydrolysis of this compound with NaH_2PO_4 (1 M, pH 5.4) was not complete after 24 h at rt (40%). An alternative method with $BF_3.Et_2O$ and *n*-PrSH in CH_2Cl_2 solved this problem and protected Lan **150** was recovered in 89% yield. Of note, the use of Fmoc protected Cys (*R*)-**86** instead of (*R*)-**36**, in this reaction, led to side products formation resulting from the low stability of (*R*)-**86** in basic

conditions. Interestingly, starting from the more hindered sulfamidate **147** and (*R*)-**36**, β-Me-Lan **151** was obtained in good yield (70%). The de of **150**-**151** was > 90%, as shown by the absence of any contaminating diastereomers in ^{13}C NMR.



Scheme 36. Synthesis of protected Lan and β-Me-Lan using sulfamidates. i) **146** (0.6 mmol, 1 eq) or **147** (0.54 mmol, 1 eq), (*R*)-**36** (1.2 eq), Cs_2CO_3 (1.2 eq), DMF, rt, 20 h, evaporation; ii) **148** or **149**, $BF_3.Et_2O$ (1.5 eq), *n*-PrSH (1.5 eq), CH_2Cl_2, 0 °C, 16 h. Yields: 89% (**150**), 70% (**151**).

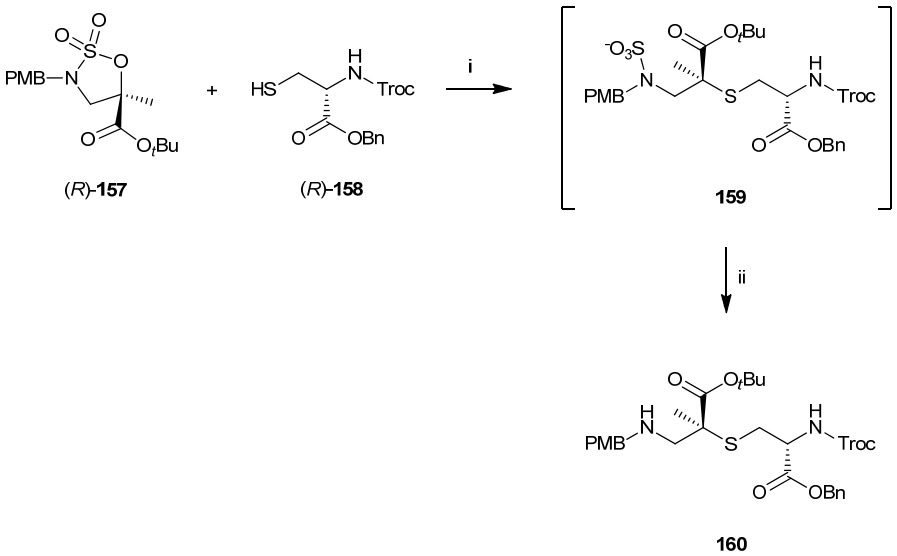
In 2006, Avenoza et al. prepared an orthogonally protected α-Me-*nor*-Lan from a β-amino acid sulfamidate derivative (*R*)-**157** (Scheme 37).[56]



Scheme 37. Synthesis of protected sulfamidate. i) **152**, AD-mix α, $MeSO_2NH_2$, *t*-BuOH/H_2O (1 : 1), 0 °C, 12 h; ii) (*R*)-**153**, Burgess reagent, THF, rt, 24 h, 78% (2 steps); iii) (*R*)-**154** (0.88 mmol, 1 eq), $LiOH.H_2O$ (10 eq), MeOH/H_2O (3 : 2), rt, 24 h, 89%; iv) (*R*)-**155** (1.57 mmol, 1 eq), allyl-OH (16 mL), AcCl (4 mL), 0 °C → 70 °C, 3 h, 92%; v) (*R*)-**156** (0.85 mmol, 1 eq), PMB-Br (1.5 eq), KO*t*-Bu (1 M in THF, 2.5 eq), THF, rt, 24 h, 93%.

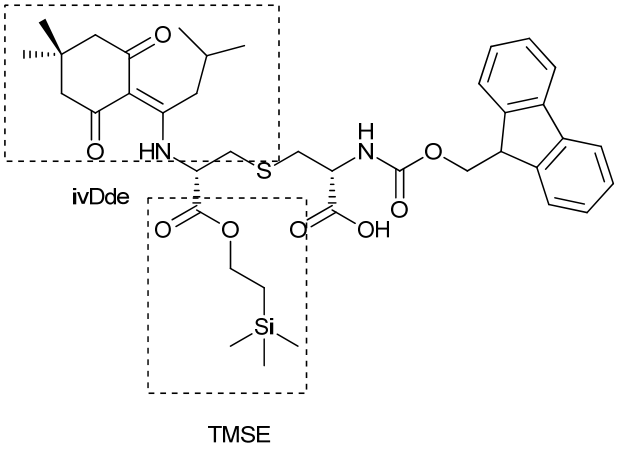
The preparation of this sulfamidate began with the Sharpless oxidation of the Weinreb amide of methacrylic acid **152**. The diol (*R*)-**153** was reacted with the Burgess reagent in THF to provide sulfamidate (*R*)-**154** in 78% yield for two steps. The carbamate and amide of (*R*)-**154** were hydrolysed with 10 eq of LiOH at rt for 24 h (89%). The acid (*R*)-**155** was converted to the allyl ester (*R*)-**156** with AcCl and allyl alcohol. The nitrogen of the sulfamidate (*R*)-**156** was then protected with PMB-Br and an excess of KO*t*-Bu in THF. During this reaction the allyl ester of (*R*)-**156** was also transesterified into a *tert*-butyl ester. Compound (*R*)-**157** was obtained in 93% yield (Scheme 37).

Sulfamidate (*R*)-**157** was reacted with (*R*)-**158** and DBU in DMF at 50 °C for 1 h to give the intermediate sulfamate **159**. Hydrolysis of **159** was done in a biphasic mixture of 20% H_2SO_4 and CH_2Cl_2 at rt for 10 h to give the orthogonally protected α-Me-*nor*-Lan **160** (91%, Scheme 38 and Figure 6).

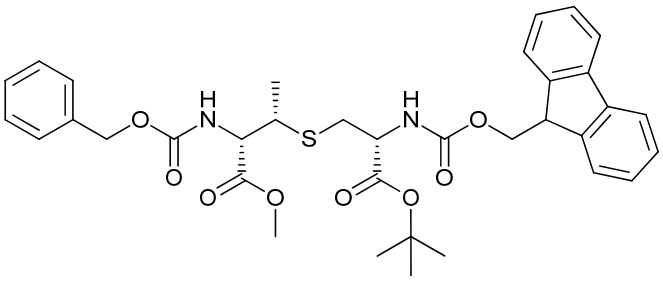


Scheme 38. Synthesis of orthogonally protected α-Me-*nor*-Lan using sulfamidate. i) (*R*)-**157** (0.15 mmol, 1 eq), (*R*)-**158** (0.17 mmol, 1.1 eq), DBU (1.05 eq), DMF, 50 °C, 1 h; ii) H_2SO_4 (20% aq), CH_2Cl_2, rt, 10 h, 91%.

136:



139:



160:

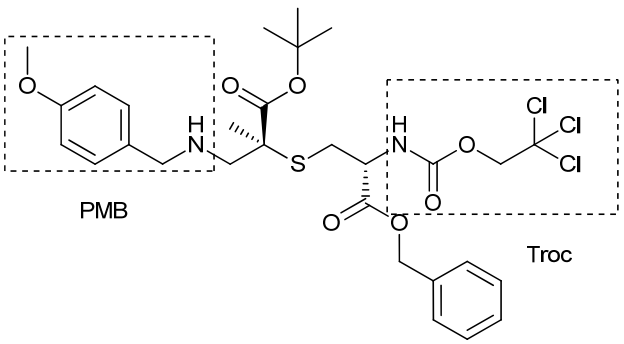


Figure 6. Detailed representation of orthogonally protected Lan **136**, β-Me-Lan **139** and *nor*-Lan **157**.

2.4 Applications

In this section two examples of applications in the lantibiotic field are presented.

2.4.1 Liquid-phase synthesis of nisin

In 1928, nisin was found in a culture broth of *Lactococcus lactis*. This was the first biologically active polypeptide containing a lanthionine (lantibiotic) to be discovered. Its isolation as a pure compound was only effected in 1947 - 1952. Structural elucidation was established by Gross and Morell in 1971 (Figure 7).[57] The proposed structure is unique for its C-terminal bicyclic 13-membered rings system containing β-Me-Lan (rings D and E). Another 13-membered ring (ring B) and two other larger rings including Lan or β-Me-Lan (rings A and C) are also presents. Altogether, nisin is composed of 34 amino acid residues, including four β-Me-Lan and one Lan.

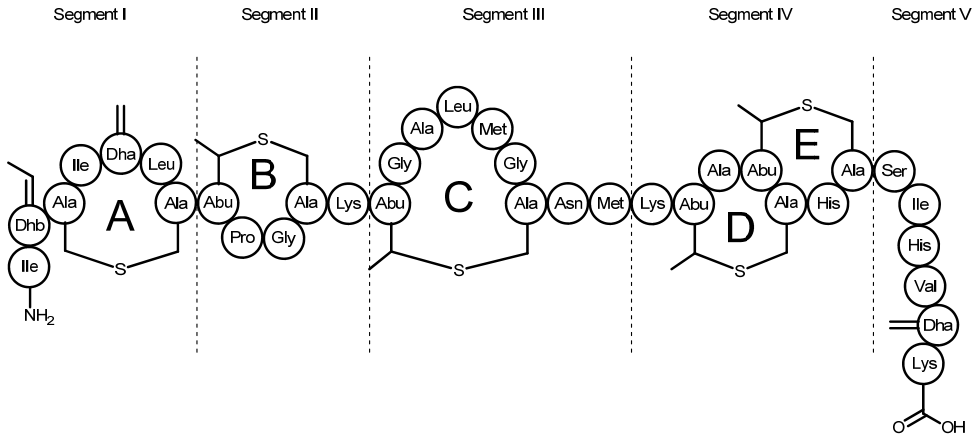
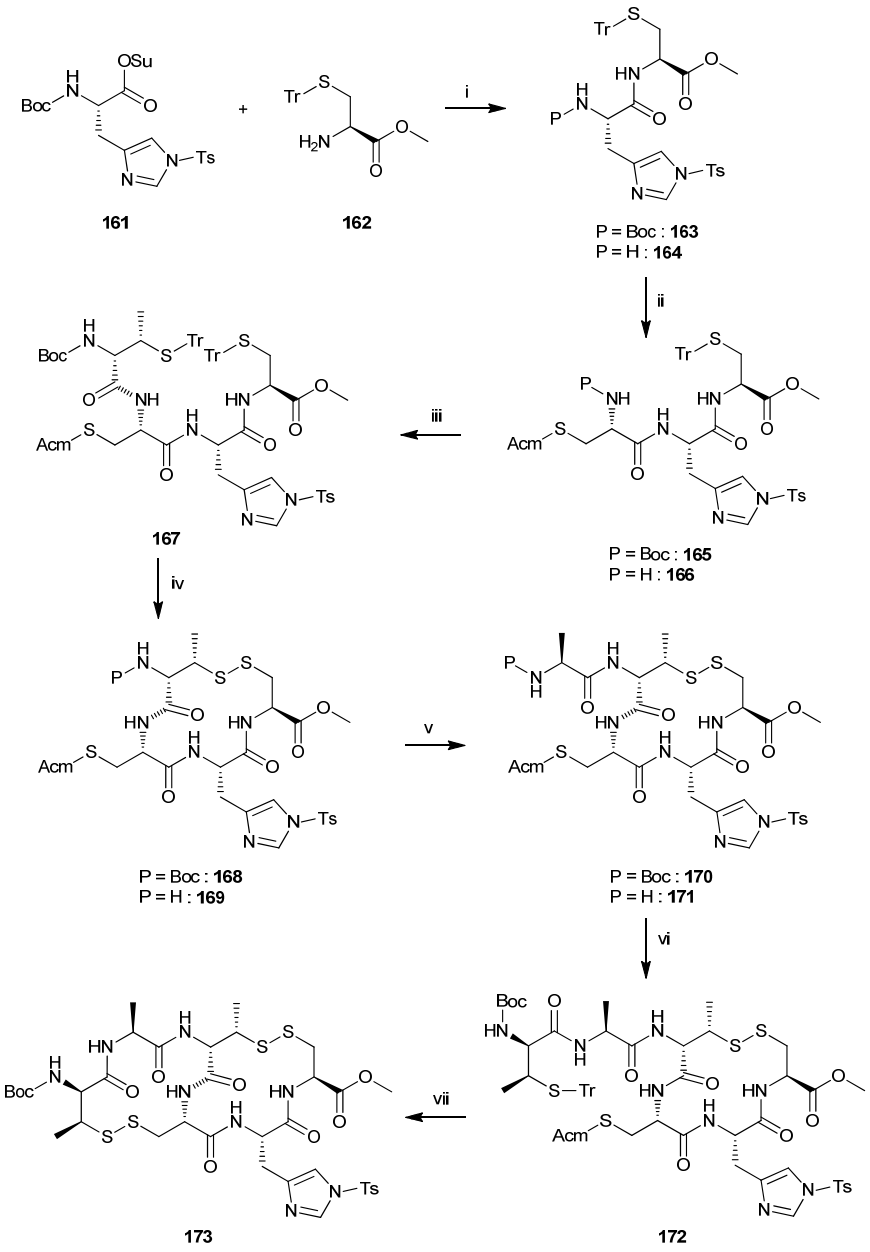


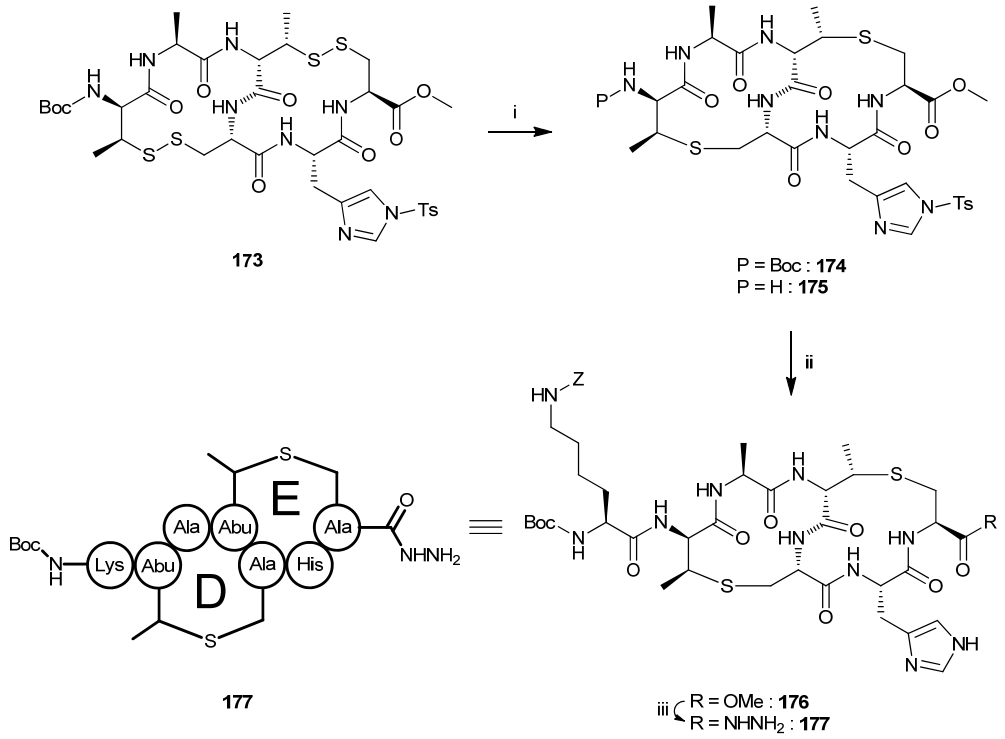
Figure 7. Five fragments in the total synthesis of nisin.

In 1988, the total synthesis of nisin was accomplished for the first time by Fukase et al..[58,59] The developed strategy is based on a convergent synthesis and involves the coupling of independently synthesized fragments. Thus, the structure of nisin was divided in five fragments (Segments I to V, Figure 7). A Boc-based liquid phase strategy was chosen, involving Cbz and benzyl groups for persistent protection of functional groups. Out of the five individual segments preparation, a focus will be done on the synthesis of segment IV containing rings D and E (Scheme 39 and 40).



Scheme 39. Synthesis of segment IV of nisin (part I). i) **161** (67.3 mmol, 1 eq), **162** (67.3 mmol, 1 eq), THF, rt, 12 h, 78%; ii) **163** (9.1 mmol, 1 eq), TFA (28 mL), evaporation, NEt_3 (2.5 eq), Boc-Cys(Acm)-OSu (14 mmol, 1.5 eq), 0 °C, 90 min, 84%; iii) **166** (30.4 mmol, 1 eq), TFA (30 mL), evaporation, $NaHCO_3$ wash, Boc-3-Me-(*S*)-Cys(Tr)-OH (30.4 mmol, 1 eq), DCC (1 eq), THF, rt, 12 h, 90%; iv) **167** (8.9 mmol, 1 eq), TFE (350 mL), CH_2Cl_2 (3.5 L), I_2 (3 eq), rt, 20 min, 78%; v) **168** (5.6 mmol, 1 eq), TFA (17 mL), evaporation, NEt_3 (3 eq), Boc-Ala-OH (16.7 mmol, 3 eq), DCC (1.5 eq), CH_2Cl_2, rt, 12 h, 91%; vi) **170** (2.25 mmol, 1 eq), TFA (10 mL), evaporation, NEt_3 (3.4 eq), Boc-3-Me-(*S*)-Cys(Tr)-OH (2.36 mmol, 1.05 eq), BDP (1.2 eq), DMF, rt, 12 h, TsCl (1 eq), NEt_3 (1 eq), rt, 1 h, 70%; vii) **172** (2.76 mmol, 1 eq), MeOH (90% aq, 1.2 L), I_2 (3 eq), rt, 1 min, Na ascorbate quench, 78%.

To begin segment IV preparation, Fukase et al. coupled tosyl-protected Boc-histidine **161** with trityl-cysteine **162**.[60] The Boc group of **163** was removed and **164** was reacted with *S*-acetamidomethyl (Acm) Boc-protected cysteine OSu ester to yield **165**. Acidolysis (TFA) of Boc was followed by treatment of **166** with Boc-3-Me-(*S*)-Cys(Tr)-OH and DCC. Compound **167**, containing three protected cysteines, was then cyclized to cystine **168** with iodine in CH_2Cl_2 containing trifluoroethanol (TFE) during 20 min at rt. In those conditions, only the trityl protecting groups are removed, leaving the Acm intact. TFA deprotection followed by the addition of Boc-Ala using DCC gave **170**. The Boc group was removed and **171** was reacted with Boc-3-Me-(*S*)-Cys(Tr)-OH and benzotriazol-1-yl diethyl phosphate (BDP) in DMF. Histidine had to be reprotected with TsCl. When **172** was treated with I_2 in MeOH, concomitant Acm deprotection and cystine **173** formation were obtained.

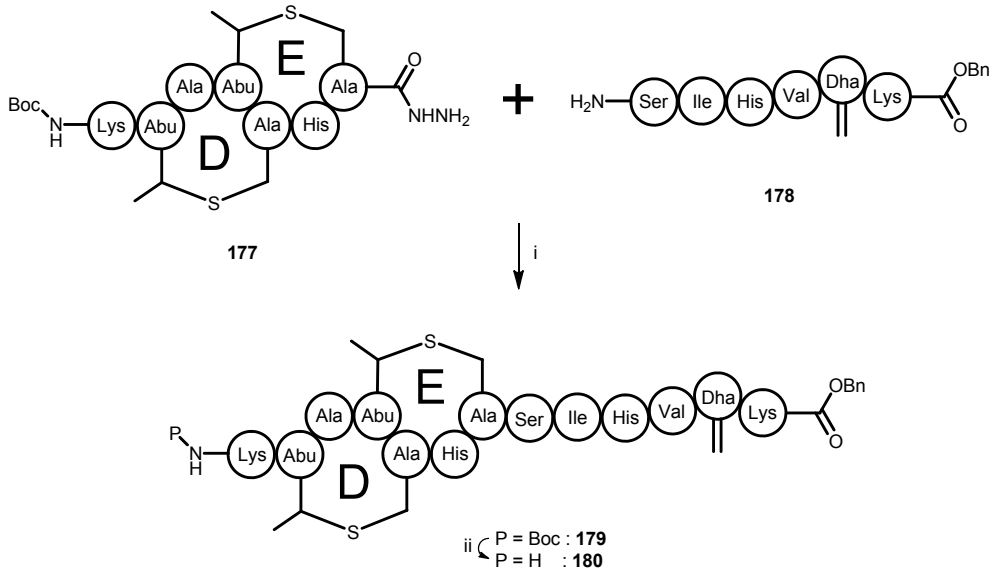


173 **177**

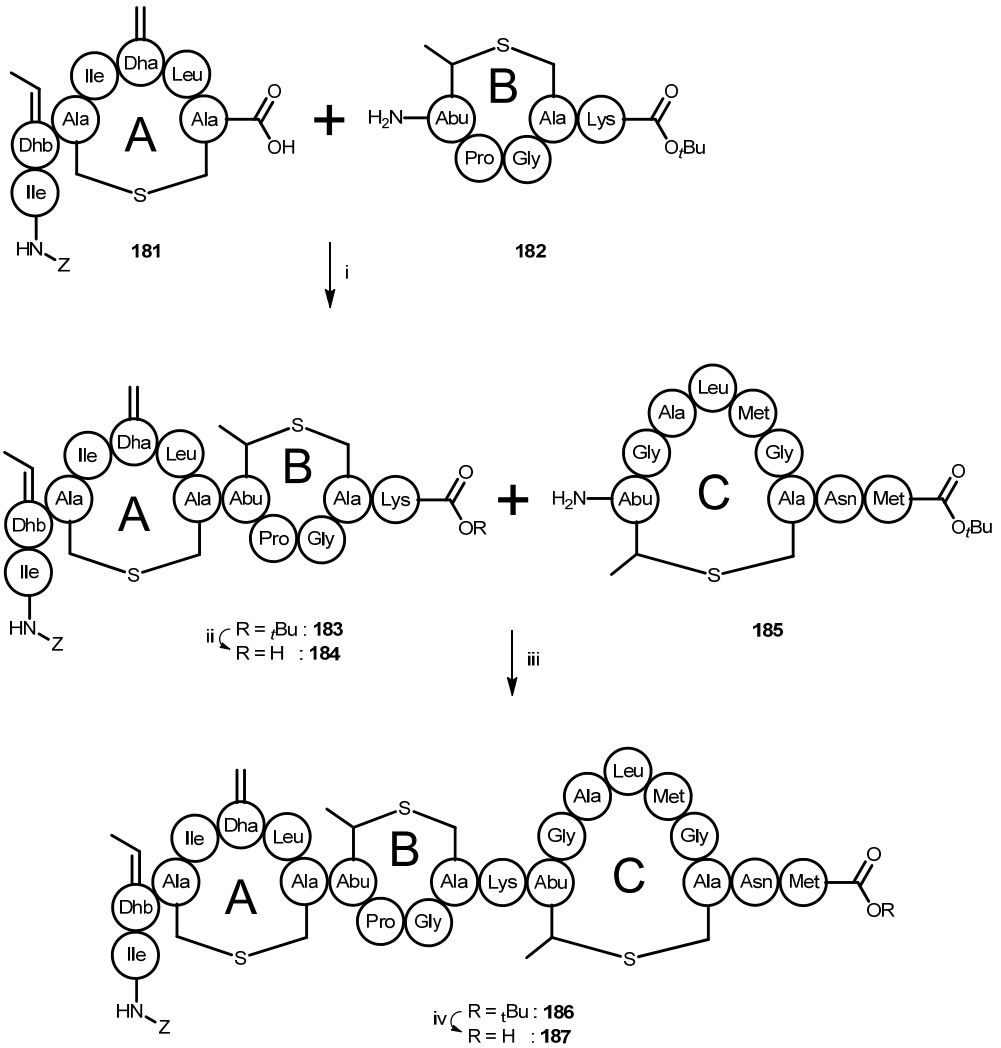
Scheme 40. Synthesis of segment IV of nisin (part II). i) **173** (0.376 mmol, 1 eq), $P(NEt_2)_3$ (15 mmol, 40 eq), benzene, rt, 72 h, 40%; ii) **174** (0.819 mmol, 1 eq), TFA (5 mL), evaporation, Boc-Lys(Z)-OH (0.86 mmol, 1.05 eq), EDC (1.1 eq), HOBt (5 eq), DMF, rt, 9 h, 69%; iii) **176** (0.41 mmol, 1 eq), $NH_2NH_2.H_2O$ (20 eq), DMF, rt, 7 h, 89%.

Bicyclic compound **173**, containing two cystines, was then contracted to **174** (Scheme 40). Using the sulfur-extrusion methodology developed by Harpp and Gleason, the two overlapping β-Me-Lan bridges of rings D and E were obtained.[26] Thus, treatment of **173** with 40 eq of amino phosphine in benzene at rt for 72 h led to **174** (40%). Boc deprotection and EDC promoted coupling with Boc-Lys(Z)-OH in the presence of HOBt gave **176**. The hydrazide **177** was then obtained after aqueous hydrazine treatment, completing segment IV synthesis.

The convergent synthesis of nisin implies the condensation of the different fragments previously synthesized. In the following steps, the individual segments I to V were thus coupled. In this way, **177** (segment IV) was treated with isopentyl nitrite in THF containing HCl and the resulting acyl azide was reacted with **178** (segment V) to give **179** in 37% yield (Scheme 41).[59] Boc deprotection of **179** with TFA at 0 °C gave the free amino **180** (58%). This product will be use in a subsequent condentation step.



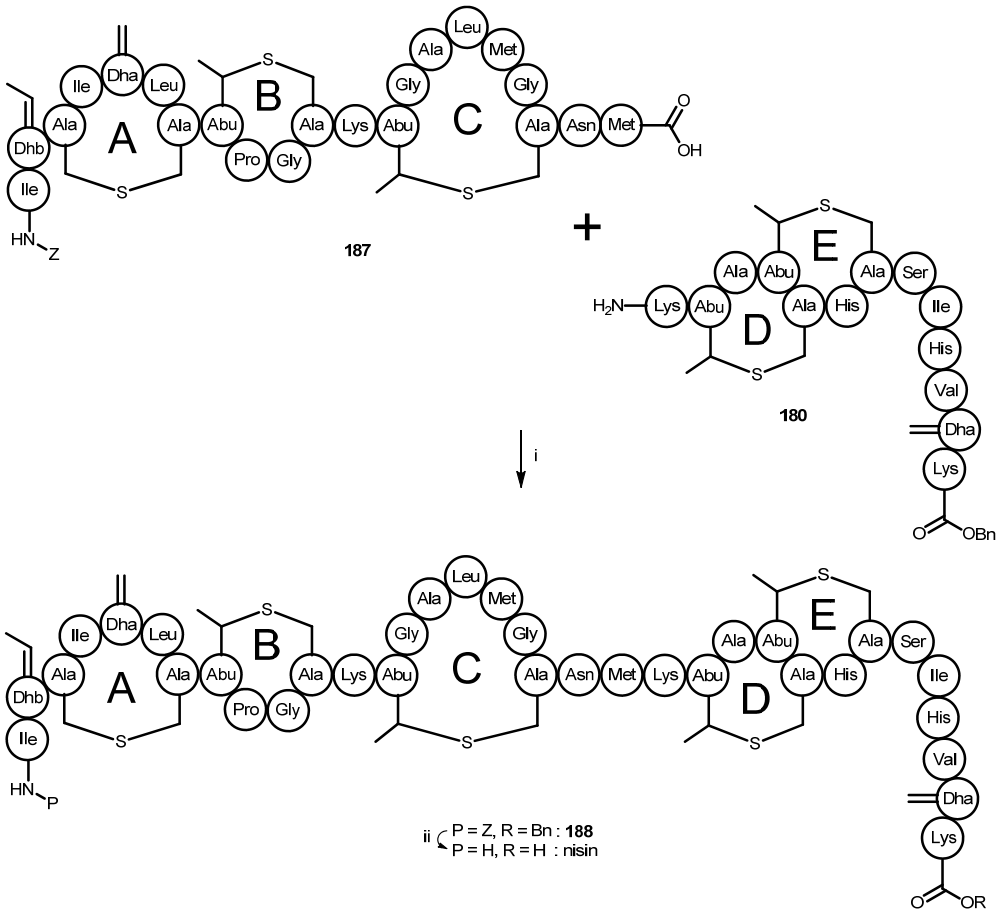
Scheme 41. Coupling of segments IV and V for the total synthesis of nisin. i) **177** (428 µmol, 1 eq), DMF, HCl (4.7 M in THF, 4 eq), isopentyl nitrite (1.5 eq), -70 °C → -20 °C, 2 h, **178** (432 µmol, 1 eq), NEt_3 (5 eq), -70 °C → -20 °C, 5 h, Boc_2O (8 eq), 37%; ii) **179** (38.2 µmol, 1 eq), TFA (2 mL), 0 °C, 90 min, 58%.



Scheme 42. Coupling of segments I, II and III for the total synthesis of nisin. i) **181** (255 µmol, 1 eq), **182** (255 µmol, 1 eq), EDC (1.5 eq), HOBt (0.9 eq), DMF, -70 °C → -20 °C, 12 h, 56%; ii) **183** (31.2 µmol, 1 eq), TFA (50% in CH_2Cl_2, 2 mL), rt, 2 h, 72%; iii) **184** (26.8 µmol, 1 eq), **185** (29.5 µmol, 1.1 eq), EDC (1.3 eq), HOBt (1.2 eq), DMF, -70 °C → -20 °C, 12 h, 61%; iv) **186** (14.8 µmol, 1 eq), TFA (600 µL with 60 µL anisole), rt, 2 h, ZOSu (1.35 eq), NEt_3 (6.8 eq), rt, 2 h, 71%.

The second half of the nisin molecule (**187**) was synthesized firstly by the EDC mediated coupling of the preliminarily prepared **181** (segment I) and **182** (segment II) to give **183** (Scheme 42). Acidolysis of the *tert*-butyl ester of **183** gave **184** which was coupled with amine **185** (segment III) using EDC. The resulting peptide **186** was obtained and its *tert*-butyl ester was removed to give the acid **187**.

Nisin was finally obtained after condensation of **187** (Segment I | Segment II | Segment III) with **180** (Segment IV | Segment V) (Scheme 43).



Scheme 43. Coupling of segments I-III and IV-V in the total synthesis of nisin. i) **184** (6.14 µmol, 1 eq), **179** (8.68 µmol, 1.4 eq), EDC (2 eq), HOBt (1.6 eq), NEt_3 (1.3 eq), DMF, -70 °C → -20 °C, 12 h, 48%; ii) **188** (1.5 µmol, 1 eq), anisole (200 µL), HF (anhydrous, 2 mL), -70 °C → 0 °C, evaporation, HPLC, 71%.

The carboxylic function on the Met residue of **187** was activated at -70 °C using EDC and HOBt and coupled with the α-amino function of the Lys residue of **180** in DMF for 12 h at -20 °C. All the persistent protecting groups (Bn and CBz) of **188** were removed with anhydrous HF to provide, after HPLC purification, pure nisin (71% yield, 3.5 mg). The synthetic nisin was indistinguishable (^{1}H-NMR, HPLC, MS) from the natural product and had the same antibacterial activities.

2.4.2 Solid-phase synthesis of lacticin 3147 A1

Nowadays, the total synthesis of lantibiotics is usually done using SPPS. In 2011, the recent developments in this field were reviewed by Tabor.[14] The importance of Lan stereochemistry in lantibiotics was recently investigated in the case of lacticin 481 by Knerr and Van der Donk.[61]

Lacticin 3147 is a lantibiotic consisting of two peptides, namely lacticin 3147 A1 and lacticin 3147 A2 (Figure 8).

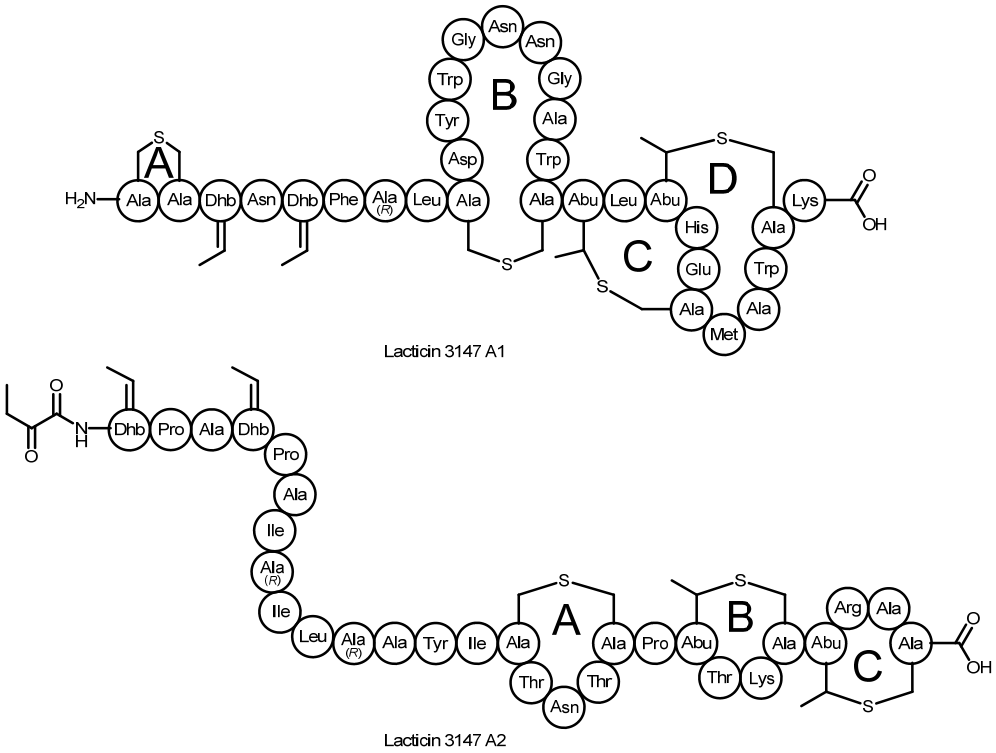
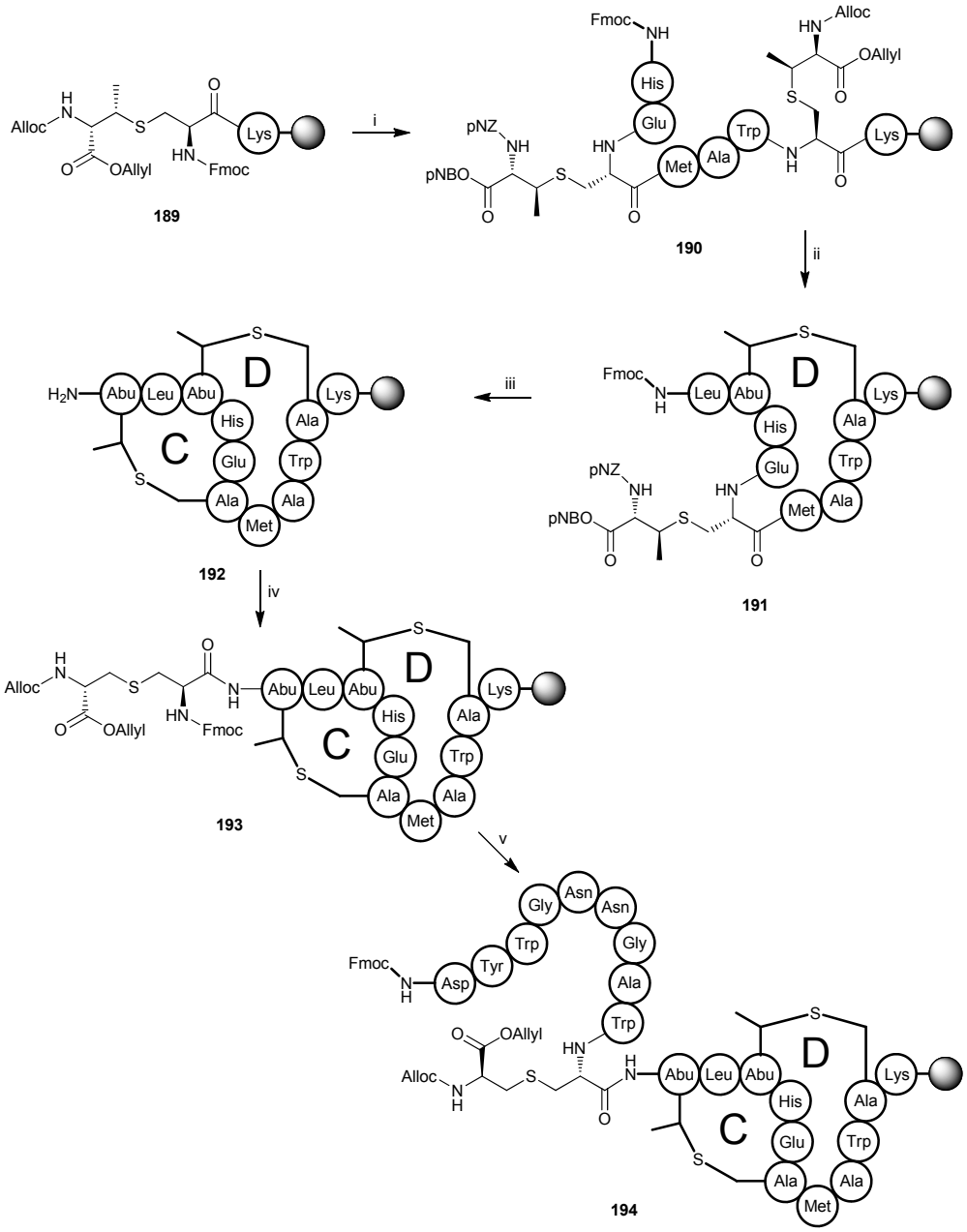


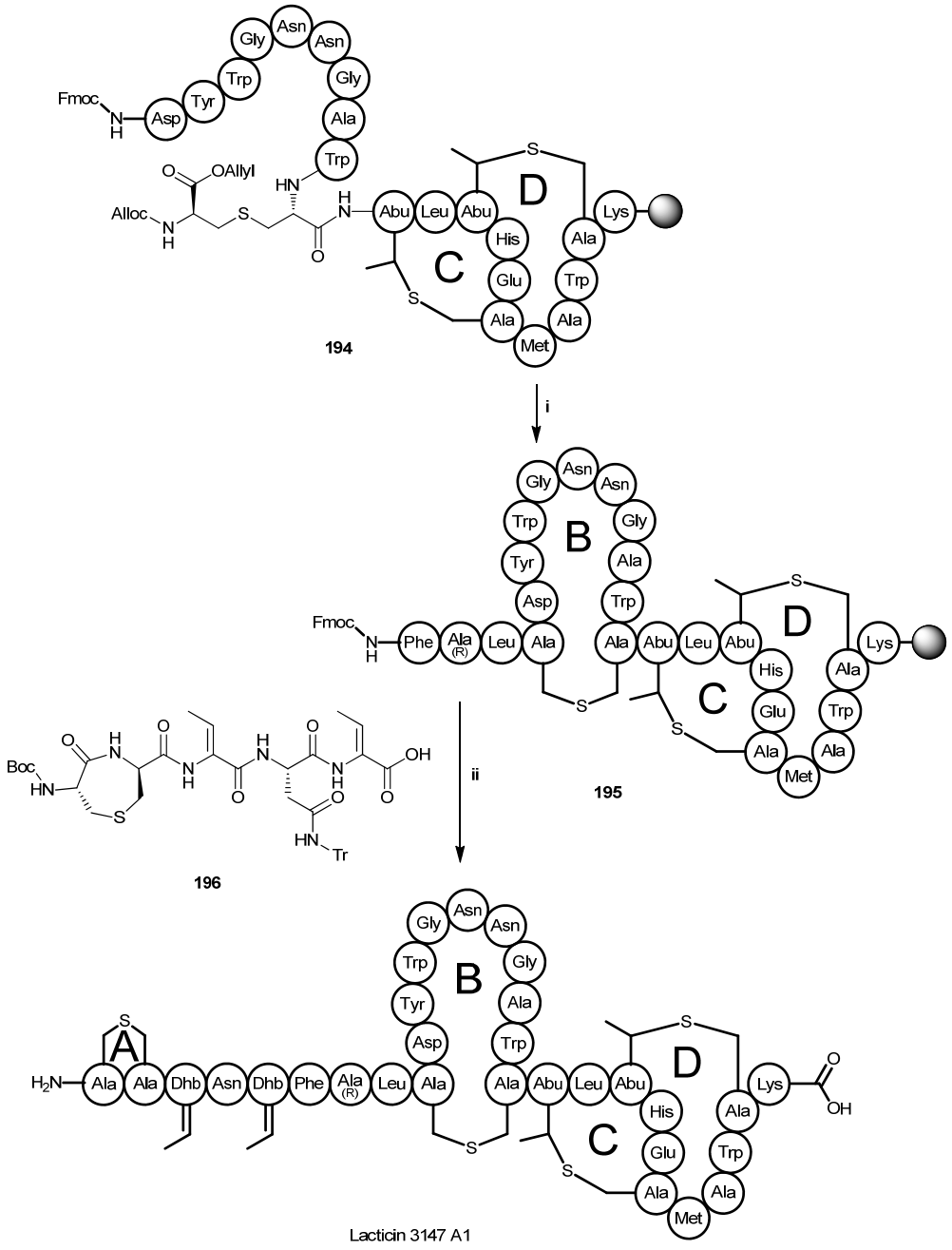
Figure 8. The two peptide-components of Lacticin 3147.

The total synthesis of lacticin 3147 A1 using SPPS was reported by Liu et al. in 2011.[37] This synthetic route involved the preliminary preparation of the two mutually orthogonally protected β-Me-Lan **82** and **84**. The synthesis of these two compounds was reviewed here above (Scheme 19 and Figure 4). Lan **97** was also prepared *via* a substituted aziridine using a similar method to **84** (Figure 5).



Scheme 44. Solid phase synthesis of lacticin 3147 A1 (part I). i) **189** (from **84**, 0.15 mmol/g on Wang resin), **82**, protected amino acids, standard Fmoc methodology [viz. Fmoc-AA (5 eq), HOBt (5 eq), NMM (5 eq), PyBOP (5 eq), DMF, 2 h, Kaiser test, end capping with 20% Ac_2O in DMF, 10 min, Fmoc-deprotection: 20% piperidine in DMF]; ii) **190** (0.075 mmol, 1 eq), $Pd(PPh_3)_4$ (2 eq), $PhSiH_3$ (10 eq), 1:1 (DMF/CH_2Cl_2), rt, 2 h, Fmoc removal, Fmoc method., Fmoc-Leu-OH, Fmoc method. iii) **191**, $SnCl_2$ (6 M in DMF, 5 mL), rt, 45 min, repeat (2 x), Fmoc removal, Fmoc method. (2 x), iv) **192**, **97**, Fmoc method., v) **193**, protected amino acids, Fmoc methodology.

The solid-phase peptide synthesis of lacticin 3147 A1 required, in a first step, the loading of Fmoc-Lys(Boc)-OH onto a Wang resin (0.15 mmol/g). After Fmoc deprotection of Lys with 20% piperidine in DMF, the Alloc/allyl protected β-Me-Lan **84** was coupled with PyBOP and a standard Fmoc methodology (Scheme 44). The resin-bound dipeptide **189** was elongated with Trp, Ala and Met. The mutually orthogonal β-Me-Lan **82** was then added followed by Glu and His to give **190**. The Alloc and allyl groups of **190** were deprotected using $Pd(PPh_3)_4$ (2 eq) and $PhSiH_3$ (10 eq) in 1:1 (DMF/CH_2Cl_2). Subsequent Fmoc removal of His and cyclization using PyBOP gave the D-ring of lacticin. After coupling of Leu, compound **191** was obtained. The pNB and pNZ groups of **191** were removed using a 6 M solution of $SnCl_2$ in DMF (twice). Fmoc deprotection of Leu was done and ring C was obtained by PyBOP cyclization. The resulting peptide **192** was reacted with Alloc/allyl protected Lan **97** to provide **193**. By continuing the standard methodology, several Fmoc-protected amino acids were then sequentially added to give **194**. The Alloc and allyl groups of **194** were subsequently deprotected using $Pd(PPh_3)_4$ and $PhSiH_3$ (Scheme 45). After Fmoc removal of Asp, cyclization to ring B was achieved using PyBOP. Leu, (*R*)-Ala and Phe were then added (**195**). In the penultimate step of the synthesis, the previously prepared ring A-tetrapeptide **196** was coupled with **195** using the fluoro formamidinium coupling agent TFFH in NMP at rt for 72 h. Following resin cleavage with 95:2.5:2.5 (TFA/anisole/H_2O) and HPLC purification, lacticin 3147 A1 was obtained in a 1.4% global yield (0.7 mg). Synthetic lacticin 3147 A1 showed the same HPLC and MS profile and identical biological activity with the natural product isolated from *Lactococcus lactis* DPC3147. The same synergism with lacticin 3147 A2 was also present.



Scheme 45. Solid phase synthesis of Lacticin 3147 A1 (part II). i) **194**, $Pd(PPh_3)_4$ (2 eq), $PhSiH_3$ (10 eq), 1:1 (DMF/CH_2Cl_2), rt, 2 h, Fmoc removal, Fmoc methodology (2 x), protected amino acids, Fmoc methodology, ii) **195** (0.015 mmol, 1 eq), Fmoc removal, **196** (0.03 mmol, 2 eq), TFFH (3 eq), DiPEA (3 eq), NMP, rt, 72 h, resin cleavage: 95:2.5:2.5 (TFA/anisole/H_2O), HPLC. Global yield of Lacticin 3147 A1 from **189**: 1.4%.

References

1. Gortner, R.A.; Hoffman, W.F. L-cystine. *Org. Synth.* **1925**, *5*, 39.
2. Hoffman, W.F. Sulfur in proteins: II. The effect of mild alkaline hydrolysis upon hair. *J. Biol. Chem.* **1925**, *65*, 251-254.
3. Horn, M.J.; Jones, D.B.; Ringel, S.J. Isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 141-149.
4. Du Vigneaud, V.; Brown, G.B. The synthesis of the new sulfur-containing amino acid (lanthionine) isolated from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 151-154.
5. Horn, M.J.; Jones, D.B.; Ringel, S.J. Isolation of dl-lanthionine from various alkali-treated proteins. *J. Biol. Chem.* **1942**, *144*, 93-98.
6. Horn, M.J.; Jones, D.B. The isolation of lanthionine from human hair, chicken feathers, and lactalbumin. *J. Biol. Chem.* **1941**, *139*, 473.
7. Horn, M.J.; Jones, D.B.; Ringel, S.J. Isolation of *meso*lanthionine from various alkali-treated proteins. *J. Biol. Chem.* **1942**, *144*, 87-91.
8. du Vigneaud, V.; Brown, G.B.; Bonsnes, R.W. The formation of lanthionine on treatment of insulin with dilute alkali. *J. Biol. Chem.* **1941**, *141*, 707-708.
9. Galande, A.K.; Trent, J.O.; Spatola, A.F. Understanding base-assisted desulfurization using a variety of disulfide-bridged peptides. *Peptide Science* **2003**, *71*, 534-551.
10. Galande, A.K.; Spatola, A.F. A facile method for the direct synthesis of lanthionine containing cyclic peptides. *Lett. Pept. Sci.* **2001**, *8*, 247-251.
11. Swan, J.M. Mechanism of alkaline degradation of cystine residues in proteins. *Nature* **1957**, *179*, 965-965.
12. Wood, L.; Mills, M.; Hatzenbuhler, N.; Vogeli, G. Serine-rich ultra high sulfur protein gene expression in murine hair and skin during the hair cycle. *J. Biol. Chem.* **1990**, *265*, 21375-21380.
13. Paul, M.; van der Donk, W.A. Chemical and Enzymatic Synthesis of Lanthionines. *Mini-Reviews in Organic Chemistry* **2005**, *2*, 23-37.
14. Tabor, A.B. The challenge of the lantibiotics: synthetic approaches to thioether-bridged peptides. *Org. Biomol. Chem.* **2011**, *9*, 7606-7628.
15. Brown, G.B.; Du Vigneaud, V. The stereoisomeric forms of lanthionine. *J. Biol. Chem.* **1941**, *140*, 767-771.
16. Brown, G.B.; du Vigneaud, V. The synthesis of *S*-(β-amino-β-carboxyethyl)-homocysteine. *J. Biol. Chem.* **1941**, *137*, 611-615.
17. Shiraiwa, T.; Nakagawa, K.; Kanemoto, N.; Kinda, T.; Yamamoto, H. Synthesis of Optically Active Homocysteine from Methionine and Its Use in Preparing Four Stereoisomers of Cystathionine. *Chem. Pharm. Bull. (Tokyo)* **2002**, *50*, 1081-1085.
18. Photaki, I.; Samouilidis, I.; Caranikas, S.; Zervas, L. Lanthionine chemistry. Part 4. Synthesis of diastereoisomeric cyclolanthionyl derivatives. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2599.
19. Zervas, L.; Ferderigos, N. On Lanthionine and Cyclolanthionyl. *Isr. J. Chem.* **1974**, *12*, 139-152.
20. Wakamiya, T.; Oda, Y.; Fukase, K.; Shiba, T. Syntheses of Four Stereoisomers of β-Methyllanthionine. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 536-539.
21. Schoberl, A.; Wagner, A. Addition of sulfhydryl carboxylic acids to unsaturated acids and a new synthesis of lanthionine. *Chem. Ber.* **1947**, *80*, 379-390.
22. Gansser, C. α,α'-Diaminodicarboxylic acids and related compounds. *Bull. Soc. Chim. Fr.* **1966**, 1713-1714.
23. Zahn, H.; Osterloh, F. Einige neue Derivate des Lanthionins. *Justus Liebigs Annalen der Chemie* **1955**, *595*, 237-241.
24. Hanus, J.; Benes, J.; Kozel, J. Synthesis of DL-lanthionine-^{35}S and DL-lanthionine selenide-^{75}Se. *Radiochem. Radioanal. Lett.* **1975**, *23*, 33-35.
25. Aydillo, C.; Avenoza, A.; Busto, J.H.; Jiménez-Osés, G.; Peregrina, J.M.; Zurbano, M.M. A Biomimetic Approach to Lanthionines. *Org. Lett.* **2011**, *14*, 334-337.

26. Harpp, D.N.; Gleason, J.G. Preparation and mass spectral properties of cystine and lanthionine derivatives. Novel synthesis of L-lanthionine by selective desulfurization. *J. Org. Chem.* **1971**, *36*, 73-80.
27. Hata, Y.; Watanabe, M. Reaction of aziridinecarboxylic acids with thiols in aqueous solution. The formation of β-amino acids. *Tetrahedron* **1987**, *43*, 3881-3888.
28. Arnold, L.D.; May, R.G.; Vederas, J.C. Synthesis of optically pure α-amino acids via salts of α-amino-β-propiolactone. *Journal of American Chemical Society* **1988**, *110*, 2237-2241.
29. Olsen, R.K.; Kini, G.D.; Hennen, W.J. Conversion of thiosulfinate derivatives of cystine to unsymmetrical cystines and lanthionines by reaction with tris(dialkylamino)phosphines. *J. Org. Chem.* **1985**, *50*, 4332-4336.
30. Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. New diastereoselective synthesis of protected *meso*-lanthionine with discrimination of the chiral centers. *Tetrahedron: Asymmetry* **1992**, *3*, 85-94.
31. Probert, J.M.; Rennex, D.; Bradley, M. Lanthionines for solid phase synthesis. *Tetrahedron Lett.* **1996**, *37*, 1101-1104.
32. Wakamiya, T.; Shimbo, K.; Sano, A.; Fukase, K.; Shiba, T. Synthetic Study on Peptide Antibiotic Nisin. I. The Synthesis of Ring A. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2044-2049.
33. Shao, H.; Wang, S.H.H.; Lee, C.-W.; Oesapay, G.; Goodman, M. A Facile Synthesis of Orthogonally Protected Stereoisomeric Lanthionines by Regioselective Ring Opening of Serine β-Lactone Derivatives. *J. Org. Chem.* **1995**, *60*, 2956-2957.
34. Narayan, R.S.; VanNieuwenhze, M.S. Versatile and Stereoselective Syntheses of Orthogonally Protected β-Methylcysteine and β-Methyllanthionine. *Org. Lett.* **2005**, *7*, 2655-2658.
35. Smith, N.D.; Goodman, M. Enantioselective Synthesis of α-Methyl-D-cysteine and Lanthionine Building Blocks via α-Methyl-D-serine-β-lactone. *Org. Lett.* **2003**, *5*, 1035-1037.
36. Nakajima, K.; Oda, H.; Okawa, K. Studies on 2-Aziridinecarboxylic Acid. IX. Convenient Synthesis of Optically Active *S*-Alkylcysteine, *threo*-*S*-Alkyl-β-methylcysteine, and Lanthionine Derivatives <I>via</I> the Ring-opening Reaction of Aziridine by Several Thiols. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 520-522.
37. Liu, W.; Chan, A.S.H.; Liu, H.; Cochrane, S.A.; Vederas, J.C. Solid Supported Chemical Syntheses of Both Components of the Lantibiotic Lacticin 3147. *J. Am. Chem. Soc.* **2011**, *133*, 14216-14219.
38. Swali, V.; Matteucci, M.; Elliot, R.; Bradley, M. The stereospecific synthesis of 'orthogonally' protected lanthionines. *Tetrahedron* **2002**, *58*, 9101-9109.
39. Mohd Mustapa, M.F.; Harris, R.; Bulic-Subanovic, N.; Elliott, S.L.; Bregant, S.; Groussier, M.F.A.; Mould, J.; Schultz, D.; Chubb, N.A.L.; Gaffney, P.R.J., *et al.* Synthesis of Orthogonally Protected Lanthionines. *J. Org. Chem.* **2003**, *68*, 8185-8192.
40. Dugave, C.; Ménez, A. Synthesis of natural and non natural orthogonally protected lanthionines from *N*-tritylserine and *allo*-threonine derivatives. *Tetrahedron: Asymmetry* **1997**, *8*, 1453-1465.
41. Dugave, C.; Ménez, A. Stereoconservative Synthesis of Orthogonally Protected γ-Functionalized Amino Acids Using *N*-Tritylserine Derivatives. *The Journal of Organic Chemistry* **1996**, *61*, 6067-6070.
42. Cherney, R.J.; Wang, L. Efficient Mitsunobu Reactions with *N*-Phenylfluorenyl or *N*-Trityl Serine Esters. *The Journal of Organic Chemistry* **1996**, *61*, 2544-2546.
43. Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T.L.; Chung, N.N.; Schiller, P.W.; Cassel, J.A.; DeHaven, R.N.; Goodman, M. Synthesis and Biological Activities of Cyclic Lanthionine Enkephalin Analogues: δ-Opioid Receptor Selective Ligands. *J. Med. Chem.* **2002**, *45*, 3746-3754.
44. Mohd Mustapa, M.F.; Harris, R.; Mould, J.; Chubb, N.A.L.; Schultz, D.; Driscoll, P.C.; Tabor, A.B. Synthesis of orthogonally protected lanthionines: a reassessment of the use of alanyl β-cation equivalents. *Tetrahedron Lett.* **2002**, *43*, 8359-8362.
45. Mohd Mustapa, M.F.; Harris, R.; Esposito, D.; Chubb, N.A.L.; Mould, J.; Schultz, D.; Driscoll, P.C.; Tabor, A.B. Synthesis of a Cyclic Peptide Containing Norlanthionine: Effect

of the Thioether Bridge on Peptide Conformation. *The Journal of Organic Chemistry* **2003**, *68*, 8193-8198.
46. Bregant, S.; Tabor, A.B. Orthogonally Protected Lanthionines: Synthesis and Use for the Solid-Phase Synthesis of an Analogue of Nisin Ring C. *J. Org. Chem.* **2005**, *70*, 2430-2438.
47. Mothia, B.; Appleyard, A.N.; Wadman, S.; Tabor, A.B. Synthesis of Peptides Containing Overlapping Lanthionine Bridges on the Solid Phase: An Analogue of Rings D and E of the Lantibiotic Nisin. *Org. Lett.* **2011**, *13*, 4216-4219.
48. Zhu, X.; Schmidt, Richard R. Efficient Synthesis of Differently Protected Lanthionines via β-Bromoalanine Derivatives. *Eur. J. Org. Chem.* **2003**, 4069-4072.
49. Seyberth, T.; Voss, S.; Brock, R.; Wiesmüller, K.-H.; Jung, G. Lipolanthionine Peptides Act as Inhibitors of TLR2-Mediated IL-8 Secretion. Synthesis and Structure-Activity Relationships. *J. Med. Chem.* **2006**, *49*, 1754-1765.
50. Küsters, E.; Allgaier, H.; Jung, G.; Bayer, E. Resolution of sulphur-containing amino acids by chiral phase gas chromatography. *Chromatographia* **1984**, *18*, 287-293.
51. Martin, N.I. Concise Preparation of Tetra-orthogonally Protected (2S,6R)-Lanthionines. *J. Org. Chem.* **2008**, *74*, 946-949.
52. Carrillo, A.K.; VanNieuwenhze, M.S. Synthesis of the AviMeCys-Containing D-Ring of Mersacidin. *Org. Lett.* **2012**, *14*, 1034-1037.
53. Pattabiraman, V.R.; McKinnie, S.M.K.; Vederas, J.C. Solid-Supported Synthesis and Biological Evaluation of the Lantibiotic Peptide Bis(desmethyl) Lacticin 3147 A2. *Angew. Chem. Int. Ed.* **2008**, *47*, 9472-9475.
54. Knerr, P.J.; van der Donk, W.A. Chemical Synthesis and Biological Activity of Analogues of the Lantibiotic Epilancin 15X. *J. Am. Chem. Soc.* **2012**, *134*, 7648-7651.
55. Cobb, S.L.; Vederas, J.C. A concise stereoselective synthesis of orthogonally protected lanthionine and β-methyllanthionine. *Org. Biomol. Chem.* **2007**, *5*, 1031-1038.
56. Avenoza, A.; Busto, J.H.; Jiménez-Osés, G.; Peregrina, J.M. Stereoselective Synthesis of Orthogonally Protected α-Methylnorlanthionine. *Org. Lett.* **2006**, *8*, 2855-2858.
57. Gross, E.; Morell, J.L. Structure of nisin. *J. Am. Chem. Soc.* **1971**, *93*, 4634-4635.
58. Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Fujita, H.; Horimoto, S.; Wakamiya, T.; Shiba, T. Total synthesis of peptide antibiotic nisin. *Tetrahedron Lett.* **1988**, *29*, 795-798.
59. Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. Synthetic Study on Peptide Antibiotic Nisin. V. Total Synthesis of Nisin. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227-2240.
60. Fukase, K.; Oda, Y.; Kubo, A.; Wakamiya, T.; Shiba, T. Synthetic Study on Peptide Antibiotic Nisin. IV. Synthesis of Ring D-E. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1758-1763.
61. Knerr, P.J.; van der Donk, W.A. Chemical Synthesis of the Lantibiotic Lacticin 481 Reveals the Importance of Lanthionine Stereochemistry. *J. Am. Chem. Soc.* **2013**, *135*, 7094-7097.

Chapter 3

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

(This chapter has been accepted the 16 July 2014 in *Bioorganic & Medicinal Chemistry*)

3.1 Introduction

meso-Lanthionine (*meso*-Lan, **1**, Figure 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]

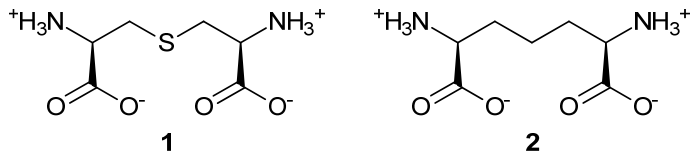


Figure 1. *meso*-Lan **1** and *meso*-A_2pm **2**.

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 catalysed by the enzymes MurA and MurB (Figure 2).

After the successive addition of different amino acids, catalysed 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 on 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 catalysed by the transglycosylase and transpeptidase activities of penicillin-binding proteins (PBPs) (Figure 2).

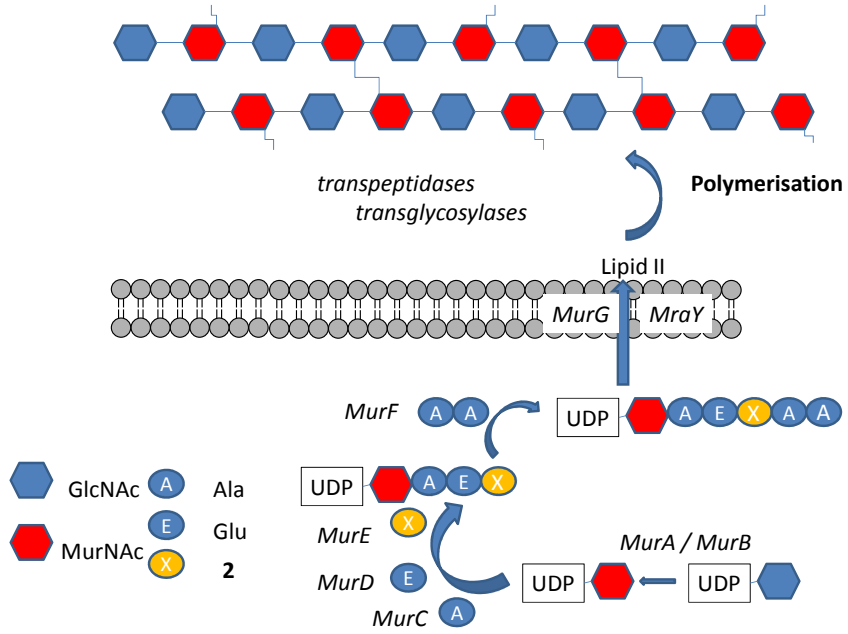


Figure 2. Metabolism of PG in *E. coli*. The biosynthesis of PG starts with the synthesis of UDP-MurNAc-pentapeptide from UDP-GlcNAc in the cytoplasm (enzymes: MurA, MurB, MurC, MurD, MurE and MurF). The second part, the synthesis of lipid II catalysed by MraY and MurG, takes place at the level of the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

In the biosynthesis of PG, MurE catalyses the formation of UDP-MurNAc-tripeptide from UDP-MurNAc-dipeptide and **2** (Figure 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* (Figure 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.

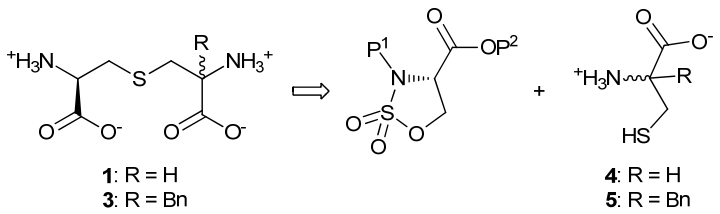
Click chemistry is an important field of research in organic chemistry. As a model for a compound bearing a substituted aromatic, the preparation in aqueous solution of the α-benzyl analogue Bn-Lan **3** (Scheme 1) is described. Results of attempts of incorporation of these molecules into *E. coli* PG are also presented.

3.2 Results and discussion

3.2.1 Chemical Synthesis

3.2.1.1 *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).



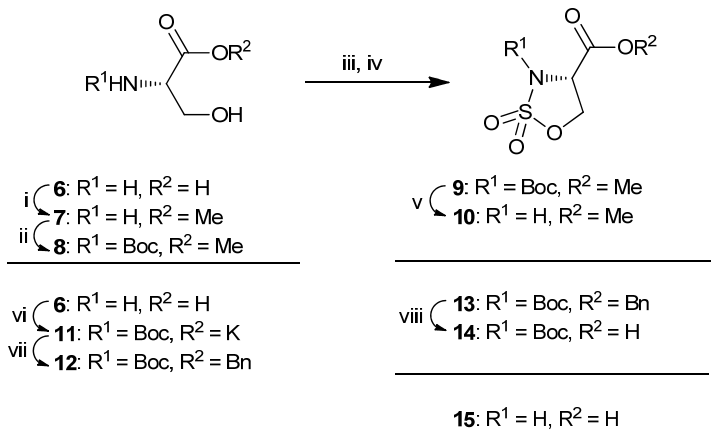
Scheme 1. Retrosynthetic approach to Lan **1** (R = H) and Bn-Lan **3** (R = Bn). P^1= H, Boc; P^2= Me, H (respectively).

3.2.1.2 *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 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-catalysed 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).

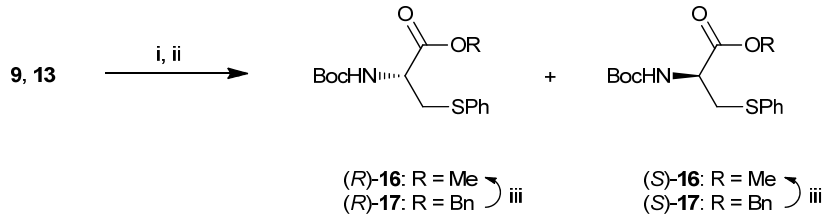
Because of the enhanced reactivity of Boc-protected sulfamidates,[31,32] compound **14** was also synthesized. Transformation of (*S*)-serine **6** into the potassium salt of Boc-serine **11** using Boc_2O and K_2CO_3 followed by a '*one pot*' alkylation with benzyl bromide in DMF, gave **12** in 93% 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-catalysed 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 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%).

3.2.1.3 *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%.

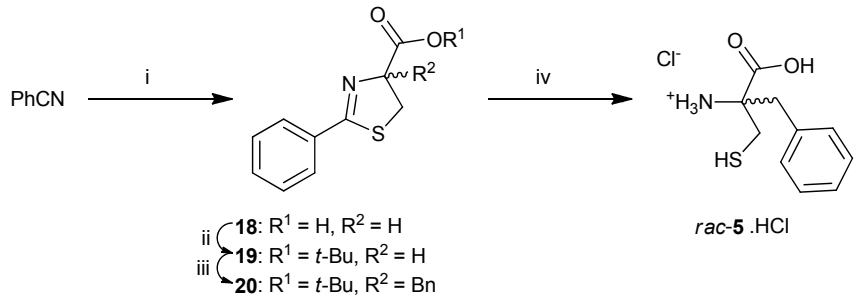


Scheme 3. Derivatization of (*S*)-**9** and (*S*)-**13**: (i) PhSNa (1 eq.), DMF, rt, 2 h (80%); (ii) 10% aq. NaH_2PO_4, EtOAc, 50 °C, 2 h; (iii) a) HCl (1 M) in MeOH, reflux, 5 h; b) Boc_2O, NEt_3, semi-preparative HPLC purification. Chiral HPLC conditions: Chiralcel OD-H 9:1 (Hexanes / *i*-PrOH), 1.0 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.

3.2.1.4 *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.

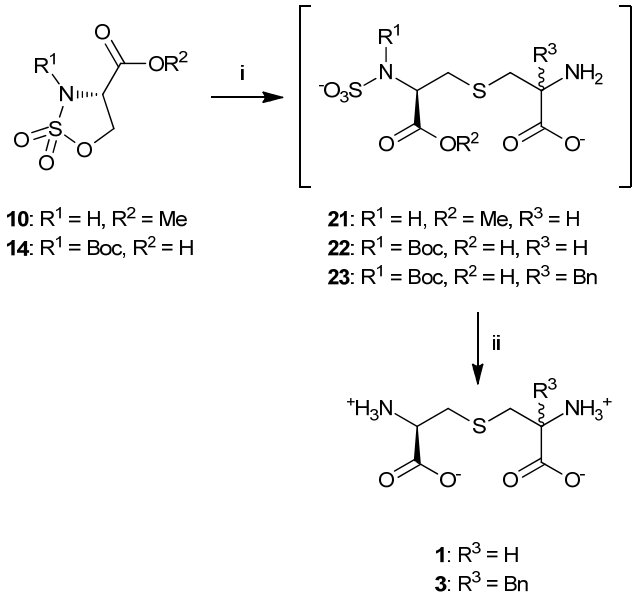


Scheme 4. Preparation of racemic α-benzyl cysteine. Reagents and conditions: (i) a) (*R*)-Cys, NaOH, MeOH, N_2, rt, 16 h; b) HCOOH, water, 0 °C (80%); (ii) a) Boc_2O (2 eq.), DMAP (10 mol%), 1:1 CH_2Cl_2/*t*-BuOH, reflux 1 h; b) imidazole, $NaHSO_4$ (97%); (iii) BnBr (5 eq.), KOH (5 eq.), TBAB (10 mol%),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

3.2.1.5 *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.



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 eq.), $NaHCO_3$ or $CsHCO_3$ (3.15 eq.), water, N_2, rt, 16 h; b) $HCl_{aq.}$, heat, isolation by Dowex 50WX8 (eluting with ammonia) or crystallization (pH 6).

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

Entry	Sulfamidate	Cys	Base [a]	H_3O^+ [b]	d.e. [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 eq.) or $CsHCO_3$ (1.6 M, 3.15 eq.) was used; [b] A: 5 M $HCl_{aq.}$, 70 °C, 5 h; B: 2.5 M $HCl_{aq.}$, 50 °C, 30 min; [c] d.e. of the crude product in an aliquot of hydrolyzed solution; [d] Yield of the crystallized product.

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 ^{1}H-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 hours 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 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).

3.2.2 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 (Figure 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).

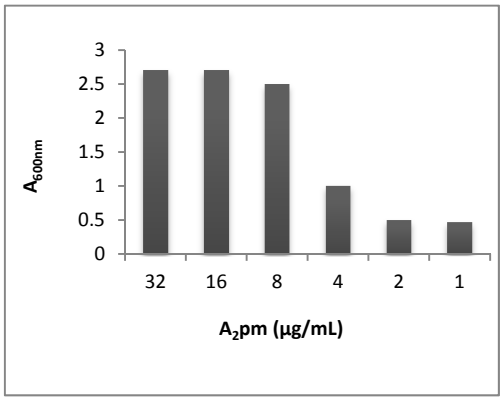


Figure 3. Optical density (600 nm) after 18 h of incubation of *E. coli* cells W7 at 37 °C in minimal medium containing 50 µg/mL (*S*)-Lys.

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) (Figure 4).

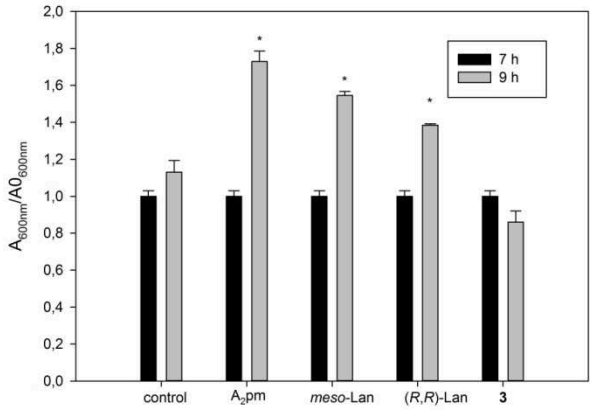


Figure 4. Bacteria were grown in the presence of 1 µg/mL A_2pm and 50 µg/mL of A_2pm, *meso*-Lan, (*R,R*)-Lan or 3 were added after 7 h when the stationary phase of bacterial growth was reached. A_{600nm}: optical density of the different cultures. $A0_{600nm}$: optical density of culture at t = 7 h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

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) (Figure 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 (Figure 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 (Figure 5).

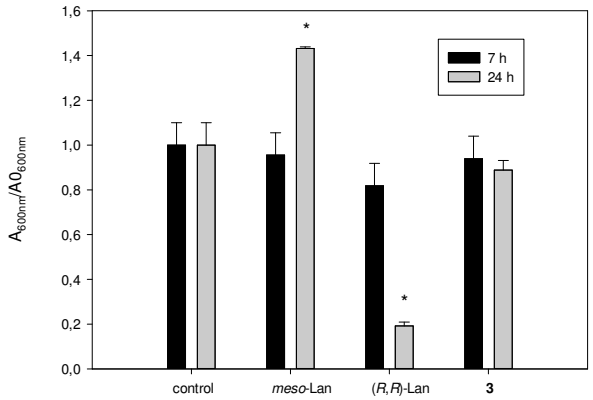


Figure 5. Bacteria were grown in the presence of 1 µg/mL A_2pm and 50 µg/mL of *meso*-Lan, (*R,R*)-Lan or 3. Control: bacteria were grown with 1 µg/mL A_2pm. A_{600nm}: optical density of the different cultures. AO_{600nm}: optical density of culture in the presence of 1 µg/mL A_2pm. * $p < 0.05$.

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 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.[17,40] No incorporation of **3** was detected in PG3.

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.

3.2.2.1 *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 catalysed 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 (Figure 4) followed by cell lysis (Figure 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]

3.3 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.

3.4 Experimental section

3.4.1 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/sulphuric 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 x 150 mm ; 3.5 µm) or for enantiomeric excess a Chiralcel OD-H column (250 x 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%.

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

A solution of Boc_2O (46.3 g, 212 mmol, 1.2 eq) 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 x 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 x 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 170.91 (CO), 155.88 (Boc-CO), 135.34 (Ar-C), 128.67 (Ar-C), 128.48 (Ar-C), 128.23 (Ar-C), 80.33 ($C(CH_3)_3$), 67.38 (CH_2Ph), 63.39 (C-β), 55.94 (C-α), 28.35 ($C(CH_3)_3$); *m/z* (ESI) 296 $[MH]^+$.

3.4.3 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 x 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.

3.4.4 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 x 200 mL) and the pooled fractions were washed with saturated $NaHCO_3$ (2 x 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.

3.4.5 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 167.58 (CO), 148.15 (Boc-CO), 86.37 ($C(CH_3)_3$), 67.58 (C-β), 57.57 (C-α), 53.69 (OCH_3), 27.93 ($C(CH_3)_3$); HRMS m/z (ES+) Calcd for $C_9H_{15}LiNO_7S$ 288.0724, found 288.0722 $[MLi]^+$.

3.4.6 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_2Ph), 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 166.99 (CO), 148.05 (Boc-CO), 134.48 (Ar-C), 128.82 (Ar-C), 128.75 (Ar-C), 128.40 (Ar-C), 86.24 ($C(CH_3)_3$), 68.49 (CH_2Ph), 67.54 (C-β), 57.66 (C-α), 27.79 ($C(CH_3)_3$); HRMS m/z (ES+) Calcd for $C_{15}H_{19}LiNO_7S$ 364.1037, found 364.1035 $[MLi]^+$.

3.4.7 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 168.84 (CO), 69.91 (C-β), 56.20 (C-α), 54.10 (OCH_3); HRMS m/z (ES+) Calcd for $C_4H_7LiNO_5S$ 188.0199, found 188.0198 $[MLi]^+$.

3.4.8 (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 (63MHz, $CDCl_3$) δ_C 170.57 (CO), 148.44 (Boc-CO), 86.97 ($C(CH_3)_3$), 67.80 (C-β), 57.50 (C-α), 27.93 ($C(CH_3)_3$); HRMS m/z (ES+) Calcd for $C_8H_{13}LiNO_7S$ 274.0567, found 274.0568 $[MLi]^+$.

3.4.9 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 x 20 mL). The combined organic layers were washed with water (2 x 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.

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

Yield 80%; ^1H 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.[34] $[\alpha]_D^{20}$ = -19° (c 1, MeOH) for (*R*)-**16**).

3.4.10 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; ^1H 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 178.11 (CO), 170.14 (CN), 133.37 (Ar-C), 132.18 (Ar-C), 128.75 (Ar-C), 127.17 (Ar-C), 59.31 (C-α), 27.05 (C-β); *m/z* (ESI) 208 $[MH]^+$.

3.4.11 *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 170.54 (CN), 170.01 (CO), 132.91 (Ar-C), 131.59 (Ar-C), 128.67 (Ar-C), 128.49 (Ar-C), 82.23 ($C(CH_3)_3$), 79.37 (C-α), 35.76 (C-β), 28.09 ($C(CH_3)_3$); *m/z* (ESI) 264 $[MH]^+$.

3.4.12 *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 (63MHz,$CDCl_3$) δ_C 171.23 (CO), 168.43 (CN), 136.18 (Ar-C), 133.24 (Ar-C), 131.45 (Ar-C), 130.62 (Ar-C), 128.63 (Ar-C), 128.48 (Ar-C), 128.16 (Ar-C), 126.89 (Ar-C), 89.27 (C-α), 82.21 ($C(CH_3)_3$), 43.18 (CH_2Ph), 39.39 (Cys–CH_2), 28.02 ($C(CH_3)_3$); *m/z* (ESI) 354 $[MH]^+$.

3.4.13 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 x 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 (250MHz, 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 (63MHz, $CDCl_3$) δ_C 171.07 (CO), 131.96 (Ar-C), 129.91 (Ar-C), 128.98 (Ar-C), 128.18 (Ar-C), 65.48 (C-α), 40.41 (CH_2Ph), 28.74 (Cys–CH_2); *m/z* (ESI) 212 $[MH]^+$.

3.4.14 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 $K_2B_4O_7.4H_2O$ (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 minutes, 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%.

3.4.14.1 *(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 (63MHz, D_2O) δ_C 170.01 (CO), 52.15 (C-α), 31.51 (CH_2); *m/z* (ESI) 209 $[MH]^+$.

3.4.14.2 *(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 (63MHz, D_2O) δ 171.06 (0.5C, CO), 171.00 (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), 128.27 (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 $[MH]^+$.

3.5 Biological experiments

3.5.1 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 $K_2HPO_4.3H_2O$, 2.95 g of KH_2PO_4, 1.05 g of $(NH_4)_2SO_4$, 1.0 g of $MgSO_4.7H_2O$, 0.1 mg of $FeSO_4.7H_2O$, 2.8 mg of $Ca(NO_3)_2.4H_2O$, 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.

3.5.2 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 minutes. 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, 50000 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, 50000 rpm). Finally, it was washed with water, with 8 M LiCl to remove any polypeptidic contamination, again with water, and lyophilized.

3.5.3 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.

References

1. Horn, M.J.; Jones, D.B.; Ringel, S.J. Isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 141-149.
2. Du Vigneaud, V.; Brown, G.B. The synthesis of the new sulfur-containing amino acid (lanthionine) isolated from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 151-154.
3. Vasstrand, E.N.; Jensen, H.B.; Miron, T.; Hofstad, T. Composition of peptidoglycans in Bacteroidaceae: determination and distribution of lanthionine. *Infect. Immun.* **1982**, *36*, 114-122.
4. Tabor, A.B. The challenge of the lantibiotics: synthetic approaches to thioether-bridged peptides. *Org. Biomol. Chem.* **2011**, *9*, 7606-7628.
5. van Heijenoort, J. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Natural Product Reports* **2001**, *18*, 503-519.
6. Auger, G.; van Heijenoort, J.; Vederas, J.C.; Blanot, D. Effect of analogues of diaminopimelic acid on the *meso*-diaminopimelate-adding enzyme from *Escherichia coli*. *FEBS Lett.* **1996**, *391*, 171-174.
7. Mengin-Lecreulx, D.; Michaud, C.; Richaud, C.; Blanot, D.; van Heijenoort, J. Incorporation of LL-diaminopimelic acid into peptidoglycan of *Escherichia coli* mutants lacking diaminopimelate epimerase encoded by dapF. *J. Bacteriol.* **1988**, *170*, 2031-2039.
8. Michaud, C.; Mengin-Lecreulx, D.; van Heijenoort, J.; Blanot, D. Over-production, purification and properties of the uridine-diphosphate-*N*-acetylmuramoyl-L-alanyl-D-glutamate: *meso*-2,6-diaminopimelate ligase from *Escherichia coli*. *Eur. J. Biochem.* **1990**, *194*, 853-861.
9. van Assche, I.; Soroka, M.; Haemers, A.; Hooper, M.; Blanot, D.; van Heijenoort, J. Synthesis and antibacterial evaluation of phosphonic acid analogues of diaminopimelic acid. *European Journal of Medicinal Chemistry* **1991**, *26*, 505-515.
10. Mengin-Lecreulx, D.; Blanot, D.; van Heijenoort, J. Replacement of diaminopimelic acid by cystathionine or lanthionine in the peptidoglycan of *Escherichia coli*. *J. Bacteriol.* **1994**, *176*, 4321-4327.
11. Rhuland, L.E. Role of α,ε-diaminopimelic acid in the cellular integrity of *Escherichia coli*. *J. Bacteriol.* **1957**, *73*, 778-783.
12. Richaud, C.; Mengin-Lecreulx, D.; Pochet, S.; Johnson, E.J.; Cohen, G.N.; Marliere, P. Directed evolution of biosynthetic pathways. Recruitment of cysteine thioethers for constructing the cell wall of *Escherichia coli*. *J. Biol. Chem.* **1993**, *268*, 26827-26835.
13. Chaloupka, J.M.; Strnadová, M.; Caslavská, J.; Veres, K. Growth and cell division of *Escherichia coli* 173-25 in the presence of some analogues of diaminopimelic acid. *Z. All. Mikrobiol.* **1974**, *14*, 283-296.
14. Arnold, L.D.; May, R.G.; Vederas, J.C. Synthesis of optically pure α-amino acids via salts of α-amino-β-propiolactone. *Journal of American Chemical Society* **1988**, *110*, 2237-2241.
15. Brown, G.B.; Du Vigneaud, V. The stereoisomeric forms of lanthionine. *J. Biol. Chem.* **1941**, *140*, 767-771.
16. Hata, Y.; Watanabe, M. Reaction of aziridinecarboxylic acids with thiols in aqueous solution. The formation of β-amino acids. *Tetrahedron* **1987**, *43*, 3881-3888.
17. Photaki, I.; Samouilidis, I.; Caranikas, S.; Zervas, L. Lanthionine chemistry. Part 4. Synthesis of diastereoisomeric cyclolanthionyl derivatives. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2599.
18. Schoberl, A.; Wagner, A. Addition of sulfhydryl carboxylic acids to unsaturated acids and a new synthesis of lanthionine. *Chem. Ber.* **1947**, *80*, 379-390.
19. Shiraiwa, T.; Nakagawa, K.; Kanemoto, N.; Kinda, T.; Yamamoto, H. Synthesis of Optically Active Homocysteine from Methionine and Its Use in Preparing Four Stereoisomers of Cystathionine. *Chem. Pharm. Bull. (Tokyo)* **2002**, *50*, 1081-1085.
20. Wakamiya, T.; Oda, Y.; Fukase, K.; Shiba, T. Syntheses of Four Stereoisomers of β-Methyllanthionine. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 536-539.
21. Harpp, D.N.; Gleason, J.G. Preparation and mass spectral properties of cystine and lanthionine derivatives. Novel synthesis of L-lanthionine by selective desulfurization. *J. Org. Chem.* **1971**, *36*, 73-80.

22. Cobb, S.L.; Vederas, J.C. A concise stereoselective synthesis of orthogonally protected lanthionine and β-methyllanthionine. *Org. Biomol. Chem.* **2007**, *5*, 1031-1038.
23. Cohen, S.B.; Halcomb, R.L. Application of Serine- and Threonine-Derived Cyclic Sulfamidates for the Preparation of *S*-Linked Glycosyl Amino Acids in Solution- and Solid-Phase Peptide Synthesis. *J. Am. Chem. Soc.* **2002**, *124*, 2534-2543.
24. White, G.J.; Garst, M.E. Cyclic sulfamate from *N*-substituted 2-amino-3-phenyl-1-propanol and its nucleophilic reactions. *J. Org. Chem.* **1991**, *56*, 3177-3178.
25. Melendez, R.E.; Lubell, W.D. Synthesis and reactivity of cyclic sulfamidites and sulfamidates. *Tetrahedron* **2003**, *59*, 2581-2616.
26. Rashid Baig, N.B.; Chandrakala, R.N.; Sudhir, V.S.; Chandrasekaran, S. Synthesis of Unnatural Selenocystines and β-Aminodiselenides via Regioselective Ring-Opening of Sulfamidates Using a Sequential, One-Pot, Multistep Strategy. *J. Org. Chem.* **2010**, *75*, 2910-2921.
27. Posakony, J.J.; Grierson, J.R.; Tewson, T.J. New Routes to *N*-Alkylated Cyclic Sulfamidates. *J. Org. Chem.* **2002**, *67*, 5164-5169.
28. Baig R. B, N.; Kanimozhi, C.K.; Sai Sudhir, V.; Chandrasekaran, S. Facile Synthesis of β-Amino Disulfides, Cystines, and Their Direct Incorporation into Peptides. *Synlett* **2009**, *2009*, 1227-1232.
29. Dondoni, A.; Perrone, D. Synthesis of 1,1-dimethylethyl (S)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate by oxidation of the alcohol. *Org. Synth.* **2000**, *77*, 64-77.
30. Boulton, L.T.; Stock, H.T.; Raphy, J.; Horwell, D.C. Generation of unnatural α,α-disubstituted amino acid derivatives from cyclic sulfamidates. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1421-1430.
31. Espino, C.G.; Wehn, P.M.; Chow, J.; Du Bois, J. Synthesis of 1,3-Difunctionalized Amine Derivatives through Selective C-H Bond Oxidation. *J. Am. Chem. Soc.* **2001**, *123*, 6935-6936.
32. Duran, F.; Leman, L.; Ghini, A.; Burton, G.; Dauban, P.; H. Dodd, R. Intramolecular PhI=O Mediated Copper-Catalyzed Aziridination of Unsaturated Sulfamates: A New Direct Access to Polysubstituted Amines from Simple Homoallylic Alcohols. *Org. Lett.* **2002**, *4*, 2481-2483.
33. Avenoza, A.; Busto, J.H.; Jiménez-Osés, G.; Peregrina, J.M. Stereoselective Synthesis of Orthogonally Protected α-Methylnorlanthionine. *Org. Lett.* **2006**, *8*, 2855-2858.
34. Sasaki, N.A.; Hashimoto, C.; Potier, P. A novel approach to the synthesis of optically pure non protein α-amino acids in both L and D configurations from L-serine. *Tetrahedron Lett.* **1987**, *28*, 6069-6072.
35. White, E.H.; McCapra, F.; Field, G.F. The Structure and Synthesis of Firefly Luciferin. *Journal of American Chemical Society* **1963**, *85*, 337-343.
36. Bergeron, R.J.; Wiegand, J.; Dionis, J.B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer, A.; Peter, H.H. Evaluation of Desferrithiocin and Its Synthetic Analogues as Orally Effective Iron Chelators. *J. Med. Chem.* **1991**, *34*, 2072-2078.
37. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.-i.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. Dicarbonates: Convenient 4-Dimethylaminopyridine Catalyzed Esterification Reagents. *Synthesis* **1994**, 1063-1066.
38. Basel, Y.; Hassner, A. Imidazole and Trifluoroethanol as Efficient and Mild Reagents for Destruction of Excess Di-tert-butyl Dicarbonate [$(BOC)_2O$]. *Synthesis* **2001**, 550-552.
39. Kim, T.-S.; Lee, Y.-J.; Jeong, B.-S.; Park, H.-g.; Jew, S.-s. Enantioselective Synthesis of (*R*)- and (*S*)-α-Alkylcysteines via Phase-Transfer Catalytic Alkylation. *J. Org. Chem.* **2006**, *71*, 8276-8278.
40. Zervas, L.; Ferderigos, N. On Lanthionine and Cyclolanthionyl. *Isr. J. Chem.* **1974**, *12*, 139-152.
41. Kenworthy, M.N.; Kilburn, J.P.; Taylor, R.J.K. Highly Functionalized Organolithium Reagents for Enantiomerically Pure α-Amino Acid Synthesis. *Org. Lett.* **2004**, *6*, 19-22.
42. McKerrow, J.; Vagg, S.; McKinney, T.; Seviour, E.M.; Maszenan, A.M.; Brooks, P.; Seviour, R.J. A simple HPLC method for analyzing diaminopimelic acid diastereomers in cell walls of Gram-positive bacteria. *Lett. Appl. Microbiol.* **2000**, *30*, 178-182.

43. Wei, L.; Lubell, W.D. Racemization in the Use of *N*-(9-(9-Phenylfluorenyl))Serine-Derived Cyclic Sulfamidates in the Synthesis of δ-Keto α-Amino Carboxylates and Prolines. *Org. Lett.* **2000**, *2*, 2595-2598.
44. Wientjes, F.B.; Pas, E.; Taschner, P.E.; Woldringh, C.L. Kinetics of uptake and incorporation of *meso*-diaminopimelic acid in different *Escherichia coli* strains. *J. Bacteriol.* **1985**, *164*, 331-337.
45. Girodeau, J.M.; Agouridas, C.; Masson, M.; Pineau, R.; Le Goffic, F. The lysine pathway as a target for a new genera of synthetic antibacterial antibiotics? *J. Med. Chem.* **1986**, *29*, 1023-1030.
46. Sundharadas, G.; Gilvarg, C. A new synthesis and some biological properties of β-hydroxy-α,ε-diaminopimelic acid. *J. Biol. Chem.* **1966**, *241*, 3276-3282.
47. Mengin-Lecreulx, D.; Falla, T.; Blanot, D.; van Heijenoort, J.; Adams, D.J.; Chopra, I. Expression of the Staphylococcus aureusUDP-*N*-Acetylmuramoyl-L-Alanyl-D-Glutamate: L-Lysine Ligase in *Escherichia coli* and Effects on Peptidoglycan Biosynthesis and Cell Growth. *J. Bacteriol.* **1999**, *181*, 5909-5914.
48. Davis, B.D. Biosynthetic interrelations of lysine, diaminopimelic acid, and threonine in mutants of *Escherichia coli*. *Nature* **1952**, *169*, 534-536.
49. Girardin, S.E.; Boneca, I.G.; Carneiro, L.A.; Antignac, A.; Jehanno, M.; Viala, J.; Tedin, K.; Taha, M.K.; Labigne, A.; Zahringer, U.*, et al.* Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **2003**, *300*, 1584-1587.

Chapter 4

Synthesis of protected α-alkyl lanthionine derivatives

(This chapter has been published in *Tetrahedron* **2014**, *70*, 4526-4533.)

4.1 Introduction

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

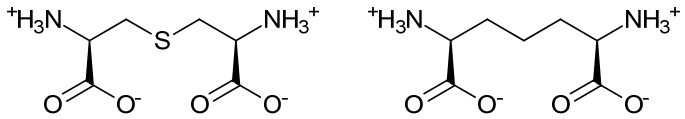


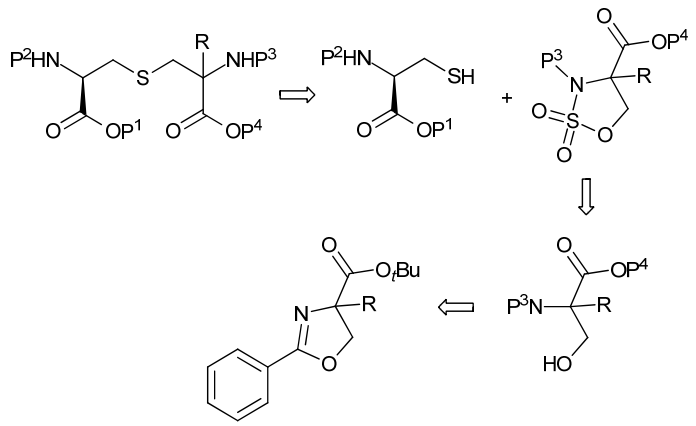
Figure 1. *meso*-Lanthionine and *meso*-diaminopimelic acid.

The aim of this work was to synthesize protected lanthionines **11a-d** (Scheme 3) bearing an α-alkyl group useful for the preparation of peptidoglycan building block analogues. Although several methods are known to produce protected lanthionines,[4-8] most are plagued with various side reactions or defects. In particular, racemization due to β-elimination[9] and subsequent Michael addition,[10-12] or regioisomerization through formation of an aziridine intermediate[13,14] have been reported. Other competing side reactions are thioester formation through O-acyl fission,[15,16] or homocoupling after disulfide exchange.[17,18] In addition, sluggish reaction[14] or lack of reactivity due to a sterically hindered α-carbon[16] have also been described. In contrast to the natural β-methyl lanthionine, the α-methyl lanthionine has been much less covered in the literature.[16] A synthetic study aiming to produce labionin, an α-alkyl lanthionine, was described but did not include the crucial thioether functionalization step.[19] Nevertheless, these interesting molecules have been used in several fields of research such as lantibiotic chemistry[4,20] and lanthionine enkephalin[16,21] analogue synthesis.

4.2 Results and discussion

4.2.1 Retrosynthetic analysis

Retrosynthesis hinted that a lanthionine could result from the S_N2 opening of a suitable cyclic sulfamidate precursor with a protected cysteine.[7,22] The sterically crowded sulfamidate can be derived from an α-alkylated serine.[22] This can be obtained from a 4-alkylated oxazoline[23] by reductive[24] opening of the ring (Scheme 1).

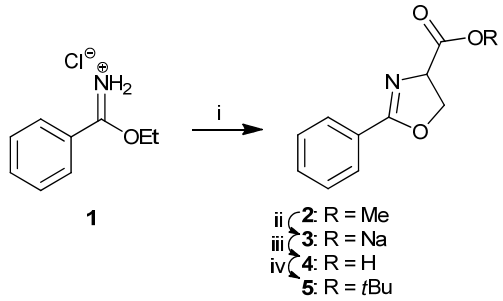


Scheme 1. Retrosynthetic approach to producing α-alkylated lanthionine derivatives. P^1= Me, P^2= Boc, P^3= Bn, P^4= *t*-Bu.

4.2.2 Preparation of precursor

An alternative route to the known[23] 4-unsubstituted phenyloxazoline precursor **5** was devised. This compound was obtained in a multigram quantity as follows: first, serine methyl ester hydrochloride[25] was reacted with ethyl benzimidate **1** (Scheme 2). The previously published procedure[26] used CH_2Cl_2 and NEt_3 which gave rise to undesired diketopiperazine by-product. In order to circumvent this problem the reaction was conducted with benzimidate freebase in MeOH. The benzimidate **1** has previously been prepared using the Pinner reaction of EtOH with benzonitrile.[27] In place of gassing the reaction mixture with HCl (g) we chose a more practical approach, involving the use of AcCl and EtOH to generate anhydrous HCl *in situ*. The methyl ester of oxazoline **2** was then hydrolyzed following the

literature method.[28] The sodium salt **3** was converted to oxazolinic acid **4** by using HCl at 0 °C according to Fry's procedure.[28] The crude acid was dried and reacted with Boc_2O and DMAP by applying the protocol reported by Takeda.[29] The poor solubility of the acid **4** was increased in a *tert*-BuOH/CH_2Cl_2 solvent mixture. Other methods such as DCC/*tert*-BuOH or *tert*-butyl trichloroacetimidate led to extensive side reactions and produced **5** in very low yields. Finally, after five steps from serine and a recrystallization in hexanes **5** was obtained on a multigram scale with a global yield of 50% (Scheme 2).



Scheme 2. Synthesis of the oxazoline precursor. *Reagents and conditions*: i) 1) Na_2CO_3, CH_2Cl_2, 2) Ser Me ester*HCl, MeOH, reflux, 2 h, 84%; ii) aq. NaOH, MeOH, acetone, rt, 1 h, 92%; iii) aq. HCl, 0 °C, 1 h, 87%; iv) Boc_2O (2 eq.), DMAP (10 mol%), 1:1 CH_2Cl_2/ *tert*-BuOH, reflux, 1 h, 75%.

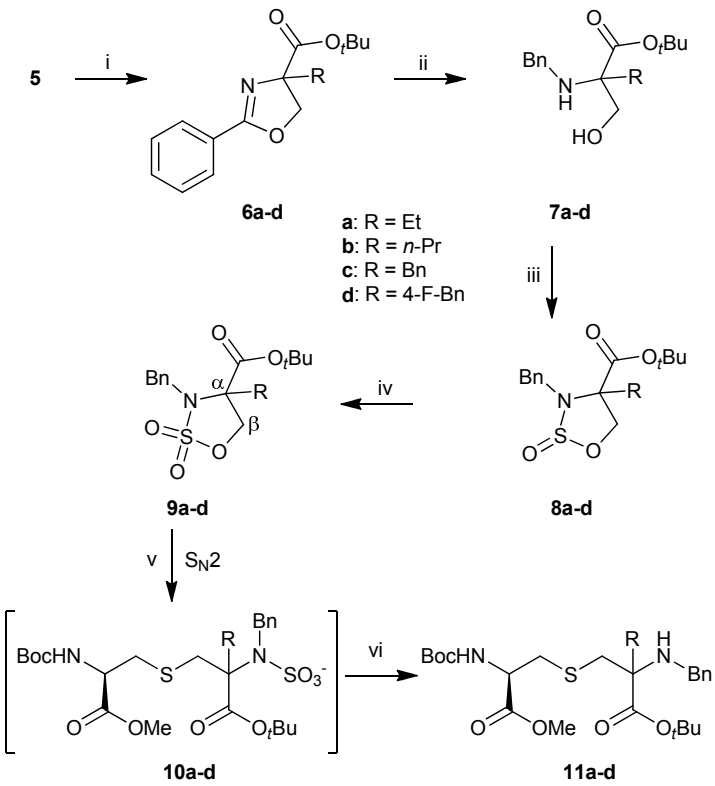
4.2.3 *N*-Benzyl lanthionines synthesis from 5

Using the oxazoline precursor **5**, various racemic 4-alkylated oxazolines **6a-d** were synthesized[23,30] (Scheme 3). In our first approach, tetrabutylammonium bromide (TBAB) was employed as an achiral phase transfer catalyst (PTC) along with solid KOH as a base.[30] Four electrophiles were used: two alkyl bromides (EtBr, *n*-PrBr) and two benzyl bromides (BnBr, 4-F-BnBr). Good yields (79 - 90%) of the racemic alkylated oxazolines **6a-d** (Scheme 3: step i) were obtained.

Next, the reductive[24] opening of the racemic oxazolines was performed. Compounds **6a-d** were protonated in acetic acid and reduced at room temperature with $NaBH_3CN$. Under these mild reaction conditions the cleavage of both the *tert*-butyl ester and the *N*-benzyl groups could be avoided, which is a significant advantage over the harsh hydrolysis conditions (6 M HCl) reported in the

literature.[23] Using this method four suitably protected racemic alkylated serines **7a-d** were obtained in near quantitative yields (94 - 98%, Scheme 3: step ii). Transformation of the *N*-benzyl protected serines **7a-d** into their respective cyclic sulfamidates[22] was then performed in two steps (Scheme 3: steps iii-iv). Amino alcohols **7a-d** were reacted with thionyl chloride and imidazole[31] to create the cyclic sulfamidites **8a-d**. These were isolated as a mixture of diastereomers in 92 - 99% yield (step iii). The crude products were then oxidized[22] by catalytic $RuCl_3.xH_2O$ and $NaIO_4$ to give the corresponding sulfamidates **9a-d** with good to excellent yields (77 - 99%, step iv).

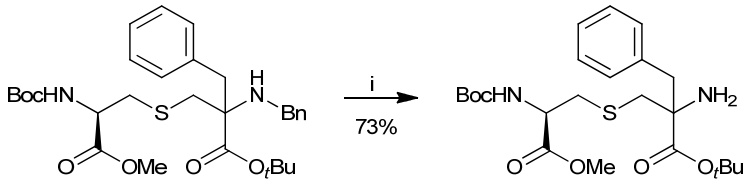
Subsequently, the sulfamidates **9a-d** were used in a coupling reaction[22] with the methyl ester of Boc-L-cysteine in CH_3CN and DBU as a base (Scheme 3: steps v). The *N*-sulfamate intermediates **10a-d** were subsequently hydrolyzed[32] at 50 °C for 2 h in aqueous 10% NaH_2PO_4 (Scheme 3: steps vi). Interestingly, the cleavage of the sulfamate group failed when the hydrolysis was carried out at room temperature.[32] It is noteworthy that despite[16] the presence of a sterically hindered α-carbon in compounds **9a-d,** the S_N2 reaction at C_β occurred remarkably well, providing protected lanthionines **11a-d** in excellent yields (90 - 99%). The outstanding reactivity[22,33] of cyclic sulfamidates with thiolate nucleophiles in basic conditions is hereby confirmed. It should be noted that an alternative mechanism based on SO_2 extrusion followed by aziridine formation was observed under neutral conditions for isomeric sulfamidates.[34] However, under our basic conditions, the formation of *N*-sulfamate products **10a-d** suggests the probable mechanism to be S_N2.



Scheme 3. Synthesis of α-alkylated lanthionines. *Reagents and conditions*: i) RX, KOH (5 eq.), TBAB (10 mol%), 1:1 toluene/CH_2Cl_2; ii) $NaBH_3CN$ (6 eq.), AcOH, rt, 16 h; iii) $SOCl_2$, imidazole, NEt_3, -10 °C → rt, 2 h; iv) $NaIO_4$ (1.2 eq.), $RuCl_3$ (1 mol%), aq. CH_3CN, rt, 4 h; v) Boc L-cysteine Me ester (1.1 eq.), DBU (1.1 eq.), CH_3CN, rt, 1 h; vi) 10% aq. NaH_2PO_4, EtOAc, 50 °C, 2 h.

4.2.4 Debenzylation of **11c**

It is known that the deprotection of a *N*-benzyl can be challenging in the presence of a sulfide group.[35] As an example, the debenzylation of the nitrogen atom of lanthionine **11c** (Scheme 4) was explored. Indeed, our initial attempts to deprotect the *N*-benzyl group of the chromatographically purified α-benzyl lanthionine **11c** by hydrogenation with Pd/C as a catalyst were unsuccessful, despite testing several reaction conditions. Furthermore, partially irreproducible *N*-debenzylation was observed when $Pd(OH)_2/C$ (Pearlman's catalyst) was substituted for Pd/C.[35] Fortunately, the use of the more active Pd black in acidic aqueous MeOH at 70 °C[36] (9/1/0.3: MeOH/H_2O/AcOH) gave the *N*-debenzylated protected α-benzyl lanthionine **12** in a good and reproducible yield (Scheme 4).



11c 12

Scheme 4. Hydrogenation of the *N*-benzyl group of **11c**. *Reagents and conditions*: i) H_2 (7 bars), Pd black (25% w/w), 9/1/0.3: MeOH/H_2O/AcOH, 70 °C, 16 h.

4.2.5 Enantioselective alkylation of **5**

By using an optically pure PTC developed by the Maruoka's group (Figure 2) compounds **6a-d** were obtained enantioselectively by Jew.[23] This approach was also used for the synthesis of the (*R*)-4-Bn analogue **6c** (entry 1, Table 1). In order to reduce the amount of the electrophilic agent required, the synthesis of which can be difficult, various reaction conditions were investigated. It was found that enantiomeric purity decreased with a smaller excess of electrophile (entries 1-2, Table 1). In contrast, higher catalyst loading (entries 2-4), or a decrease in temperature (entries 4-7), increased the enantiomeric excess (ee). At a lower temperature (-10 °C) and at a high catalyst loading (10 mol%) we obtained an excellent ee (97%) by using only 1.5 molar equivalent of the electrophile (entry 7). The high price of the catalyst and high loading was a drawback of this alkylation approach.

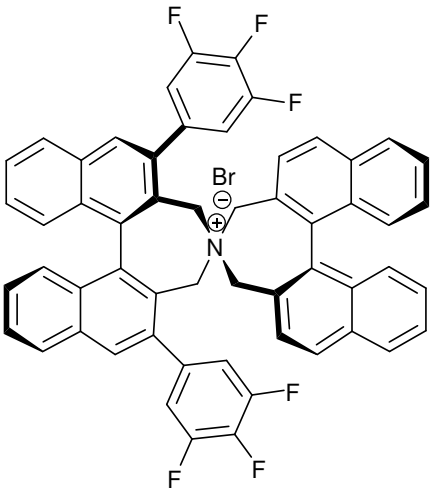


Figure 2. Maruoka's chiral PTC (*R*,*R*)-3,4,5-Trifluorophenyl-NAS bromide used for the alkylation of **5** in Jew's paper[23] and in Table 1 of this work.

Table 1. Enantioselective alkylation of 5 with BnBr to (*R*)-6c[a]

Entry	BnBr (Eq.)	PTC (mol%)	T (°C)	Ee (%)	Yield (%)
1	5	2.5	-5	97	85
2	3	2.5	-5	77	75
3	3	5	-5	85	77
4	1.5	10	5	90	82
5	1.5	10	0	92	81
6	1.5	10	-5	94	80
7	1.5	10	-10	97	84

[a] Reaction conditions: **5**, toluene, BnBr, Maruoka's chiral PTC, KOH (5 eq.), 1200 rpm stir under N_2 at the chosen temperature until completion (HPLC).

4.3 Conclusion

In this paper, a straightforward synthesis of various protected α-alkyl lanthionines **11a-d** was developed. This synthetic scheme started with the disclosure of a multigram preparation of the known oxazoline precursor **5**. Alkylation of **5** gave the compounds **6a-d** in good yields (79 - 90%) by using TBAB as an achiral PTC. A smooth reductive cleavage provided protected serines **7a-d** in excellent yields (94 - 98%). Cyclization with $SOCl_2$ and oxidation gave sulfamidates **9a-d** in 75 - 92% yields in two steps. An efficient S_N2 reaction on the cyclic sulfamidates was highly successful, despite the sterically crowded α-carbon, in producing protected lanthionines **11a-d** in excellent yields (90 - 99%). The challenging debenzylation of **11c** was overcome by means of Pd black in warm acidic aqueous MeOH. Finally, for the enantioselective alkylation of **5**, an enantiomeric excess of 97% was obtained with fewer equivalents (1.5 vs 5) of the electrophilic agent. These debenzylated products are excellent starting blocks for peptide synthesis.

4.4 Experimental section

4.4.1 Reagents

All solvents and chemicals were of analytical grade and used without further purification. CH_3CN was dried on 3 Å molecular sieves. TLC: Macherey-Nagel Polygram SIL G/UV_{254} using UV light or a stain anisaldehyde/sulphuric acid/AcOH/EtOH: 1/1/0.04/18:v/v/v/v. CC: silica gel Acros, 0.060-0.200 mm, 60 Å. HPLC: Waters system (600 pump, 717 autosampler, 996 PDA detector) with a column XTerra RP18 (4.6 x 150 mm ; 3.5 µm). Chiral HPLC: Chiracel OD-H column (Daicel, 150 mm x 4 mm, 5 µm); mobile phase: *n*-Hex/*i*-PrOH:98/2; 0.8 mL/min; 37 °C. Mp: Büchi Melting Point B-545 calibrated on three points (83, 136 and 237 °C). ^1H and ^{13}C NMR: Bruker Avance DRX 400 (^1H at 400 MHz and ^{13}C at 101 MHz) and Bruker AM 250 (^1H 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).

4.4.2 Ethyl benzimidate 1

AcCl (75 mL, 1.05 mol) was added dropwise over 30 min at 0 °C to a solution of dry EtOH (124 mL, 2.1 mol) in dry Et_2O (200 mL) under nitrogen and protected from moisture. The mixture was left to stir for 15 min and benzonitrile (103 g, 1 mol) was added. The solution was poured into a brown glass reagent bottle which was closed and left at 2 °C for one month. The bottle was shattered and the crystals were suspended in Et_2O and decanted from glass fragments. The product was filtered and dried *in vacuo* to constant weight to give the *title compound* (101 g, 54%) as colorless crystals. Mp 123-124 °C (lit.[37] 122-123 °C); ^{1}H NMR (250 MHz, MeOD) δ_H 8.13–8.03 (m, 2H, Ar-H), 7.84–7.76 (m, 1H, Ar-H), 7.71–7.58 (m, 2H, Ar-H), 4.69 (q, J = 7.0 Hz, 2H, OCH_2), 1.62 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, MeOD) δ_C 174.24 (CN), 136.82 (Ar-C), 130.50 (Ar-C), 130.07 (Ar-C), 127.13 (Ar-C), 71.40 (CH_2), 13.91 (CH_3); *m/z* (ESI) 150 $[MH]^+$.

4.4.3 Methyl 2-phenyloxazoline-4-carboxylate 2

The protocol of Huang[26] was modified as follows: The freebase of ethyl benzimidate **1** (37.25 g, 250 mmol), generated from the salt by basification with saturated aqueous Na_2CO_3 and extraction in CH_2Cl_2, was dissolved in 250 mL of MeOH. To this solution serine methyl ester hydrochloride (39 g, 250 mmol) was added and this was heated under reflux for 2 h. The mixture was cooled and diluted with acetone (250 mL). NH_4Cl was filtered and washed with acetone. The solvents were evaporated, the residue was dissolved in CH_2Cl_2 (50 mL) and Et_2O (200 mL), and filtered again. The organic layer was washed twice with water, with brine and dried over MgSO4. After filtration and removal of the solvents *in vacuo*, the *title compound* (43 g, 84%) was obtained as a pale pink oil. NMR data were in agreement with the literature;[38] *m/z* (ESI) 206 $[MH]^+$.

4.4.4 Sodium 2-phenyloxazoline-4-carboxylate 3

The synthesis was done by following the protocol described by Fry.[28] A cold solution of NaOH (32 g, 800 mmol) in water (200 mL) was added to the methyl ester **2** (152 g, 741 mmol). The mixture was stirred vigorously with intermittent cooling in an ice bath for 20 min. The suspension was diluted with MeOH (200 mL) and left to stir for 1 h. The suspension was diluted with acetone (2 L) and left at 2 °C for 2 h. The product was filtered on Büchner and washed with acetone (1 L) then dried *in vacuo* to constant weight. The *title compound* (169 g, 92%) was obtained as pink crystals. ^{1}H NMR (250 MHz, MeOD) δ_H 8.04-7.90 (m, 2H, Ar-H), 7.58–7.36 (m, 3H, Ar-H), 4.76 (dd, J = 10.4, 8.6 Hz, 1H, H-α),

4.65 (dd, J = 10.4, 7.9 Hz, 1H, CH_2), 4.53 (dd, J = 8.6, 7.9 Hz, 1H, CH_2); ^{13}C NMR (63 MHz, MeOD) δ_C 178.83 (CO), 166.68 (CN), 132.73 (Ar-C), 129.43 (Ar-C), 129.41 (Ar-C), 128.71 (Ar-C), 72.54 (C-β), 71.94 (C-α); *m/z* (ESI) 190 $[M]^-$.

4.4.5 2-Phenyloxazoline-4-carboxylic acid 4

The synthesis was done by following the protocol described by Fry.[28] The sodium salt **3** (169 g, 679 mmol) was suspended in water (1.2 L) and cooled to 0 °C. After cooling to 0 °C, 3 N aqueous HCl solution (220 mL), was added dropwise until pH < 3 to precipitate the acid. The product was filtered on Büchner and washed with cold water (500 mL) then dried by repeated suspension in CH_3CN and evaporation *in vacuo* to constant weight. The *title compound* (112.8 g, 87%) was obtained as pink crystals that were pure enough for the next step. Mp 156-157 °C; ^1H NMR (250 MHz, DMSO) δ_H 7.95 – 7.82 (m, 2H, Ar-H), 7.58 - 7.42 (m, 3H, Ar-H), 4.88 (t, J = 9.1 Hz, 1H, H-α), 4.57 (d, J = 9.1 Hz, 2H, CH_2); ^{13}C NMR (63 MHz, DMSO) δ_C 172.61 (CO), 164.26 (CN), 131.86 (Ar-C), 128.69 (Ar-C), 128.05 (Ar-C), 127.00 (Ar-C), 69.92 (C-β), 68.60 (C-α); *m/z* (ESI) 190 $[M]^-$.

4.4.6 *tert*-Butyl 2-phenyloxazoline-4-carboxylate 5

The esterification was done as proposed by Takeda[29] with special emphasis on the poor solubility of the acid **4**. Compound **4** (19.1 g, 100 mmol), DMAP (2.44 g, 20 mol%), CH_2Cl_2 (125 mL) and *t*-BuOH (125 mL) were placed under nitrogen in a 500 mL round bottom flask equipped with an efficient condenser. The solution was heated at reflux and Boc_2O (43.6 g, 200 mmol) was added *via* syringe in two portions. **CAUTION**: This step produces substantial gas evolution. The mixture was then heated for 1 h and the solvents were evaporated. The yellow oil was dissolved in CH_2Cl_2 (200 mL) and then filtered through 40 g of silica. The solvent was removed *in vacuo*, the oil was dissolved in 1:9 (Et_2O/hexanes) and filtered through 40 g of silica then eluted with the same mixture (500 mL). The yellow oil (25 g) that remained after evaporation of the solvents was crystallized from hexanes to give the *title compound* (18.5 g, 75%) as off-white crystals. Mp 42-44 °C (lit.[30] 42-45 °C); Anal (C,H,N) $C_{14}H_{17}NO_3$; NMR data were in agreement with the literature;[30] *m/z* (ESI) 248 $[MH]^+$.

4.4.7 General method for the preparation of 4-alkyl oxazolines 6a-d

The protocol of Jew[23] was modified as follows: Phenyloxazoline **5** (4.94 g, 20 mmol) was dissolved in toluene (10 mL) and CH_2Cl_2 (10 mL) and the alkyl bromide and TBAB (644 mg, 2 mmol, 10 mol%) were added. The flask was cooled to 0 °C and flushed with nitrogen then finely powdered KOH (6.6 g, 85%, 100 mmol) was added. The flask was capped and the mixture was vigorously stirred at rt until the end of the reaction, which was monitored by TLC (1:4 EtOAc/hexanes, anisaldehyde stain). The suspension was diluted with CH_2Cl_2 (50 mL) and then filtered. The organic layer was washed with water and the solvent was evaporated. The residue was flash chromatographied on 40 g of silica first with hexanes to remove excess electrophile then with 1:4 (EtOAc/hexanes) and the solvent was removed *in vacuo* to give the product.

4.4.7.1 *tert-Butyl 4-ethyl-2-phenyloxazoline-4-carboxylate* **6a** *(rac)*

Following the general method for the preparation of 4-alkyl oxazolines and using EtBr (15 mL, 200 mmol) as an electrophile, the *title compound* was obtained as a pale yellow oil (4.7 g, 85%). NMR data were in agreement with the literature;[30] *m/z* (ESI) 276 $[MH]^+$.

4.4.7.2 *tert-Butyl 2-phenyl-4-propyloxazoline-4-carboxylate* **6b** *(rac)*

Following the general method for the preparation of 4-alkyl oxazolines and using *n*-PrBr (18 mL, 200 mmol) as an electrophile, the *title compound* was obtained as a pale yellow oil (4.7 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.20–7.75 (m, 2H, Ar-H), 7.64–7.28 (m, 3H, Ar-H), 4.70 (d, J = 8.8 Hz, 1H, CH_2O), 4.21 (d, J = 8.8 Hz, 1H, CH_2O), 2.03–1.77 (m, 2H, CH_2), 1.49 (s, 9H, $C(CH_3)_3$), 1.42–1.22 (m, 2H, CH_2CH_3), 0.95 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 172.17 (CO), 164.12 (CN), 131.59 (Ar-C), 128.66 (Ar-C), 128.32 (Ar-C), 127.61 (Ar-C), 81.84 ($C(CH_3)_3$), 78.64 (C-α), 73.89 (CH_2O), 40.45 (CH_2), 28.09 ($C(CH_3)_3$), 17.22 (CH_2CH_3), 14.36 (CH_3); *m/z* (ESI) 290 $[MH]^+$.

4.4.7.3 *tert-Butyl 4-benzyl-2-phenyloxazoline-4-carboxylate* **6c** *(rac)*

Following the general method for the preparation of 4-alkyl oxazolines and using BnBr (7.1 mL, 60 mmol) as an electrophile, the *title compound* was obtained as a pale yellow solid (6.1 g, 90%). This can be recrystallized from 1:4 (Et_2O/hexanes) to give a colorless solid (4.9 g, 73%). Mp 68-69 °C; NMR data were in agreement with the literature;[30] *m/z* (ESI) 338 $[MH]^+$.

4.4.7.4 *tert-Butyl 4-(4-fluorobenzyl)-2-phenyloxazoline-4-carboxylate* **6d** *(rac)*

Following the general method for the preparation of 4-alkyl oxazolines and using 4-F-BnBr (7.5 mL, 60 mmol) as an electrophile, the *title compound* was obtained as a pale yellow solid (5.6 g, 79%). This can be recrystallized from 1:4 (Et_2O/hexanes) to give a colorless solid (4.1 g, 58%). Mp 69-71 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.09–7.97 (m, 2H, Ar-H), 7.64–7.40 (m, 3H, Ar-H), 7.40–7.24 (m, 2H, Ar-H), 7.08–6.94 (m, 2H, Ar-H), 4.74 (d, J = 8.9 Hz, 1H, CH_2O), 4.36 (d, J = 8.9 Hz, 1H, CH_2O), 3.34 (d, J = 13.9 Hz, 1H, CH_2Ar), 3.27 (d, J = 13.9 Hz, 1H, CH_2Ar), 1.56 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.46 (CO), 164.79 (CN), 162.03 (d, $^1J_{C\text{-}F}$ = 245.1 Hz, Ar-C), 132.01 (d, $^3J_{C\text{-}F}$ = 7.9 Hz, Ar-C), 131.70 (Ar-C), 131.52 (d, $^4J_{C\text{-}F}$ = 3.3 Hz, Ar-C), 128.60 (Ar-C), 128.34 (Ar-C), 127.38 (Ar-C), 115.04 (d, $^2J_{C\text{-}F}$ = 21.1 Hz, Ar-C), 82.33 ($C(CH_3)_3$), 78.88 (d, $^6J_{C\text{-}F}$ = 1.2 Hz, C-α), 73.08 (CH_2O), 42.50 (CH_2Ar), 28.04 ($C(CH_3)_3$); *m/z* (ESI) 356 $[MH]^+$.

4.4.8 General method for the reduction of oxazolines **7a-d**

The protocol of Reddy[24] was modified as follows: A freshly prepared solution of $NaBH_3CN$ (3.8 g, 60 mmol) in AcOH (60 mL), cooled to 0 °C, was added to the oxazoline **6a-d** (10 mmol) and the mixture was stirred at rt for 16 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc and an excess of a saturated Na_2CO_3 solution. The pH was adjusted to >12 with 10% NaOH and the organic layer was decanted. The basic aqueous layer was extracted two more times with EtOAc and the pooled fractions were washed twice with brine. The solution was dried over $MgSO_4$, was filtered and the solvent was removed *in vacuo* to give the product.

4.4.8.1 *tert-Butyl 2-(benzylamino)-2-(hydroxymethyl)butanoate* **7a** *(rac)*

Following the general method for the reduction of oxazolines and using **6a** (2.75 g, 10 mmol), the *title compound* was obtained as a colorless solid (2.66 g, 95%). Mp 52-53 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.48 – 7.05 (m, 5H, Ar-H), 3.73 (d, J = 11.0 Hz, 1H, CH_2O), 3.65 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.59 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.58 (d, J = 11.0 Hz, 1H, CH_2O), 2.30 (b, 2H, NH, OH), 1.75 – 1.56 (m, 2H, CH_2), 1.49 (s, 9H, $C(CH_3)_3$), 0.88 (t, J = 7.5 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.53 (CO), 140.05 (Ar-C), 128.66 (Ar-C), 128.31 (Ar-C), 127.35 (Ar-C), 81.78 ($C(CH_3)_3$), 66.70 (C-α), 61.85 (CH_2O), 46.90 (CH_2Ph), 28.21 ($C(CH_3)_3$), 26.38 (CH_2), 7.97 (CH_3); *m/z* (ESI) 280 $[MH]^+$.

4.4.8.2 *tert-Butyl 2-(benzylamino)-2-(hydroxymethyl)pentanoate* ***7b*** *(rac)*

Following the general method for the reduction of oxazolines and using **6b** (2.89 g, 10 mmol), the *title compound* was obtained as a colorless solid (2.76 g, 94%). Mp 44-46 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37 – 7.29 (m, 4H, Ar-H), 7.29 – 7.20 (m, 1H, Ar-H), 3.73 (d, J = 11.0 Hz, 1H, CH_2O), 3.65 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.60 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.57 (d, J = 11.0 Hz, 1H, CH_2O), 2.31 (br, 2H, NH, OH), 1.70 – 1.51 (m, 2H, CH_2), 1.49 (s, 9H, $C(CH_3)_3$), 1.35 – 1.23 (m, 2H, CH_2CH_3), 0.92 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.60 (CO), 140.01 (Ar-C), 128.63 (Ar-C), 128.29 (Ar-C), 127.32 (Ar-C), 81.76 ($C(CH_3)_3$), 66.32 (C-α), 62.26 (CH_2O), 46.93 (CH_2Ph), 35.92 (CH_2), 28.20 ($C(CH_3)_3$), 16.98 (CH_2CH_3), 14.51 (CH_3); *m/z* (ESI) 294 $[MH]^+$.

4.4.8.3 *tert-Butyl 2-benzyl-2-(benzylamino)-3-hydroxypropanoate* ***7c*** *(rac)*

Following the general method for the reduction of oxazolines and using **6c** (3.37 g, 10 mmol), the *title compound* was obtained as a colorless solid (3.31 g, 97%). Mp 69-70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 – 7.25 (m, 10H, Ar-H), 3.87 (d, J = 11.4 Hz, 1H, CH_2O), 3.86 (d, J = 12.0 Hz, 1H, CH_2N), 3.80 (d, J = 12.0 Hz, 1H, CH_2N), 3.74 (d, J = 11.4 Hz, 1H, CH_2O), 3.15 (d, J = 13.4 Hz, 1H, CH_2Ph), 3.04 (d, J = 13.4 Hz, 1H, CH_2Ph), 2.45 (br, 2H, NH, OH), 1.54 (s, 9H, $C(CH_3)_3$).; ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.60 (CO), 140.01 (Ar-C), 128.63 (Ar-C), 128.29 (Ar-C), 127.32 (Ar-C), 81.76 ($C(CH_3)_3$), 66.32 (C-α), 62.26 (CH_2O), 46.93 (CH_2Ph), 35.92 (CH_2), 28.20 ($C(CH_3)_3$), 16.98 (CH_2CH_3), 14.51 (CH_3); *m/z* (ESI) 342 $[MH]^+$.

4.4.8.4 *tert-Butyl 2-(benzylamino)-2-(4-fluorobenzyl)-3-hydroxy propanoate* ***7d*** *(rac)*

Following the general method for the reduction of oxazolines and using **6d** (3.55 g, 10 mmol), the *title compound* was obtained as a colorless solid (3.52 g, 98%). Mp 67-69 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38–7.29 (m, 4H, Ar-H), 7.29–7.23 (m, 1H, Ar-H), 7.21–7.15 (m, 2H, Ar-H), 6.99–6.91 (m, 2H, Ar-H), 3.71 (d, J = 11.5 Hz, 1H, CH_2O), 3.71 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.66 (d, J = 12.0 Hz, 1H, CH_2Ph), 3.57 (d, J = 11.5 Hz, 1H, CH_2O), 2.99 (d, J = 13.5 Hz, 1H, CH_2Ar), 2.87 (d, J = 13.5 Hz, 1H, CH_2Ar), 1.42 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 172.78 (CO), 162.09 (d, $^{1}J_{C\text{-}F}$ = 245.2 Hz, Ar-C), 139.72 (Ar-C), 131.98 (d, $^{3}J_{C\text{-}F}$ = 7.9 Hz, Ar-C), 131.39 (d, $^{4}J_{C\text{-}F}$ = 3.3 Hz, Ar-C), 128.79 (Ar-C), 128.28 (Ar-C), 127.54 (Ar-C), 115.15 (d, $^{2}J_{C\text{-}F}$ = 21.2 Hz, Ar-C), 82.43 ($C(CH_3)_3$), 67.16 (d,

$^{6}J_{C\text{-}F}$ = 1.2 Hz, C-α), 60.67 (CH_2O), 47.59 (CH_2Ph), 39.55 (CH_2Ar), 28.27 ($C(CH_3)_3$); *m/z* (ESI) 360 $[MH]^+$.

4.4.9 General method for the formation of sulfamidites 8a-d

The protocol of Dolence[31] was modified as follows: In a dry round bottom flask under nitrogen *N*-benzyl serine **7a-d** (5 mmol), imidazole (1.36 g, 20 mmol), NEt_3 (2.1 mL, 15 mmol) and CH_2Cl_2 (25 mL) were added. The mixture was cooled to -10 °C and then with stirring $SOCl_2$ (0.54 mL, 7.5 mmol) was added dropwise *via* syringe. The solution was kept for 30 min at -10 °C then allowed to return to rt during 2 h. The mixture was diluted with water (10 mL) and then with 10% aqueous $NaHSO_4$ (30 mL). The organic layer was separated and the aqueous layer was extracted once more with CH_2Cl_2. The pooled fractions were washed four times with water and azeotropically dried by evaporation with CH_3CN *in vacuo* to give the product.

4.4.9.1 *tert-Butyl 3-benzyl-4-ethyl-2-oxo-1,2,3-oxathiazolidine-4-carboxylate 8a*

Following the general method for the formation of sulfamidites and using **7a** (1.40 g, 5 mmol), the *title compound* was obtained as a yellow oil (1.49 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.47–7.26 (m, 5H, Ar-H), 5.35 (d, *J* = 8.3 Hz, 0.5H, CH_2O), 5.01 (d, *J* = 9.0 Hz, 0.5H, CH_2O), 4.71 (d, *J* = 9.0 Hz, 0.5H, CH_2O), 4.61 (d, *J* = 14.2 Hz, 0.5H, CH_2Ph), 4.47 (d, *J* = 14.9 Hz, 0.5H, CH_2Ph), 4.41 (d, *J* = 14.9 Hz, 0.5H, CH_2Ph), 4.26 (d, *J* = 14.2 Hz, 0.5H, CH_2Ph), 4.14 (d, *J* = 8.3 Hz, 0.5H, CH_2O), 2.19 – 2.01 (m, 1H, CH_2), 1.92 – 1.81 (m, 0.5H, CH_2), 1.78 – 1.63 (m, 0.5H, CH_2), 1.53 (s, 4.5H, $C(CH_3)_3$), 1.49 (s, 4.5H, $C(CH_3)_3$), 0.93 (t, *J* = 7.5 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.05 (0.5C, CO), 169.98 (0.5C, CO), 137.22 (0.5C, Ar-C), 137.11 (0.5C, Ar-C), 128.92 (Ar-C), 128.83 (Ar-C), 128.72 (Ar-C), 128.66 (Ar-C), 127.97 (Ar-C), 83.18 (0.5C, $C(CH_3)_3$), 82.88 (0.5C, $C(CH_3)_3$), 77.81 (0.5C, CH_2O), 75.60 (0.5C, CH_2O), 71.55 (0.5C, C-α), 69.51 (0.5C, C-α), 46.26 (0.5C, CH_2Ph), 46.20 (0.5C, CH_2Ph), 28.04 (1.5C, $C(CH_3)_3$), 28.00 (1.5C, $C(CH_3)_3$), 27.51 (0.5C, CH_2), 27.07 (0.5C, CH_2), 9.28 (0.5C, CH_3), 8.51 (0.5C, CH_3).

4.4.9.2 *tert-Butyl 3-benzyl-2-oxo-4-propyl-1,2,3-oxathiazolidine-4-carboxylate 8b*

Following the general method for the formation of sulfamidites and using **7b** (1.46 g, 5 mmol), the *title compound* was obtained as a yellow oil (1.59 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.50–7.27 (m, 5H, Ar-H), 5.34 (d, *J* = 8.2 Hz, 0.5H, CH_2O), 5.01 (d, *J* = 9.0 Hz,

0.5H, CH_2O), 4.71 (d, *J* = 9.0 Hz, 0.5H, CH_2O), 4.61 (d, *J* = 14.2 Hz, 0.5H, CH_2Ph), 4.47 (d, *J* = 15.0 Hz, 0.5H, CH_2Ph), 4.42 (d, *J* = 15.0 Hz, 0.5H, CH_2Ph), 4.26 (d, *J* = 14.2 Hz, 0.5H, CH_2Ph), 4.12 (d, *J* = 8.2 Hz, 0.5H, CH_2O), 2.14–1.96 (m, 1H, CH_2), 1.82–1.70 (m, 0.5H, CH_2), 1.67–1.58 (m, 0.5H, CH_2), 1.52 (s, 4.5H, $C(CH_3)_3$), 1.48 (s, 4.5H, $C(CH_3)_3$), 1.36–1.23 (m, 2H, CH_2CH_3), 0.94 (t, *J* = 7.3 Hz, 1.5H, CH_3), 0.88 (t, *J* = 7.3 Hz, 1.5H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.12 (0.5C, CO), 170.01 (0.5C, CO), 137.20 (0.5C, Ar-C), 137.07 (0.5C, Ar-C), 128.92 (Ar-C), 128.83 (Ar-C), 128.71 (Ar-C), 128.65 (Ar-C), 127.97 (Ar-C), 83.15 (0.5C, $C(CH_3)_3$), 82.85 (0.5C, $C(CH_3)_3$), 78.08 (0.5C, CH_2O), 75.73 (0.5C, CH_2O), 71.01 (0.5C, C-α), 68.96 (0.5C, C-α), 46.31 (0.5C, CH_2Ph), 46.21 (0.5C, CH_2Ph), 36.75 (0.5C, CH_2), 36.20 (0.5C, CH_2), 28.04 (1.5C, $C(CH_3)_3$), 27.98 (1.5C, $C(CH_3)_3$), 18.40 (0.5C, CH_2CH_3), 17.67 (0.5C, CH_2CH_3), 14.47 (0.5C, CH_3), 14.42 (0.5C, CH_3).

4.4.9.3 *tert-Butyl 3,4-dibenzyl-2-oxo-1,2,3-oxathiazolidine-4-carboxylate* **8c**

Following the general method for the formation of sulfamidites and using **7c** (1.71 g, 5 mmol), the *title compound* was obtained as a yellow oil (1.94 g, 99%). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.57 – 7.11 (m, 10H, Ar-H), 5.10 (d, *J* = 8.5 Hz, 0.5H, CH_2O), 4.90 (d, *J* = 9.2 Hz, 0.5H, CH_2O), 4.83 (d, *J* = 9.2 Hz, 0.5H, CH_2O), 4.75 (d, *J* = 14.2 Hz, 0.5H, CH_2N), 4.63 (d, *J* = 14.9 Hz, 0.5H, CH_2N), 4.52 (d, *J* = 14.9 Hz, 0.5H, CH_2N), 4.44 (d, *J* = 14.2 Hz, 0.5H, CH_2N), 4.34 (d, *J* = 8.5 Hz, 0.5H, CH_2O), 3.61 (d, *J* = 13.3 Hz, 0.5H, CH_2Ph), 3.47 (d, *J* = 13.3 Hz, 0.5H, CH_2Ph), 3.15 (d, *J* = 13.3 Hz, 0.5H, CH_2Ph), 2.87 (d, *J* = 13.3 Hz, 0.5H, CH_2Ph), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.80 (0.5C, CO), 168.74 (0.5C, CO), 136.97 (0.5C, Ar-C), 136.86 (0.5C, Ar-C), 134.97 (0.5C, Ar-C), 134.21 (0.5C, Ar-C), 129.84 (Ar-C), 129.71 (Ar-C), 128.88 (Ar-C), 128.83 (Ar-C), 128.67 (Ar-C), 127.98 (0.5C, Ar-C), 127.95 (0.5C, Ar-C), 127.63 (0.5C, Ar-C), 127.49 (0.5C, Ar-C), 83.42 (0.5C, $C(CH_3)_3$), 83.22 (0.5C, $C(CH_3)_3$), 77.91 (0.5C, CH_2O), 76.04 (0.5C, CH_2O), 70.73 (0.5C, C-α), 69.36 (0.5C, C-α), 46.27 (0.5C, CH_2N), 46.21 (0.5C, CH_2N), 40.84 (CH_2Ph), 27.90 (1.5C, $C(CH_3)_3$), 27.78 (1.5C, $C(CH_3)_3$).

4.4.9.4 *tert-Butyl 3-benzyl-4-(4-fluorobenzyl)-2-oxo-1,2,3-oxathiazolidine-4-carboxylate 8d*

Following the general method for the formation of sulfamidites and using **7d** (1.80 g, 5 mmol), the *title compound* was obtained as a yellow oil (1.99 g, 98%) that was used 'as is' for the next step.

4.4.10 General method for the formation of sulfamidates 9a-d

The protocol of Boulton[22] was modified as follows: The crude sulfamidite **8a-d** (ca. 5 mmol) was dissolved in CH_3CN (50 mL) and the solution was cooled to 0 °C. Next, $RuCl_3.xH_2O$ (11 mg, 1 mol%) was added, followed by $NaIO_4$ (1.2 g, 5.6 mmol) and water (25 mL). The greenish-brown solution with a white precipitate was stirred for 15 min at 0 °C and was allowed to return to rt. After 4 h the mixture was diluted with Et_2O (50 mL) and brine (50 mL). The aqueous layer was extracted with Et_2O (3 x 50 mL) and the pooled fractions were washed twice with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo* to yield the crude product. This was flash chromatographied on silica with 1:4 (EtOAc/hexanes) and the solvents were removed *in vacuo* to give the pure compound.

4.4.10.1 *tert-Butyl 3-benzyl-4-ethyl-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 9a (rac)*

Following the general method for the formation of sulfamidates and using **8a** (1.41 g, 4.3 mmol), the *title compound* was obtained as a colorless oil (1.45 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.47–7.39 (m, 2H, Ar-H), 7.39–7.27 (m, 3H, Ar-H), 4.92 (d, *J* = 8.7 Hz, 1H, CH_2O), 4.61 (d, *J* = 15.8 Hz, 1H, CH_2Ph), 4.49 (d, *J* = 15.8 Hz, 1H, CH_2Ph), 4.31 (d, *J* = 8.7 Hz, 1H, CH_2O), 2.01 – 1.90 (m, 1H, CH_2), 1.75 – 1.63 (m, 1H, CH_2), 1.50 (s, 9H, $C(CH_3)_3$), 0.84 (t, *J* = 7.5 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.20 (CO), 136.10 (Ar-C), 128.54 (Ar-C), 128.29 (Ar-C), 127.95 (Ar-C), 84.17 ($C(CH_3)_3$), 72.28 (CH_2O), 69.45 (C-α), 46.84 (CH_2Ph), 27.84 ($C(CH_3)_3$), 26.93 (CH_2), 7.46 (CH_3).

4.4.10.2 *tert-Butyl 3-benzyl-2,2-dioxo-4-propyl-1,2,3-oxathiazolidine-4-carboxylate 9b (rac)*

Following the general method for the formation of sulfamidates and using **8b** (1.5 g, 4.4 mmol), the *title compound* was obtained as a colorless oil (1.54 g, 98%). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.50–7.38 (m, 2H, Ar-H), 7.38–7.23 (m, 3H, Ar-H), 4.90 (d, *J* = 8.7 Hz,

1H, CH_2O), 4.60 (d, *J* = 15.9 Hz, 1H, CH_2Ph), 4.48 (d, *J* = 15.9 Hz, 1H, CH_2Ph), 4.31 (d, *J* = 8.7 Hz, 1H, CH_2O), 1.94–1.75 (m, 1H, CH_2), 1.73–1.52 (m, 1H, CH_2), 1.49 (s, 9H), 1.32–1.09 (m, 2H, CH_2CH_3), 0.82 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.35 (CO), 136.05 (Ar-C), 128.56 (Ar-C), 128.33 (Ar-C), 127.97 (Ar-C), 84.20 ($C(CH_3)_3$), 72.49 (CH_2O), 69.11 (C-α), 46.91 (CH_2Ph), 35.80 (CH_2), 27.87 ($C(CH_3)_3$), 16.60 (CH_2CH_3), 14.04 (CH_3); HRMS *m/z* (ES+) Calcd for $C_{17}H_{25}LiNO_5S$ 362.1608, found 362.1607 $[MLi]^+$.

4.4.10.3 *tert-Butyl 3,4-dibenzyl-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 9c (rac)*

Following the general method for the formation of sulfamidates and using **8c** (1.94 g, 5 mmol), the *title compound* was obtained as a colorless solid (1.66 g, 82%). Mp 88-90 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.67–7.55 (m, 2H, Ar-H), 7.55–7.29 (m, 6H, Ar-H), 7.29-7.10 (m, 2H, Ar-H), 4.80 (d, *J* = 15.9 Hz, 1H, CH_2N), 4.77 (d, *J* = 8.8 Hz, 1H, CH_2O), 4.73 (d, *J* = 15.9 Hz, 1H, CH_2N), 4.56 (d, *J* = 8.8 Hz, 1H, CH_2O), 3.50 (d, *J* = 13.4 Hz, 1H, CH_2Ph), 2.92 (d, *J* = 13.4 Hz, 1H, CH_2Ph), 1.54 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.13 (CO), 136.11 (Ar-C), 132.57 (Ar-C), 130.00 (Ar-C), 128.95 (Ar-C), 128.74 (Ar-C), 128.51 (Ar-C), 128.16 (Ar-C), 128.05 (Ar-C), 84.71 ($C(CH_3)_3$), 72.40 (CH_2O), 69.18 (C-α), 47.01 (CH_2N), 40.10 (CH_2Ph), 27.89 ($C(CH_3)_3$); HRMS *m/z* (ES+) Calcd for $C_{21}H_{25}LiNO_5S$ 410.1608, found 410.1607 $[MLi]^+$.

4.4.10.4 *tert-Butyl 3-benzyl-4-(4-fluorobenzyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 9d (rac)*

Following the general method for the formation of sulfamidates and using **8d** (1.99 g, 4.9 mmol), the *title compound* was obtained as a colorless solid (1.59 g, 77%). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.52–7.44 (m, 2H, Ar-H), 7.42–7.30 (m, 3H, Ar-H), 7.09–6.92 (m, 4H, Ar-H), 4.68 (d, *J* = 15.8 Hz, 1H, CH_2Ph), 4.65 (d, *J* = 8.8 Hz, 1H, CH_2O), 4.58 (d, *J* = 15.8 Hz, 1H, CH_2Ph), 4.42 (d, *J* = 8.8 Hz, 1H, CH_2O), 3.36 (d, *J* = 13.6 Hz, 1H, CH_2Ar), 2.80 (d, *J* = 13.6 Hz, 1H, CH_2Ar), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.06 (CO), 162.51 (d, $^1J_{C\text{-}F}$ = 247.4 Hz, Ar-C), 136.00 (Ar-C), 131.66 (d, $^3J_{C\text{-}F}$ = 8.1 Hz, Ar-C), 128.77 (Ar-C), 128.48 (Ar-C), 128.38 (d, $^4J_{C\text{-}F}$ = 3.4 Hz, Ar-C), 128.21 (Ar-C), 115.90 (d, $^2J_{C\text{-}F}$ = 21.4 Hz, Ar-C), 84.90 ($C(CH_3)_3$), 72.18 (CH_2O), 69.14 (d, $^6J_{C\text{-}F}$ = 1.5 Hz, C-α), 47.03 (CH_2Ph), 39.17 (CH_2Ar), 27.88 ($C(CH_3)_3$); HRMS *m/z* (ES+) Calcd for $C_{21}H_{24}FLiNO_5S$ 428.1514, found 428.1513 $[MLi]^+$.

4.4.11 General method for the formation of lanthionines **11a-d**

To a degassed solution of sulfamidate **9a-d** (1 mmol) and Boc cysteine methyl ester (259 mg, 1.1 mmol) in CH_3CN (10 mL), DBU (182 mg, 1.2 mmol) in degassed CH_3CN (1 mL) was added *via* syringe and the reaction mixture was stirred for 1 h at rt under nitrogen. The solution was diluted with EtOAc (25 mL) and 10% aqueous NaH_2PO_4 (25 mL) and this was heated with stirring at 50 °C for 2 h. The reaction mixture was then basified by pouring in an excess of saturated Na_2CO_3 solution. The organic layer was decanted and the aqueous layer was extracted with EtOAc (3 x 50 mL). The pooled fractions were washed with brine and dried over $MgSO_4$, were filtered and the solvent was evaporated *in vacuo* to yield the crude product. This was chromatographied on silica with 1:3 (EtOAc/hexanes) and the solvents were removed *in vacuo* to give the pure compound.

4.4.11.1 *tert-Butyl 2-benzylamino-3-((R)-2-(tert-butoxycarbonyl amino)-3-methoxy-3-oxopropylthio)-2-ethyl propanoate* ***11a***

Following the general method for the formation of lanthionines and using **9a** (341 mg, 1 mmol), the *title compound* was obtained as a colorless oil (495 mg, 99%). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.45 – 7.13 (m, 5H, Ar-H), 5.82 (br, 0.5H, NH-Boc), 5.52 (br, 0.5H, NH-Boc), 4.52 (br, 1H, H-α), 3.72 (s, 1.5H, OCH_3), 3.68 (s, 1.5H, OCH_3), 3.68 – 3.59 (m, 1H, CH_2Ph), 3.59 – 3.50 (m, 1H, CH_2Ph), 3.04 (d, J = 12.9 Hz, 1H, SCH_2C), 3.04 – 2.94 (m, 2H, $CHCH_2S$), 2.90 (d, J = 12.9 Hz, 0.5H, SCH_2C), 2.89 (d, J = 12.9 Hz, 0.5H, SCH_2C), 2.17 (br, 1H, NH), 1.90 – 1.62 (m, 2H, CH_2CH_3), 1.50 (s, 9H, $C(CH_3)_3$), 1.44 (s, 4.5H, $C(CH_3)_3$), 1.43 (s, 4.5H, $C(CH_3)_3$), 0.96 – 0.80 (m, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.23 (0.5C, CO), 173.16 (0.5C, CO), 171.42 (CO), 155.11 (Boc-CO), 140.00 (0.5C, Ar-C), 139.90 (0.5C, Ar-C), 128.37 (Ar-C), 128.28 (Ar-C), 128.26 (Ar-C), 126.96 (0.5C, Ar-C), 126.93 (0.5C, Ar-C), 81.43 (0.5C, $C(CH_3)_3$), 81.42 (0.5C, $C(CH_3)_3$), 79.82 (0.5C, $C(CH_3)_3$), 79.75 (0.5C, $C(CH_3)_3$), 65.86 (0.5C, C-α), 65.73 (0.5C, C-α), 53.63 (0.5C, CH-α), 53.48 (0.5C, CH-α), 52.30 (0.5C, OCH_3), 52.26 (0.5C, OCH_3), 47.23 (0.5C, CH_2Ph), 47.00 (0.5C, CH_2Ph), 37.79 (0.5C, SCH_2C), 37.55 (0.5C, SCH_2C), 35.70 (0.5C, $CHCH_2S$), 35.49 (0.5C, $CHCH_2S$), 28.21 (1.5C, $C(CH_3)_3$), 28.19 (1.5C, $C(CH_3)_3$), 28.07 (0.5C, CH_2), 27.99 ($C(CH_3)_3$), 27.83 (0.5C, CH_2), 7.89 (0.5C, CH_3), 7.86 (0.5C, CH_3); *m/z* (ESI) 497 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{25}H_{41}N_2O_6S$ 497.2680, found 497.2677 $[MH]^+$.

4.4.11.2 *tert-Butyl 2-benzylamino-3-((R)-2-(tert-butoxycarbonyl amino)-3-methoxy-3-oxopropylthio)-2-(1-propyl) propanoate* ***11b***

Following the general method for the formation of lanthionines and using **9b** (355 mg, 1 mmol), the *title compound* was obtained as a colorless oil (511 mg, 99%).^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.40–7.17 (m, 5H, Ar-H), 5.79 (br, 0.5H, NH-Boc), 5.44 (br, 0.5H, NH-Boc), 4.51 (br, 1H, H-α), 3.72 (s, 1.5H, OCH_3), 3.67 (s, 1.5H, OCH_3), 3.63 (d, J = 11.7 Hz, 1H, CH_2Ph), 3.54 (d, J = 11.7 Hz, 1H, CH_2Ph), 3.00 (m, 3H, $CHCH_2S$, SCH_2C), 2.88 (d, J = 12.8 Hz, 0.5H, SCH_2C), 2.86 (d, J = 12.8 Hz, 0.5H, SCH_2C), 1.92 (br, 1H, NH), 1.81–1.56 (m, 2H, CH_2), 1.48 (s, 9H, $C(CH_3)_3$), 1.43 (s, 4.5H, $C(CH_3)_3$), 1.42 (s, 4.5H, $C(CH_3)_3$), 1.33–1.22 (m, 2H, CH_2CH_3), 0.95–0.85 (m, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.49 (0.5C, CO), 173.42 (0.5C, CO), 171.56 (CO), 155.17 (Boc-CO), 140.15 (0.5C, Ar-C), 140.04 (0.5C, Ar-C), 128.48 (Ar-C), 128.39 (Ar-C), 127.07 (0.5C, Ar-C), 127.04 (0.5C, Ar-C), 81.61 (0.5C, $C(CH_3)_3$), 81.58 (0.5C, $C(CH_3)_3$), 80.03 (0.5C, $C(CH_3)_3$), 79.93 (0.5C, $C(CH_3)_3$), 65.63 (0.5C, C-α), 65.50 (0.5C, C-α), 53.75 (0.5C, CH-α), 53.57 (0.5C, CH-α), 52.46 (0.5C, OCH_3), 52.42 (0.5C, OCH_3), 47.41 (0.5C, CH_2Ph), 47.16 (0.5C, CH_2Ph), 38.43 (0.5C, SCH_2C), 38.15 (0.5C, SCH_2C), 37.76 (0.5C, CH_2), 37.51 (0.5C, CH_2), 35.91 (0.5C, $CHCH_2S$), 35.71 (0.5C, $CHCH_2S$), 28.33 (1.5C, $C(CH_3)_3$), 28.31 (1.5C, $C(CH_3)_3$), 28.12 ($C(CH_3)_3$), 16.98 (0.5C, CH_2CH_3), 16.93 (0.5C, CH_2CH_3), 14.42 (0.5C, CH_3), 14.40 (0.5C, CH_3); *m/z* (ESI) 511 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{26}H_{43}N_2O_6S$ 511.2836, found 511.2834 $[MH]^+$.

4.4.11.3 *tert-Butyl 2-benzyl-2-benzylamino-3-((R)-2-(tert-butoxy carbonylamino)-3-methoxy-3-oxopropylthio)propanoate* ***11c***

Following the general method for the formation of lanthionines and using **9c** (403 mg, 1 mmol), the *title compound* was obtained as a colorless oil (503 mg, 90%); ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.47 – 7.15 (m, 10H, Ar-H), 5.57 (br, 0.5H, NH-Boc), 5.43 (br, 0.5H, NH-Boc), 4.55 (br, 1H, H-α), 3.81 (d, J = 11.5 Hz, 0.5H, CH_2N), 3.80 (d, J = 11.5 Hz, 0.5H, CH_2N), 3.74 (s, 1.5H, OCH_3), 3.71 (s, 1.5H, OCH_3), 3.66 (d, J = 11.5 Hz, 0.5H, CH_2N, 3.65 (d, J = 11.5 Hz, 0.5H, CH_2N), 3.20 – 3.04 (m, 3H, SCH_2C, CH_2Ph), 3.04 – 2.96 (m, 2H, $CHCH_2S$), 2.81 (d, J = 12.5 Hz, 0.5H, SCH_2C), 2.79 (d, J = 12.7 Hz, 0.5H, SCH_2C), 1.87 (br, 1H, NH), 1.45 (m, 18H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 172.85 (0.5C, CO), 172.79 (0.5C, CO), 171.58 (0.5C, CO), 171.50 (0.5C, CO), 155.16 (Boc-CO), 139.86 (0.5C, Ar-C), 139.79 (0.5C, Ar-C), 136.01 (0.5C, Ar-C), 130.27 (Ar-C), 130.25 (Ar-C), 128.43 (Ar-C), 128.41 (Ar-C), 128.38 (Ar-C), 128.34 (Ar-C), 128.17 (Ar-C), 128.15 (Ar-C), 127.13 (0.5C,

Ar-C), 127.09 (0.5C, Ar-C), 126.89 (0.5C, Ar-C), 126.86 (0.5C, Ar-C), 82.08 (0.5C, $C(CH_3)_3$), 82.05 (0.5C, $C(CH_3)_3$), 80.02 ($C(CH_3)_3$), 66.40 (C-α), 53.53 (CH-α), 52.48 (0.5C, OCH_3), 52.44 (0.5C, OCH_3), 47.54 (0.5C, CH_2N), 47.48 (0.5C, CH_2N), 41.39 (CH_2Ph), 37.52 (0.5C, SCH_2C), 37.32 (0.5C, SCH_2C), 35.65 (CHCH_2S), 28.31 (1.5C, C(CH_3)$_3$), 28.28 (1.5C, C(CH_3)$_3$), 28.10 (C(CH_3)$_3$); *m/z* (ESI) 559 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{30}H_{43}N_2O_6S$ 559.2836, found 559.2838 $[MH]^+$.

4.4.11.4 *tert-Butyl 2-benzylamino-3-((R)-2-(tert-butoxycarbonyl amino)-3-methoxy-3-oxopropylthio)-2-(4-fluorobenzyl) propanoate* **11d**

Following the general method for the formation of lanthionines and using **9d** (421 mg, 1 mmol), the *title compound* was obtained as a colorless oil (564 mg, 98%); ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.44–7.10 (m, 7H, Ar-H), 7.05–6.81 (m, 2H, Ar-H), 5.53 (br, 0.5H, NH-Boc), 5.41 (br, 0.5H, NH-Boc), 4.51 (br, 1H, H-α), 3.77 (d, J = 11.4 Hz, 1H, CH_2Ph), 3.72 (s, 1.5H, OCH_3), 3.70 (s, 1.5H, OCH_3), 3.64 (d, J = 11.4 Hz, 0.5H, CH_2Ph), 3.62 (d, J = 11.4 Hz, 0.5H, CH_2Ph), 3.24–2.83 (m, 5H, CHCH_2S, SCH_2C, CH_2Ph), 2.76 (d, J = 12.6 Hz, 1H, SCH_2C), 1.88 (br, 1H, NH), 1.48–1.36 (m, 18H, C(CH_3)$_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 172.77 (0.5C, CO), 172.72 (0.5C, CO), 171.53 (0.5C, CO), 171.46 (0.5C, CO), 161.90 (d, $^1J_{C\text{-}F}$ = 245.1 Hz, Ar-C), 155.14 (Boc-CO), 139.76 (0.5C, Ar-C), 139.70 (0.5C, Ar-C), 131.77 (d, $^4J_{C\text{-}F}$ = 4.3 Hz, Ar-C), 131.75 (d, $^3J_{C\text{-}F}$ = 7.7 Hz, Ar-C), 128.46 (Ar-C), 128.45 (Ar-C), 128.33 (Ar-C), 128.30 (Ar-C), 127.18 (0.5C, Ar-C), 127.15 (0.5C, Ar-C), 114.95 (d, $^2J_{C\text{-}F}$ = 21.1 Hz, 0.5C, Ar-C), 114.92 (d, $^2J_{C\text{-}F}$ = 21.1 Hz, 0.5C, Ar-C), 82.21 (0.5C, $C(CH_3)_3$), 82.18 (0.5C, $C(CH_3)_3$), 80.04 ($C(CH_3)_3$), 66.34 (C-α), 53.57 (CH-α), 52.49 (0.5C, OCH_3), 52.46 (0.5C, OCH_3), 47.48 (0.5C, CH_2Ph), 47.44 (0.5C, CH_2Ph), 40.35 (0.5C, CH_2Ar), 40.30 (0.5C, CH_2Ar), 37.48 (0.5C, SCH_2C), 37.25 (0.5C, SCH_2C), 35.62 (CHCH_2S), 28.29 (C(CH_3)$_3$), 28.10 (C(CH_3)$_3$); *m/z* (ESI) 577 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{30}H_{42}FN_2O_6S$ 577.2742, found 577.2747 $[MH]^+$.

4.4.12 *tert*-Butyl 2-amino-2-benzyl-3-((*R*)-2-(tert-butoxycarbonylamino)-3-methoxy-3-oxopropylthio) propanoate **12**

Compound **11c** (300 mg, 0.54 mmol) was dissolved in MeOH (90 mL) and water (10 mL) and the solution was placed in a hydrogenator containing a magnetic stir bar. Pd black (75 mg, wet with water) was then added cautiously followed by AcOH (3 mL). The reactor was closed and purged four times with H_2 then placed under H_2 pressure (7 bars). The vessel was externally heated to 70 °C and the reaction was allowed to proceed for 16 h with stirring.

The vessel was cooled to rt, the H_2 was removed and the reactor was opened. The solution was decanted from palladium and filtered on a fritted funnel. The solvent was evaporated *in vacuo* and the residue was basified with saturated Na_2CO_3 and extracted twice with CH_2Cl_2. The solvent was removed and the crude oil was purified by column chromatography on silica with 1:1 (EtOAc/hexanes + 1% NEt_3) to produce, after evaporation of the solvents *in vacuo*, the *title compound* as a pale oil (73%). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.23 – 7.03 (m, 5H, Ar-H), 6.32 (br, 0.5H, NH-Boc), 5.77 (br, 0.5H, NH-Boc), 4.44 (br, 1H, H-α), 3.63 (s, 1.5H, OCH_3), 3.62 (s, 1.5H, OCH_3), 3.09 (d, J = 13.0 Hz, 0.5H, SCH_2C), 3.02 (d, J = 13.0 Hz, 0.5H, SCH_2C), 3.01 – 2.89 (m, 3H, $CHCH_2S$, CH_2Ph), 2.71 (d, J = 13.1 Hz, 1H, CH_2Ph), 2.63 (d, J = 13.0 Hz, 0.5H, SCH_2C), 2.59 (d, J = 13.0 Hz, 0.5H, SCH_2C), 1.75 (br, 2H, NH_2), 1.36 (s, 4.5H, $C(CH_3)_3$), 1.35 (s, 4.5H, $C(CH_3)_3$), 1.34 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.64 (0.5C, CO), 173.59 (0.5C, CO), 171.14 (0.5C, CO), 171.12 (0.5C, CO), 155.12 (0.5C, Boc-CO), 155.01 (0.5C, Boc-CO), 135.51 (0.5C, Ar-C), 135.48 (0.5C, Ar-C), 129.93 (Ar-C), 129.90 (Ar-C), 128.07 (Ar-C), 128.03 (Ar-C), 126.85 (0.5C, Ar-C), 126.80 (0.5C, Ar-C), 81.71 (0.5C, $C(CH_3)_3$), 81.69 (0.5C, $C(CH_3)_3$), 79.59 (0.5C, $C(CH_3)_3$), 79.49 (0.5C, $C(CH_3)_3$), 62.78 (0.5C, C-α), 62.66 (0.5C, C-α), 53.85 (0.5C, CH-α), 53.57 (0.5C, CH-α), 52.18 (0.5C, OCH_3), 52.14 (0.5C, OCH_3), 45.26 (0.5C, CH_2Ph), 45.08 (0.5C, CH_2Ph), 43.29 (0.5C, SCH_2C), 43.08 (0.5C, SCH_2C), 36.31 (0.5C, $CHCH_2S$), 36.08 (0.5C, $CHCH_2S$), 28.09 (1.5C, $C(CH_3)_3$), 28.05 (1.5C, $C(CH_3)_3$), 27.70 ($C(CH_3)_3$); *m/z* (ESI) 469 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{23}H_{37}N_2O_6S$ 469.2367, found 469.2367 $[MH]^+$.

References

1. Horn, M.J.; Jones, D.B.; Ringel, S.J. Isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 141-149.
2. Du Vigneaud, V.; Brown, G.B. The synthesis of the new sulfur-containing amino acid (lanthionine) isolated from sodium carbonate-treated wool. *J. Biol. Chem.* **1941**, *138*, 151-154.
3. Vasstrand, E.N.; Jensen, H.B.; Miron, T.; Hofstad, T. Composition of peptidoglycans in Bacteroidaceae: determination and distribution of lanthionine. *Infect. Immun.* **1982**, *36*, 114-122.
4. Tabor, A.B. The challenge of the lantibiotics: synthetic approaches to thioether-bridged peptides. *Org. Biomol. Chem.* **2011**, *9*, 7606-7628.
5. Zhu, X.; Schmidt, Richard R. Efficient Synthesis of Differently Protected Lanthionines via β-Bromoalanine Derivatives. *Eur. J. Org. Chem.* **2003**, 4069-4072.
6. Paul, M.; van der Donk, W.A. Chemical and Enzymatic Synthesis of Lanthionines. *Mini-Reviews in Organic Chemistry* **2005**, *2*, 23-37.
7. Cobb, S.L.; Vederas, J.C. A concise stereoselective synthesis of orthogonally protected lanthionine and β-methyllanthionine. *Org. Biomol. Chem.* **2007**, *5*, 1031-1038.
8. Aydillo, C.; Avenoza, A.; Busto, J.H.; Jiménez-Osés, G.; Peregrina, J.M.; Zurbano, M.M. A Biomimetic Approach to Lanthionines. *Org. Lett.* **2011**, *14*, 334-337.
9. Narayan, R.S.; VanNieuwenhze, M.S. Versatile and Stereoselective Syntheses of Orthogonally Protected β-Methylcysteine and β-Methyllanthionine. *Org. Lett.* **2005**, *7*, 2655-2658.
10. Bregant, S.; Tabor, A.B. Orthogonally Protected Lanthionines: Synthesis and Use for the Solid-Phase Synthesis of an Analogue of Nisin Ring C. *J. Org. Chem.* **2005**, *70*, 2430-2438.
11. Martin, N.I. Concise Preparation of Tetra-orthogonally Protected (2*S*,6*R*)-Lanthionines. *J. Org. Chem.* **2008**, *74*, 946-949.
12. Probert, J.M.; Rennex, D.; Bradley, M. Lanthionines for solid phase synthesis. *Tetrahedron Lett.* **1996**, *37*, 1101-1104.
13. Dugave, C.; Ménez, A. Synthesis of natural and non natural orthogonally protected lanthionines from *N*-tritylserine and *allo*-threonine derivatives. *Tetrahedron: Asymmetry* **1997**, *8*, 1453-1465.
14. Mohd Mustapa, M.F.; Harris, R.; Bulic-Subanovic, N.; Elliott, S.L.; Bregant, S.; Groussier, M.F.A.; Mould, J.; Schultz, D.; Chubb, N.A.L.; Gaffney, P.R.J., *et al.* Synthesis of Orthogonally Protected Lanthionines. *J. Org. Chem.* **2003**, *68*, 8185-8192.
15. Shao, H.; Wang, S.H.H.; Lee, C.-W.; Oesapay, G.; Goodman, M. A Facile Synthesis of Orthogonally Protected Stereoisomeric Lanthionines by Regioselective Ring Opening of Serine β-Lactone Derivatives. *J. Org. Chem.* **1995**, *60*, 2956-2957.
16. Smith, N.D.; Goodman, M. Enantioselective Synthesis of α-Methyl-D-cysteine and Lanthionine Building Blocks via α-Methyl-D-serine-β-lactone. *Org. Lett.* **2003**, *5*, 1035-1037.
17. Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. New diastereoselective synthesis of protected *meso*-lanthionine with discrimination of the chiral centers. *Tetrahedron: Asymmetry* **1992**, *3*, 85-94.
18. Harpp, D.N.; Gleason, J.G. Preparation and mass spectral properties of cystine and lanthionine derivatives. Novel synthesis of L-lanthionine by selective desulfurization. *J. Org. Chem.* **1971**, *36*, 73-80.
19. Sambeth, G.M.; Süssmuth, R.D. Synthetic studies toward labionin, a new α,α-disubstituted amino acid from type III lantibiotic labyrinthopeptin A2. *J. Pept. Sci.* **2011**, *17*, 581-584.
20. Knerr, P.J.; van der Donk, W.A. Chemical Synthesis of the Lantibiotic Lacticin 481 Reveals the Importance of Lanthionine Stereochemistry. *J. Am. Chem. Soc.* **2013**, *135*, 7094-7097.
21. Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T.L.; Chung, N.N.; Schiller, P.W.; Cassel, J.A.; DeHaven, R.N.; Goodman, M. Synthesis and Biological Activities of Cyclic Lanthionine Enkephalin Analogues: δ-Opioid Receptor Selective Ligands. *J. Med. Chem.* **2002**, *45*, 3746-3754.

22. Boulton, L.T.; Stock, H.T.; Raphy, J.; Horwell, D.C. Generation of unnatural α,α-disubstituted amino acid derivatives from cyclic sulfamidates. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1421-1430.
23. Jew, S.-s.; Lee, Y.-J.; Lee, J.; Kang, M.J.; Jeong, B.-S.; Lee, J.-H.; Yoo, M.-S.; Kim, M.-J.; Choi, S.-h.; Ku, J.-M*., et al.* Highly Enantioselective Phase-Transfer-Catalytic Alkylation of 2-Phenyl-2-oxazoline-4-carboxylic Acid tert-Butyl Ester for the Asymmetric Synthesis of α-Alkyl Serines. *Angew. Chem. Int. Ed.* **2004**, *43*, 2382-2385.
24. Reddy, L.R.; Saravanan, P.; Corey, E.J. A Simple Stereocontrolled Synthesis of Salinosporamide A. *J. Am. Chem. Soc.* **2004**, *126*, 6230-6231.
25. Dondoni, A.; Perrone, D. Synthesis of 1,1-dimethylethyl (S)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate by oxidation of the alcohol. *Org. Synth.* **2000**, *77*, 64-77.
26. Huang, Y.; Dalton, D.R.; Carroll, P.J. The Efficient, Enantioselective Synthesis of Aza Sugars from Amino Acids. 1. The Polyhydroxylated Pyrrolidines. *J. Org. Chem.* **1997**, *62*, 372-376.
27. Roger, R.; Neilson, D.G. The Chemistry of Imidates. *Chem. Rev.* **1961**, *61*, 179-211.
28. Fry, E.M. Syntheses of Cystine. *J. Org. Chem.* **1950**, *15*, 438-447.
29. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.-i.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. Dicarbonates: Convenient 4-Dimethylaminopyridine Catalyzed Esterification Reagents. *Synthesis* **1994**, 1063-1066.
30. Park, H.-g.; Lee, J.; Kang, M.J.; Lee, Y.-J.; Jeong, B.-S.; Lee, J.-H.; Yoo, M.-S.; Kim, M.-J.; Choi, S.-h.; Jew, S.-s. An unusual base-dependent α-alkylation and β-elimination of *tert*-butyl 2-phenyl-2-oxazoline-4-carboxylate: practical synthesis of (±)-α-alkylserines and *tert*-butyl benzamidoacrylate. *Tetrahedron* **2004**, *60*, 4243-4249.
31. Dolence, E.K.; Mayer, G.; Kelly, B.D. Use of optically active cyclic diethyl sulfamidate 2-phosphonates as chiral synthons for the synthesis of β-substituted α-amino phosphonates. *Tetrahedron: Asymmetry* **2005**, *16*, 1583-1594.
32. Wei, L.; Lubell, W.D. Racemization in the Use of *N*-(9-(9-Phenylfluorenyl))Serine-Derived Cyclic Sulfamidates in the Synthesis of δ-Keto α-Amino Carboxylates and Prolines. *Org. Lett.* **2000**, *2*, 2595-2598.
33. Avenoza, A.; Busto, J.H.; Jiménez-Osés, G.; Peregrina, J.M. S_N2 Reaction of Sulfur Nucleophiles with Hindered Sulfamidates: Enantioselective Synthesis of α-Methylisocysteine. *J. Org. Chem.* **2006**, *71*, 1692-1695.
34. Jiménez-Osés, G.; Avenoza, A.; Busto, J.H.; Rodríguez, F.; Peregrina, J.M. A Novel Multistep Mechanism for the Stereocontrolled Ring Opening of Hindered Sulfamidates: Mild, Green, and Efficient Reactivity with Alcohols. *Chemistry – A European Journal* **2009**, *15*, 9810-9823.
35. Angle, S.R.; Henry, R.M. Synthesis of Enantiopure Indolizidine Alkaloids from α-Amino Acids: Total Synthesis of (−)-Indolizidine 167B. *J. Org. Chem.* **1997**, *62*, 8549-8552.
36. McNair, R.J. Catalytic Deprotection—DEPRO™ Catalyst Kit/Catalytic Deprotection. *Aldrich ChemFiles* **2009**, *9*, 21.
37. Shine, H.J.; Hoque, A.K.M.M. Formation of 1,2,4-triazoles by cation radical-induced oxidative addition of arylhydrazones of benzaldehyde and butyraldehyde to nitriles. *J. Org. Chem.* **1988**, *53*, 4349-4353.
38. Meyer, F.; Laaziri, A.; Papini, A.M.; Uziel, J.; Jugé, S. Efficient synthesis of β-halogeno protected L-alanines and their β-phosphonium derivatives. *Tetrahedron: Asymmetry* **2003**, *14*, 2229-2238.

Chapter 5

Enantioselective synthesis of α-benzylated lanthionines and related tripeptides for biological incorporation into *E. coli* peptidoglycan

(This chapter has been submitted for publication in *Organic & Biomolecular Chemistry*)

5.1 Introduction

Peptidoglycan (PG) is unique to bacteria and essential for cell survival. It lies outside of the cytoplasmic membrane. It provides the cells with osmotic stability and has also a vital function in bacterial morphogenesis. Enzymes catalysing the biosynthesis of PG are attractive targets for the development of new antibiotics.[1] The biosynthesis starts in the cytoplasm by the formation of UDP-MurNAc from UDP-GlcNAc through the involvement of Mur ligases MurA and MurB. The successive addition of different amino acids is catalysed by different Mur ligases ((*S*)-Ala/MurC, (*R*)-Glu/MurD, *meso*-A_2pm/MurE, (*R*)-Ala-(*R*)-Ala/MurF). Then the MurNAc-peptide moiety of UDP-MurNAc-pentapeptide is transferred onto membrane-bound bactoprenyl phosphate by MraY. The next step is the formation of lipid II by reaction with UDP-GlcNAc under catalysis by MurG. This molecule is translocated to the periplasmic side of the plasma membrane and used there as a substrate for the polymerisation reactions, transglycosylation followed by transpeptidation, catalysed by penicillin-binding proteins (PBPs) (Figure 1).

During growth and cell division a high fraction of PG is broken down by the action of lytic transglycosylases, amidases and endopeptidases. Besides *de novo* biosynthesis of PG, recycling of PG fragments generated during the growth and division process is observed. Thus the end-product of the degradation process, the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm is transported by the MppA (murein peptide permease A) into the cytoplasm where it is directly bound to UDP-MurNAc under catalysis by Mpl (murein peptide ligase) (Figure 1).

Herein we report the recycling of PG fragments as a novel labeling approach of the PG of *E. coli* through the replacement of natural A_2pm **1** (Figure 2) in the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm by derivatives of lanthionine **2** (Figure 2). Modified amino acids (A_2pm and Lys derivatives) in the form of tripeptide analogs of (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm can be introduced *in vivo* by the use of the permeases and Mpl activity of *E. coli* to allow the biosynthesis of modified UDP-MurNAc-tripeptides.[2,3][4] The incorporation of the diastereomers of tripeptides **3** and **4**

(Figure 2), containing α-substituted lanthionines, may open ways to the study of PG synthesis and turnover in relation with cell growth and division.

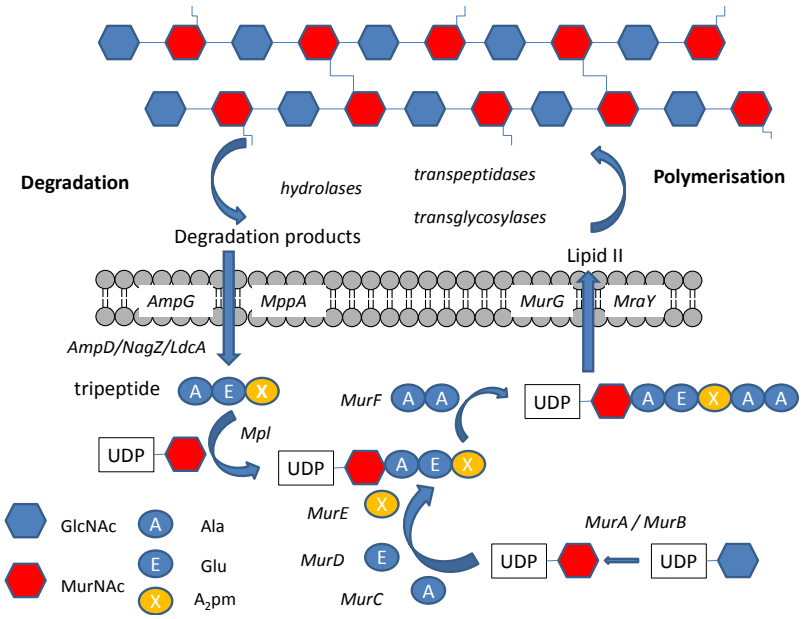


Figure 1. Metabolism of PG in *E. coli*. The *de novo* biosynthesis of PG starts with the synthesis of UDP-MurNAc-pentapeptide from UDP-GlcNAc in the cytoplasm (enzymes: MurA, MurB, MurC, MurD, MurE and MurF). The second part, the synthesis of lipid II catalysed by MraY and MurG, takes place at the level of the cytoplasmic membrane. Finally, the polymerisation reactions (transglycosylation and transpeptidation), take place in the periplasm. During the cell growth and cell division the PG is partly degraded by numerous hydrolases. The degradation products are re-imported by permeases (AmpG, MppA) and further degraded (AmpD, NagZ, LdcA). The end-product, the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm, is re-introduced in the biosynthesis of PG by murein peptide ligase (Mpl).

Olrichs et al.[5,6] have reported a lysine tagged with a fluorescent dye in the form of the tripeptide (*S*)-Ala-γ-(*R*)-Glu-(*S*)-Lys-NBD (**5**, Figure 2). Tripeptides containing [^{35}S]lanthionine and a photoactivatable group situated on an α-carbon could potentially be used to study PG biosynthesis. *In vivo*, upon UV irradiation, identification of proteins involved in the biosynthesis or biodegradation of the PG or in the cell division should be feasible.

In this report, we describe a diastereoselective synthesis of α-substituted tripeptides **3** and **4** (Figure 2). Moreover, results of biological incorporation of those molecules into *E. coli* PG are presented.

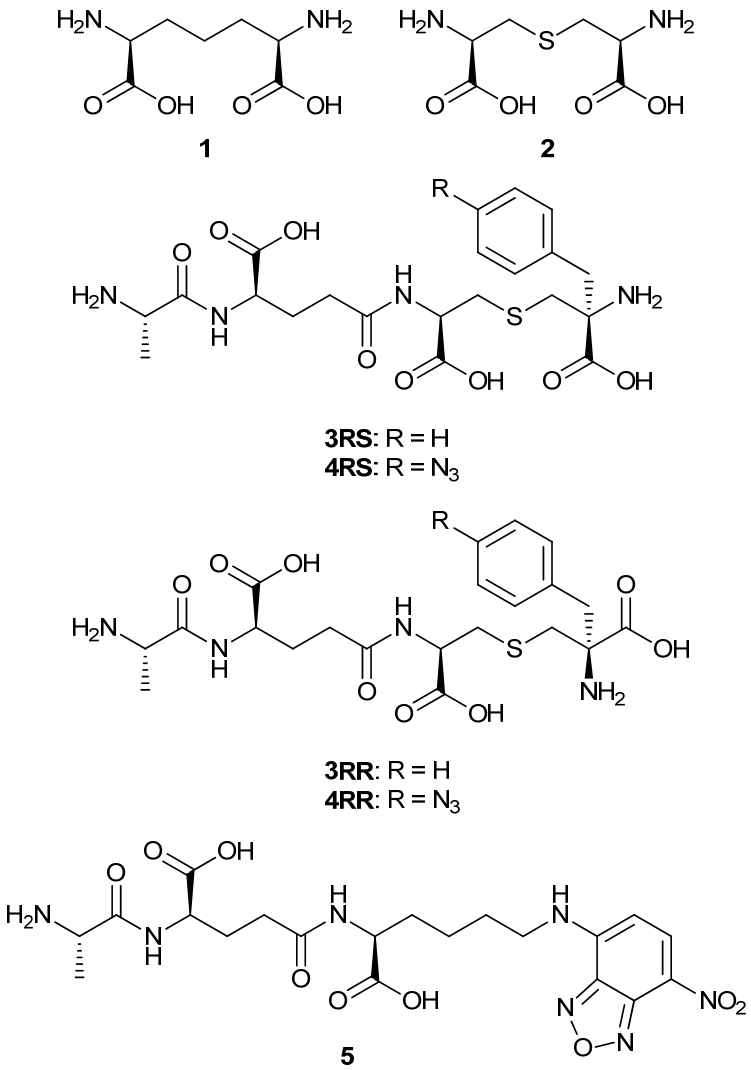


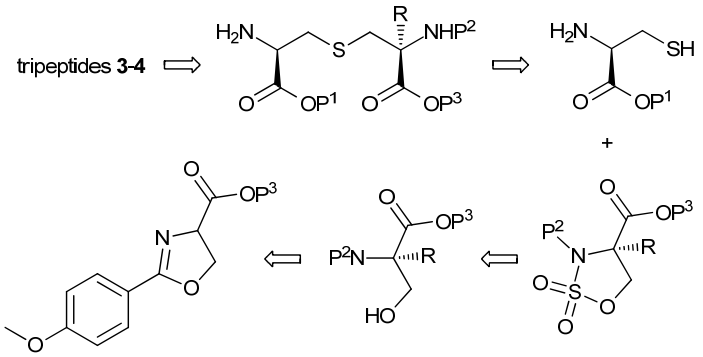
Figure 2. Structural formulas of representative molecules.

5.2 Results and discussion

5.2.1 Chemical synthesis

5.2.1.1 *Retrosynthetic analysis*

The synthesis of tripeptides **3** and **4** (Figure 2) could be envisioned from a protected lanthionine through amide coupling and usual deprotection (Scheme 1). The lanthionine derivative could be obtained *via* S_N2 opening of a sterically crowded sulfamidate with a cysteine derivative (Scheme 1).[7,8] The sulfamidate could arise from an α-alkylated serine[9] available in an enantioselective way by alkylation of an oxazoline precursor.[8,10] A phenyloxazoline, substituted with a *para*-methoxy group, will provide a sulfamidate carrying a *para*-methoxy benzyl (PMB) protective group (removable[11] by oxidative treatment with CAN) (Scheme 1).



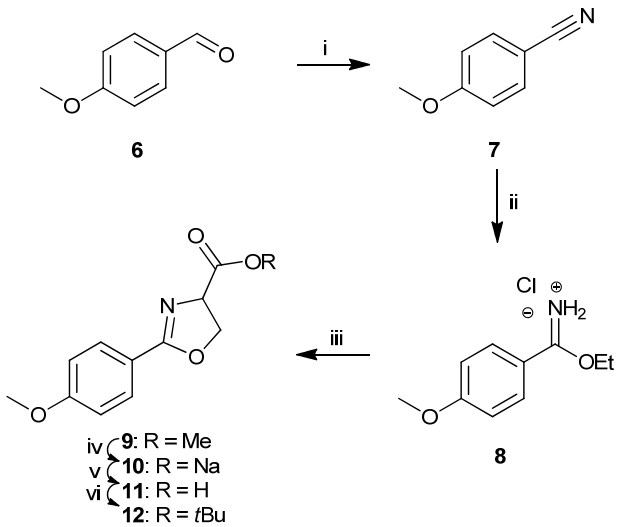
Scheme 1. Retrosynthetic approach to α-alkylated lanthionine derivatives. P^1= Me, P^2= Boc, PMB, P^3= *t*Bu.

5.2.1.2 *Preparation of precursor 12*

Phenyloxazoline **12** was prepared from anisaldehyde **6** in six steps (Scheme 2). First, reaction of **6** with hydroxylamine in the presence of formic acid afforded nitrile **7** on a multimolar scale (step i, Scheme 2).[12] Likewise, conversion to the imidate **8** was done at the molar scale (step ii). The reaction conditions previously described[13] were modified by using AcCl to generate HCl *in situ.* The amount of EtOH had to be increased in order to solubilize **7**. As indicated by NMR, a small amount of amide that does not disturb the subsequent reaction (step iii, Scheme 2) was formed. To

get **9,** the free base of **8** was reacted with serine methyl ester hydrochloride in MeOH (step iii).[8]

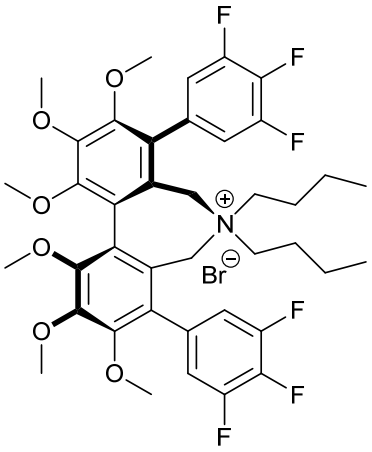
After saponification with aqueous NaOH, the salt of **10** was precipitated in acetone (step iv).[14] To minimize the protonation of the oxazoline ring and subsequent hydrolysis,[14] acidification of **10** was conducted at 0 °C with formic acid to yield **11** (step v). The *tert*-butyl ester **12** was then synthesized with Boc_2O/DMAP (step vi).[8] Starting from **8**, 62 g of **12** were obtained (four steps, 51%).



Scheme 2. Synthesis of the oxazoline precursor. i) $H_2NOH.HCl$, pyridine, HCOOH, reflux, 1 h, 85%; ii) EtOH, AcCl, Et_2O, 2 °C, 1 month, 53%; iii) Na_2CO_3, H-(*S*)-Ser-OMe*HCl, MeOH, reflux, 2 h, 68%; iv) aq. NaOH, MeOH, acetone, rt, 1 h, 99%; v) HCOOH, water, 0 °C, 1 h, 85%; vi) Boc_2O (2 eq.), DMAP (10 mol%), 1:1 CH_2Cl_2/ *t*-BuOH, reflux, 1 h, 90%.

5.2.1.3 *Preparation of Maruoka's catalyst 13*

In order to get a high diastereoselectivity in the synthesis of the α-substituted tripeptides **3** and **4**, enantioselective alkylation of oxazoline precursor **12** is mandatory. Among all the chiral phase transfer catalysts (PTC) described in the literature,[15] the Maruoka's **13** (Figure 3) seems the most active while exhibiting high enantioselectivity.[16] Unfortunately, it is presently commercially unavailable. The two enantiomers of catalyst **13** were thus prepared according to a slightly modified procedure (see supplementary material).[17]



(*R*)-**13**

Figure 3. Phase transfer catalyst (PTC) (*R*)-**13** that was used for the enantioselective alkylation of **12**.

5.2.1.4 *Enantioselective alkylation of 12*

With both antipodes of the chiral catalyst **13** in hands, we proceeded to the enantioselective alkylation of **12** with benzyl bromide and *para*-bromo benzyl bromide (step i, Scheme 3).[8,10] The reaction was investigated in CH_2Cl_2 or toluene with 5 eq. of $CsOH_{(s)}$ as a base at various temperatures (Table 1). First, alkylation of **12** was realized with *para*-bromo benzyl bromide and catalyst (*S*)-**13** (Table 1, entries 1-9). When reaction was carried out at rt, in CH_2Cl_2, the enantiomeric excesses (ee) of (*S*)-**20** were poor (Table 1, entries 1-4). By substituting toluene for CH_2Cl_2 and operating at -20 °C the ee increased from 63% (entries 1-4) to 96% (entries 5-7), using only 1.5 eq. of electrophile. Decreasing the amount of catalyst from 0.5 mol% to 0.1 mol% resulted in a decrease in ee from 97% to 90% (entries 7-8). A decrease of ten fold of that amount of catalyst (0.01 mol%) lead to a drastic drop in ee (65%, entry 9).

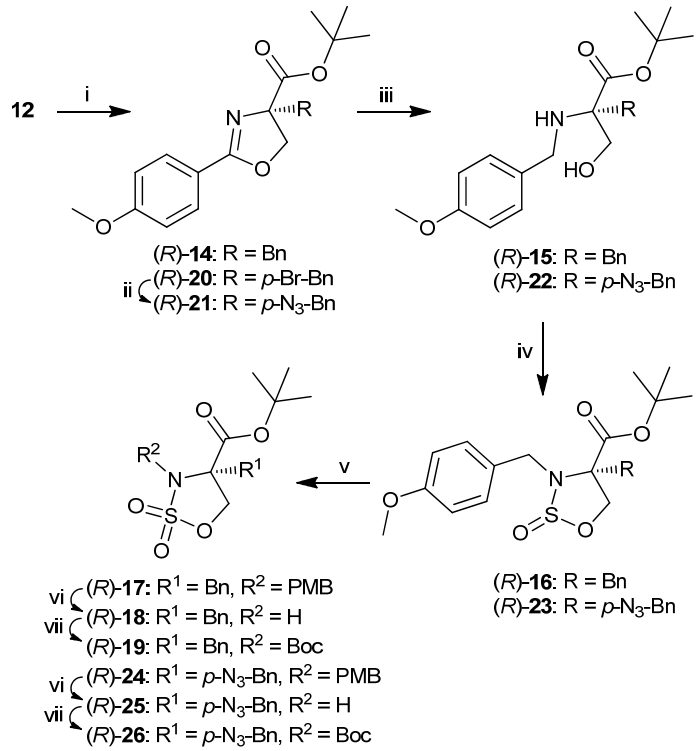
Thus, alkylation of **12** was performed in multigram quantity with 1 mol% of (*S*)-**13**. After reaction for 72 h at -20 °C, (*S*)-**20** was obtained in 87% yield and an ee of 96% (entry 6). However, a slightly lower ee (90%) was observed when the reaction was conducted with antipode (*R*)-**13** and the same conditions (entry 10).

In the same manner, by using benzyl bromide as electrophile, (*S*)-**14** and (*R*)-**14** were also obtained in a multigram scale (entries 11-12). Excellent yields (93-99%) and high ee were observed (89-97%).

Table 1. Enantioselective alkylation of 12

RX[a] (eq.)	Solvent	T (°C)	CTP (mol%)	Product (ee%)[b]	Yield (%)[c]
A (3)	CH_2Cl_2	rt	(*S*)-**13** (1)	(*S*)-**20** (60)	79
A (1.5)	CH_2Cl_2	rt	(*S*)-**13** (1)	(*S*)-**20** (63)	73
A (1.5)	CH_2Cl_2	rt	(*S*)-**13** (2)	(*S*)-**20** (66)	-
A (1.5)	CH_2Cl_2	rt	(*S*)-**13** (10)	(*S*)-**20** (64)	-
A (1.5)	toluene	-20 °C	(*S*)-**13** (2)	(*S*)-**20** (96)	-
A (1.5)	toluene	-20 °C	(*S*)-**13** (1)	(*S*)-**20** (96)	87
A (1.5)	toluene	-20 °C	(*S*)-**13** (0.5)	(*S*)-**20** (97)	-
A (1.5)	toluene	-20 °C	(*S*)-**13** (0.1)	(*S*)-**20** (90)	-
A (1.5)	toluene	-20 °C	(*S*)-**13** (0.01)	(*S*)-**20** (65)	-
A (1.5)	toluene	-20 °C	(*R*)-**13** (1)	(*R*)-**20** (90)	99
B (1.5)	toluene	-20 °C	(*S*)-**13** (1)	(*S*)-**14** (97)	93
B (3)	toluene	-20 °C	(*R*)-**13** (0.5)	(*R*)-**14** (89)	99

[a] A: 4-Br-BnBr, B: BnBr (in molar eq.); [b] Enantiomeric excesses were determined by HPLC on a Chiracel OD-H column (Daicel, 150 mm x 4 mm, 5 μm) with 98:2 (*n*-Hex/*i*-PrOH) as an eluent at 1 mL/min, 37 °C; retention times for isomers *S* and *R* were 9.0 min and 15.5 min, respectively; [c] Isolated yields.



Scheme 3. Synthesis of sulfamidates **19** and **26**. i) RX, CsOH (5 eq.), (*R*)-**13**; ii) NaN_3 (2 eq.), sodium ascorbate (15 mol%), CuI (20 mol%), DMEDA (30 mol%), aq. EtOH, reflux, 5 h, 99%, iii) $NaBH_3CN$ (6 eq.), AcOH, rt, 16 h, 90-99%; iv) $SOCl_2$, imidazole, NEt_3, -10 °C → rt, 2 h, 98%; v) $NaIO_4$ (1.2 eq.), $RuCl_3$ (1 mol%), aq. CH_3CN, rt, 4 h (75-80%); vi) $(NH_4)_2Ce(NO_3)_6$ (CAN) (3 eq.), rt, 20 min, 53-80%; vii) Boc_2O, DMAP (10 mol%), NEt_3 (cat.), CH_3CN, rt, 16 h, 90-93%.

The bromine atom carried by (*R*)-**20** was then substituted by an azido group. The reaction was catalysed by a Cu(I) salt and *N*,*N*'-dimethylethylenediamine (DMEDA) as ligand (step ii, Scheme 3).[18]

5.2.1.5 *Synthesis of sulfamidates 18 and 25*

The sulfamidates **18** and **25** were synthesized in four steps from the 4-alkylated oxazolines (*R*)-**14** and (*R*)-**21** (steps iii-vi, Scheme 3). Steps iii-v were conducted in accordance to our recent publication.[8] Briefly, reduction[19] of oxazoline (*R*)-**14** with $NaBH_3CN$ in acetic acid afforded the α-alkylated serines (*R*)-**15** (step iii). Cyclization into sulfamidites (*R*)-**16** occurred with $SOCl_2$ in the presence of

imidazole (step iv).[20] The crude intermediate was then oxidized into the sulfamidate (*R*)-**17** with a catalytic amount of RuO_4 (step v).[9] The PMB protecting group of (*R*)-**17** was then removed by using CAN in CH_3CN (step vi).[11] The sulfamidate (*R*)-**18** was purified by recrystallization.

Using the same approach, sulfamidates (*S*)-**18**, (*R*)-**25** and (*S*)-**25** were obtained in an overall yield of 42-60% (four steps) from (*S*)-**14**, (*R*)-**21** and (*S*)-**21**, respectively. These crystalline compounds can be stored at 2 °C for several years without decomposition. Notably, the azido group was stable under those reaction conditions.

The solid-state structures of both compounds were determined by X-ray diffraction analysis (XRD) (Figure 4). Single crystals of both compounds were crystallized in chiral space groups (*R*)-**18** (CCDC958602) in monoclinic P_{21} and (*R*)-**25** (CCDC958603) in orthorhombic P_{212121}. The absolute configuration of both structures were determined by the Flack parameter,[21] 0.03(4) for (*R*)-**18** and 0.10(8) for (*R*)-**25**.

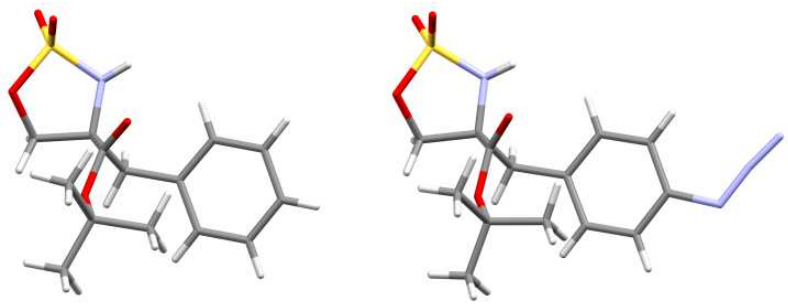


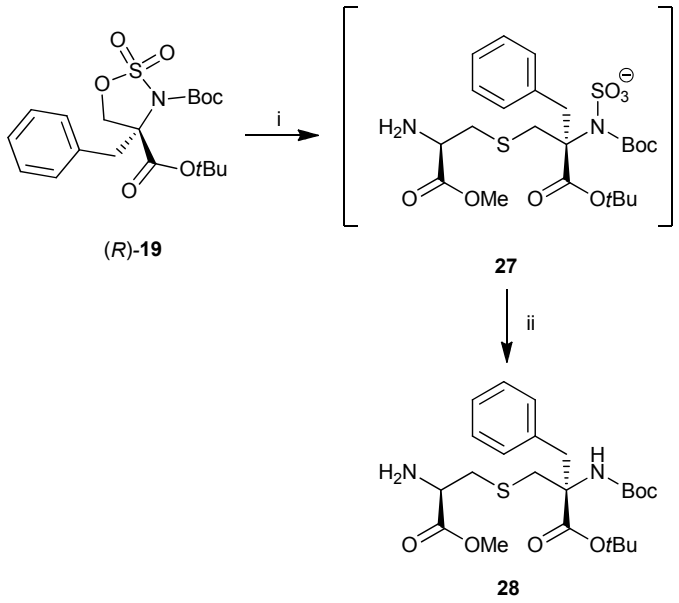
Figure 4. Capped sticks representation of compounds (*R*)-**18** and (*R*)-**25** solved by single crystal X-ray diffraction.

5.2.1.6 *Synthesis of protected lanthionine 28*

Attempts to open[7,9] the sulfamidate (*R*)-**18** by the methyl ester of (*R*)-cysteine in CH_3CN with DBU failed to afford the desired product. As carbamate-protected sulfamidates are more reactive towards nucleophiles,[22,23] the nitrogen of the sulfamidates (*R*)-**18** and (*R*)-**25** was protected with a *tert*-butyl carbamate group.[24] For both compounds, the reaction proceeded with Boc_2O in the presence of DMAP in CH_3CN (step vii, Scheme 3). Using these conditions, described in the literature to

convert an amide into an amido-carbamate,[25] Boc-sulfamidates (*R*)-**19** and (*R*)-**26** were obtained in excellent yields.

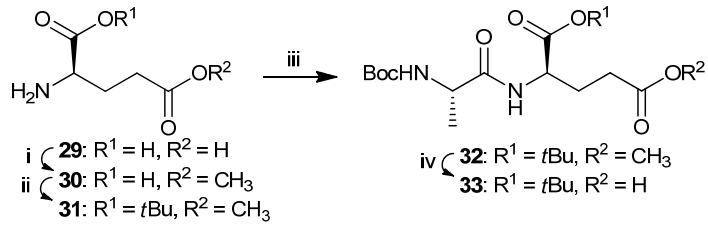
Lanthionine **27** was formed after reaction of (*R*)-**19** with the methyl ester of (*R*)-cysteine (step i, Scheme 4) and used in the next step without purification. Removal[8] of the sulfamate group by aqueous NaH_2PO_4 at 50 °C afforded the protected lanthionine **28** in good yield (78%, steps i-ii, Scheme 4).



Scheme 4. Synthesis of protected lanthionine **28**. i) Cysteine Me ester*HCl (1 eq.), DBU (1.1 eq.), CH_3CN, rt, 1 h; ii) 10% aq. NaH_2PO_4, EtOAc, 50 °C, 2 h, 78% (2 steps).

5.2.1.7 *Synthesis of dipeptide 33*

To prepare tripeptide **34** from lanthionine **27** (Scheme 6), dipeptide **33** was necessary (Scheme 5). The γ-carboxylic acid function of (*R*)-Glutamic acid **29** was selectively esterified with methanolic HCl during a period of 2 h at rt (step i, Scheme 5).[26] Treatment with pyridine in aqueous MeOH gave the zwitterion **30**, that was converted to the α-*tert*-butyl ester **31** by transesterification with *tert*-butyl acetate (step ii).[26] After basification, diester **31** was reacted with Boc-(*S*)-alanine using carbonyl diimidazole (CDI) (step iii). The methyl ester of **32** was then deprotected with LiOH to give dipeptide **33** (step iv, Scheme 5).

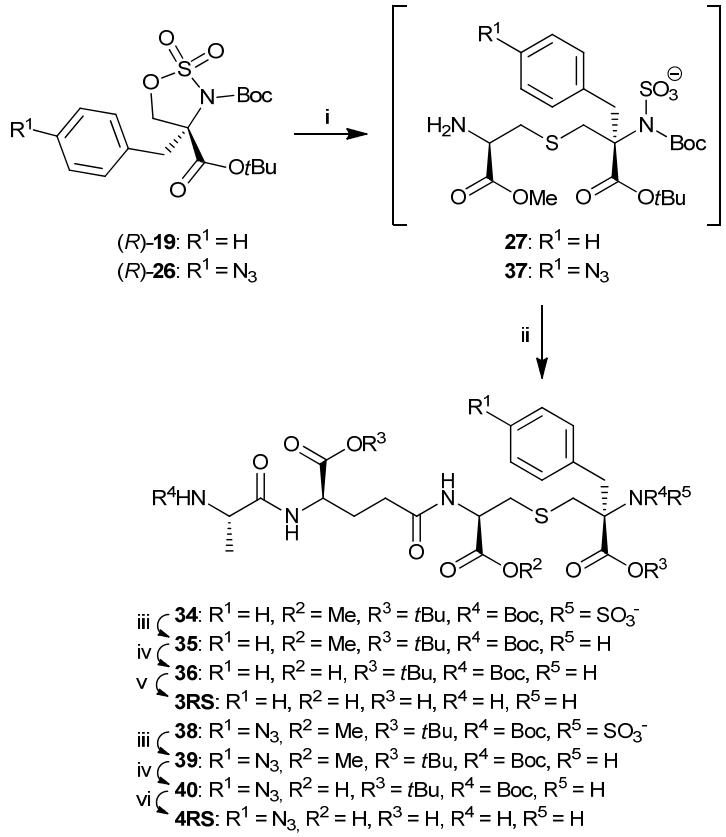


Scheme 5. Synthesis of dipeptide **33**. i) MeOH, AcCl (1.2 eq), rt, 2 h then pyridine (1.3 eq), aq. MeOH, 0 °C, 16 h, 50%; ii) *t*-BuOAc, H_2SO_4 (1.5 eq), rt, 24 h, 49%; iii) (*S*)-Boc-Ala, CDI, CH_2Cl_2, rt, 16 h, 45%; iv) $LiOH.H_2O$ (1.2 eq.), aq. CH_3CN, rt, 4 h then aq. $NaHSO_4$, 74%.

5.2.1.8 *Synthesis of tripeptides 3RS, 3RR, 4RS, 4RR*

To prepare tripeptides **35** and **39**, a three steps one pot reaction was considered (steps i, ii and iii; Scheme 6). First, the sulfamidates (*R*)-**19** and (*R*)-**26** were opened in CH_3CN with cysteine methyl ester and DBU (step i, Scheme 6). After solvent evaporation, coupling of **27** and **37** was realized in CH_2Cl_2 with activated **33** to afford tripeptides **34** and **38**, respectively (step ii). Activation of dipeptide **33** was previously done with HBTU in CH_2Cl_2. Substitution of CH_2Cl_2 for CH_3CN was required for high-yielding amide formation. The sulfamate function of **34** and **38** was then hydrolyzed (step iii) to provide, after chromatography, the protected tripeptides **35** and **39** in 78-84% (three steps). Removal of the methyl ester of the tripeptides **35** and **39** using LiOH in aqueous CH_3CN (16 h, rt) also gave partial hydrolysis of the *tert*-butyl ester on the glutamic acid moiety (step iv). The target tripeptide **3RS** was obtained by treatment of the crude product **36** with a large excess of TFA in CH_2Cl_2 (10%) (step v). Deprotection of the azido tripeptide **40** with this reagent resulted in partial *tert*-butylation of the phenylazido group even in the presence of triethylsilane.[27] Thus, an alternative deprotection method was selected, involving the use of HCl in dioxane, followed by a trituration of the dihydrochloride of **4RS** in Et_2O was selected (step vi, Scheme 6). The synthesis of the epimers **3RR** and **4RR** (figure 2) was done in the same way starting from sulfamidates (*S*)-**19** and (*S*)-**26**, respectively. The global yield was 55-69% (five steps). The proposed synthesis (Scheme 6), featuring a one pot procedure and only

one chromatography purification, should be suitable for future preparation of [^{35}S]tripeptides from commercially available [^{35}S]cysteine.



Scheme 6. Synthesis of tripeptides **3RS** and **4RS**. i) Cysteine Me ester*HCl (1 eq.), DBU (1.1 eq.), CH_3CN, rt, 1 h; ii) **33**, HBTU, DiPEA, CH_2Cl_2, rt, 16 h; iii) 10% aq. NaH_2PO_4, EtOAc. 50 °C, 2 h, 78-84% (3 steps); iv) LiOH (1.5 eq.), aq. CH_3CN, rt, 16 h, 94-97%; v) TFA, CH_2Cl_2, rt, 16 h; 73%; vi) HCl 10 M / dioxane : 1/3, 50 °C, 8 h; 88%.

5.2.2 Results of biological experiments

5.2.2.1 *In vitro assays with Mpl*

Tripeptides **3RS** ((*S*)-Ala-γ-(*R*)-Glu-(*R,S*)-α-benzyl-lanthionine) and its corresponding diastereomer **3RR** ((*S*)-Ala-γ-(*R*)-Glu-(*R,R*)-α-benzyl-lanthionine) (Figure 2) were incubated with ATP, UDP-[^{14}C]MurNAc and purified murein peptide ligase (Mpl, Figure 1). HPLC analysis of the reaction mixtures showed the appearance of new radioactive products (70% conversion with **3RS** and 40% with **3RR**, with respect to UDP-MurNAc). However, prolonged incubation (16 h) and a large amount of enzyme (2 µg/50 µL) were necessary (Table 2). To identify these new compounds, the reactions were repeated with non-labeled UDP-MurNAc and the products were purified by HPLC. Mass spectrometry analysis confirmed that they were UDP-MurNAc-tripeptides containing the respective lanthionine derivatives (Figure 5). A similar spectrum was obtained from **3RR**.

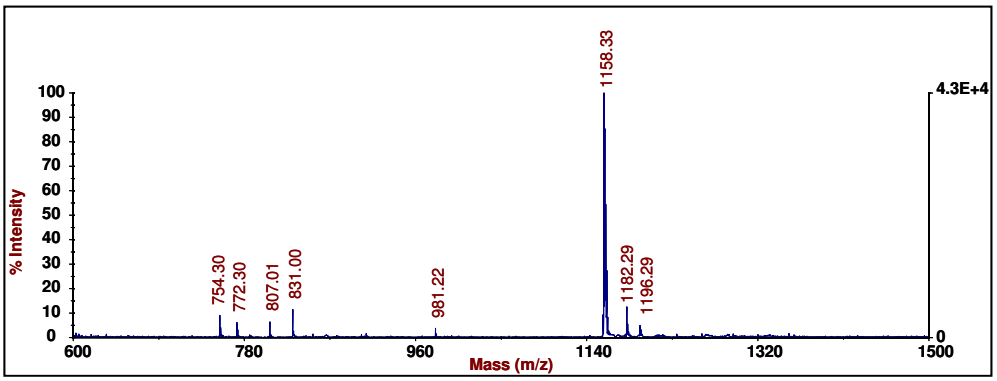


Figure 5. MALDI-TOF mass spectrometry (negative mode) of UDP-MurNAc-tripeptide. Theoretical mass $C_{41}H_{58}N_7O_{26}P_2S$: m/z = 1158.26.

5.2.2.2 *In vivo assays of bacterial growth*

Tripeptide **3RS** and **3RR** were evaluated in incorporation experiments. No growth of *E. coli* cells W7 was observed in minimal medium[28] (supplemented with 50 µg/mL (*S*)-Lys) in the presence of lanthionine **1** (50 µg/mL) or tripeptide **3RS** or **3RR** (50 µg/mL or 500 µg/mL). It has been shown that A_2pm auxotrophy in *E. coli* can be complemented by exogenous lanthionine in the presence of lysine in minimal medium after a preliminary growth with small amounts of A_2pm.[29] It is shown in Figure 6 that a minimal concentration of 1 µg/mL A_2pm is sufficient for the growth of bacterial cells.

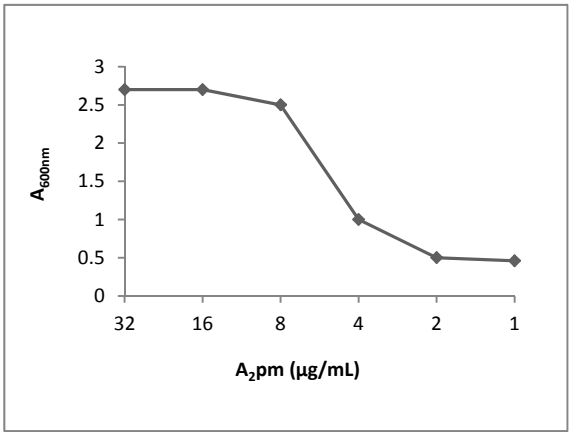


Figure 6. Optical density (600 nm) after 18 h of incubation at 37 °C in minimal medium. In the absence of A_2pm no growth was observed.

Therefore, bacteria were first grown in the presence of 1 µg/mL A_2pm. When the stationary phase was reached at t = 7 h, A_2pm, **3RS** or **3RR** (50 µg/mL) was added (Figure 7).

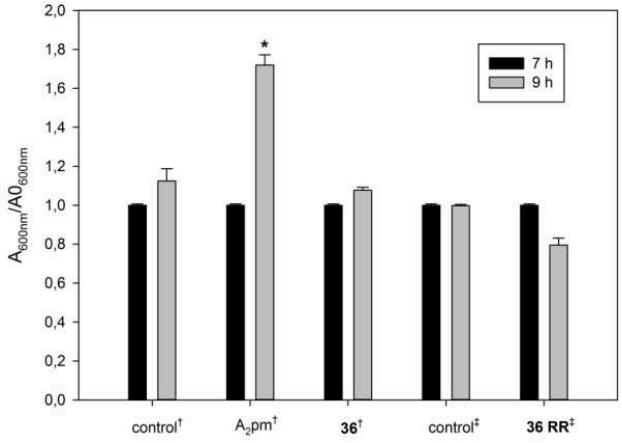


Figure 7. Compounds (A_2pm, **3RS**, **3RR**) were added at 50 µg/mL after the stationary phase of bacterial growth was reached. A_{600nm}: optical density of the different cultures, AO_{600nm}: optical density of culture at t = 7 h with 1 µg/mL A_2pm. Control: no addition at t = 7 h. * $p < 0.05$. † Experiment 1. ‡ Experiment 2 done under the same conditions.

At t = 9 h, with A_2pm, an increase of bacterial growth was observed. In contrast, with **3RS** or **3RR**, a small decrease was noticed. These last results are probably due to a coincidence arising from random sampling ($p > 0.05$) and not an indication of a poor incorporation or a toxic effect of these tripeptides (Figure 7).

5.2.2.3 *PG production and analysis with 3RS and 3RR*

The *in vivo* assays analyzing bacterial growth may not be sensitive enough to study the incorporation of **3RS** and **3RR**. Thus, one-liter cultures have been performed in minimal medium supplemented with 0.5% glucose, 1 µg/mL A_2pm and 50 µg/mL **3RS** or **3RR**. PGs of the different cultures have been purified, hydrolyzed and submitted to amino acid analysis. However, under these conditions, no incorporation of compound **3RS** or **3RR** was observed (data not shown).

Indeed, even if **3RS** and **3RR** are substrates of Mpl *in vitro*, as a high enzyme concentration and a long incubation time were necessary, these are poor substrates. Therefore, the tripeptide concentration (50 µg/mL, 0.1 mM) in our culture conditions was maybe too low. Thus, the following incorporation experiment with

3RS was done in the presence of a high tripeptide concentration (500 µg/mL, 1 mM).

Moreover, the transport of tripeptides from the culture medium into cells depends on murein peptide permease A (MppA, Figure 1).[30] If tripeptides are poor ligands of this permease, the tripeptide concentration inside the cells will be very low. The periplasmic protein MppA binds the tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm, which is then transported into the cytoplasm *via* membrane components of the oligopeptide permease (Opp) pathway.[30] In constrast to most transport systems, which are tightly regulated, the oligopeptide permease genes are expressed constitutively.[31] It is well known that the glucose concentration can significantly change the expression level of genes in *E. coli*.[32] The effect of glucose amount on the expression of MppA and Mpl is not known but incorporation experiments with natural and modified murein tripeptides described in literature (Table 2) are mostly done in minimal media supplemented with only 0.2% glucose.[4,31]

Thus, an incorporation experiment with **3RS** was done with a high tripeptide concentration (500 µg/mL, 1 mM) and a lower glucose concentration (0.2% instead of 0.5%) using minimal medium. However, once more no incorporation was observed (data not shown). Because of these negative results, no incorporation experiments were attempted with **4RS** or **4RR**.

5.2.2.4 *Discussion*

In *E. coli*, the first steps of incorporation into PG of tripeptides analogous to (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm depend on the MppA and Mpl activities, catalysing the tripeptide uptake and the synthesis of UDP-MurNAc-tripeptide, respectively.[4] The importance of the MppA activity is nicely shown by the results obtained by Hervé et al. with the tripeptide (*S*)-Ala-γ-(*R*)-Glu-ε-(*R*)-Lys.[3] This tripeptide is a good substrate of Mpl (Table 2), but probably a very poor ligand of MppA owing to the lack of the proximal carboxylate at the C-terminus, and thus was not incorporated *in vivo*.[4]

We have shown that the tripeptides, (*S*)-Ala-γ-(*R*)-Glu-(*R,S*)-α-benzyl-lanthionine **3RS** and (*S*)-Ala-γ-(*R*)-Glu-(*R,R*)-α-benzyl-lanthionine **3RR** are poor substrates of Mpl *in vitro* (Table 2). However, *in vivo*, no incorporation into the PG was observed, even in the presence of a high tripeptide concentration (1 mM). In the literature, the tagged (*S*)-Ala-γ-(*R*)-Glu-(*S*)-Lys analogue, (*S*)-Ala-γ-(*R*)-Glu-(*S*)-Lys-ε-NBD (**5**, Figure 2), is incorporated at a concentration of 1 mM.[5] Compared to the natural (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm tripeptide, the NBD-tripeptide **5** lacks one primary amino and one carboxylate. However, the presence of a bulky substituent like NBD bound to the ε-amino group is accepted.[5] Despite the fact that **3RS** and **3RR** possess the same amino and carboxylate functions as the natural tripeptide, they were not incorporated into PG even at 1 mM. It has been shown that (*R*,*R*) and (*R*,*S*)-lanthionine can effectively replace *meso*-A_2pm *in vivo*.[33-37] Thus, the sulfur group in the tripeptides is probably not responsible for the disappointing results of our *in vivo* experiments. However, the bulky benzyl group at the C_α carbon of the C-terminal amino acid probably disturbed the incorporation of **3RS** and **3RR**.

Table 2. Specificity of Mpl for tripeptide substrates

Tripeptide	Mpl
(S)-Ala-γ-(*R*)-Glu-*meso*-A_2pm	k_{cat}/Km = 2900 $mM^{-1}min^{-1}$ [3]
(S)-Ala-γ-(*R*)-Glu-(S)-Lys	k_{cat}/Km = 3.9 $mM^{-1}min^{-1}$ [3]
(S)-Ala-γ-(*R*)-Glu-ε-(*R*)-Lys	k_{cat}/Km = 2400 $mM^{-1}min^{-1}$ [4]
(S)-Ala-γ-(*R*)-Glu-*p*-amino-(S)-phenylalanine	50% conversion [4][a]
(S)-Ala-γ-(*R*)-Glu-*p*-nitro-(S)-phenylalanine	10% conversion [4][a]
(S)-Ala-γ-(*R*)-Glu-(*R,S*)-α-benzyl-lanthionine **3RS**	70% conversion [a]
(S)-Ala-γ-(*R*)-Glu-(*R,R*)-α-benzyl-lanthionine **3RR**	40% conversion [a]

[a] 1 mM tripeptide, 2 µg Mpl, 0.4 mM UDP-[^{14}C]MurNAc in 50 µL, 16 h at 37 °C.

5.3 Conclusion

In conclusion, we have developed a stereoselective synthesis of α-alkylated sulfamidates **19** and **26**. These efficient electrophiles were transformed into protected lanthionines **27** and **37** which, in turn, were readily converted into tripeptides **3RS, 3RR, 4RS** and **4RR** (Scheme 4). The tripeptides, (*S*)-Ala-γ-(*R*)-Glu-(*R,S*)-α-benzyl-lanthionine **3RS** and (*S*)-Ala-γ-(*R*)-Glu-(*R,R*)-α-benzyl-lanthionine **3RR**, are poor substrates of Mpl *in vitro.* No incorporation of these tripeptides in the PG of *E. coli* was observed *in vivo*.

5.4 Experimental section

5.4.1 Reagents

All solvents and chemicals were of analytical grade and used without further purification. DMF and CH_3CN were dried on 3 Å molecular sieves. TLC: Macherey-Nagel Polygram SIL G/UV_{254} using UV light or an anisaldehyde/sulphuric acid/AcOH in EtOH stain. CC: silica gel Acros, 0.060-0.200 mm, 60 Å. HPLC: Waters system (600 pump, 717 autosampler, 996 PDA detector) with a column XTerra RP18 (4.6 × 150 mm; 3.5 μm). 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. 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).

5.4.2 4-Methoxybenzonitrile 7

Compound **7** was synthesized according to a process previously described[12] but in a multimolar scale. In a 10 L round bottom flask fitted with an efficient condenser were placed *p*-anisaldehyde **6** (544 g, 4 mol), hydroxylamine.HCl (345 g, 5 mol) and pyridine (400 mL, 5 mol) (exothermic). The flask was left to cool to rt and formic acid (800 mL) was added with cooling. Boiling stones were added and the flask was carefully heated until an exothermic reaction began (ca. 80 °C) and heating was *immediately* stopped. **CAUTION**: the

reaction is very exothermic at this point and the solvents can be projected out of the flask if the condenser is not efficient enough or the flask is too small. After 1 h the reaction was over as indicated by a drop in temperature to 40 °C. The reaction mixture was poured on crushed ice (2 kg). The precipitated solid was filtered, washed with water and recrystallized in boiling MeOH. The product **7** was filtered on Büchner and dried *in vacuo* to constant weight. The *title compound* (450 g, 85%) was obtained as colourless needles. Mp 57-59 °C (lit.[38] 59 °C, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.70 – 7.53 (m, 2H, Ar-H), 7.08 – 6.85 (m, 2H, Ar-H), 3.89 (s, 3H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 162.83 (Ar-C), 133.91 (Ar-C), 119.21 (Ar-C), 114.75 (Ar-C), 103.84 (CN), 55.53 (OCH_3); *m/z* (ESI) 134 $[MH]^+$.

5.4.3 Ethyl 4-methoxybenzimidate hydrochloride 8

A solution of compound **7** (266 g, 2 mol) in dry EtOH (600 mL), under nitrogen and protected from moisture, was cooled to 0 °C. Through a pressure equalized addition funnel, AcCl (300 mL, 4.2 mol) was added dropwise followed by dry Et_2O (1 L). The solution was kept in a closed flask at 2 °C for one month. The product was filtered, washed with Et_2O and dried *in vacuo* to constant weight to give the *title compound* (230 g, 53%) as colourless crystals. Mp: 115 - 116 °C (dec.) (lit.[39] 132 - 134 °C); ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 12.18 (s, 1H, NH_2), 11.51 (s, 1H, NH_2), 8.38 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.01 (d, *J* = 9.0 Hz, 2H, Ar-H), 4.88 (q, *J* = 7.0 Hz, 2H, CH_2), 3.87 (s, 3H, OCH_3), 1.58 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 169.94 (CN), 165.25 (Ar-C), 131.84 (Ar-C), 116.69 (Ar-C), 114.32 (Ar-C), 70.42 (CH_2), 55.53 (OCH_3), 13.62 (CH_3); *m/z* (ESI) 180 $[MH]^+$.

5.4.4 Methyl 2-(4-methoxyphenyl)-oxazoline-4-carboxylate 9

Compound **9** was synthesized according to a process previously described for an analogous oxazoline.[9] The free base of **8** (179 g, 1 mol), generated from the salt by basification with saturated aqueous Na_2CO_3 and extraction in CH_2Cl_2, was dissolved in MeOH (1 L). To this solution was added serine methyl ester.HCl (155 g, 1 mol). After heating under reflux for 2 h, the suspension was cooled and diluted with acetone (1 L). The precipitated NH_4Cl was filtered and washed with acetone. The solvents were evaporated, the residue was dissolved in CH_2Cl_2 (500 mL) and filtered. The solvent was removed *in vacuo* and the product recrystallized in aqueous MeOH to give the *title compound* (160 g, 68%) as a colourless powder. Mp 119 - 121 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.98 - 7.78 (m, 2H, Ar-H), 6.98 - 6.79 (m, 2H, Ar-H), 4.90 (dd, *J* = 10.5, 7.8 Hz, 1H, H-α), 4.63 (dd, *J* = 8.7, 7.8 Hz, 1H, CH_2), 4.53 (dd, *J* = 10.5, 8.7 Hz, 1H, CH_2), 3.81 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, OCH_3);

^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.88 (CO), 166.14 (CN), 162.56 (Ar-C), 130.45 (Ar-C), 119.44 (Ar-C), 113.77 (Ar-C), 69.50 (C-β), 68.62 (C-α), 55.42 (ArO*C*H$_3$), 52.72 (OCH_3); *m/z* (ESI) 236 $[MH]^+$.

5.4.5 Sodium 2-(4-methoxyphenyl)-oxazoline-4-carboxylate 10

The synthesis was realized by slightly modifying the protocol described by Fry for an analogous derivative.[14] To a suspension of the methyl ester **9** (141 g, 600 mmol) in MeOH (500 mL), cooled to 0 °C, was added a cold solution of NaOH (26.4 g, 660 mmol) in water (220 mL). The mixture was stirred vigorously for 1 h. The suspension was diluted with acetone (500 mL) and left at 2 °C for 2 h. The product was filtered on Büchner and washed with acetone (1 L) then dried *in vacuo* to constant weight. The *title compound* (167 g, 99%) was obtained as rose crystals. Mp 220 °C; ^{1}H NMR (250 MHz, MeOD) δ_H 7.97 – 7.82 (m, 2H, Ar-H), 7.02 – 6.89 (m, 2H, Ar-H), 4.72 (dd, *J* = 10.3, 8.2 Hz, 1H, H-α), 4.62 (dd, *J* = 10.3, 7.7 Hz, 1H, CH_2O), 4.49 (dd, *J* = 8.2, 7.7 Hz, 1H, CH_2O), 3.83 (s, 3H, OCH_3); ^{13}C NMR (63 MHz, MeOD) δ_C 179.08 (CO), 166.66 (CN), 163.92 (Ar-C), 131.26 (Ar-C), 121.00 (Ar-C), 114.71 (Ar-C), 72.50 (C-β), 71.86 (C-α), 55.89 (OCH_3); *m/z* (ESI) 220 $[M]^-$.

5.4.6 2-(4-Methoxyphenyl)-oxazoline-4-carboxylic acid 11

The synthesis was realized by slightly modifying the protocol described by Fry for an analogous derivative.[14] The sodium salt **10** (167 g, 598 mmol) was suspended in water (500 mL) and this was cooled to 0 °C. A 30% aqueous formic acid solution (100 mL, 660 mmol) cooled to 0 °C was added dropwise to precipitate acid **11**. The product was filtered on Büchner and washed with cold water (500 mL), then dried by repeated suspension in CH_3CN and evaporation *in vacuo* to constant weight. The *title compound* (113 g, 85%) was obtained as rose crystals that were pure enough for the next step. Mp 142 °C (dec.); *m/z* (ESI) 220 $[M]^-$.

5.4.7 *tert*-Butyl 2-(4-methoxyphenyl)-oxazoline-4-carboxylate 12

The esterification was done as previously described for an analogous derivative.[8] Under nitrogen, in a 500 mL round bottom flask equipped with an efficient condenser, were placed **11** (55.2 g, 250 mmol), DMAP (6.1 g, 20 mol%), CH_2Cl_2 (400 mL) and *t*-BuOH (200 mL). The solution was heated at reflux and Boc_2O (108 g, 500 mmol) was added *via* syringe in two portions (20 minutes apart). **CAUTION:** This step produces substantial gas evolution. The mixture was heated for 1 h and the solvents were evaporated. The resulting yellow oil was dissolved in CH_2Cl_2 (400 mL) and MeOH (20 mL). The solution was prepurified by

passing through 80 g of silica which was washed with the same eluent (300 mL). The solvents were removed *in vacuo* and the oil was crystallized in Et_2O/hexanes to give the *title compound* (62 g, 90%) as pale yellow crystals. Mp 64 – 66 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.89 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.87 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.77 (dd, *J* = 10.2, 8.0 Hz, 1H, H-α), 4.63 – 4.36 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 170.70 (CO), 165.85 (CN), 162.39 (Ar-C), 130.39 (Ar-C), 119.71 (Ar-C), 113.67 (Ar-C), 82.01 ($C(CH_3)_3$), 69.75 (C-β), 69.45 (C-α), 55.37 (OCH_3), 28.06 ($C(CH_3)_3$); *m/z* (ESI) 278 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{15}H_{20}NO_4$ 278.1387, found 278.1385 $[MH]^+$.

5.4.8 (*R*)-*tert*-Butyl 4-benzyl-2-(4-methoxyphenyl)-oxazoline-4-carboxylate 14

Phenyloxazoline **12** (6.93 g, 25 mmol) was dissolved in toluene (250 mL). Benzyl bromide (12.8 g, 75 mmol) and (*R*)-**13** (102 mg, 0.5 mol%) were then added. The flask was flushed with nitrogen and cooled to -20 °C. $CsOH.H_2O$ (21 g, 125 mmol) was added, the flask capped and the mixture vigorously stirred for 72 h at -20 °C. The suspension was diluted with hexanes (250 mL), filtered and the organic layer flash chromatographied on 100 g of silica. Elution was done first with hexanes to remove excess BnBr, then with 2:3 (EtOAc/hexanes) to recover **14**. The solvent was evaporated *in vacuo* to give the *title compound* (9.2 g, 99%) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.95 – 7.82 (m, 2H, Ar-H), 7.30 – 7.11 (m, 5H, Ar-H), 6.91 – 6.79 (m, 2H, Ar-H), 4.63 (d, *J* = 8.9 Hz, 1H, CH_2O), 4.27 (d, *J* = 8.9 Hz, 1H, CH_2O), 3.77 (s, 3H, OCH_3), 3.27 (d, *J* = 13.8 Hz, 1H, CH_2Ph), 3.19 (d, *J* = 13.8 Hz, 1H, CH_2Ph), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.43 (CO), 164.27 (CN), 162.18 (Ar-C), 135.76 (Ar-C), 130.28 (Ar-C), 130.18 (Ar-C), 128.03 (Ar-C), 126.69 (Ar-C), 119.75 (Ar-C), 113.49 (Ar-C), 81.86 ($C(CH_3)_3$), 78.73 (C-α), 72.72 (CH_2O), 55.16 (OCH_3), 43.21 (CH_2Ph), 27.82 ($C(CH_3)_3$); *m/z* (ESI) 368 $[MH]^+$.

5.4.9 (*R*)-*tert*-Butyl 2-benzyl-3-hydroxy-2-(4-methoxybenzylamino) propanoate 15

A freshly prepared solution of $NaBH_3CN$ (10 g, 159 mmol) in AcOH (200 mL), cooled to 0 °C, was added to the oxazoline **14** (25 mmol) and the mixture was stirred at rt for 16 h. The solvent was removed *in vacuo* and the residue partitioned between EtOAc and a saturated Na_2CO_3 solution. The basic aqueous layer was extracted twice with EtOAc and the pooled organic fractions were washed twice with water and brine. After drying over $MgSO_4$ and

filtration, the solvent was removed *in vacuo* to give the *title compound* (9.2 g, 99%) as colourless oil that crystallized on standing at 2 °C. Mp 49 – 52 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.33 – 7.23 (m, 7H, Ar-H), 6.94 – 6.85 (m, 2H, Ar-H), 3.79 (s, 3H, OCH_3), 3.79 (d, J = 11.5 Hz, 1H, CH_2O), 3.71 (d, J = 11.8 Hz, 1H, CH_2N), 3.66 (d, J = 11.5 Hz, 1H, CH_2O), 3.65 (d, J = 11.8 Hz, 1H, CH_2N), 3.07 (d, J = 13.4 Hz, 1H, CH_2Ph), 2.95 (d, J = 13.4 Hz, 1H, CH_2Ph), 2.46 (br, 2H, NH, OH), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 172.72 (CO), 158.82 (Ar-C), 135.68 (Ar-C), 131.90 (Ar-C), 130.32 (Ar-C), 129.28 (Ar-C), 128.10 (Ar-C), 126.79 (Ar-C), 113.93 (Ar-C), 81.85 ($C(CH_3)_3$), 66.97 (C-α), 60.82 (CH_2O), 55.15 (OCH_3), 46.74 (CH_2N), 40.13 (CH_2Ph), 28.03 ($C(CH_3)_3$); *m/z* (ESI) 372 $[MH]^+$.

5.4.10 (4*R*)-*tert*-Butyl 4-benzyl-3-(4-methoxybenzyl)-2-oxo-1,2,3-oxathiazolidine-4-carboxylate 16

In a dry round bottom flask under nitrogen were added compound **15** (25 mmol), imidazole [20] (6.8 g, 100 mmol), NEt_3 (10.4 mL, 75 mmol) and CH_2Cl_2 (125 mL). The mixture was cooled to -10 °C and $SOCl_2$ (2.7 mL, 37.5 mmol) was added dropwise *via* syringe. The solution was kept for 30 min at -10 °C then allowed to return to rt during 2 h. The mixture was diluted with water (50 mL) and thereafter with 10% aqueous $NaHSO_4$ (150 mL). The organic layer was separated and the aqueous layer extracted once with CH_2Cl_2. The pooled organic fractions were washed twice with water and brine. The solution was dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to give the *title compound* (10.2 g, 98%) as yellow oil, mixture of diastereoisomers.

5.4.11 (4*R*)-*tert*-Butyl 4-benzyl-3-(4-methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 17

The crude sulfamidite **16** (10.2 g, 24 mmol) was dissolved in CH_3CN (500 mL) and the solution cooled to 0 °C. $RuCl_3.x\ H_2O$ (52 mg, 1 mol%) was added followed by $NaIO_4$ (6 g, 28 mmol) and water (250 mL).[9] 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 (250 mL) and brine (250 mL). The aqueous layer was extracted with Et_2O (3× 250 mL). The pooled organic fractions were washed twice with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents evaporated *in vacuo* to yield crude **17**. After flash chromatography on silica with 1:4 (EtOAc/hexanes), the solvents were removed *in vacuo* to give the *title compound* (8.5 g, 80%) as a golden oil. ^{1}H NMR

(250 MHz, $CDCl_3$) δ_H 7.51 – 7.34 (m, 2H, Ar-H), 7.34 – 7.16 (m, 3H, Ar-H), 7.16 – 7.00 (m, 2H, Ar-H), 7.00 – 6.77 (m, 2H, Ar-H), 4.63 (d, *J* = 8.8 Hz, 1H, CH_2O), 4.62 (d, *J* = 15.4 Hz, 1H, CH_2N), 4.55 (d, *J* = 15.4 Hz, 1H, CH_2N), 4.43 (d, *J* = 8.8 Hz, 1H, CH_2O), 3.77 (s, 3H, OCH_3), 3.38 (d, *J* = 13.4 Hz, 1H, CH_2Ph), 2.83 (d, *J* = 13.4 Hz, 1H, CH_2Ph), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.00 (CO), 159.35 (Ar-C), 132.41 (Ar-C), 129.81 (Ar-C), 128.72 (Ar-C), 127.82 (Ar-C), 127.64 (Ar-C), 113.89 (Ar-C), 84.38 ($C(CH_3)_3$), 72.20 (CH_2O), 69.02 (C-α), 55.10 (OCH_3), 46.49 (CH_2N), 39.85 (CH_2Ph), 27.66 ($C(CH_3)_3$).

5.4.12 (4*R*)-*tert*-Butyl 4-benzyl-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 18

To a stirred solution of sulfamidate **17** (1.0 g, 2.3 mmol) in CH_3CN (23 mL) and water (6 mL) was added CAN[11] (3.8 g, 6.9 mmol). After stirring at rt for 20 min, the solution was diluted with CH_2Cl_2 (100 mL) and decanted. The organic layer was washed with water (100 mL), saturated $NaHCO_3$ (100 mL) and dried over $MgSO_4$. After filtration, the solvents were evaporated *in vacuo* to yield crude **18**. Chromatography on silica with 3:7 (EtOAc/hexanes) followed by removal of solvents afforded an oil which was crystallized in Et_2O/hexanes. The *title compound* (380 mg, 53%) was obtained as colourless crystals. Mp 78 – 80 °C; ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.38 (s, 5H, Ar-H), 5.50 (b, 1H, NH), 4.82 (d, *J* = 9.2 Hz, 1H, CH_2O), 4.52 (d, *J* = 9.2 Hz, 1H, CH_2O), 3.38 (d, *J* = 13.7 Hz, 1H, CH_2Ph), 3.22 (d, *J* = 13.7 Hz, 1H, CH_2Ph), 1.51 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.65 (CO), 133.82 (Ar-C), 130.22 (Ar-C), 128.43 (Ar-C), 127.78 (Ar-C), 85.65 ($C(CH_3)_3$), 74.69 (CH_2O), 68.57 (C-α), 42.49 (CH_2Ph), 27.75 ($C(CH_3)_3$); HRMS *m/z* (ES+) Calcd for $C_{14}H_{19}LiNO_5S$ 320.1139, found 320.1138 $[MLi]^+$.

5.4.13 (4*R*)-*tert*-Butyl 4-benzyl-3-(*tert*-butyloxycarbonyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 19

To a solution of sulfamidate **18** (207 mg, 0.66 mmol) in CH_3CN (4 mL) were added DMAP (8 mg, 10 mol%), Boc_2O (174 mg, 0.66 mmol) and one drop of NEt_3. After stirring at rt for 16 h, the solvent was evaporated and the resulting oil was chromatographied on silica with 1:9 (EtOAc/hexanes). Evaporation of the solvent give the *title compound* (253 mg, 93%) as colourless crystals. ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.43 – 7.24 (m, 3H, Ar-H), 7.24 – 7.06 (m, 2H, Ar-H), 4.45 (d, *J* = 9.7 Hz, 1H, CH_2O), 4.41 (d, *J* = 9.7 Hz, 1H, CH_2O), 3.68 (d, *J* = 14.7 Hz, 1H, CH_2Ph), 3.24 (d, *J* = 14.7 Hz, 1H, CH_2Ph), 1.60 (s, 9H, $C(CH_3)_3$), 1.52 (s,

9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.82 (CO), 148.50 (Boc-CO), 133.41 (Ar-C), 130.55 (Ar-C), 128.73 (Ar-C), 127.79 (Ar-C), 85.64 ($C(CH_3)_3$), 84.25 ($C(CH_3)_3$), 70.00 (CH_2O), 68.55 (C-α), 37.30 (CH_2Ph), 27.97 ($C(CH_3)_3$), 27.72 ($C(CH_3)_3$).

5.4.14 (*R*)-*tert*-Butyl 4-(4-bromobenzyl)-2-(4-methoxyphenyl)-oxazoline-4-carboxylate 20

Phenyloxazoline **12** (2.77 g, 10 mmol) was dissolved in toluene (100 mL). *p*-Bromo benzyl bromide (3.75 g, 15 mmol) and (*R*)-**13** (83 mg, 1 mol%) were then added. The flask was flushed with nitrogen and cooled to -20 °C. $CsOH.H_2O$ (8.4 g, 50 mmol) was added, the flask was capped and the mixture was vigorously stirred for 72 h at -20 °C. The suspension was diluted with hexanes (100 mL) and filtered on a Büchner funnel. The organic layer was flash chromatographed on 100 g of silica that was eluted first with hexanes to remove excess electrophile. The product was eluted with 2:3 (EtOAc/hexanes). The solvent was removed *in vacuo* to give the title compound (4.4 g, 99%) as a yellow oil. ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.91 - 7.81 (m, 2H, Ar-H), 7.36 - 7.27 (m, 2H, Ar-H), 7.15 - 7.06 (m, 2H, Ar-H), 6.90 - 6.80 (m, 2H, Ar-H), 4.60 (d, J = 8.9 Hz, 1H, CH_2O), 4.20 (d, J = 8.9 Hz, 1H, CH_2O), 3.77 (s, 3H, OCH_3), 3.18 (d, J = 13.8 Hz, 1H, CH_2Ar), 3.11 (d, J = 13.8 Hz, 1H, CH_2Ar), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.25 (CO), 164.47 (CN), 162.23 (Ar-C), 134.81 (Ar-C), 132.06 (Ar-C), 131.06 (Ar-C), 130.19 (Ar-C), 120.79 (Ar-C), 119.50 (Ar-C), 113.53 (Ar-C), 82.04 ($C(CH_3)_3$), 78.40 (C-α), 72.79 (CH_2O), 55.18 (OCH_3), 42.51 (CH_2Ar), 27.83 ($C(CH_3)_3$); *m/z* (ESI) 446/448 $[MH]^+$.

5.4.15 (*R*)-*tert*-Butyl 4-(4-azidobenzyl)-2-(4-methoxyphenyl)-oxazoline-4-carboxylate 21

The synthesis was done following the general protocol described by Andersen.[18] To compound **20** (4.2 g, 9.4 mmol) in EtOH (100 mL) were added NaN_3 (1.3 g, 20 mmol), sodium ascorbate (300 mg, 15 mol%), CuI (380 mg, 20 mol%), DMEDA (264 mg, 30 mol%) and water (43 mL). The flask was purged with nitrogen and heated under reflux for 5 h. After EtOH evaporation, the aqueous layer was extracted three times with EtOAc. The pooled fractions were washed twice with 12.5% ammonia and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent removed to give the *title compound* (3.88 g, 99%) as a yellow oil. ^1H NMR (250 MHz, $CDCl_3$) δ_H 8.01 – 7.88 (m, 2H, Ar-H), 7.39 – 7.22 (m, 2H, Ar-H), 7.02 – 6.85 (m, 4H, Ar-H), 4.68 (d, J = 8.9 Hz, 1H, CH_2O), 4.31 (d, J = 8.9 Hz, 1H, CH_2O), 3.89 (s, 3H, OCH_3), 3.31 (d, J = 13.8 Hz, 1H, CH_2Ar), 3.23 (d, J = 13.8 Hz, 1H,

CH_2Ar), 1.54 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.49 (CO), 164.48 (CN), 162.31 (Ar-C), 138.56 (Ar-C), 132.62 (Ar-C), 131.80 (Ar-C), 130.26 (Ar-C), 119.68 (Ar-C), 118.69 (Ar-C), 113.60 (Ar-C), 82.11 ($C(CH_3)_3$), 78.70 (C-α), 72.82 (CH_2O), 55.30 (OCH_3), 42.58 (CH_2Ar), 27.93 ($C(CH_3)_3$); *m/z* (ESI) 409 $[MH]^+$.

5.4.16 (*R*)-*tert*-Butyl 2-(4-azidobenzyl)-3-hydroxy-2-(4-methoxy benzylamino)propanoate 22

A freshly prepared solution of $NaBH_3CN$ (4 g, 159 mmol) in AcOH (80 mL), was cooled to 0 °C and added to the oxazoline **21** (3.7 g, 10 mmol). After stirring at rt for 16 h, the solvent was removed *in vacuo.* The residue was partitioned between a saturated Na_2CO_3 solution and EtOAc. The basic aqueous layer was extracted twice with EtOAc and the pooled organic layers were washed twice with water and brine. The solution was dried over $MgSO_4$. After filtration, the solvent was removed to give the *title compound* (3.3 g, 89%) as an amber oil. ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.34 – 7.10 (m, 4H, Ar-H), 7.02 – 6.76 (m, 4H, Ar-H), 3.78 (s, 3H, OCH_3), 3.72 (d, J = 11.5 Hz, 1H, CH_2O), 3.66 (d, J = 11.9 Hz, 1H, CH_2N), 3.60 (d, J = 11.9 Hz, 1H, CH_2N), 3.57 (d, J = 11.5 Hz, 1H, CH_2O), 2.99 (d, J = 13.5 Hz, 1H, CH_2Ar), 2.88 (d, J = 13.5 Hz, 1H, CH_2Ar), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 172.69 (CO), 158.85 (Ar-C), 138.56 (Ar-C), 132.49 (Ar-C), 131.79 (Ar-C), 131.71 (Ar-C), 129.27 (Ar-C), 118.68 (Ar-C), 113.95 (Ar-C), 82.05 ($C(CH_3)_3$), 66.96 (C-α), 60.62 (CH_2O), 55.19 (OCH_3), 46.80 (CH_2N), 39.42 (CH_2Ar), 28.09 ($C(CH_3)_3$); *m/z* (ESI) 413 $[MH]^+$.

5.4.17 (4*R*)-*tert*-Butyl 4-(4-azidobenzyl)-3-(4-methoxybenzyl)-2-oxo-1,2,3-oxathiazolidine-4-carboxylate 23

In a dry round bottom flask under nitrogen and stirring were added compound **22** (3 g, 7.3 mmol), imidazole (2.0 g, 29 mmol), NEt_3 (3 mL, 22 mmol) and CH_2Cl_2 (40 mL). To this mixture previously cooled to -10 °C, $SOCl_2$ (0.79 mL, 11.0 mmol) was added dropwise *via* syringe. The solution was kept for 30 min at -10 °C and allowed to return to rt during 2 h. The mixture was diluted with water (20 mL), thereafter with 10% aqueous $NaHSO_4$ (40 mL). The organic layer was recovered and the aqueous layer was extracted once with CH_2Cl_2. The pooled organic fractions were washed twice with water and brine. After drying over $MgSO_4$, and filtration, the solvent was evaporated to give the *title compound* (3.3 g, 98%) as a yellow oil, mixture of diastereoisomers. ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.41 – 7.26 (m, 2H, Ar-H), 7.23 – 7.05 (m, 2H, Ar-H), 7.00 – 6.78 (m, 4H, Ar-H), 5.04 (d, J = 8.4 Hz, 0.5H, CH_2O),

4.81 (d, *J* = 9.2 Hz, 0.5H, CH_2O), 4.76 (d, *J* = 9.2 Hz, 0.5H, CH_2O), 4.60 (d, *J* = 13.6 Hz, 0.5H, CH_2N), 4.45 (d, *J* = 15.0 Hz, 0.5H, CH_2N), 4.39 (d, *J* = 15.0 Hz, 0.5H, CH_2N), 4.28 (d, *J* = 13.6 Hz, 0.5H, CH_2N), 4.26 (d, *J* = 8.4 Hz, 0.5H, CH_2O), 3.75 (s, 3H, OCH_3), 3.54 (d, *J* = 13.4 Hz, 0.5H, CH_2Ar), 3.39 (d, *J* = 13.4 Hz, 0.5H, CH_2Ar), 3.09 (d, *J* = 13.4 Hz, 0.5H, CH_2Ar), 2.82 (d, *J* = 13.4 Hz, 0.5H, CH_2Ar), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.57 (0.5C, CO), 168.48 (0.5C, CO), 159.28 (0.5C, Ar-C), 159.25 (0.5C, Ar-C), 139.24 (0.5C, Ar-C), 139.11 (0.5C, Ar-C), 131.57 (0.5C, Ar-C), 131.10 (Ar-C), 130.99 (Ar-C), 130.88 (0.5C, Ar-C), 130.15 (Ar-C), 130.12 (Ar-C), 128.37 (0.5C, Ar-C), 128.25 (0.5C, Ar-C), 119.04 (Ar-C), 119.01 (Ar-C), 113.89 (Ar-C), 83.29 (0.5C, $C(CH_3)_3$), 83.11 (0.5C, $C(CH_3)_3$), 77.67 (0.5C, CH_2O), 75.89 (0.5C, CH_2O), 70.42 (0.5C, C-α), 69.15 (0.5C, C-α), 55.06 (0.5C, OCH_3), 55.05 (0.5C, OCH_3), 45.74 (0.5C, CH_2N), 45.66 (0.5C, CH_2N), 39.87 (0.5C, CH_2Ar), 39.80 (0.5C, CH_2Ar), 27.74 (0.5C, $C(CH_3)_3$), 27.62 (0.5C, $C(CH_3)_3$).

5.4.18 (4*R*)-*tert*-Butyl 4-(4-azidobenzyl)-3-(4-methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 24

The crude sulfamidite **23** (3.3 g, 7.2 mmol) was dissolved in CH_3CN (200 mL) and the solution was cooled to 0 °C. $RuCl_3.xH_2O$ (15 mg, 1 mol%) was added, followed by $NaIO_4$ (1.73 g, 8.1 mmol) and water (100 mL). The green-brown solution with a white precipitate was stirred for 15 min at 0 °C. After 4 h of stirring at rt, the mixture was diluted with Et_2O (100 mL) and brine (100 mL). The aqueous layer was extracted with Et_2O (3 × 100 mL) and the pooled organic fractions were washed twice with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$. After filtration, the solvents were evaporated to yield the crude product **24** that was purified by flash chromatography on silica with 1:4 (EtOAc/hexanes). Evaporation of the solvents gave the *title compound* (2.6 g, 75%) as a golden oil. ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.43 – 7.34 (m, 2H, Ar-H), 7.12 – 7.03 (m, 2H, Ar-H), 6.98 – 6.84 (m, 4H, Ar-H), 4.61 (d, *J* = 8.8 Hz, 1H, CH_2O), 4.60 (d, *J* = 15.1 Hz, 1H, CH_2N), 4.50 (d, *J* = 15.1 Hz, 1H, CH_2N), 4.39 (d, *J* = 8.8 Hz, 1H, CH_2O), 3.79 (s, 3H, OCH_3), 3.36 (d, *J* = 13.5 Hz, 1H, CH_2Ar), 2.80 (d, *J* = 13.5 Hz, 1H, CH_2Ar), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.05 (CO), 159.50 (Ar-C), 139.87 (Ar-C), 131.38 (Ar-C), 129.93 (Ar-C), 129.21 (Ar-C), 127.61 (Ar-C), 119.39 (Ar-C), 114.05 (Ar-C), 84.76 ($C(CH_3)_3$), 71.99 (CH_2O), 69.09 (C-α), 55.27 (OCH_3), 46.67 (CH_2N), 39.28 (CH_2Ar), 27.85 ($C(CH_3)_3$).

5.4.19 (4*R*)-*tert*-Butyl 4-(4-azidobenzyl)-2,2-dioxo-1,2,3-oxathiazol idine-4-carboxylate 25

To a solution of compound **24** (2.6 g, 5.5 mmol) in CH_3CN (60 mL) and water (20 mL) was added CAN (9.0 g, 16.4 mmol). The mixture was stirred at rt for 30 min and then diluted with CH_2Cl_2 (100 mL). After decantation, the organic layer was washed with water (100 mL) and saturated $NaHCO_3$ (100 mL). Evaporation *in vacuo* yielded a wet oil which was taken up in CH_2Cl_2 and flash chromatographied on silica with CH_2Cl_2 as an eluent. The yellow oil obtained after solvent removal was crystallized in 1:2 (Et_2O/hexanes) to give the *title compound* (1.6 g, 80%) as beige needles. Mp 100 – 101 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.26 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.95 (d, *J* = 8.1 Hz, 2H, Ar-H), 5.44 (br, 1H, NH), 4.69 (d, *J* = 9.2 Hz, 1H, CH_2O), 4.40 (d, *J* = 9.2 Hz, 1H, CH_2O), 3.25 (d, *J* = 13.8 Hz, 1H, CH_2Ar), 3.11 (d, *J* = 13.8 Hz, 1H, CH_2Ar), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 168.53 (CO), 139.66 (Ar-C), 131.71 (Ar-C), 130.55 (Ar-C), 119.00 (Ar-C), 85.91 ($C(CH_3)_3$), 74.65 (CH_2O), 68.49 (C-α), 41.80 (CH_2Ar), 27.87 ($C(CH_3)_3$); HRMS *m*/*z* (ES+) Calcd for $C_{14}H_{18}LiN_4O_5S$ 361.1153, found 361.1151 $[MLi]^+$.

5.4.20 (4*R*)-*tert*-Butyl 4-(4-azidobenzyl)-3-(*tert*-butyloxycarbonyl)-2,2-dioxo-1,2,3-oxathiazolidine-4-carboxylate 26

To compound **25** (234 mg, 0.66 mmol) in CH_3CN (4 mL) were added DMAP (8 mg, 10 mol%), Boc_2O (174 mg, 0.66 mmol) and one drop of NEt_3. The solution was stirred at rt for 16 h. The solvent was evaporated *in vacuo* and the obtained oil was chromatographed on silica with 1:9 (EtOAc/hexanes). After solvent evaporation, the *title compound* (269 mg, 90%) was recovered as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.22 – 7.08 (m, 2H, Ar-H), 7.08 – 6.93 (m, 2H, Ar-H), 4.45 (d, *J* = 9.4 Hz, 1H, CH_2O), 4.37 (d, *J* = 9.4 Hz, 1H, CH_2O), 3.65 (d, *J* = 14.8 Hz, 1H, CH_2Ar), 3.21 (d, *J* = 14.8 Hz, 1H, CH_2Ar), 1.59 (s, 9H, $C(CH_3)_3$), 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.55 (CO), 148.42 (Boc-CO), 139.60 (Ar-C), 131.91 (Ar-C), 130.14 (Ar-C), 119.32 (Ar-C), 85.79 ($C(CH_3)_3$), 84.38 ($C(CH_3)_3$), 70.03 (CH_2O), 68.47 (C-α), 36.80 (CH_2Ar), 27.96 ($C(CH_3)_3$), 27.71 ($C(CH_3)_3$).

5.4.21 (*S*)-*tert*-Butyl 3-((*R*)-2-amino-3-methoxy-3-oxopropylthio)-2-benzyl-2-(*tert*-butoxycarbonylamino)propanoate 28

Under nitrogen, to a degassed solution of sulfamidate **19** (100 mg, 0.24 mmol) and cysteine methyl ester.HCl (42 mg, 0.24 mmol) in CH_3CN (4 mL) was added *via* syringe, a solution of

DBU (84 mg, 0.55 mmol) in degassed CH_3CN (1 mL). After stirring for 1 h at rt, EtOAc (20 mL) and 10% aqueous NaH_2PO_4 (20 mL) were added. The biphasic mixture containing **27** was heated with stirring at 50 °C for 2 h. After basification by pouring in an excess of saturated Na_2CO_3 solution, the organic layer was recovered and the aqueous layer was extracted with EtOAc (3 × 20 mL). The pooled organic fractions were washed with brine and dried over $MgSO_4$. After filtration, evaporation and chromatography on silica with 1:1 (EtOAc/hexanes), the *title compound* (88 mg, 78%) was obtained as a colourless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.49 – 7.14 (m, 3H, Ar-H), 7.14 – 6.91 (m, 2H, Ar-H), 5.60 (s, 1H, NH-Boc), 3.80 – 3.71 (m, 1H, H-α), 3.69 (s, 3H, OCH_3), 3.65 (d, *J* = 13.5 Hz, 1H, SCH_2C), 3.55 (d, *J* = 13.5 Hz, 1H, CH_2Ph), 3.05 (d, *J* = 13.5 Hz, 1H, SCH_2C), 2.97 (d, *J* = 13.5 Hz, 1H, CH_2Ph), 2.95 (dd, *J* = 13.3, 4.6 Hz, 1H, $CHCH_2S$), 2.77 (dd, *J* = 13.3, 8.0 Hz, 1H, $CHCH_2S$), 1.80 (br, 2H, NH_2), 1.44 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 174.30 (CO), 170.27 (CO), 154.22 (CO-Boc), 135.86 (Ar-C), 129.99 (Ar-C), 128.10 (Ar-C), 127.00 (Ar-C), 83.44 ($C(CH_3)_3$), 79.40 ($C(CH_3)_3$), 65.85 (C-α), 54.56 (CH-α), 52.16 (OCH_3), 40.82 (CH_2Ph), 38.40 (SCH_2C), 38.05 ($CHCH_2S$), 28.44 ($C(CH_3)_3$), 28.01 ($C(CH_3)_3$); *m/z* (ESI) 469 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{23}H_{37}N_2O_6S$ 469.2367, found 469.2367 $[MH]^+$.

5.4.22 (*R*)-1-*tert*-Butyl 5-methyl 2-aminopentanedioate 31

In a reactor equipped with a mechanical stirrer were placed (*R*)-2-amino-5-methoxy-5-oxopentanoic acid **30** (8.05 g) and *tert*-BuOAc (150 mL). The reactor was cooled to 0 °C and concentrated H_2SO_4 (4.1 mL, 75 mmol) was added. The reactor was closed and stirring was continued at rt for 24 h. The mixture was poured on an excess of 10% aqueous Na_2CO_3. After extraction with Et_2O (3 x 200 mL), the pooled organic layers were washed with water and brine. Drying over Na_2CO_3, followed by filtration and solvent evaporation gave the *title compound* (5.3 g, 49 %) as a colourless oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 3.52 (s, 3H, OCH_3), 3.18 (dd, *J* = 8.2, 5.3 Hz, 1H), 2.31 (t, *J* = 7.6 Hz, 2H), 1.95 - 1.79 (m, 1H), 1.74 - 1.55 (m, 1H), 1.37 (b, 2H), 1.31 (s, 9H); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 174.69 (CO), 173.48 (CO), 80.90 ($C(CH_3)_3$), 54.16 (CH), 51.37 (OCH_3), 30.27 (CH_2), 29.70 (CH_2), 27.84 ($C(CH_3)_3$); *m/z* (ESI) 218 $[MH]^+$.

5.4.23 (*R*)-1-*tert*-Butyl 5-methyl 2-((*S*)-2-(*tert*-butoxycarbonylamino) propanamido)pentanedioate 32

(*S*)-Boc-alanine (4.73 g, 25 mmol) and CDI (4.05 g, 25 mmol) were stirred for 1 h in CH_2Cl_2 (75 mL). A solution of **31** (5.2 g, 24 mmol) in CH_2Cl_2 was then added. After stirring at rt for 16 h, the organic layer was washed successively with 10% aqueous $NaHSO_4$, water, saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was removed to give the crude product **32**. After recrystallisation from Et_2O/hexanes the *title compound* (4.2 g, 45%) was obtained as off-white crystals. Mp 109 – 110 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 6.87 (br d, *J* = 6.7 Hz, 1H, NH), 5.23 (br d, *J* = 6.9 Hz, 1H, NH-Boc), 4.50 - 4.33 (m, 1H, H-α-Glu), 4.14 (b, 1H, H-α-Ala), 3.59 (s, 3H, OCH_3), 2.45 - 2.21 (m, 2H, CH_2CO), 2.21 - 2.01 (m, 1H, CH_2), 2.01 - 1.75 (m, 1H, CH_2), 1.38 (s, 9H, $C(CH_3)_3$), 1.37 (s, 9H, $C(CH_3)_3$), 1.29 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 173.15 (CO-γ-Glu), 172.70 (CO-Ala), 170.75 (CO-Glu), 155.41 (CO-Boc), 82.29 (*C*-$(CH_3)_3$), 79.94 (*C*$(CH_3)_3$), 51.95 (C-α-Glu), 51.68 (OCH_3), 50.16 (C-α-Ala), 29.89 (*C*H_2CO), 28.27 (C(*C*$H_3)_3$), 27.93 (C(*C*$H_3)_3$), 27.56 (CH_2), 18.39 (CH_3); *m/z* (ESI) 389 $[MH]^+$.

5.4.24 (*R*)-5-*tert*-Butoxy-4-((*S*)-2-(*tert*-butoxycarbonylamino) propanamido)-5-oxopentanoic acid 33

Compound **32** (4.66 g, 12 mmol) was dissolved in CH_3CN (36 mL). A solution of $LiOH.H_2O$ (605 mg, 14.4 mmol) in water (36 mL) was added. After stirring at rt for 4 h, the CH_3CN was evaporated. The aqueous layer was washed twice with Et_2O and acidified with 10% aqueous $NaHSO_4$. The precipitated solid was filtered and washed with cold water. After drying *in vacuo* to constant weight, the *title compound* (3.3 g, 74%) was obtained as a colourless solid. Mp 168 – 170 °C; ^{1}H NMR (250 MHz, MeOD) δ_H 8.01 (b, 1H, NH), 4.41 – 4.22 (m, 1H, H-α-Glu), 4.07 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 2.37 (t, *J* = 7.5 Hz, 2H, CH_2CO), 2.24 – 2.03 (m, 1H, CH_2), 2.03 – 1.80 (m, 1H, CH_2), 1.47 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.31 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, MeOD) δ_C 176.05 (CO-γ-Glu), 175.85 (CO-Ala), 172.06 (CO-Glu), 157.37 (CO-Boc), 82.95 (*C*$(CH_3)_3$), 80.57 (*C*$(CH_3)_3$), 53.64 (C-α-Glu), 51.63 (C-α-Ala), 30.94 (*C*H_2CO), 28.69 (C(*C*$H_3)_3$), 28.22 (C(*C*$H_3)_3$), 27.73 (CH_2), 18.43 (CH_3); *m/z* (ESI) 373 $[M]^-$; HRMS *m/z* (ES+) Calcd for $C_{17}H_{31}N_2O_7$ 375.2126, found 375.2125 $[MH]^+$.

5.4.25 (*S*)-*tert*-Butyl 3-((*R*)-2-((*R*)-5-*tert*-Butoxy-4-((*S*)-2-(*tert*-butoxy carbonylamino)propanamido)-5-oxopentanamido)-3-methoxy-3-oxopropylthio)-2-benzyl-2-(*tert*-butoxycarbonylamino) propanoate 35

Under nitrogen, dipeptide **33** (374 mg, 1 mmol), HBTU (379 mg, 1 mmol) and diisopropylethylamine (DiPEA, 190 μL, 1.1 mmol) were suspended in dry CH_2Cl_2 (20 mL) and stirred for 1 h at rt (ie activated ester).

Meanwhile in another flask, containing a degassed suspension of sulfamidate **19** (373 mg, 0.9 mmol) and cysteine methyl ester.HCl (154 mg, 0.9 mmol) in CH_3CN (10 mL), was added, *via* syringe, a degassed solution of DBU (304 mg, 2 mmol) in CH_3CN (10 mL). The resulting solution was stirred for 1 h at rt under nitrogen and then concentrated to about one fourth of its volume.

To this last solution of **27**, was added, *via* syringe, the previously synthesized activated ester solution. Stirring was continued for 16 h at rt to afford **34**. After solvents evaporation, the solution was diluted with EtOAc (20 mL). Aqueous NaH_2PO_4 (10%, 20 mL) was added and the biphasic mixture was heated with stirring at 50 °C for 2 h. The organic layer was recovered and the aqueous layer was extracted with EtOAc (3 × 20 mL). The pooled organic fractions were washed with brine, dried over $MgSO_4$ and filtered. After solvent evaporation, the crude product **35** was chromatographied on silica with 7:3 (EtOAc/hexanes). Solvents evaporation afforded the *title compound* (580 mg, 78%) as a colourless oil. ^{1}H NMR (250 MHz, MeOD) δ_H 7.46 – 7.25 (m, 3H, Ar-H), 7.25 – 7.07 (m, 2H, Ar-H), 6.26 (br, 1H, NH-Boc), 4.73 – 4.56 (m, 1H, H-α-Lan), 4.37 (dd, *J* = 9.0, 4.6 Hz, 1H, H-α-Glu), 4.16 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.80 (s, 3H, OCH_3), 3.44 (d, *J* = 13.5 Hz, 1H, SCH_2C), 3.41 (d, *J* = 14.1 Hz, 1H, CH_2Ph), 3.17 (br d, *J* = 13.5 Hz, 2H, SCH_2C, CH_2Ph), 3.14 – 3.05 (m, 1H, $CHCH_2S$), 2.98 (dd, *J* = 13.5, 8.1 Hz, 1H, $CHCH_2S$), 2.40 (t, *J* = 7.1 Hz, 2H, CH_2CO), 2.34 - 2.14 (m, 1H, CH_2CH_2CO), 2.14 – 1.88 (m, 1H, CH_2CH_2CO), 1.66 – 1.44 (m, 36H, $C(CH_3)_3$), 1.40 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, MeOD) δ_C 175.79 (CO), 174.55 (CO), 172.29 (CO), 172.00 (CO), 171.83 (CO), 157.49 (Boc-CO), 155.81 (Boc-CO), 136.86 (Ar-C), 131.19 (Ar-C), 129.09 (Ar-C), 128.03 (Ar-C), 84.00 ($C(CH_3)_3$), 82.93 ($C(CH_3)_3$), 80.63 ($C(CH_3)_3$), 80.49 ($C(CH_3)_3$), 65.95 (C-α), 54.13 (CH-α-Lan), 53.76 (CH-α-Glu), 52.93 (OCH_3), 51.73 (CH-α-Ala), 41.09 (CH_2Ph), 38.58 (SCH_2C), 35.59 ($CHCH_2S$), 32.73 (CH_2CO), 28.85 ($C(CH_3)_3$), 28.78 ($C(CH_3)_3$), 28.30 ($C(CH_3)_3$), 28.28 (CH_2CH_2CO), 28.25 ($C(CH_3)_3$), 18.38 (CH_3); *m/z* (ESI) 825 $[MH]^+$.

5.4.26 (*S*)-*tert*-Butyl 3-((*R*)-2-((*R*)-5-*tert*-Butoxy-4-((*S*)-2-(*tert*-butoxy carbonylamino)propanamido)-5-oxopentanamido)-3-hydroxy -3-oxopropylthio)-2-benzyl-2-(*tert*-butoxycarbonylamino) propanoate 36

To tripeptide **35** (540 mg, 0.65 mmol) dissolved in CH_3CN (10 mL) was added $LiOH.H_2O$ (41 mg, 0.98 mmol) in water (10 mL). After stirring for 16 h at rt under nitrogen and acidification with 10% aqueous $NaHSO_4$ (2 mL), **36** was extracted three times with Et_2O. The pooled organic layers were washed with brine and dried over $MgSO_4$. Filtration and solvent evaporation yielded the *title compound* (515 mg, 97%) as a colourless foam. ^{1}H NMR (250 MHz, MeOD) δ_H 7.09 – 6.91 (m, 3H, Ar-H), 6.91 – 6.74 (m, 2H, Ar-H), 4.32 (dd, *J* = 7.5, 4.4 Hz, 1H, H-α-Lan), 4.12 – 3.95 (m, 1H, H-α-Glu), 3.86 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.25 – 2.99 (m, 2H, SCH_2C, CH_2Ph), 2.94 – 2.75 (m, 3H, SCH_2C, CH_2Ph, $CHCH_2S$), 2.68 (dd, *J* = 12.7, 8.2 Hz, 1H, $CHCH_2S$), 2.18 – 2.01 (m, 2H, CH_2CO), 2.01 - 1.84 (m, 1H, CH_2CH_2CO), 1.84 – 1.63 (m, 1H, CH_2CH_2CO), 1.32 – 1.10 (m, 36H, $C(CH_3)_3$), 1.08 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, MeOD) δ_C 175.54 (CO), 174.34 (CO), 173.40 (CO), 171.78 (CO), 171.59 (CO), 157.21 (Boc-CO), 155.52 (Boc-CO), 136.71 (Ar-C), 131.01 (Ar-C), 128.95 (Ar-C), 127.89 (Ar-C), 83.87 ($C(CH_3)_3$), 82.76 ($C(CH_3)_3$), 80.47 ($C(CH_3)_3$), 80.34 ($C(CH_3)_3$), 65.87 (C-α), 54.00 (CH-α-Lan), 53.69 (CH-α-Glu), 51.46 (CH-α-Ala), 40.98 (CH_2Ph), 38.78 (SCH_2C), 35.84 ($CHCH_2S$), 32.67 (CH_2CO), 28.81 ($C(CH_3)_3$), 28.75 ($C(CH_3)_3$), 28.27 ($C(CH_3)_3$), 28.22 ($C(CH_3)_3$), 28.06 (CH_2CH_2CO), 18.37 (CH_3); *m/z* (ESI) 811 $[MH]^+$.

5.4.27 (*R*)-5-((*R*)-2-((*S*)-2-amino-2-carboxy-3-phenylpropylthio)-1-carboxyethylamino)-2-((*S*)-2-aminopropanamido)-5-oxo pentanoic acid bis(trifluoroacetate) salt 3RS

Compound **36** (489 mg, 0.6 mmol) was dissolved in CH_2Cl_2 (50 mL) and the solution was purged with nitrogen for 15 min. TFA (5 mL) was added. The flask was closed with a glass stopper and the mixture was stirred for 16 h at rt. After solvents evaporation, the residue was repetitively taken up in CH_3CN and evaporated. The product was transferred in a centrifugation vial with the help of CH_3CN (15 mL). Then, Et_2O (15 mL) was added to precipitate it. After centrifugation, the pellet was washed with Et_2O. The product **3RS** was taken up in water and lyophilised to yield the *title compound* (318 mg, 73%) as a colourless solid. ^{1}H NMR (250 MHz, D_2O + MeOD) δ_H 7.37 – 7.14 (m, 3H, Ar-H, Ar-H), 7.14 – 6.90 (m, 2H, Ar-H), 4.36 (dd, *J* = 7.1, 5.2 Hz, 1H, H-α-Lan), 4.22 (dd, *J* = 8.6, 4.8 Hz, 1H,

H-α-Glu), 3.96 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.23 (d, *J* = 14.4 Hz, 1H, SCH_2C), 3.17 (d, *J* = 14.4 Hz, 1H, CH_2Ph), 3.08 – 2.83 (m, 3H, $CHCH_2S$, CH_2Ph), 2.78 (d, *J* = 14.4 Hz, 1H, SCH_2C), 2.28 (t, *J* = 7.3 Hz, 2H, CH_2CO), 2.18 – 1.98 (m, 1H, CH_2CH_2CO), 1.98 – 1.73 (m, 1H, CH_2CH_2CO), 1.39 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, D_2O) δ_C 176.81 (CO), 175.82 (CO), 175.73 (CO), 174.26 (CO), 171.65 (CO), 134.31 (Ar-C), 131.08 (Ar-C), 130.05 (Ar-C), 129.06 (Ar-C), 66.51 (C-α), 54.61 (CH-α-Lan), 54.10 (CH-α-Glu), 50.10 (CH-α-Ala), 42.39 (CH_2Ph), 39.25 (SCH_2C), 35.86 ($CHCH_2S$), 32.72 (CH_2CO), 27.75 (CH_2CH_2CO), 17.60 (CH_3); *m/z* (ESI) 499 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{21}H_{31}N_4O_8S$ 499.1857, found 499.1855 $[MH]^+$.

5.4.28 (*S*)-*tert*-Butyl 3-((*R*)-2-((*R*)-5-*tert*-Butoxy-4-((*S*)-2-(*tert*-butoxy carbonylamino)propanamido)-5-oxopentanamido)-3-methoxy -3-oxopropylthio)-2-(4-azidobenzyl)-2-(*tert*-butoxycarbonyl amino)propanoate 39

Under nitrogen, dipeptide **33** (249 mg, 0.66 mmol), HBTU (250 mg, 0.66 mmol) and DiPEA (127 µL, 1.1 mmol) were suspended in dry CH_2Cl_2 (15 mL) and stirred for 1 h at rt (ie activated ester).

Meanwhile in another flask, containing a degassed suspension of sulfamidate **26** (269 mg, 0.59 mmol) and cysteine methyl ester.HCl (103 mg, 0.6 mmol) in CH_3CN (7 mL), was added, *via* syringe, a degassed solution of DBU (203 mg, 1.33 mmol) in CH_3CN (7 mL). The resulting solution was stirred for 1 h at rt under nitrogen and then concentrated to about one fourth of its volume.

To this last solution of **37**, was added, *via* syringe, the previously synthesized activated ester solution. Stirring was continued for 16 h at rt to afford **38**. After solvents evaporation, the solution was diluted with EtOAc (20 mL). Aqueous NaH_2PO_4 (10%, 20 mL) was added and the biphasic mixture was heated with stirring at 50 °C for 2 h. The organic layer was recovered and the aqueous layer was extracted with EtOAc (3 × 20 mL). The pooled organic fractions were washed with brine, dried over $MgSO_4$ and filtered. After solvent evaporation, the crude product **39** was chromatographied on silica with 7:3 (EtOAc/hexanes). Solvents evaporation afforded the *title compound* (429 mg, 84%) as a colourless foam. ^1H NMR (250 MHz, MeOD) δ_H 7.20 – 7.08 (m, 2H, Ar-H), 7.00 – 6.90 (m, 2H, Ar-H), 4.59 (dd, *J* = 8.5, 4.8 Hz, 1H, H-α-Lan), 4.29 (dd, *J* = 8.8, 4.4 Hz, 1H, H-α-Glu), 4.08 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.73 (s, 3H, OCH_3), 3.40 – 3.31 (m, 2H, SCH_2C, CH_2Ar), 3.16 – 2.97 (m, 3H,

CHC*H*$_2$S, SCH$_2$C, CH$_2$Ar), 2.89 (dd, *J* = 13.5, 8.5 Hz, 1H, CHC*H*$_2$S), 2.39 – 2.25 (m, 2H, CH$_2$CO), 2.23 – 2.07 (m, 1H, C*H*$_2$CH$_2$CO), 2.00 – 1.84 (m, 1H, C*H*$_2$CH$_2$CO), 1.59 – 1.37 (m, 36H, C(CH$_3$)$_3$), 1.32 (d, *J* = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (63 MHz, MeOD) δ_C 175.66 (CO), 174.51 (CO), 172.20 (CO), 171.94 (CO), 171.70 (CO), 157.41 (Boc-CO), 155.71 (Boc-CO), 155.67 (Boc-CO, rotamer), 140.05 (Ar-C), 133.82 (Ar-C), 132.61 (Ar-C), 119.63 (Ar-C), 84.02 (*C*(CH$_3$)$_3$), 82.84 (*C*(CH$_3$)$_3$), 80.54 (*C*(CH$_3$)$_3$), 80.48 (*C*(CH$_3$)$_3$), 66.01 (C-α), 65.94 (C-α, rotamer), 53.90 (CH-α-Lan), 53.66 (CH-α-Glu), 52.91 (OCH$_3$), 51.65 (CH-α-Ala), 40.30 (CH$_2$Ar), 38.31 (SCH$_2$C), 35.62 (CH*C*H$_2$S), 32.70 (*C*H$_2$CO), 28.82 (C(*C*H$_3$)$_3$), 28.77 (C(*C*H$_3$)$_3$, *C*H$_2$CH$_2$CO), 28.31 (C(*C*H$_3$)$_3$), 28.23 (C(*C*H$_3$)$_3$), 18.36 (CH$_3$); *m/z* (ESI) 866 [MH]$^+$; HRMS *m/z* (ES+) Calcd for $C_{40}H_{64}N_7O_{12}S$ 866.4328, found 866.4319 [MH]$^+$.

5.4.29 (*S*)-*tert*-Butyl 3-((*R*)-2-((*R*)-5-*tert*-Butoxy-4-((*S*)-2-(*tert*-butoxy carbonylamino)propanamido)-5-oxopentanamido)-3-hydroxy -3-oxopropylthio)-2-(4-azidobenzyl)-2-(*tert*-butoxycarbonyl amino)propanoate 40

To tripeptide **39** (225 mg, 0.26 mmol) dissolved in CH$_3$CN (4 mL) was added LiOH.H$_2$O (17 mg, 0.4 mmol) in water (4 mL). After stirring for 16 h at rt under nitrogen and acidification with 10% aqueous NaHSO$_4$ (1 mL), **40** was extracted three times with Et$_2$O. The pooled organic layers were washed with brine and dried over MgSO$_4$. Filtration and solvent evaporation yielded the *title compound* (205 mg, 93%) as a colourless foam. ^{1}H NMR (250 MHz, MeOD) δ_H 7.14 (m, 2H, Ar-H), 6.95 (m, 2H, Ar-H), 4.56 (dd, *J* = 8.2, 4.6 Hz, 1H, H-α-Lan), 4.38 – 4.20 (m, 1H, H-α-Glu), 4.09 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.44 – 3.27 (m, 2H, SCH$_2$C, CH$_2$Ar), 3.18 – 3.01 (m, 3H, CHC*H*$_2$S, SCH$_2$C, CH$_2$Ar), 2.91 (dd, *J* = 13.5, 8.2 Hz, 1H, CHC*H*$_2$S), 2.41 – 2.27 (m, 2H, CH$_2$CO), 2.27 – 2.09 (m, 1H, C*H*$_2$CH$_2$CO), 2.01 - 1.87 (m, 1H, C*H*$_2$CH$_2$CO), 1.46 (m, 36H, C(CH$_3$)$_3$), 1.32 (d, *J* = 7.1 Hz, 3H, CH$_3$); ^{13}C NMR (63 MHz, MeOD) δ_C 175.73 (CO), 174.57 (CO), 173.28 (CO), 171.95 (CO), 171.74 (CO), 157.43 (Boc-CO), 155.76 (Boc-CO), 140.09 (Ar-C), 133.87 (Ar-C), 132.65 (Ar-C), 119.65 (Ar-C), 84.11 (*C*(CH$_3$)$_3$), 82.91 (*C*(CH$_3$)$_3$), 80.57 (*C*(CH$_3$)$_3$), 65.93 (C-α), 53.97 (CH-α-Lan), 53.86 (CH-α-Glu), 51.62 (CH-α-Ala), 40.30 (CH$_2$Ar), 38.77 (SCH$_2$C), 35.86 (CH*C*H$_2$S), 32.78 (*C*H$_2$CO), 28.82 (C(*C*H$_3$)$_3$), 28.76 (C(*C*H$_3$)$_3$), 28.30 (C(*C*H$_3$)$_3$), 28.23 (C(*C*H$_3$)$_3$, *C*H$_2$CH$_2$CO), 18.39 (CH$_3$); *m/z* (ESI) 852 [MH]$^+$.

5.4.30 (*R*)-5-((*R*)-2-((*S*)-2-amino-2-carboxy-3-(4-azidophenyl)-propyl thio)-1-carboxyethylamino)-2-((*S*)-2-aminopropanamido)-5-oxopentanoic acid dihydrochloride 4RS

Compound **40** (111 mg, 0.13 mmol) was dissolved in a mixture of aqueous HCl (10 M, 10 mL) and dioxane (30 mL). The mixture was purged with N_2 and stirred under nitrogen at 50 °C for 8 h. The solution was repeatedly evaporated *in vacuo* with the help of water to dryness. After lyophilisation and trituration with Et_2O (to remove the traces of diethylene glycol), the *title compound* **4RS** was obtained as a beige solid that was dried *in vacuo* to constant weight (70 mg, 88%). ^{1}H NMR (250 MHz, D_2O) δ_H 7.19 – 7.05 (m, 2H, Ar-H), 6.99 - 6.85 (m, 2H, Ar-H), 4.52 (dd, *J* = 8.5, 4.5 Hz, 1H, H-α-Lan), 4.30 (dd, *J* = 8.9, 5.2 Hz, 1H, H-α-Glu), 4.02 (q, *J* = 7.1 Hz, 1H, H-α-Ala), 3.33 – 3.14 (m, 2H, SCH_2C, CH_2Ar), 3.14 - 2.96 (m, 2H, $CHCH_2S$, CH_2Ar), 2.92 (d, *J* = 14.8 Hz, 1H, SCH_2C), 2.82 (dd, *J* = 14.1, 8.5 Hz, 1H, $CHCH_2S$), 2.32 (t, *J* = 7.3 Hz, 2H, CH_2CO), 2.21 – 2.01 (m, 1H, CH_2CH_2CO), 2.01 – 1.78 (m, 1H, CH_2CH_2CO), 1.43 (d, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (63 MHz, D_2O) δ_C 174.67 (CO), 174.49 (CO), 173.22 (CO), 171.61 (CO), 170.80 (CO), 139.77 (Ar-C), 131.55 (Ar-C), 128.81 (Ar-C), 119.45 (Ar-C), 64.48 (C-α), 52.10 (CH-α-Lan), 52.03 (CH-α-Glu), 49.03 (CH-α-Ala), 40.44 (CH_2Ar), 36.84 (SCH_2C), 33.96 ($CHCH_2S$), 31.30 (CH_2CO), 26.28 (CH_2CH_2CO), 16.61 (CH_3); *m/z* (ESI) 540 $[MH]^+$; HRMS *m/z* (ES+) Calcd for $C_{21}H_{30}N_7O_8S$ 540.1871, found 540.1869 $[MH]^+$.

5.5 Biological experiments

5.5.1 Strains and media

W7 (*dap lysA*) [40] was obtained from A. Derouaux (Newcastle University, Newcastle, United Kingdom). The cells were grown at 37 °C in a minimal salts medium [41] containing (per liter) 6.33 g of $K_2HPO_4.3H_2O$, 2.95 g of KH_2PO_4, 1.05 g of $(NH_4)_2SO_4$, 1.0 g of $MgSO_4.7\ H_2O$, 0.1 mg of $FeSO_4.7\ H_2O$, 2.8 mg of $Ca(NO_3)_2.4\ H_2O$, 4 mg of thiamine, 5 g (0.5%) or 2 g (0.2%) of glucose, and 50 mg of (*S*)-lysine. Incorporation experiments were done in the presence of 50 µg/mL or 500 µg/mL **3RS** and **3RR**, respectively.

5.5.2 Purification of PG

The preparation of highly purified PG has been done from one-liter culture medium by modifying a protocol described by Girardin.[42] 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 pellet was suspended in 20 mL ice-cold water, and the suspension was added dropwise to 8% boiling SDS (20 mL). The sample was boiled for 45 min. SDS treatment removes 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, 50000 rpm) and washed five times with water in order to remove SDS. PG was 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, 50000 rpm). Finally, it was washed with water, with 8 M LiCl to remove any polypeptidic contamination, again with water, and lyophilized.

5.5.3 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 (Science-Tec). 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*)-α-benzyl-lanthionine, 30.7 min, (*R,S*)-α-benzyl-lanthionine, 31.3min.

5.5.4 *In vitro* assay with Mpl

The assay mixture (50 µL) contained 100 mM Tris-HCl buffer (pH 8.4), 5 mM ATP, 15 mM, $MgCl_2$, 0.4 mM UDP-[^{14}C]MurNAc (500 Bq), 1 mM tripeptide, and purified Mpl enzyme (2 µg of protein). Mixtures were incubated at 37 °C for 16 h and reactions were stopped by the addition of 10 µL of acetic acid, followed by lyophilization. The residue was dissolved in 50 mM ammonium formate, pH 3.8 (buffer A), and injected onto a Nucleosil 100 C_{18} 5 µm column (4.6 × 150 mm; Grace Davison Discovery Sciences). Elution was performed at 0.6 mL.min^{-1} with buffer A (isocratic, 10 min) followed by a gradient of methanol in buffer A (from 0 to 40 % over 35 min). Detection was performed with a radioactive flow detector (model LB506-C1, Berthold) using the Quicksafe Flow 2 scintillator (Zinsser Analytic) at 0.6 mL.min^{-1}. Quantification was carried out with the Winflow software (Berthold).

References

1. Zervosen, A.; Sauvage, E.; Frère, J.M.; Charlier, P.; Luxen, A. Development of new drugs for an old target: the penicillin binding proteins. *Molecules* **2012**, *17*, 12478-12505.
2. Goodell, E.W. Recycling of murein by *Escherichia coli*. *J. Bacteriol.* **1985**, *163*, 305-310.
3. Hervé, M.; Boniface, A.; Gobec, S.; Blanot, D.; Mengin-Lecreulx, D.; Hervé, M.; Kovač, A.; Cardoso, C.; Patin, D.; Brus, B.*, et al.* Biochemical characterization and physiological properties of *Escherichia coli* UDP-*N*-acetylmuramate:L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate ligase. *J. Bacteriol.* **2007**, *189*, 3987-3995.
4. Hervé, M.; Kovač, A.; Cardoso, C.; Patin, D.; Brus, B.; Barreteau, H.; Mengin-Lecreulx, D.; Gobec, S.; Blanot, D. Synthetic tripeptides as alternate substrates of murein peptide ligase (Mpl). *Biochimie* **2013**, *95*, 1120-1126.
5. Olrichs, N.K.; Aarsman, M.E.; Verheul, J.; Arnusch, C.J.; Martin, N.I.; Hervé, M.; Vollmer, W.; de Kruijff, B.; Breukink, E.; den Blaauwen, T. A novel *in vivo* cell-wall labeling approach sheds new light on peptidoglycan synthesis in *Escherichia coli*. *ChemBioChem* **2011**, *12*, 1124-1133.
6. van Dam, V.; Olrichs, N.; Breukink, E. Specific labeling of peptidoglycan precursors as a tool for bacterial cell wall studies. *ChemBioChem* **2009**, *10*, 617-624.
7. Cobb, S.L.; Vederas, J.C. A concise stereoselective synthesis of orthogonally protected lanthionine and β-methyllanthionine. *Org. Biomol. Chem.* **2007**, *5*, 1031-1038.
8. Denoël, T.; Zervosen, A.; Lemaire, C.; Plenevaux, A.; Luxen, A. Synthesis of protected α-alkyl lanthionine derivatives. *Tetrahedron* **2014**, *70*, 4526-4533.
9. Boulton, L.T.; Stock, H.T.; Raphy, J.; Horwell, D.C. Generation of unnatural α,α-disubstituted amino acid derivatives from cyclic sulfamidates. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1421-1430.
10. Jew, S.-s.; Lee, Y.-J.; Lee, J.; Kang, M.J.; Jeong, B.-S.; Lee, J.-H.; Yoo, M.-S.; Kim, M.-J.; Choi, S.-h.; Ku, J.-M.*, et al.* Highly Enantioselective Phase-Transfer-Catalytic Alkylation of 2-Phenyl-2-oxazoline-4-carboxylic Acid *tert*-Butyl Ester for the Asymmetric Synthesis of α-Alkyl Serines. *Angew. Chem. Int. Ed.* **2004**, *43*, 2382-2385.
11. Cohen, S.B.; Halcomb, R.L. Application of Serine- and Threonine-Derived Cyclic Sulfamidates for the Preparation of *S*-Linked Glycosyl Amino Acids in Solution- and Solid-Phase Peptide Synthesis. *J. Am. Chem. Soc.* **2002**, *124*, 2534-2543.
12. Olah, G.A.; Keumi, T. Synthetic methods and reaction ; 60. Improved one-step conversion of aldehydes into nitriles with hydroxylamine in formic acid solution. *Synthesis* **1979**, 112.
13. Roger, R.; Neilson, D.G. The Chemistry of Imidates. *Chem. Rev.* **1961**, *61*, 179-211.
14. Fry, E.M. Syntheses of Cystine. *J. Org. Chem.* **1950**, *15*, 438-447.
15. Shirakawa, S.; Maruoka, K. Recent Developments in Asymmetric Phase-Transfer Reactions. *Angew. Chem. Int. Ed.* **2013**, *52*, 4312-4348.
16. Wang, Y.-G.; Ueda, M.; Wang, X.; Han, Z.; Maruoka, K. Convenient preparation of chiral phase-transfer catalysts with conformationally fixed biphenyl core for catalytic asymmetric synthesis of α-alkyl- and α,α-dialkyl-α-amino acids: application to the short asymmetric synthesis of BIRT-377. *Tetrahedron* **2007**, *63*, 6042-6050.
17. Maruoka, K.; Nishimoto, Y.; Yamamoto, K. Optically active quaternary ammonium salt having axial asymmetry and process for producing α-amino acid and derivative thereof with the same. *US2009270614* **2009**.
18. Andersen, J.; Madsen, U.; Björkling, F.; Liang, X. Rapid Synthesis of Aryl Azides from Aryl Halides under Mild Conditions. *Synlett* **2005**, *2005*, 2209-2213.
19. Reddy, L.R.; Saravanan, P.; Corey, E.J. A Simple Stereocontrolled Synthesis of Salinosporamide A. *J. Am. Chem. Soc.* **2004**, *126*, 6230-6231.
20. Dolence, E.K.; Mayer, G.; Kelly, B.D. Use of optically active cyclic diethyl sulfamidate 2-phosphonates as chiral synthons for the synthesis of β-substituted α-amino phosphonates. *Tetrahedron: Asymmetry* **2005**, *16*, 1583-1594.
21. Flack, H. On enantiomorph-polarity estimation. *Acta Crystallogr. Sect. A: Found. Crystallogr.* **1983**, *39*, 876-881.
22. Duran, F.; Leman, L.; Ghini, A.; Burton, G.; Dauban, P.; H. Dodd, R. Intramolecular PhI=O Mediated Copper-Catalyzed Aziridination of Unsaturated Sulfamates: A New Direct Access

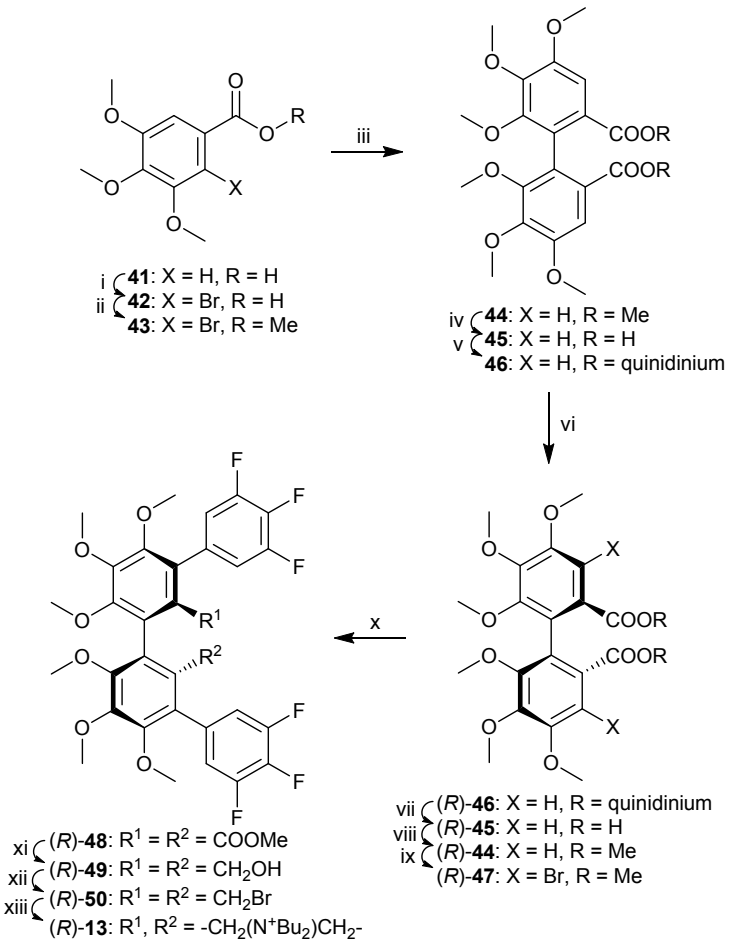
to Polysubstituted Amines from Simple Homoallylic Alcohols. *Org. Lett.* **2002**, *4*, 2481-2483.

23. Espino, C.G.; Wehn, P.M.; Chow, J.; Du Bois, J. Synthesis of 1,3-Difunctionalized Amine Derivatives through Selective C-H Bond Oxidation. *J. Am. Chem. Soc.* **2001**, *123*, 6935-6936.
24. Huang, C.; Yuan, L.; Rich, K.M.; McConathy, J. Radiosynthesis and biological evaluation of alpha-[F-18]fluoromethyl phenylalanine for brain tumor imaging. *Nucl. Med. Biol.* **2013**, *40*, 498-506.
25. Burk, M.J.; Allen, J.G. A Mild Amide to Carbamate Transformation. *J. Org. Chem.* **1997**, *62*, 7054-7057.
26. Baldwin, J.E.; North, M.; Flinn, A.; Moloney, M.G. Synthesis of nonproteinogenic amino acids part 2: preparation of a synthetic equivalent of the γ anion synthon for asymmetric amino acid synthesis. *Tetrahedron* **1989**, *45*, 1453-1464.
27. Lundt, B.; Johansen, N.; Vølund, A.; Markussen, J. Removal of *t*-butyl and *t*-butoxycarbonyl protecting groups with trifluoroacetic acid. Mechanisms, biproduct formation and evaluation of scavengers. *Int. J. Pept. Protein Res.* **1978**, *12*, 258-268.
28. Wientjes, F.B.; Pas, E.; Taschner, P.E.; Woldringh, C.L. Kinetics of uptake and incorporation of *meso*-diaminopimelic acid in different *Escherichia coli* strains. *J. Bacteriol.* **1985**, *164*, 331-337.
29. Rhuland, L.E. Role of α,ε-diaminopimelic acid in the cellular integrity of *Escherichia coli*. *J. Bacteriol.* **1957**, *73*, 778-783.
30. Park, J.T.; Raychaudhuri, D.; Li, H.; Normark, S.; Mengin-Lecreulx, D. MppA, a Periplasmic Binding Protein Essential for Import of the Bacterial Cell Wall Peptide L-Alanyl-γ-D-Glutamyl-*meso*-Diaminopimelate. *J. Bacteriol.* **1998**, *180*, 1215-1223.
31. Goodell, E.W.; Higgins, C.F. Uptake of cell wall peptides by Salmonella typhimurium and *Escherichia coli*. *J. Bacteriol.* **1987**, *169*, 3861-3865.
32. Khankal, R.; Chin, J.W.; Ghosh, D.; Cirino, P.C. Transcriptional effects of CRP* expression in *Escherichia coli*. *J. Biol. Eng.* **2009**, *3*, 1-14.
33. Mengin-Lecreulx, D.; Blanot, D.; van Heijenoort, J. Replacement of diaminopimelic acid by cystathionine or lanthionine in the peptidoglycan of *Escherichia coli*. *J. Bacteriol.* **1994**, *176*, 4321-4327.
34. Rhuland, L.E. Role of α,ε-diaminopimelic acid in the cellular integrity of *Escherichia coli*. *J. Bacteriol.* **1957**, *73*, 778-783.
35. Richaud, C.; Mengin-Lecreulx, D.; Pochet, S.; Johnson, E.J.; Cohen, G.N.; Marliere, P. Directed evolution of biosynthetic pathways. Recruitment of cysteine thioethers for constructing the cell wall of *Escherichia coli*. *J. Biol. Chem.* **1993**, *268*, 26827-26835.
36. Chaloupka, J.M.; Strnadová, M.; Caslavská, J.; Veres, K. Growth and cell division of *Escherichia coli* 173-25 in the presence of some analogues of diaminopimelic acid. *Z. All. Mikrobiol.* **1974**, *14*, 283-296.
37. Denoël, T.; Zervosen, A.; Gerards, T.; Lemaire, C.; Joris, B.; Blanot, D.; Luxen, A. Stereoselective synthesis of lanthionine derivatives in aqueous solution and their incorporation into the peptidoglycan of *Escherichia coli*. *Biorg. Med. Chem.* **2014**, *Submitted*.
38. Liebscher, J. Formylation Products of Thioamides; Part II1. Synthesis of *N*-Thioacylformamidines, *N*-Thioacylformamides, or Nitriles from Thioamides and Formamide Chlorides. *Synthesis* **1982**, 1084-1086.
39. Tarzia, G.; Schiatti, P.; Selva, D.; Favara, D.; Ceriani, S. 4,5-Dicarbamyl-2-aryloxazoles. Synthesis and anti-inflammatory properties. *Eur. J. Med. Chem. - Chim. Ther.* **1976**, *11*, 263-270.
40. Davis, B.D. Biosynthetic interrelations of lysine, diaminopimelic acid, and threonine in mutants of *Escherichia coli*. *Nature* **1952**, *169*, 534-536.
41. Wientjes, F.B.; Pas, E.; Taschner, P.E.; Woldringh, C.L. Kinetics of uptake and incorporation of *meso*-diaminopimelic acid in different *Escherichia coli* strains. *Journal of bacteriology* **1985**, *164*, 331-337.
42. Girardin, S.E.; Boneca, I.G.; Carneiro, L.A.; Antignac, A.; Jehanno, M.; Viala, J.; Tedin, K.; Taha, M.K.; Labigne, A.; Zahringer, U., *et al.* Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **2003**, *300*, 1584-1587.

5.6 Appendix to chapter 5

5.6.1 Preparation of Maruoka's catalyst 13

The catalyst **13** described by the Maruoka group was prepared as follow (Scheme 1).[1,2] Trimethoxybenzoic acid **41** was brominated in CH_3CN with NBS (step i) followed by conversion of the carboxylic acid **42** to the methyl ester **43** with TMS-Cl in MeOH (step ii).[2,3] The Ullmann biaryl coupling of **43** to form bicyclic **44** was performed in NMP with activated copper bronze by adapting a general procedure (step iii).[4,5] Saponification and precipitation by acidification yielded diacid **45**, in multigram quantity, which was purified by recrystallization (step iv). The diacid **45** was treated with two equivalents of quinidine forming the double salt **46** (step v). Resolution of **46** by three fractional crystallizations was done by following the literature procedure (step vi).[6] In this case, after resolution, the acid (*R*)-**45** was obtained with high enantiopurity (≥ 99%, step vii). Treatment of (*R*)-**45** with TMS-Cl in MeOH for 72 h, at rt, gave the diester (*R*)-**44** (step viii).[3] From this product the catalyst was made by following the Maruoka's patented method (steps ix-xiii).[2] After purification by preparative HPLC, the overall yield of (*R*)-**13** from (*R*)-**45** was 30% (steps viii-xiii). In the same way (*S*)-**13** was also obtained from (*S*)-**45**.



Scheme 1. Synthesis of Maruoka's catalyst. i) NBS, CH_3CN, 2 °C, 16 h, 93%; ii) TMS-Cl, MeOH, rt, 48 h, 94%; iii) Cu, NMP, 170 °C, 2 h; iv) NaOH, aq. MeOH, reflux, 16 h, then aq. HCl, 0 °C, 77%; v) quinidine (2 eq), aq. EtOH; vi) fractional crystallizations (3 x); vii) NaOH, HCl; viii) TMS-Cl, MeOH, rt, 48 h, 96%; ix) Br_2, CH_3CN, rt, 16 h, 80%; x) 3,4,5-F_3-PhB$(OH)_2$ (3 eq.), Pd$(OAc)_2$ (20 mol%), tri-*o*-tolyl-P (80 mol%), NaOMe (3 eq.), DME, 80 °C, 16 h, 75% ; xi) $LiAlH_4$, rt, 4 h, 85%; xii) PBr_3 (3 eq.), CH_2Cl_2, 0 °C, 2 h, 91%; Bu_2NH (1.3 eq), K_2CO_3, CH_3CN, 85 °C, 16 h, 67%.

5.6.2 Experimental section

5.6.2.1 *2-Bromo-3,4,5-trimethoxybenzoic acid 42*

The procedure described in Maruoka's patent was modified as follows by using CH_3CN in place of $CHCl_3$.[2] Small portions of NBS (89 g, 500 mmol) was added during 10 min to an ice-cold solution of 3,4,5-trimethoxybenzoic acid **41** (106 g, 500 mmol) in CH_3CN (1 L). The resulting mixture was stirred for 16 h at 2 °C. The solvent was evaporated *in vacuo* and

the resulting solids were dissolved in boiling water (1 L) containing NaOH (22 g, 550 mmol). The solution was cooled to 10 °C and acidified with an excess of HCl (pH: 3). The precipitate was filtered, washed with water and dried *in vacuo* to constant weight. The *title compound* (135.5 g, 93%) was obtained as a beige solid. Mp 145-148 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 12.29 (s, 1H, OH), 7.38 (s, 1H, Ar-H), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 171.20 (CO), 152.19 (Ar-C), 151.68 (Ar-C), 147.05 (Ar-C), 125.37 (Ar-C), 111.31 (Ar-C), 110.97 (Ar-C), 61.24 (OCH_3), 61.07 (OCH_3), 56.29 (OCH_3); *m/z* (ESI) 289/291 $[M]^-$.

5.6.2.2 *Methyl 2-bromo-3,4,5-trimethoxybenzoate 43*

The esterification was done as proposed by Nakao.[3] To compound **42** (135.5 g, 466 mmol) in MeOH (500 mL) was added TMS-Cl (250 mL, 1.97 mol). The solution was stirred for 48 h at rt. The solvents were evaporated and the residue was dissolved in CH_2Cl_2. The organic layer was washed with water, aqueous saturated $NaHCO_3$ and water. The organics were dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to give the *title compound* (134 g, 94%) as a golden oil. ^{1}H NMR (250 MHz, D_2O) δ_H 7.10 (s, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 166.37 (CO), 152.30 (Ar-C), 151.47 (Ar-C), 145.98 (Ar-C), 127.41 (Ar-C), 110.06 (Ar-C), 109.43 (Ar-C), 61.11 (OCH_3), 60.97 (OCH_3), 56.21 (OCH_3), 52.45 (OCH_3); *m/z* (ESI) 305/307 $[MH]^+$.

5.6.2.3 *4,4',5,5',6,6'-Hexamethoxybiphenyl-2,2'-dicarboxylic acid 45*

In order to conduct the reaction at lower temperature and to increase yield, the synthesis was done by modifying the known literature method and a solvent, NMP, was added.[4,5] Compound **43** (133 g, 436 mmol) was dissolved in NMP (150 mL) and the solution was heated to 170 °C under nitrogen. Activated copper bronze (115 g, [iodine (2%) in acetone, aqueous HCl (10 M)/acetone:1/1][7]) was added in one portion and the suspension was stirred for 2 h. The dark brown mixture was cooled to 100 °C and the copper was filtered on celite and washed with boiling toluene. After solvents evaporation *in vacuo* (0.1 mm Hg) at 95 °C, a dark brown oil contaminated by solids was obtained. The crude product **44** was dissolved in EtOAc and washed twice with ammonium hydroxide (6 M) and water. After evaporation, a brown oil was obtained (100 g). The crude diester **44** was saponified by heating under reflux for 16 h with a solution of NaOH (50 g) in MeOH/H_2O (1/1; 400 mL). The MeOH was evaporated *in vacuo*. The volume of the solution was adjusted to 500 mL with water and hydrochloric acid was added under stirring until pH 3. The suspension was

cooled to 0 °C, the precipitate **45** was filtered and washed with water. The wet solid was recrystallized twice from boiling aqueous MeOH. After filtration and drying to constant weight the *title compound* (71 g, 77 %) was obtained as an off-white solid. Mp 248-249 °C; ^{1}H NMR (250 MHz, DMSO) δ_H 12.24 (s, 2H, COOH), 7.32 (s, 2H, Ar-H), 3.87 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 3.49 (s, 6H, OCH_3); ^{13}C NMR (63 MHz, DMSO) δ_C 167.29 (CO), 151.44 (Ar-C), 150.81 (Ar-C), 144.49 (Ar-C), 126.61 (Ar-C), 125.80 (Ar-C), 108.94 (Ar-C), 60.37 (OCH_3), 60.11 (OCH_3), 55.74 (OCH_3); *m/z* (ESI) 421 $[M]^-$.

5.6.2.4 *(R)-4,4',5,5',6,6'-Hexamethoxybiphenyl-2,2'-dicarboxylic acid (R)-45*

Resolution of compound **45** was realized through the diquinidinium salt **46** by following the known method.[6] Ee was determined on a Chiracel OD-H column (Daicel, 150 mm × 4 mm, 5 µm); mobile phase: *n*-Hex/*i*-PrOH/TFA:90/10/0.1; 0.8 mL/min; 37 °C. Retention times for isomers *R* and *S* were 8.3 min and 11.0 min, respectively. Enantiomeric purity of (*R*)-**45** was ≥ 99%. Enantiomer (*S*)-**45** was also obtained with an ee ≥ 99%.

5.6.2.5 *Dimethyl (R)-4,4',5,5',6,6'-hexamethoxybiphenyl-2,2'-dicarboxylate (R)-44*

A mixture of (*R*)-**45** (7.4 g, 17.5 mmol) and TMS-Cl (19 mL, 150 mmol) in MeOH (200 mL) was stirred for 72 h at rt. The solvents were evaporated and the residue was dissolved in CH_2Cl_2. The organic layer was washed with water, aqueous saturated $NaHCO_3$ and water. The organics were dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo* to give the *title compound* (7.8 g, 95%) as a golden oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.34 (s, 2H, Ar-H), 3.91 (s, 6H, OCH_3), 3.90 (s, 6H, OCH_3), 3.57 (s, 12H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 166.89 (CO), 152.04 (Ar-C), 151.23 (Ar-C), 145.40 (Ar-C), 126.58 (Ar-C), 124.96 (Ar-C), 108.86 (Ar-C), 60.77 (OCH_3), 60.51 (OCH_3), 55.93 (OCH_3), 51.80 (OCH_3); *m/z* (ESI) 451 $[MH]^+$.

5.6.2.6 *Dimethyl (R)-3,3'-dibromo-4,4',5,5',6,6'-hexamethoxy biphenyl-2,2'-dicarboxylate (R)-47*

The *title compound* was obtained as colourless needles as previously described.[2] Mp 114-115 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 3.92 (s, 6H, OCH_3), 3.91 (s, 6H, OCH_3), 3.76 (s, 6H, OCH_3), 3.61 (s, 6H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 166.23 (CO), 151.83 (Ar-C), 151.49 (Ar-C), 148.00 (Ar-C), 130.90 (Ar-C), 125.06 (Ar-C), 109.53 (Ar-C), 61.16 (OCH_3), 61.03 (OCH_3), 60.94 (OCH_3), 52.08 (OCH_3); *m/z* (ESI) 607 (1)/609 (2)/611 (1) $[MH]^+$.

5.6.2.7 *Dimethyl (R)-3,3'-bis(3,4,5-trifluorophenyl)-4,4',5,5',6,6'-hexa methoxybiphenyl-2,2'-dicarboxylate (R)-48*

The *title compound* was obtained as an amber oil as previously described.[2] ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.03 – 6.83 (m, 4H, Ar-H), 4.00 (s, 6H, OCH_3), 3.88 (s, 6H, OCH_3), 3.72 (s, 6H, OCH_3), 3.29 (s, 6H, OCH_3); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 167.11 (CO), 152.31 (Ar-C), 151.01 (Ar-C), 150.54 (Ar-C) (ddd, $J_{C\text{-}F}$ = 249.4, 9.8, 4.1 Hz, Ar-C), 147.62 (Ar-C), 139.00 (dt, $J_{C\text{-}F}$ = 251.8, 15.2 Hz, Ar-C), 132.37 (td, $J_{C\text{-}F}$ = 8.1, 4.8 Hz, Ar-C), 128.91 (Ar-C), 127.08 (Ar-C), 125.24 (Ar-C), 114.30 – 113.91 (m, Ar-C), 113.82 (Ar-C), 61.11 (OCH_3), 60.81 (OCH_3), 51.55 (OCH_3); *m/z* (ESI) 711 $[MH]^+$.

5.6.2.8 *(R)-3,3'-bis(3,4,5-trifluorophenyl)-4,4',5,5',6,6'-hexamethoxy biphenyl-2,2'-dimethanol (R)-49*

The *title compound* was obtained as a beige powder as previously described.[2] ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.18 – 7.00 (m, 4H, Ar-H), 4.02 (d, J = 11.3 Hz, 2H, CH_2), 3.94 (s, 6H, OCH_3), 3.93 (d, J = 11.1 Hz, 2H, CH_2), 3.75 (s, 6H, OCH_3), 3.70 (s, 6H, OCH_3), 3.01 (b, 2H, OH); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 151.40 (Ar-C), 151.06 (Ar-C), 150.56 (ddd, $J_{C\text{-}F}$ = 249.5, 9.8, 4.2 Hz, Ar-C), 146.05 (Ar-C), 139.18 (dt, $J_{C\text{-}F}$ = 251.5, 15.3 Hz, Ar-C), 133.49 (Ar-C), 132.20 (td, $J_{C\text{-}F}$ = 8.3, 5.2 Hz, Ar-C), 130.48 (Ar-C), 126.51 (Ar-C), 114.95 (b, Ar-C), 61.13 (OCH_3), 60.88 (OCH_3), 60.80 (OCH_3), 59.65 (CH_2); *m/z* (ESI) 655 $[MH]^+$.

5.6.2.9 *(R)-3,3'-bis(3,4,5-trifluorophenyl)-4,4',5,5',6,6'-hexamethoxybiphenyl-2,2'-dimethyl bromide (R)-50*

The *title compound* was obtained as a white solid as previously described[2] and was used without purification for the next step; *m/z* (ESI) 779 (1)/781 (2)/783 (1) $[MH]^+$.

5.6.2.10 *Chiral quaternary ammonium salt (R)-13*

The *title compound* was obtained as a white solid as previously described.[2] ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.39 – 7.09 (m, 4H, Ar-H), 4.45 (d, J = 13.7 Hz, 2H, CH_2Ar), 4.08 (s, 6H, OCH_3), 3.94 (s, 6H, OCH_3), 3.84 (d, J = 14.1 Hz, 2H, CH_2Ar), 3.78 (s, 6H, OCH_3), 3.06 (t, J = 12.5 Hz, 2H, NCH_2), 2.80 (b, 2H, NCH_2), 1.26 – 0.96 (m, 6H, CH_2), 0.80 (t, J = 5.7 Hz, 6H, CH_3), 0.24 (b, 2H, CH_2); ^{13}C NMR (63 MHz, $CDCl_3$) δ_C 152.40 (Ar-C), 152.00 (Ar-C), 151.00 (dtd, $J_{C\text{-}F}$ = 252.1, 9.2, 3.7 Hz, Ar-C), 148.11 (Ar-C), 139.54 (dt, $J_{C\text{-}F}$ = 253.9, 15.0 Hz, Ar-C), 130.47 (td, J = 8.0, 5.3 Hz, Ar-C), 130.05 (Ar-C), 126.80 (Ar-C), 120.29 (Ar-C), 115.75 (dd, $J_{C\text{-}F}$ = 17.9, 2.3 Hz, Ar-C), 61.63 (OCH_3), 61.18 (OCH_3), 61.01 (OCH_3), 57.76 (CH_2Ar), 57.17 (NCH_2), 24.32 (CH_2), 19.34 (CH_2), 13.24 (CH_3); *m/z* (ESI) 748 $[M]^+$.

References

1. Wang, Y.-G.; Ueda, M.; Wang, X.; Han, Z.; Maruoka, K. Convenient preparation of chiral phase-transfer catalysts with conformationally fixed biphenyl core for catalytic asymmetric synthesis of α-alkyl- and α,α-dialkyl-α-amino acids: application to the short asymmetric synthesis of BIRT-377. *Tetrahedron* **2007**, *63*, 6042-6050.
2. Maruoka, K.; Nishimoto, Y.; Yamamoto, K. Optically active quaternary ammonium salt having axial asymmetry and process for producing α-amino acid and derivative thereof with the same. *US2009270614* **2009**.
3. Nakao, R.; Oka, K.; Fukumoto, T. A Simple Method for the Esterification of Carboxylic Acids Using Chlorosilanes. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1267-1268.
4. Fanta, P.E. The Ullmann Synthesis of Biaryls. *Synthesis* **1974**, 9-21.
5. Hassan, J.; Sévignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M. Aryl-Aryl Bond Formation One Century after the Discovery of the Ullmann Reaction. *Chem. Rev.* **2002**, *102*, 1359-1470.
6. Schmidt, O.T.; Demmler, K. Natural tannins. XIII. Optically active 4,4',5,5',6,6'-hexamethoxy-2,2'-biphenyl-dicarboxylic acids. *Justus Liebigs Ann. Chem.* **1952**, *576*, 85-93.
7. Vogel , A.I. *Practical Organic Chemistry*. Third ed.; Longman: 1956.

Chapter 6

General discussion and prospects

6.1 General discussion and prospects

In this work, we report a novel labeling approach of the PG of *E. coli* through the replacement of natural A_2pm by derivatives of lanthionine (Figure 1). One possible entry for these derivatives in PG biosynthesis is the enzymatic addition of the modified amino acids **1**-**2** onto UDP-(*S*)-Ala-γ-(*R*)-Glu catalysed by MurE. Another possibility is the replacement of the natural tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm by the analogues **3**-**4** and their incorporation *via* the recycling pathway (Figure 3, Chapter 1).

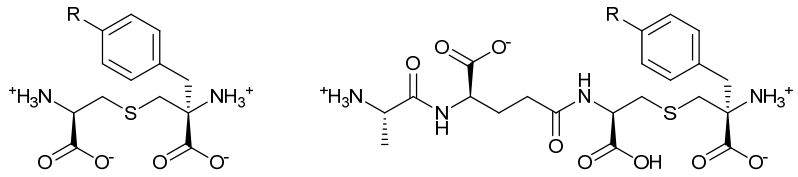
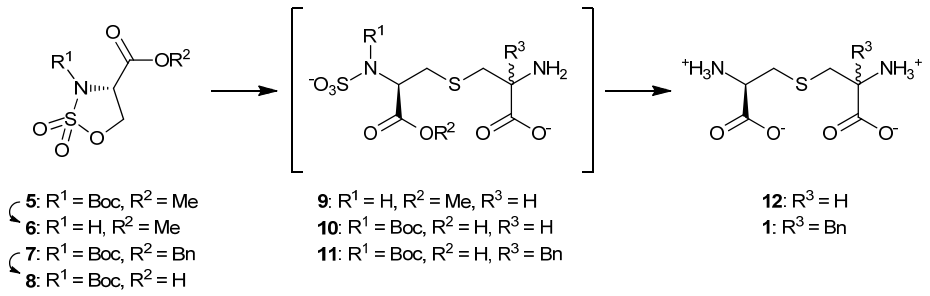


Figure 1. Structural formulas of natural and labelled tripeptides.

In our *Bioorganic & Medicinal Chemistry* publication, we reported the aqueous synthesis of the three diastereoisomers - (*R,R*), (*S,S*) and *meso* - of lanthionine (Scheme 1). This approach proceeds with high diastereoselectivity (> 99%) without any enrichment step. The synthesis of the starting (*S*) and (*R*) enantiomers of two differently protected water-soluble sulfamidates **6** and **8** was realized in five steps from serine. This multigram (> 10 g) synthesis afforded global yields of 38% (**6**) and 30% (**8**).



Scheme 1. Preparation of Lan **12** and (*R,R/S*)-α-Bn-Lan **3**.The three diastereomers of Lan **12** were prepared in de 94-99% starting from both enantiomers of **6** or both enantiomers of **8** and (*R*) or (*S*)-cysteine.

The four sulfamidates (*R*) or (*S*)-**6** and (*R*) or (*S*)-**8** were then opened by nucleophilic attack of (*R*) or (*S*)-cysteine at room temperature in the presence of alkaline aqueous bicarbonate (Scheme 1). Acidification and controlled heating (50 - 70 °C) liberated the free (*R,R*), (*S,S*) and *meso*- lanthionines **12**. The nature of the cysteine counter-ion (Na^+, Cs^+) influenced the diastereomeric excesses. The cesium salt led to higher diastereomeric excesses (86 - 88% *vs* 94 - 99%). The highest diastereomeric purity (> 99%) was obtained with the Boc- protected sulfamidates and the cesium salt. After elution on a Dowex 50WX8, all three diastereomers of Lan **12** were isolated with yields > 95% and chemical purity > 95%. Using the same chemistry, α-benzyl lanthionine **1** was also prepared from previously synthesized *rac*-cysteine α-benzyl. As an expedient purification process, **1** was easily precipitated at pH 6 in 60% yield.

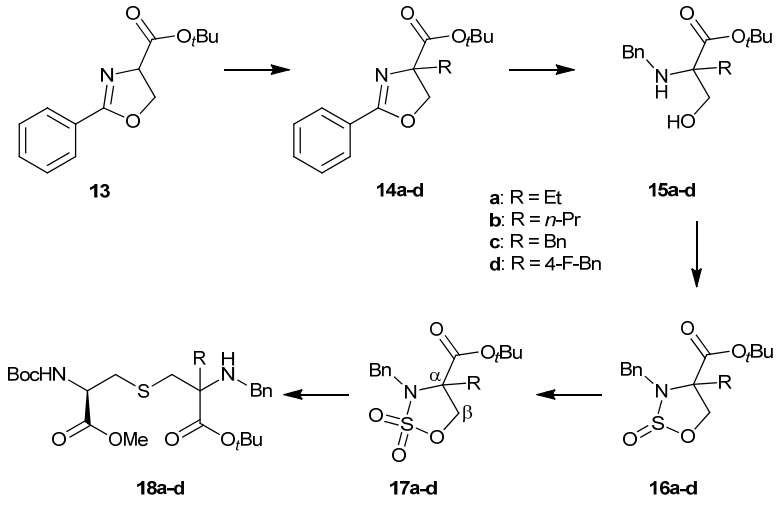
At the time of this writing, the three pure diastereoisomers (*R,R*), (*S,S*) and *meso* of Lan are not commercially available. Our gram-scale procedure could find widespread application in the synthesis of unprotected lanthionine or analogues.

In addition, the proposed method, which avoids the need of enrichment by recrystallization, is convenient for the labeling of these compounds with ^{35}S. Starting from commercially available (*R*)-[^{35}S]cysteine, this straightforward aqueous approach could be applicable for the preparation of diastereomerically pure (*R,R*) and *meso*-[^{35}S]Lan.

Moreover, this synthesis opens the way to the preparation of the (*S*)-Ala-γ-(*R*)-Glu-(*R,S*)-[^{35}S]Lan tripeptide and furthermore, under Mpl catalysis, to the synthesis of the labelled [^{35}S]UDP-MurNac-tripeptide. All these [^{35}S]-labelled molecules could be used to study PG biosynthesis.

The same strategy starting from sulfamidates **5** and **7**, but in aprotic solvent, could be useful for the preparation, with excellent de, of differently protected lanthionines. Likewise, β-alkylated Lan could also be obtained. In the future, all these compounds will be useful for peptide synthesis such as lantibiotics.

In our *Tetrahedron* paper, we described an efficient synthesis of various differently protected α-alkyl lanthionines **18a-d** (Scheme 2). This synthetic scheme started with the multigram preparation of the known oxazoline precursor **13** (four steps, 50%). Alkylation of **13** by PTC with TBAB gave the racemic compounds **14a-d** in good yields (79 - 90%). The enantioselective benzylation of **13**, with 1.5 equivalents of benzyl bromide in the presence of a chiral binaphthyl PTC, afforded **14c** with an enantiomeric excess of 97%. The smooth reductive cleavage of **14a-d** provided protected serines **15a-d** in excellent yields (94 - 98%). Cyclization with $SOCl_2$ and oxidation gave sulfamidates **17a-d** in 75 - 92% yields (two steps). An efficient S_N2 reaction on the cyclic sulfamidates was highly successful, despite the sterically crowded α-carbon, in producing protected lanthionines **18a-d** in excellent yields (90 - 99%). Finally, the challenging *N*-debenzylation of **18c** was overcome by means of Pd black in warm acidic aqueous MeOH. The corresponding *N*-debenzylated lanthionines are promising building blocks for α-alkyl lanthionines-containing peptides synthesis.

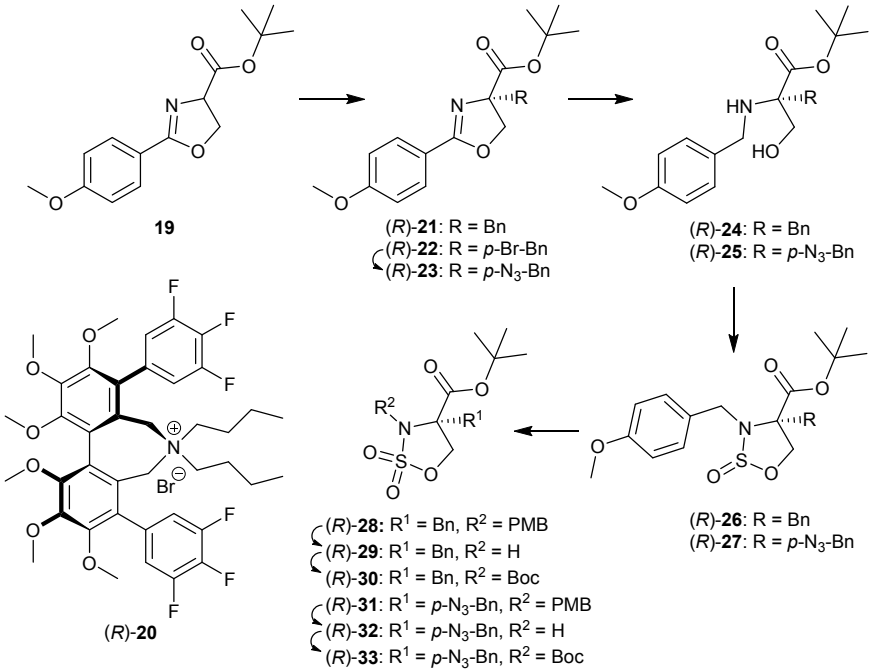


Scheme 2. Preparation of differently protected α-alkyl-Lan **18a-d**.

In our *Organic & Biomolecular Chemistry* publication, the synthesis of modified tripeptides (*S*)-Ala-γ-(*R*)-Glu-X where X = (*R*,*S*) and (*R*,*R*) diastereomers of α-benzyl or α-(4-azidobenzyl)lanthionine, was carried out (**3** and **4**, Figure 1). These

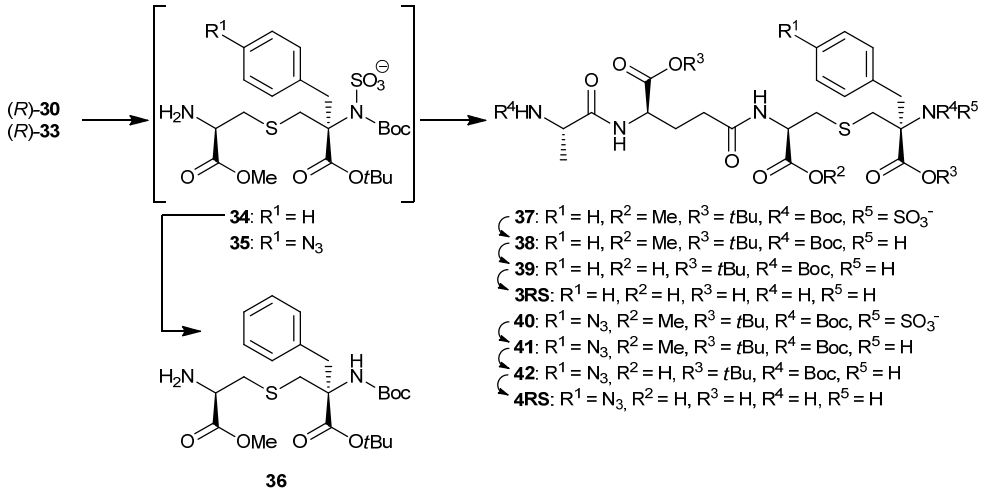
four tripeptides (**3**, **4** and their α-benzyl Lan epimers) were prepared as potential analogues of the natural tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm.

The chemical strategy involved the multigram preparation of the 4-MeO-phenyloxazoline **19** (four steps, 51%). Enantioselective alkylation of **19** using the chiral PTC (*R*)-**20** (previously synthesized in six steps, 30%) afforded the (*R*) enantiomer of 4-alkylated oxazolines **21**-**22** with > 89% ee (87 - 99% yield, Scheme 3). Subsequently, 4-bromo-benzyl of (*R*)-**22** was substituted with an azido to give (*R*)-**23** (99%). Reductive opening of the oxazolines (*R*)-**21** and (*R*)-**23** gave α-benzyl serines (*R*)-**24** and (*R*)-**25** (90 - 99%), respectively. Cyclization and oxidation, led to PMB protected sulfamidates (*R*)-**28** and (*R*)-**31** (73 - 78%, two steps). In the next reactions, the PMB group was removed to yield (*R*)-**29** and (*R*)-**32** (53 - 80%). These structures were confirmed by X-ray diffraction analyses.



Scheme 3. Preparation of enantiopure (*R*)-α-benzyl and (*R*)-α-(4-azidobenzyl) sulfamidates **28**-**33**. The (*S*) sulfamidates were similarly obtained using (*S*)-**20**.

Subsequent Boc protection of (*R*)-**29** and (*R*)-**32** (90 - 93%) and regioselective opening of the resulting (*R*)-**30** and (*R*)-**33** with cysteine methyl ester led to protected lanthionines **34** and **35**, respectively (Scheme 4). These compounds were either isolated (**36**, 78%) or further elaborated in a one-pot process to the corresponding protected tripeptides **38** and **41** in 78 - 84% yields. Hydrolysis of the methyl ester (94 - 97%) and acidolysis of the Boc groups afforded the final tripeptides **3RS** (73%) and **4RS** (88%). Using the same approach, the epimers **3RR** and **4RR** were also obtained from sulfamidates (*S*)-**30** and (*S*)-**33**, respectively. The tripetides **3RR** and **3RS** were recovered at the gram scale required for the PG incorporation experiments.



Scheme 4. Preparation of differenty protected (*S*)-α-benzyl Lan 36 and tripeptides 3RS and 4RS. The tripeptides 3RR and 4RR were similarly prepared from the (*S*)-α-benzyl sulfamidate (*S*)-30 and the (*S*)-α-(4-azidobenzyl) sulfamidate (*S*)-33.

As the synthesis described in Schemes 3 and 4 employs mild, non reductive conditions, photoactivatable groups such as phenylazido or trifluoromethyldiazirine can be introduced in these protected Lan. Those compounds can be used for synthesis of peptides, for example modified lantibiotics. In addition, the route is suitable for incorporation of ^{35}S. So, radioactive labelled peptides containing a photocrosslinker are accessible targets. Furthermore, the phenyl azido group can also be useful for click-chemistry with fluorescent dyes.

In another prospect, the α-benzyl sulfamidates (*R*)-**30** and (*S*)-**30** are known precursors in ^{18}F chemistry giving access to enantiopure (*S*) or (*R*)-[^{18}F]fluoromethyl-phenylalanine, respectively.

Biological experiments showed the incorporation of *meso*-Lan and (*R*,*R*)-Lan into PG. 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 **1** was not incorporated. This result indicates that the introduction in the PG biosynthesis of lanthionines with a substituted aromatic group at the α-position *via* the MurE catalysis is not feasible.

Successful *in vitro* biosynthesis of UDP-MurNAc-tripeptides was achieved. The tripeptides (*S*)-Ala-γ-(*R*)-Glu-(*R,S*)-α-benzyl-lanthionine **3RS** and (*S*)-Ala-γ-(*R*)-Glu-(*R,R*)-α-benzyl-lanthionine **3RR**, are substrates of Mpl *in vitro*. No incorporation of these tripeptides in the PG of *E. coli* was observed *in vivo*. Nevertheless, this result shows that it is probably possible to introduce non-modified lanthionines in PG biosynthesis by the recycling pathway.

Summary

The widespread use of antibiotics has caused bacteria to become drug resistant. Efforts have been done by many research groups to identify new molecules as inhibitors or inactivators of resistant proteins like Penicillin Binding Proteins (PBPs) or to find new targets which allow putting peptidoglycan (PG) biosynthesis under control. The aim of this work is to develop a new labeling method for PG allowing to identify novel antibiotics and worthy target for inhibition.

In *Escherichia coli*, *meso*-diaminopimelic acid (A_2pm) is a key molecule for reticulation of PG. During this work, we synthesized *meso*-lanthionine, the monosulfur analogue of *meso*-A_2pm, and some of its derivatives. As these compounds are susceptible to enter PG biosynthesis via MurE enzymatic addition onto UDP-(*S*)-Ala-γ-(*R*)-Glu, their use in a new labeling method has been investigated. Another approach was the introduction of labeled lanthionines in PG *via* the recycling pathway by replacement of the natural tripeptide (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm with tripeptide analogues containing modified lanthionines.

We have developed a new stereoselective synthesis of the three lanthionine diastereomers (*meso*-Lan, (*R,R*)-Lan and (*S,S*)-Lan) and of an α-benzylated analogue. Good yields at the gram scale and excellent diastereomeric excesses (>99%), without any enrichment step, were obtained in aqueous solution. Biological experiments showed the incorporation of *meso*-Lan and (*R,R*)-Lan into PG. Upon this results, [^{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 was not incorporated. This result indicates that the introduction of lanthionines with a substituted aromatic group at this position is not feasible *via* the reaction catalysed by MurE.

We have also developed a synthesis of protected lanthionines bearing an α-alkyl group useful for the preparation of PG building block analogues. An enantiomeric excess of 97% was obtained for the alkylation of an oxazoline precursor. An S_N2 reaction with a cysteine on the β-carbon of cyclic sulfamidate precursors was highly successful, despite the sterically crowded α-carbon. In this way, we have obtained several protected α-alkyl lanthionines. These products are promising building blocks for peptide synthesis.

In this report, we also describe a diastereoselective synthesis of α-substituted tripeptides. These compounds, containing a fluorescent tag or a photoactivatable group situated on an α-carbon, could potentially be used to study PG biosynthesis. We have obtained chiral α-alkyl sulfamidates with enantiomeric excess of 89 - 97%. Those sulfamidates are excellent electrophiles with cysteine to provide lanthionines. The lanthionines were then readily converted into tripeptides (*S*)-Ala-γ-(*R*)-Glu-(*R,S/R,R*)-α-benzyl-lanthionine and phenylazido analogues in a one pot reaction. Those tripeptides were substrates of Mpl *in vitro.* We did not observe any incorporation of these tripeptides in the PG of *E. coli*.

Résumé

L'utilisation généralisée des antibiotiques a rendu beaucoup de bactéries résistantes à ces médicaments. De nombreux groupes de recherches se sont intéressés à ce problème. Il est donc primordial d'identifier de nouvelles molécules inhibitrices de protéines résistantes telles les *Penicillin Binding Proteins* (PBPs) et de trouver de nouvelles cibles qui permettent de mettre la biosynthèse du peptidoglycane (PG) sous contrôle. Mettre au point une méthode originale de marquage du PG constituerait une nouvelle stratégie qui permettrait d'identifier de nouveaux antibiotiques et cibles dignes d'inhibition.

Dans *Escherichia coli*, l'acide *méso*-diaminopimélique (A_2pm) est une molécule clé pour la réticulation du PG. Au cours de ce travail, nous avons synthétisé la *méso*-lanthionine, l'analogue soufré du *méso*-A_2pm, et certains de ses dérivés. Leur utilisation en vue du marquage a ensuite éte étudiée car ces composés sont succeptibles d'entrer dans la biosynthèse du PG par addition enzymatique sur UDP-(*S*)-Ala-γ-(*R*)-Glu catalysée par l'enzyme MurE. Une autre approche investiguée consistait à introduire les lanthionines *via* la voie de recyclage du PG en remplaçant le tripeptide naturel (*S*)-Ala-γ-(*R*)-Glu-*meso*-A_2pm par des analogues de tripeptide contenant des lanthionines modifiées.

Nous avons donc développé une nouvelle synthèse stéréosélective des trois diastéréoisomères de la lanthionine (*meso*-Lan, (*R*,*R*)-Lan et (*S*,*S*)-Lan) et d'un analogue α-benzylé. De bons rendements à l'échelle du gramme et d'excellents excès diastéréomériques (> 99%), sans aucune étape d'enrichissement, ont été obtenus en solution aqueuse. Les expériences biologiques ont montré l'incorporation des *méso*-Lan et (*R*,*R*)-Lan dans le PG. Sur base de ces résultats, les diastéréoisomères de la [^{35}S]lanthionine pourraient être utilisés pour étudier la biosynthèse du PG et son renouvellement lors de la croissance et de la division cellulaire. Malheureusement, la lanthionine α-benzylée n'a pas été incorporée. Ce résultat indique que l'introduction de lanthionines avec un groupe aromatique substitué à cette position n'est pas réalisable *via* la réaction catalysée par MurE.

Nous avons également développé une synthèse de lanthionines protégées portant un groupe α-alkyle utile pour la préparation d'analogues de précurseurs du PG. Un excès énantiomérique de 97% a été obtenu lors de l'alkylation d'un précurseur de type oxazoline. Une réaction S_N2 d'une cystéine sur le carbone β de sulfamidates cycliques a été couronnée de succès, et ce, en dépit du carbone α stériquement encombré. Ainsi, nous avons pu obtenir diverses lanthionines protégées α-alkylées. Ces produits sont des molécules prometteuses pour la synthèse peptidique.

Dans ce rapport, nous décrivons également une synthèse diastéréosélective de tripeptides α-substitués. Ces composés contenant un marqueur fluorescent ou un groupe photoactivable situé sur un carbone α pourraient être utilisés pour étudier la biosynthèse du PG. Nous avons obtenu des sulfamidates chiraux α-alkylés avec un excès énantiomérique de 89 à 97%. Ces sulfamidates constituent d'excellents électrophiles avec la cystéine pour fournir des lanthionines. Les lanthionines ont ensuite été converties aisément en tripeptides (*S*)-Ala-γ-(*R*)-Glu-(*R,S/R,R*)-α-benzyl-lanthionine et en analogues porteurs d'un phénylazido lors d'une réaction monotope. Ces tripeptides sont des substrats de Mpl *in vitro*. Nous n'avons cependant pas observé l'incorporation de ces tripeptides dans le PG d'*E. coli*.