

# Synthesis of natural and non natural orthogonally protected lanthionines from *N*-tritylserine and *allo*-threonine derivatives

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**Abstract:** The reactivity of electrophiles derived from *N*-tritylserine, threonine and *allo*-threonine esters toward a selection of nucleophiles was investigated. Best yields from substitution products were obtained with *N*-trityliodoalanine and soft nucleophiles such as thiols. This strategy was applied to the synthesis of lanthionines, the monosulfide analogs of cysteine. Orthogonally protected sulfide adducts from L- and D-cysteines, *threo*- $\beta$ -methyl-L-cysteine and D-penicillamine were isolated in 81–88% yield (ee>98%). This strategy was applied to the preparation of lanthionine and cyclolanthionine suitably protected for peptide synthesis. © 1997 Elsevier Science Ltd

## Introduction

In a previous paper,<sup>1</sup> we investigated the potential of electrophiles derived from *N*-tritylserine esters<sup>2</sup> as novel alanyl  $\beta$ -cation equivalents towards basic soft nucleophiles. We showed that *N*-trityl-3-iodoalanine esters obtained from the corresponding mesylates can be substituted by malonate related anions in good yields and almost without epimerization. The trityl group not only prevents  $\alpha$ -proton abstraction but also protects the  $\alpha$ -ester from saponification and can be selectively removed when various protecting groups are used. We planed to extend this strategy to other nucleophiles in particular for the preparation of orthogonally protected and enantiomerically pure lanthionines starting from the serine and threonine series.

Lanthionines are the major constituents of peptides lantibiotics which possess interesting antibacterial, cytotoxic and immunomodulating activities, and promising biomedical applications (Figure 1).<sup>3</sup> Lanthionines are monosulfide cysteine analogs which result from the 1,4-addition of cysteine or homocysteine residues, or a thioenol precursor on to the  $\alpha,\beta$ -dehydroamino acids dehydroalanine and dehydroaminobutyric acid.<sup>3a-c</sup>

Cysteine monosulfides are widely distributed in natural products such as peptides isolated from plants (carboxypropylcysteine)<sup>4</sup> or antibiotics (griseoviridin, penicillin family).<sup>5</sup> Furthermore, syn-

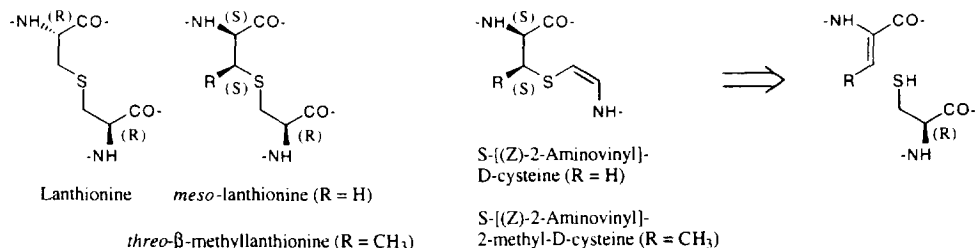
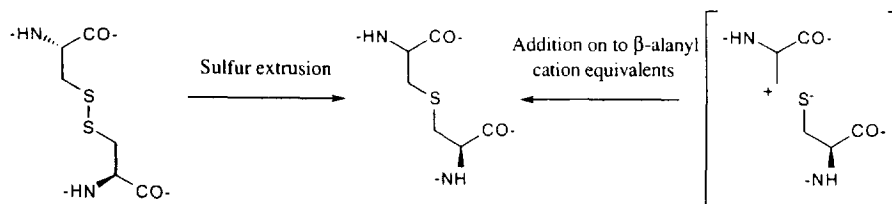


Figure 1.

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thetic orthogonally protected cyclolanthionines have been recently proposed as constrained building blocks for the design of novel peptide mimetics.<sup>6</sup>

Two strategies have been previously considered for synthesising lanthionines and lantibiotics (Scheme 1). Sulfur extrusion of protected cystine, or cystine containing peptides, led to the corresponding monosulfides, without racemisation.<sup>7</sup> However, conversion yields were low and this strategy could not be transposed to the synthesis of peptides containing several sulfide bridges, due to presumed disulfide exchange.<sup>8</sup> The biomimetic conjugate addition of cysteine derivatives on to protected dehydroamino acids has been extensively used though it proceeded non diastereospecifically, except in the case of intramolecular cyclization of peptides.<sup>3d,9</sup>



Scheme 1.

In order to overcome the lack of stereospecificity of the 1,4-addition, several alanyl β-cation equivalents have been previously tested as lanthionine precursors (Figure 2). The basic conditions required for generating thiolates precluded the use of halogenoalanine, since connection results from an elimination–addition process which causes epimerisation.<sup>10</sup> The ring-opening of aziridine-2-carboxylates **1** and **2** did not give satisfactory yields (12–37%).<sup>11</sup> Recently, Goodman and coworkers<sup>12</sup> described an interesting use of the serine β-lactone **3** developed by Vederas *et al.*<sup>13</sup> However, a competitive formation of thioester was observed with cysteine. Moreover, attempts for opening compound **3** with cysteine<sup>14</sup> or Fmoc-cysteine allyl ester have failed.<sup>15</sup> The conjugate addition of the Fmoc-cysteine allyl ester to the protected dehydroalanine **4** followed by a chromatographic separation of the two diastereomers has been proposed as an alternative route.<sup>14</sup>

In the present paper, we describe the synthesis of lanthionines suitably protected for solid phase peptide synthesis and orthogonally protected cyclolanthionines, starting from the easily available *N*-tritylserine methyl ester **5** and benzyl ester **6**.

## Results and discussion

### Preliminary study

We evaluated the reactivity of several *N*-trityl-β-hydroxy-amino acids derived electrophiles toward a selection of nucleophiles. As already noticed with the soft nucleophilic malonates, mesylate derivative of *N*-tritylserine methyl ester was inert at room temperature.<sup>1</sup> Above 40°C, it reacted with hard nucleophiles (F<sup>-</sup>, N<sub>3</sub><sup>-</sup>, CN<sup>-</sup>) or a mild nucleophile such as benzylamine, and gave the corresponding aziridine-2-carboxylate in high yields (91–96%). In the case of thiolates, a mixture of thioether and aziridine was obtained whereas, with sodium iodide in acetone, *N*-trityl-3-iodoalanine was isolated in quantitative yield.<sup>16</sup> As suggested by previous observations,<sup>1</sup> inter- and intramolecular substitutions

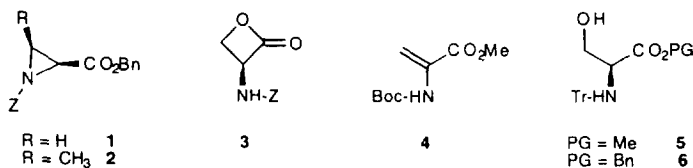
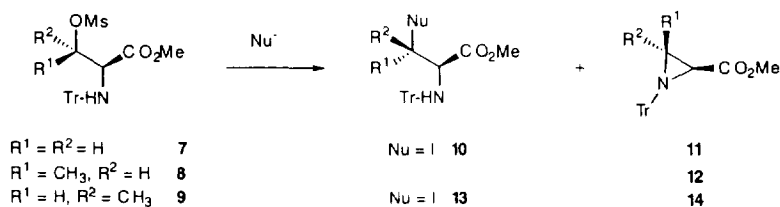


Figure 2.

are competitive reactions: cyclization was favored by high temperatures, basic reagents and strong nucleophiles.  $^1\text{H}$  NMR analysis of compounds **7** and **10** clearly indicated the existence of at least two rotamers for iodide **10** whereas the more hindered mesylate **7** appeared as a single population in the same conditions ( $\text{CDCl}_3$ ,  $20^\circ\text{C}$ ).<sup>17,18</sup> The methanesulfonyl group which should adopt an *anti* position relative to the triphenylmethyl moiety, disfavors substitution versus cyclization (Scheme 2).



Scheme 2. (see associated table in ref<sup>16</sup>)

As anticipated, the mesylates derived from *N*-tritylthreonine **8** and *allo*-threonine **9** were less reactive (no reaction below  $60^\circ\text{C}$ ). In all cases, compound **8** led exclusively to the *cis*-aziridine **12** in 81–99% yields, a result which should be correlated to the marked tendency of classical threonine derived electrophiles to generate the corresponding  $\alpha,\beta$ -didehydroamino acids.<sup>19,20</sup> In the same conditions, substitution of the *allo*-threonine mesylate **9** by sodium iodide in refluxing acetone led to a non easily separable mixture of iodide **13** (36%) and *cis*-aziridine **12** (31%) beside traces of its *trans*-isomer **14**.<sup>16</sup> Unfortunately, we failed to improve both the yield and purity of iodide at the preparative scale. Moreover, all our attempts for substituting iodide **13** with thiolates were unsuccessful: *cis*-aziridine **12** was obtained as the major product beside a mixture of non identified compounds.

The more polarisable *N*-trityl-3-iodoalanine **10** was tested toward nucleophiles. Even though it poorly reacted with benzylamine to give the protected 2,3-diaminopropionate **15** (20%) (Figure 3), coupling with ethanedithiol (67%, dithioether **16**)<sup>21</sup> was obtained with considerably higher yield than those observed with mesylate **7** (8%).<sup>16</sup>

We focused our attention on the *N*-trityl-3-iodoalanine benzyl ester **17**, as common lanthionines precursor since it provides a complete orthogonality of protection: the trityl group is removed under acidic conditions (5% formic acid in 1,2-dichloroethane or TFA) while the benzyl ester is saponified after prior removal of trityl;<sup>10</sup> both groups can be hydrogenolyzed quantitatively. Moreover, this reagent can be easily prepared at the molar scale and stored for several months at  $-20^\circ\text{C}$  without detectable degradation.

### Synthesis of lanthionines

Iodide **17** was substituted by conveniently protected L-cysteine, L-*threo*-3-methylcysteine and D-penicillamine. The corresponding lanthionines were isolated in good yields together with the aziridines (Scheme 3, Table 1, entries 1–4). Best results were obtained in DMF with cesium carbonate as a base.<sup>15</sup> *threo*- $\beta$ -Methyl cysteine was obtained from Boc-*allo*-threonine methyl ester according to Baldwin *et al.*<sup>20b</sup> (its (2*R*,3*S*) epimer can be prepared by the procedure of Wakamiya *et al.*).<sup>20a</sup> Results obtained with penicillamine derivative Boc-D-Pen-OMe are equivalent to those reported by Goodman and

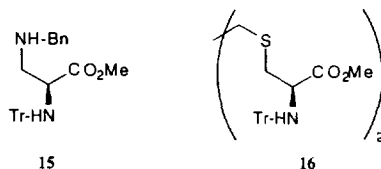
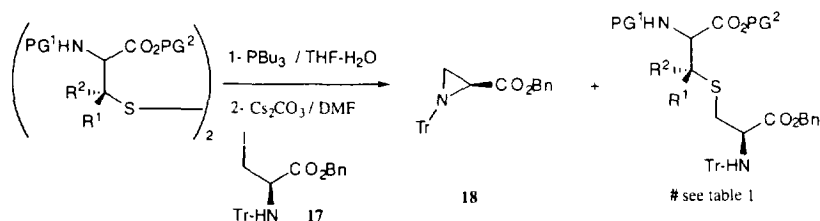


Figure 3.

coworkers with serine- $\beta$ -lactones. In the case of protected cysteines, yields have been improved since *N*-tritylamino esters cannot undergo an O-acyl fission.<sup>12</sup>



Scheme 3.

Fmoc-protected cysteine was allowed to react with compound **17** under the conditions described above, however, lanthionine **23** was obtained in moderate yields (Table 1, entry 5). The relatively hydrophobic penicillamine reacted with iodide **17** in a mixture of DMF:DMSO 9:1 and gave the derivative **22** in low yields (13%) after trapping the intermediate with di-*tert*-butyl dicarbonate and diazomethane. The reaction could not be transposed to the Fmoc series due to the basic conditions employed (Scheme 4).

#### Determination of the enantiomeric excesses

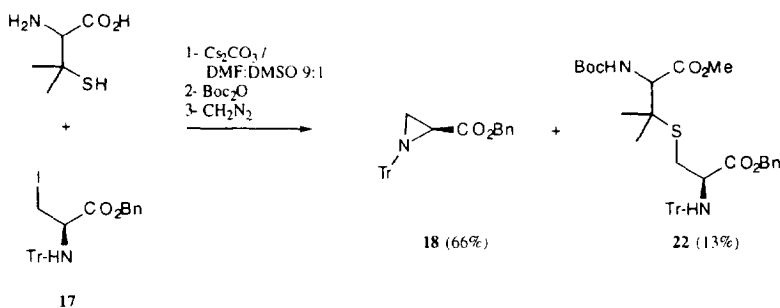
On the basis of previous results obtained in our laboratory, we anticipated that the stereochemistry was conserved at the  $\alpha$ -position of the *N*-tritylalanine moiety.<sup>1</sup> In order to ascertain the absolute configuration of each stereogenic center, compounds **19**, **20** and **23** were treated with formic acid (5% in dichloroethane) and desulfurized (Raney Nickel)<sup>22</sup> into alanine derivatives which were deprotected by acidic hydrolysis (refluxing 6N HCl, 1 hour). The residues were quantitatively derivatised with an excess of (+)-FLEC-Cl<sup>®</sup> and analysed by HPLC as previously reported.<sup>23</sup> Elution of (+)-FLEC-Ala and comparison with authentic samples of (+)-FLEC-(D,L)-Ala and (+)-FLEC-(L)-Ala, established the good enantiomeric purity (*ee*>98%) of these lanthionines.<sup>24</sup> However desulfurization was slow and incomplete after 24 hours at 40°C. We could not, therefore, rule out a diastereoselective desulfurization and hence a bias in the *ee* determination. To overcome this difficulty, the data were independently confirmed by a chemical correlation: starting from *N*-trityl-3-iodoalanine methyl esters of the D and L series, we synthesized lanthionines **24** (the methyl ester equivalent of compound **19**) and its epimer **25** which were transformed into the corresponding *N*-Boc, *N'*-Boc-lanthionine dimethyl ester **26** and its *meso* isomer **27** (Figure 4).

<sup>1</sup>H and <sup>13</sup>C NMR and DCI mass spectra of these products displayed only very slight differences; however, for each diastereomer, no trace of the other isomer could be detected by NMR. In order to confirm this result, compounds **26** was degraded under mild conditions: acidolysis of the Boc groups (TFA, 0°C), thus desulfurization followed by cautious saponification (lithium hydroxide) provided enantiomerically pure L-alanine (no trace of the D-isomer was observed by HPLC elution of the (+)-

Table 1.

entry	Disulfides configurations	R <sup>1</sup>	R <sup>2</sup>	PG <sup>1</sup>	PG <sup>2</sup>	<b>18</b> (%) <sup>a</sup>	Cmpd. (%) <sup>a</sup>	<i>ee</i> (%) <sup>b</sup>
1	2R	H	H	Fmoc	<i>t</i> Bu	8	<b>19</b> (88)	> 98
2	2R	H	H	Boc	Me	17	<b>20</b> (79)	> 98
3	2R,3R <sup>c</sup>	H	CH <sub>3</sub>	Boc	Me	7	<b>21</b> (83)	ND
4	2S	CH <sub>3</sub>	CH <sub>3</sub>	Boc	Me	13	<b>22</b> (81)	ND
5	2R	H	H	Fmoc	H	35	<b>23</b> (41)	> 98

<sup>a</sup> Isolated yields; all reactions were carried out at the 1 mmol scale. <sup>b</sup> Determined after derivatization (see text). <sup>c</sup> Diastereomer of the natural *threo*- $\beta$ -methylanthionine.



Scheme 4.

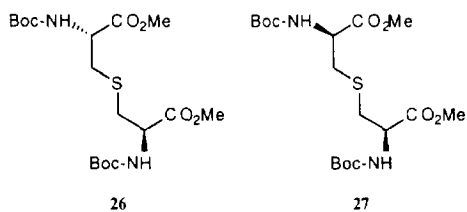


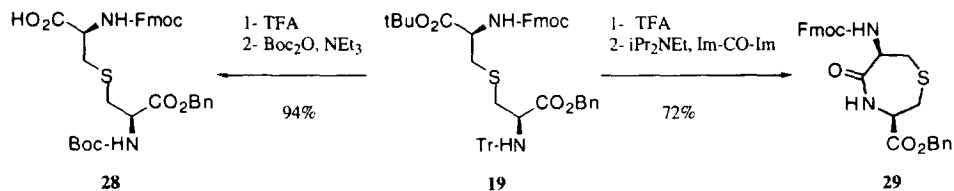
Figure 4.

FLEC derivative.<sup>27</sup> Compound **27**, treated in the same way, led to an equimolar mixture of D- and L-alanine. These data clearly indicated that the slight racemisation observed by HPLC (<1%) after degradation of trityllanthionines, may be attributed to a parasitic effect of the acidic hydrolysis.<sup>25</sup>

<sup>1</sup>H NMR spectra of the constrained compounds **16** and **19–25** confirmed the existence of at least two rotamers.<sup>1,18</sup> The unusual chemical shifts observed in some cases for the methyl ester ( $\delta$  3.55–3.23 ppm) suggest a shielding effect due to the bulky trityl group<sup>2a</sup> whereas less hindered compounds **16**, **26**, **27** display standard values.

### Applications

Treatment of the *N*-trityllanthionine **19** with trifluoroacetic acid and protection of the amine moiety with a Boc group generated lanthionine **28** which was suitably protected for solid phase peptide synthesis<sup>4c,9a,10</sup> in high yield (94%). Orthogonal deprotection of *N*-Boc,*N'*-trityl-lanthionine diesters (compounds **20**, **24** or **25**) has been already reported by another group.<sup>10</sup> The 7-membered cyclolanthionine **29** was isolated in 72% yield (ee>88%) after acidic deprotection, and thus intramolecular coupling using carbonyldiimidazole in DMF (Scheme 5). The decrease in enantiomeric excess may be considered as an artefact rather than a partial racemization of the product during the coupling with carbonyldiimidazole. As a matter of fact, the strong acidic conditions (6N HCl, 110°C, 4 hours) used for opening and deprotecting the cyclic dipeptide are well-known for racemising lanthionines.<sup>10</sup>



Scheme 5.

## Conclusion

*N*-Trityl-3-iodoalanine esters are readily available reagents which display valuable reactivities toward heteroatom nucleophiles. We observed that iodine may be substituted in good yields with thiolates. In addition to prevent the epimerization of the stereogenic center ( $ee > 98\%$ ), the bulky and easily removed trityl group enabled us to reach a complete orthogonality of protection. These new alanyl  $\beta$ -cation equivalents have been used for the synthesis of lanthionines derived from cysteine, *threo*-methyl-cysteine and penicillamine. We demonstrated that lanthionine and cyclolanthionine derivatives, suitably protected for peptide synthesis, can be easily obtained from the corresponding trityl protected compounds. In contrast, mesylates derived from *N*-tritylthreonine and *allo*-threonine cyclised preferentially into the aziridine-2-carboxylates; the corresponding iodides could not be synthesised and isolated in satisfactory yields. Work is now in progress to design synthetic equivalents for homoalanyl  $\beta$ -cations with the view of synthesising  $\beta, \beta'$ -methylcyclolanthionines which are interesting elements to build constrained peptidomimetic scaffold.

## Experimental section

### General procedures

All reagents employed were of analytical grade and were purchased from Aldrich Chemical Co and Lancaster Synthesis Ltd. THF was distilled before use from sodium-benzophenone. Amino acids derivatives were purchased from Aldrich Chemical Co and Bachem. Flash chromatography was performed on 40–60  $\mu\text{m}$  (230–400 mesh) Merck silica gel. High pressure liquid chromatography was performed on a Waters 625 LC HPLC system coupled to a Waters 991 Photodiode Array detector. Melting points were measured on a hot stage Kofler apparatus and are uncorrected. Optical rotations were measured on a Schmidt & Haensch Polartronic-E polarimeter.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker WM 250 NMR spectrometer with tertamethylsilane as an internal reference:  $\delta$  and  $J$  values are given in ppm and Hz respectively. Mass spectra were recorded on a Nermag 10–10 mass spectrometer coupled to a Digital PDP 11 computer. Mass Combustion analysis were carried out by the 'Service Central d'Analyse' of the CNRS (Vernaison, France).

### (*S*)-Benzyl-*O*-methanesulfonyl-*N*-triphenylmethyl-serinate **7**

Compound **7** was synthesized as reported previously.<sup>1</sup> **7**:  $[\alpha]_{\text{D}}^{20} = +20$  ( $c = 1.0$ , chloroform);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.54–7.43 (m) +7.32–7.17 (m) (20H, CH Ar Tr+Bn), 4.75 (d,  $J = 12.1$ , 1H, HCH–Ph), 4.52 (d,  $J = 12.1$ , 1H, HCH–Ph), 4.41 (dd,  $J = 4.2$ ,  $J' = 9.9$ , 1H, HCH–OSO<sub>2</sub>), 4.20 (dd,  $J = 6.2$ ,  $J' = 9.9$ , 1H, HCH–OSO<sub>2</sub>), 3.69 (m, dd,  $J = 4.2$ ,  $J' = 6.2$ , 1H, CH–CO<sub>2</sub>), 2.95 (b, 1H, NH), 2.79+2.75+2.71 (app. t, inequiv 3H, OSO<sub>2</sub>CH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  171.4 (CO<sub>2</sub>), 145.3+135.0+128.6–126.7 (complex) (C+CH Ar), 71.1 (CPh<sub>3</sub>), 70.5 (OSO<sub>2</sub>CH<sub>3</sub>), 67.2 (CH<sub>2</sub>–Ph), 55.6 (CH–CO<sub>2</sub>), 37.3 (CH<sub>2</sub>OSO<sub>2</sub>); MS (DCI, NH<sub>3</sub>)  $m/z = 548$  (MNH<sub>4</sub><sup>+</sup>, 30%); 243 (Tr<sup>+</sup>, 100%).

### (*2S,3R*)-Methyl-*O*-methanesulfonyl-*N*-triphenylmethyl-threoninate **8**<sup>1</sup>

$[\alpha]_{\text{D}}^{20} = +52$  ( $c = 1.0$ , chloroform);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.54–7.44 (m, 6H) +7.31–7.16 (m, 9H) (CH Tr), 5.10 (m, 1H, CH–OSO<sub>2</sub>), 3.66 (dd,  $J = 5.0$ ,  $J' = 10.9$ , 1H, CH–CO<sub>2</sub>), 3.19 (s, 3H, OCH<sub>3</sub>), 2.94 (s, 3H, OSO<sub>2</sub>CH<sub>3</sub>), 2.75 (bd,  $J = 10.9$ , 1H, NH), 1.53 (d,  $J = 6.5$ , 3H, C–CH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  171.6 (CO<sub>2</sub>), 145.2 (C Ar), 128.7+127.9+126.6 (CH Ar), 79.1 (OSO<sub>2</sub>CH<sub>3</sub>), 70.9 (CPh<sub>3</sub>), 59.1 (CH–CO<sub>2</sub>), 52.0 (OCH<sub>3</sub>), 38.6 (CH–OSO<sub>2</sub>), 17.2 (C–CH<sub>3</sub>); MS (DCI, NH<sub>3</sub>)  $m/z = 454$  (MH<sup>+</sup>, 10%), 358 (MH<sup>+</sup>–CH<sub>3</sub>SO<sub>3</sub>H, 100% or **12**, H<sup>+</sup>).

### (*2S,3S*)-Methyl-*O*-methanesulfonyl-*N*-triphenylmethyl-threoninate **9**<sup>1</sup>

Mp = 132–139°C (Dec.);  $[\alpha]_{\text{D}}^{20} = +5$  ( $c = 1.0$ , chloroform);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.57–7.46 (m, 6H) +7.30–7.15 (m, 9H) (CH Tr), 5.04 (dt,  $J = 3.2$ ,  $J' = 6.6$ ,  $J'' = 6.6$ , 1H, CH–OSO<sub>2</sub>), 3.53 (dd,  $J = 3.1$ ,  $J' = 10.3$ , 1H, CH–CO<sub>2</sub>), 3.21 (s, 3H, OCH<sub>3</sub>), 3.07 (s, 3H, OSO<sub>2</sub>CH<sub>3</sub>), 2.98 (bd,  $J = 10.3$ , 1H, NH), 1.43 (d,  $J = 6.6$ , 3H, C–CH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  170.9 (CO<sub>2</sub>), 145.3 (C Ar), 128.8+128.0+126.6 (CH

Ar), 80.8 (OSO<sub>2</sub>CH<sub>3</sub>), 71.1 (CPh<sub>3</sub>), 60.3 (CH-CO<sub>2</sub>), 51.9 (OCH<sub>3</sub>), 38.8 (CH-OSO<sub>2</sub>), 18.2 (C-CH<sub>3</sub>); MS (DCI, NH<sub>3</sub>) m/z=454 (MH<sup>+</sup>, 1%), 243 (Tr<sup>+</sup>, 100%), 212 (M<sub>2</sub>H<sup>+</sup>, 10%).

*(2S,3S)-Methyl-N-triphenylmethyl-3-methyl-aziridine-2-carboxylate 12<sup>16</sup>*

Mp=108–110°C (lit. 109–110°C);<sup>20,26</sup> [α]<sub>D</sub><sup>20</sup>=−101 (c=1.0, chloroform);<sup>20,26</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.57–7.49 (m, 6H) +7.33–7.18 (m, 9H) (CH Ar Tr), 3.74 (s, 3H, OCH<sub>3</sub>), 1.88 (d, *J*=6.5, 1H, CH-CO<sub>2</sub>), 1.63 (m, 1H, HC-N), 1.37 (d, *J*=5.5, 3H, C-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.7 (CO<sub>2</sub>), 143.9 (C Ar), 129.4+127.6+126.8 (CH Ar), 75.0 (CPh<sub>3</sub>), 51.8 (OCH<sub>3</sub>), 35.9+34.8 (CH-CO<sub>2</sub>+CH-CH<sub>3</sub>), 13.3 (C-CH<sub>3</sub>); MS (DCI, NH<sub>3</sub>): m/z=358 (MH<sup>+</sup>, 25%), 243 (Tr<sup>+</sup>, 100%); Anal. calcd for C<sub>24</sub>H<sub>23</sub>NO<sub>2</sub>: C, 80.64; H, 6.48; N, 3.92. Found: C, 80.49; H, 6.54; N, 3.78.

*(2S,3S)-Methyl-N-triphenylmethyl-2-amino-3-iodo-butanoate 13<sup>16</sup>*

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.57–7.49 (m) +7.32–7.18 (m) (15H, CH Ar Tr), 4.52 (dtt, *J*=4.0, *J'*=7.3, *J''*=7.4, 1H, CH-I), 3.21 (s, 3H, OCH<sub>3</sub>), 3.03 (dd, *J*=4.0, *J'*=10.3, 1H, CH-CO<sub>2</sub>), 1.56 (b, 1H, NH), 1.84 (d, *J*=7.3, 3H, C-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 171.1 (CO<sub>2</sub>), 146.8 (C Ar), 128.7+127.9+127.2+126.6 (CH Ar), 70.8 (CPh<sub>3</sub>), 61.0 (OCH<sub>3</sub>), 51.5 (CH-CO<sub>2</sub>), 33.0 (CH-I), 26.0 (C-CH<sub>3</sub>); MS (DCI, NH<sub>3</sub>) m/z=486 (MH<sup>+</sup>, <1%), 243 (MH<sup>+</sup> and MH<sup>+</sup>-Tr<sup>+</sup>, 100%).

*(2S,3R)-Methyl-N-triphenylmethyl-3-methyl-aziridine-2-carboxylate 14<sup>16</sup>*

Mp=141–143°C; [α]<sub>D</sub><sup>20</sup>=+10 (c=1.0, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.52–7.47 (m, 6H) +7.35–7.19 (m, 9H) (CH Ar Tr), 3.69 (s, 3H, OCH<sub>3</sub>), 2.948 (dq, *J*=2.3, *J'*=6.2, 1H, CH-CH<sub>3</sub>), 2.35 (d, *J*=2.3, 1H, CH-CO<sub>2</sub>), 0.60 (d, *J*=6.2, 1H, CH-CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.3 (CO<sub>2</sub>), 146.8 (C Ar), 127.9+127.5+127.2 (CH Ar), 73.0 (CPh<sub>3</sub>), 51.9 (OCH<sub>3</sub>), 41.9+38.2 (CH-CO<sub>2</sub>+C-CH<sub>3</sub>), 13.7 (C-CH<sub>3</sub>); MS (DCI, NH<sub>3</sub>) m/z=358 (MH<sup>+</sup>, <1%), 243 (Tr<sup>+</sup>, 100%); Anal. calcd for C<sub>24</sub>H<sub>23</sub>NO<sub>2</sub>: C, 80.64; H, 6.48; N, 3.92. Found: C, 80.95; H, 6.24; N, 3.63.

*(S)-Methyl-N<sup>α</sup>-triphenylmethyl-N<sup>β</sup>-benzyl-diaminopropionate 15*

Compound **10** (451 mg, 1 mmol) was treated with benzylamine (220 μL, 2 mmol) in THF (10 mL) overnight at 40°C. After evaporation of the solvent, the product, dissolved in ethyl acetate, was washed with water. The organic layer was dried over sodium sulfate and the solvent was eliminated under reduced pressure. Silica gel flash chromatography (solvent ethyl acetate–cyclohexane 10–90) afforded **11** (241 mg, 70%) and **15** (92 mg, 20%): [α]<sub>D</sub><sup>25</sup>=−12 (c=1.0, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.46 (m, 6H), 7.30–7.13 (m, 14H), 3.74 (s, 3H), 3.61 (AB, *J*<sub>AB</sub>=13.1, *v*<sub>A</sub>=3.68, *v*<sub>B</sub>=3.55, 2H), 3.37 (dd, *J*=4.8, *J'*=6.3, 1H), 2.48 (dd, *J*=4.8, *J'*=11.6, 1H), 2.38 (dd, *J*=6.3, *J'*=11.6, 1H), 2.17 (b, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 174.6, 145.8, 139.6, 129.3, 128.6, 128.3+128.2, 127.8, 127.1, 126.3, 70.5, 60.5, 51.8+51.7, 45.1; MS (DCI, NH<sub>3</sub>): m/z 451 (MH<sup>+</sup>, 15%), 344 (MH<sup>+</sup>-Bn-NH<sub>2</sub>, 2%), 243 (Tr<sup>+</sup>, 100%), 209 (MH<sub>2</sub><sup>+</sup>-Tr<sup>+</sup>, 3%); Anal. calcd for C<sub>30</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub>: C, 79.97; H, 6.71; N, 6.22. Found: C, 79.89; H, 6.97; N, 6.38.

*1,2-Bis(methyl-N-triphenylmethyl-alanyl-thio) ethane 16*

Compound **10** (451 mg, 1 mmol) in DMF (10 mL) was treated with ethanedithiol (47 μL, 0.5 mmol) and cesium carbonate (326 mg, 1 mmol) 2 hours, at room temperature. After reduction of the volume, the product was dissolved in diethyl ether, washed twice with water and the organic layer was dried over sodium sulfate. Evaporation of the solvent under reduced pressure and silica gel flash chromatography (solvent: ethyl acetate–cyclohexane 15–85) yielded **11** (20 mg, 6%) and **16** (524 mg, 67%): [α]<sub>D</sub><sup>25</sup>=+33 (c=1.0, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.54–7.44 (m, 12H) +7.29–7.14 (m, 18H) (CH Ar Tr), 3.75 (4 peaks, 3H, OCH<sub>3</sub>), 3.51 (b, 1H, CH-CO<sub>2</sub>), 3.39 (m, 1H, CH-CO<sub>2</sub>), 3.23 (4 peaks, 3H, OCH<sub>3</sub>), 2.95–2.57 (m, 9H, SCH<sub>2</sub>CH<sub>2</sub> S +2 CH<sub>2</sub>S+NH), 2.45 (m, 1H, NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.2+171.9 (CO<sub>2</sub>), 145.6 (2 peaks, C Ar), 129.3–126.4 (complex, CH Ar), 71.1+70.7 (CPh<sub>3</sub>), 56.3+52.3+51.7+47.5+44.2+38.2+37.1+32.3+31.9+31.0 (CH-CO<sub>2</sub>+OCH<sub>3</sub>+SCH<sub>2</sub>CH<sub>2</sub>S); MS (DCI, NH<sub>3</sub>): m/z 781 (MH<sup>+</sup>, <1%), 539 (M<sub>2</sub>H<sup>+</sup>-Tr<sup>+</sup>, <1%), 438 (M<sub>2</sub>H<sup>+</sup>-Tr<sup>+</sup>-Azy-OMe, <1%), 378

( $M_2H^+ - Tr^+ - Azy-OMe-C_2H_4S$ , <1%), 344 ( $MH_2^+ - Tr^+ - Azy-OMe-C_2H_4S_2$ , 2%), 243 ( $Tr^+$ , 100%); Anal. calcd for  $C_{48}H_{48}N_2O_4S_2$ : C, 73.81; H, 6.19; N, 3.59. Found: C, 73.95; H, 6.50; N, 3.88.

#### Synthesis of lanthionines 19–23 from disulfides; general procedure

Disulfide (0.5 mmol) was treated with tributylphosphine (125  $\mu$ L, 1 equiv.) and in wet THF for 15 min (cystine) or 2 hours (methylcysteine disulfide) at room temperature. After reduction of the volume under reduced pressure, the product, dissolved in ethyl acetate, was washed with 10% citric acid and with brine and the solvent was evaporated in *vacuo*. The resulting oil was used immediately without further purification. For penicillamine disulfide (0.75 mmol), the reduction was achieved in good yield with a 10-fold excess of tributylphosphine in refluxing wet THF for 2 days. The product was isolated as described above and was promptly purified by silica gel flash-chromatography (eluent: ethyl acetate–hexane 25–75).

A mixture of thiol (1 mmol th.) and compound **17** (547 mg, 1 mmol) was treated with cesium carbonate (326 mg, 1 equiv.) in dry DMF, for 4 hours at room temperature. After dilution with diisopropyl ether, the solution was washed with 10% citric acid. The aqueous layer was extracted with diisopropyl ether. The combined organic layers were washed with 10% citric acid and with brine. After evaporation of the solvent, the product was purified by silica gel flash-chromatography (eluent: see below).

#### (*R*)-Benzyl-*N*-triphenylmethyl-alanyl (*R*)-tert-butyl-*N*-fluorenylmethylloxycarbonyl-alanyl sulfide **19**

Flash-chromatography (eluent: ethyl acetate–hexane 15–85) afforded **18** (33 mg, 8%)<sup>14</sup> and **19** (719 mg, 88%):  $[\alpha]_D^{20} = -6$  ( $c=0.5$ , ethyl acetate);  $^1H$  NMR ( $CDCl_3$ ): (2 rotamers 1:2)  $\delta$  7.75 (d,  $J=7.3$ , 2H) +7.58 (t,  $J=7.8$ , 2H) +7.49 (m, 2H) +7.40 (m, 6H) +7.35–7.14 (m, 16H) (CH Ar), 5.66+5.57 (2b, 1H, NH–Fmoc); 5.19 (AB) +4.61 (AB,  $J_{AB}=12.2$ ,  $\nu_A=4.70$ ,  $\nu_B=4.52$ ) (2H,  $CH_2$  Bn), 4.49–4.11 (m) +4.34 (d,  $J=6.8$ ) +4.20 (t,  $J=6.8$ ) (4H,  $CH-CO_2+CH-CH_2$  Fmoc), 3.56 (m, 1H,  $CH-CO_2$ ), 3.10–2.68 (m) +2.59 (m) +2.45 (m) (4H,  $CH_2-S-CH_2$ ), 2.05 (b, 1H, NH–Tr), 1.46+1.44 (2s, 9H,  $CH_3$  *t*Bu);  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  173.1+171.5+169.3 ( $CO_2$ Bn+ $CO_2$ *t*Bu), 155.7 (OCON), 145.5+143.8+141.2+135.4+135.3+128.5–125.1 (complex) +119.9 (C+CH Ar), 82.9 ( $CMe_3$ ), 71.1+70.7+67.1 ( $CPh_3+CH-CH_2$  Fmoc+ $CH_2$  Bn), 56.3+54.2+53.9 (2  $CH-CO_2$ ), 47.0 ( $CH-CH_2$  Fmoc), 44.5+44.3 ( $CH_2-S$ ), 34.3+33.8 ( $S-CH_2$ ), 27.9 ( $CH_3$  *t*Bu); MS (DCI,  $NH_3$ ):  $m/z$  819 ( $MH^+$ , 5%), 577 ( $MH_2^+ - Tr^+$ , 15%), 243 ( $Tr^+$ , 100%); Anal. calcd for  $C_{51}H_{50}N_2O_6S$ : C, 74.79; H, 6.15; N, 3.42; S, 3.92. Found: C, 74.91; H, 6.06; N, 3.10; S, 4.04.

#### (*R*)-Benzyl-*N*-triphenylmethyl-alanyl (*R*)-methyl-*N*-tert-butylloxycarbonyl-alanyl sulfide **20**

Flash-chromatography (eluent: ethyl acetate–hexane 15–85) afforded **18** (73 mg, 17%)<sup>14</sup> and **20** (516 mg, 79%):  $[\alpha]_D^{20} = +6.5$  ( $c=1.0$ , ethyl acetate);  $^1H$  NMR ( $CDCl_3$ ): (3 rotamers 4:3:3)  $\delta$  7.52–7.14 (m, 20H, CH Ar), 5.38–5.27 (b, 1H, NH–Boc); 5.20 (AB) +4.60 (AB,  $J_{AB}=12.2$ ,  $\nu_A=4.71$ ,  $\nu_B=4.50$ ) +4.50 (b) (3H,  $CH_2$  Bn+ $CH-CO_2$ ), 3.71+3.68 (2s, 3H,  $OCH_3$ ), 3.58 (dd,  $J=4.9$ ,  $J'=6.8$ ) +3.49 (dd,  $J=6.1$ ,  $J'=7.3$ ) (1H,  $CH-CO_2$ ), 3.02–2.79 (m) +2.59 (m) +2.44 (m) (4H,  $CH_2-S-CH_2$ ), 2.2 (b, 1H, NH–Tr), 1.44+1.43+1.39 (3s, 9H,  $CH_3$  Boc);  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  173.1+171.4+171.3+171.2 ( $CO_2$ Bn+ $CO_2$ Me), 155.1 (OCON), 145.6+145.5+135.4+135.2+128.7–127.9 (complex) (CH Ar), 80.2 ( $CMe_3$ ), 71.2+70.8+70.7+67.0+66.9+66.8 ( $CPh_3+CH_2$  Bn), 56.3+53.1 (2  $CH-CO_2$ ), 52.6+52.5 ( $OCH_3$ ), 37.9+35.4+33.8+31.9 ( $CH_2-S-CH_2$ ) 29.6+28.2 ( $CH_3$  Boc); MS (DCI,  $NH_3$ ):  $m/z$  655 ( $MH^+$ , 25%), 413 ( $MH_2^+ - Tr^+$ , 10%), 243 ( $Tr^+$ , 100%); Anal. calcd for  $C_{38}H_{42}N_2O_6S$ : C, 69.70; H, 6.46; N, 4.28; S, 4.90. Found: C, 69.89; H, 6.61; N, 4.06; S, 4.49.

#### (2*R*,3*R*)-Methyl-*N*-tert-butylloxycarbonyl-homoalanyl (*R*)-benzyl-*N*-triphenylmethyl-alanyl sulfide **21**

Flash-chromatography (eluent: ethyl acetate–hexane 10–90) afforded **18** (30 mg, 7%)<sup>14</sup> and **21** (555 mg, 83%):  $[\alpha]_D^{20} = +37.5$  ( $c=1.0$ , ethyl acetate);  $^1H$  NMR ( $CDCl_3$ ): (3 rotamers 5:3:2)  $\delta$  7.50–7.13 (m, 20H, CH Ar), 5.3 (b, 1H, NH–Boc); 5.21+5.20 (2s) +4.60 (AB,  $J_{AB}=12.2$ ,  $\nu_A=4.71$ ,  $\nu_B=4.50$ ) (2H,  $CH_2$  Bn), 4.44 (dt,  $J=2.9$ ,  $J'=9.7$ ,  $CH-CO_2$ ), 3.68+3.64+3.61 (3s, 3H,  $OCH_3$ ), 3.52 (m, 1H,



CH-CO<sub>2</sub>), 2.93–2.34 (m, 3H, CH<sub>2</sub>-S-CH), 2.04 (b, 1H, NH-Tr), 1.44+1.43+1.42 (3s, 9H, CH<sub>3</sub> Boc), 1.28 (*J*=7.1) +1.21 (d) +1.19 (d, *J*=6.9) (3H, CH<sub>3</sub>-C-S); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.0+171.9+171.3 (CO<sub>2</sub>Bn+CO<sub>2</sub>Me), 155.7 (OCON), 145.6+145.5+145.4+135.4+135.2+128.7–127.9 (complex) +1126.5+126.3 (CH Ar), 80.1+80.0 (CMe<sub>3</sub>), 71.2+70.8+70.7+67.0+66.8 (CPh<sub>3</sub>+CH<sub>2</sub> Bn), 58.2+57.8+56.3 (2 CH-CO<sub>2</sub>), 52.4 (2 peaks) +52.3 (OCH<sub>3</sub>), 47.3+44.9+43.8+43.6+43.1+36.7 (CH-S-CH<sub>2</sub>) 28.2+26.8 (CH<sub>3</sub> Boc), 19.6+19.5 (CH<sub>3</sub>-C-S); MS (DCI, NH<sub>3</sub>): *m/z* 669 (MH<sup>+</sup>, 35%), 427 (MH<sub>2</sub><sup>+</sup> - Tr<sup>+</sup>, 5%), 243 (Tr<sup>+</sup>); Anal. calcd for C<sub>39</sub>H<sub>44</sub>N<sub>2</sub>O<sub>6</sub>S: C, 70.03; H, 6.63; N, 4.19; S, 4.79. Found: C, 70.27; H, 6.69; N, 4.03; S, 4.59.

(*S*)-Methyl-*N*-tert-butylloxycarbonyl-valinyl (*R*)-benzyl-*N*-triphenylmethyl-alanyl sulfide **22** (from Boc-*D*-Pen-OMe)<sub>2</sub>)

Flash-chromatography (eluent: ethyl acetate–hexane 10–90) afforded **18** (55 mg, 13%)<sup>14</sup> and **22** (548 mg, 81%): [α]<sub>D</sub><sup>20</sup>=+7 (c=1.0, ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>): (3 rotamers) δ 7.51 (d, *J*=7.5, 2H, CH Ar), 7.46–7.12 (m, 18H, CH Ar), 5.68 (bd) +5.54 (bd) +5.40 (bd) (1H, NH-Boc); 5.22 (AB, *J*<sub>AB</sub>=12.3, *v*<sub>A</sub>=5.26, *v*<sub>B</sub>=5.18) +5.19 (s) +4.62 (AB, *J*<sub>AB</sub>=12.3, *v*<sub>A</sub>=4.72, *v*<sub>B</sub>=4.52) (2H, CH<sub>2</sub> Bn), 4.35 (d, *J*=9.5) +4.28 (d, *J*=8.9) (1H, CH-CO<sub>2</sub>), 3.72–3.52 (m, 1H, CH-CO<sub>2</sub>), 3.66+3.61+3.55 (3s, 3H, OCH<sub>3</sub>), 2.95–2.7 (m) +2.60 (m) +2.39 (m) (2H, CH<sub>2</sub>-S-C), 2.16 (b, 1H, NH-Tr), 1.43–1.22 (m, 15H, CH<sub>3</sub> Boc+CH<sub>3</sub> Pen); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.1+172.6+172.4+171.0+170.6 (CO<sub>2</sub>Bn+CO<sub>2</sub>Me), 155.2 (OCON), 145.6+145.5+145.4+138.6+135.4+135.2+130.7+128.7–127.8 (complex) +126.5+126.4+126.2 (CH Ar), 80.0 (CMe<sub>3</sub>), 71.1+70.8+70.6+67.0+66.8+66.6 (CPh<sub>3</sub>+CH<sub>2</sub> Bn), 61.0+60.4+56.0 (2 CH-CO<sub>2</sub>), 51.9+51.8 (OCH<sub>3</sub>), 49.1–44.9 (CH<sub>2</sub>-S), 33.3+31.8 ((CH<sub>3</sub>)<sub>2</sub>C-S) 29.6+29.2+28.2 (CH<sub>3</sub> Boc), 26.6+26.2+25.7+22.6+15.2+14.0 ((CH<sub>3</sub>)<sub>2</sub>C-S); MS (DCI, NH<sub>3</sub>): *m/z* 683 (MH<sup>+</sup>, 30%), 243 (Tr<sup>+</sup>); Anal. calcd for C<sub>40</sub>H<sub>46</sub>N<sub>2</sub>O<sub>6</sub>S: C, 70.35; H, 6.79; N, 4.10; S, 4.70. Found: C, 70.62; H, 6.72; N, 3.77; S, 4.35.

(*R*)-Benzyl-*N*-triphenylmethyl-alanyl (*R*)-*N*-fluorenylmethyloxycarbonyl-alanyl sulfide **23**

Flash-chromatography (eluent: ethyl acetate–hexane 15–85 then chloroform–methanol 95–5) afforded **18** (148 mg, 35%)<sup>14</sup> and **23** (304 mg, 41%): [α]<sub>D</sub><sup>20</sup>=+12 (c=0.5, ethyl acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.00 (bs, 1H, CO<sub>2</sub>H), 7.66 (bd, 2H, CH Ar), 7.6–7.0 (m, 26H, CH Ar), 6.1 (b, 1H, NH-Fmoc); 5.1 (b, 2H, CH<sub>2</sub> Bn), 4.5–4.0 (m, 4H, CH-CO<sub>2</sub>+CH-CH<sub>2</sub> Fmoc), 3.55 (m, 1H, CH-CO<sub>2</sub>), 3.1–2.8 (m) +2.51 (m) +2.40 (m) (4H, CH<sub>2</sub>-S-CH<sub>2</sub>), 2.05 (b, 1H, NH-Tr); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 180.8 (CO<sub>2</sub>H), 172.2 (CO<sub>2</sub>Bn), 162.7 (OCON), 145.4+145.3+143.8+41.1+135.4+128.4–125.3 (complex) +119.7 (CH Ar), 70.7+67.0 (CPh<sub>3</sub>+CH<sub>2</sub> Bn+CH Fmoc), 53.4 (CH-CO<sub>2</sub>), 46.9 (CH<sub>2</sub> Fmoc), 36.5+31.4 (CH<sub>2</sub>-S-CH<sub>2</sub>); MS (DCI, NH<sub>3</sub>): *m/z* 764 (MH<sup>+</sup>, <1%), 521 (MH<sub>2</sub><sup>+</sup> - Tr<sup>+</sup>, 3%), 243 (Tr<sup>+</sup>, 100%).

(*S*)-Methyl-*N*-tert-butylloxycarbonyl-valinyl (*R*)-benzyl-*N*-triphenylmethyl-alanyl sulfide **22** (from *D*-Pen)

*D*-Penicillamine (224 mg, 1.5 equiv.) was dissolved in DMSO (1 mL). Compound **17** (547 mg, 1 mmol) in DMF (9 mL) and cesium carbonate (326 mg, 1 equiv.) were added and the mixture was stirred overnight at room temperature. Diterbutyl dicarbonate (250 mg, 1.1 equiv.) was added and the mixture was stirred for 5 hours. The volume was reduced under reduced pressure and the product, dissolved in ethyl acetate, was washed 3 times with 10% citric acid. After evaporation of the solvent *in vacuo*, the product was esterified with diazomethane in diethyl ether. The reaction mixture was diluted in diisopropyl ether and washed 6 times with 10% citric acid. Solvent removal under reduced pressure and purification by silica gel flash-chromatography yielded **22** (87 mg, 13%): [α]<sub>D</sub><sup>20</sup>=+6.5 (c=1.0, ethyl acetate); <sup>1</sup>H and <sup>13</sup>C NMR spectra identical to compound **22** obtained from Boc-Pen-OMe.

(*R*)-Methyl-*N*-triphenylmethyl-alanyl (*R*)-methyl-*N*-tert-butylloxycarbonyl-alanyl sulfide **24** and (*S*)-Methyl-*N*-triphenylmethyl-alanyl (*R*)-methyl-*N*-tert-butylloxycarbonyl-alanyl sulfide **25**

Compounds **24** and **25** were prepared from iodide **10** (471 mg, 1 mmol) and purified as described for compound **19**.

**24** (470 mg, 81%):  $[\alpha]_{\text{D}}^{20} = +11.5$  ( $c=1.0$ , ethyl acetate);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): (2 rotamers 1:2)  $\delta$  7.48 (m, 6H) +7.34–7.15 (m, 9H) (CH Ar), 5.38 (bm, 1H, NH–Boc); 4.52 (m, 1H, CH–CO<sub>2</sub>), 3.76+3.75+3.72+3.23 (4s, 6H, 2 OCH<sub>3</sub>), 3.53+3.44 (2m, 1H, CH–CO<sub>2</sub>), 3.1–2.8 (m) +2.76–2.56 (m) +2.40 (m) (4H, CH<sub>2</sub>–S–CH<sub>2</sub>), 2.25 (b, 1H, NH–Tr), 1.44+1.42 (2s, 9H, CH<sub>3</sub> Boc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  173.8+172.1+171.3+171.2 (2 CO<sub>2</sub>Me), 155.1 (OCON), 145.6+145.5+128.7+128.5 (2 peaks) +128.1+127.9+126.5+126.4 (C+CH Ar), 80.2+80.0 (CMe<sub>3</sub>), 71.1+70.7 (CPh<sub>3</sub>), 56.3+52.5+52.4+51.8 (2 OCH<sub>3</sub>), 53.3+53.1 (2 CH–CO<sub>2</sub>), 44.2+44.1+37.9+35.3 (CH<sub>2</sub>–S–CH<sub>2</sub>), 28.2 (CH<sub>3</sub> Boc); MS (DCI, NH<sub>3</sub>):  $m/z$  579 (MH<sup>+</sup>, 15%), 344 (MH, NH<sub>4</sub><sup>+</sup> – Tr<sup>+</sup>, 10%), 243 (Tr<sup>+</sup>, 100%).

**25** (448 mg, 78%):  $[\alpha]_{\text{D}}^{20} = -6$  ( $c=1.0$ , ethyl acetate);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): (2 rotamers 1:2)  $\delta$  7.47–7.41 (m, 6H) +7.31–7.16 (m, 9H) (CH Ar), 5.38 (bm, 1H, NH–Boc); 4.53 (m, 1H, CH–CO<sub>2</sub>), 3.76+3.75 (2s, 3H, OCH<sub>3</sub>), 3.72 (s, 3H, OCH<sub>3</sub>), 3.55 (t,  $J=6.1$ ) +3.44 (t,  $J=6.7$ ) (1H, CH–CO<sub>2</sub>), 3.09–2.84 (m, 2H, CH<sub>2</sub>–S–CH<sub>2</sub>), 2.61 (m, 1H) +2.40 (m, 1H) (CH<sub>2</sub>–S–CH<sub>2</sub>), 2.20 (b, 1H, NH–Tr), 1.44+1.42+1.40 (3s, 9H, CH<sub>3</sub> Boc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  172.1+171.2 (2 CO<sub>2</sub>Me), 145.5+128.7+128.5+128.4+127.9+126.4 (C+CH Ar), 80.2 (CMe<sub>3</sub>), 70.7 (CPh<sub>3</sub>), 53.2+52.5+52.4 (2 OCH<sub>3</sub>), 44.3+44.1+33.9 (CH<sub>2</sub>–S–CH<sub>2</sub>), 28.2 (CH<sub>3</sub> Boc); MS (DCI, NH<sub>3</sub>):  $m/z$  579 (MH<sup>+</sup>, 25%), 344 (MH, NH<sub>4</sub><sup>+</sup> – Tr<sup>+</sup>, 5%), 243 (Tr<sup>+</sup>, 100%).

*(R,R)*-Dimethyl-*N,N'*-tert-butylloxycarbonyl-lanthioninate **26** and *(R,S)*-dimethyl-*N,N'*-tert-butylloxycarbonyl-lanthioninate **27**

Compounds **25** or **26** (58 mg, 1 mmol) were treated with 5% formic acid in dichloroethane (5 mL) 10 min at room temperature. After removal of the solvent under reduced pressure, the product was resuspended in dichloromethane (5 mL) and the mixture was neutralized by addition of triethylamine. An equivalent of triethylamine (30  $\mu\text{L}$ ) then di-tert-butyl dicarbonate (22 mg, 1 equiv.) were added. The mixture was stirred overnight. Standard work-up and purification by silica gel flash-chromatography afforded:

**26** (39 mg, 97%):  $[\alpha]_{\text{D}}^{20} = -12$  ( $c=0.5$ , ethyl acetate);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.41 (b, 2H, NH–Boc); 4.56 (bm, 2H, CH–CO<sub>2</sub>), 3.78 (s, 6H, OCH<sub>3</sub>), 3.19–2.91 (m, 4H, CH<sub>2</sub>–S–CH<sub>2</sub>), 1.46 (s, 18H, CH<sub>3</sub> Boc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ): 171.2+171.1 (CO<sub>2</sub>Me), 155.1 (OCON Boc), 80.3 (CMe<sub>3</sub>), 53.2 (CH–CO<sub>2</sub>), 52.7 (OCH<sub>3</sub>), 35.3+35.2 (CH<sub>2</sub>–S–CH<sub>2</sub>), 28.2 (CH<sub>3</sub> Boc); MS (DCI, NH<sub>3</sub>):  $m/z$  454 (MNH<sub>4</sub><sup>+</sup>, 45%), 437 (MH<sup>+</sup>, 100%), 398 (MNH<sub>4</sub><sup>+</sup> – C<sub>4</sub>H<sub>8</sub>, 15%), 381 (MNH<sub>4</sub><sup>+</sup> – C<sub>4</sub>H<sub>8</sub>, 25%), 337 (MH<sup>+</sup> – C<sub>4</sub>H<sub>8</sub> – CO<sub>2</sub>, 5%); 253 (MH, NH<sub>4</sub><sup>+</sup> – 2 C<sub>4</sub>H<sub>8</sub> – 2 CO<sub>2</sub>, 20%), 236 (MH<sub>2</sub><sup>+</sup> – 2 C<sub>4</sub>H<sub>8</sub> – 2 CO<sub>2</sub>, 25%); Anal. calcd for C<sub>18</sub>H<sub>32</sub>N<sub>2</sub>O<sub>8</sub>S: C, 53.44; H, 7.97; N, 6.92; S, 7.93. Found: C, 53.05; H, 7.56; N, 6.94; S, 7.56.

**27** (39 mg, 97%):  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.41 (b, 2H, NH–Boc); 4.54 (bm, 2H, CH–CO<sub>2</sub>), 3.77 (s, 6H, OCH<sub>3</sub>), 3.08–2.97 (m, 4H, CH<sub>2</sub>–S–CH<sub>2</sub>), 1.44 (2s, 18H, CH<sub>3</sub> Boc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ): 171.1 (CO<sub>2</sub>Me), 155.2 (OCON Boc), 80.3 (CMe<sub>3</sub>), 53.3 (CH–CO<sub>2</sub>), 52.7 (OCH<sub>3</sub>), 35.2 (CH<sub>2</sub>–S–CH<sub>2</sub>), 28.2 (CH<sub>3</sub> Boc); MS (DCI, NH<sub>3</sub>):  $m/z$  454 (MNH<sub>4</sub><sup>+</sup>, 60%), 437 (MH<sup>+</sup>, 100%), 398 (MNH<sub>4</sub><sup>+</sup> – C<sub>4</sub>H<sub>8</sub>, 20%), 381 (MNH<sub>4</sub><sup>+</sup> – C<sub>4</sub>H<sub>8</sub>, 25%), 337 (MH<sup>+</sup> – C<sub>4</sub>H<sub>8</sub> – CO<sub>2</sub>, 5%), 253 (MH, NH<sub>4</sub><sup>+</sup> – 2 C<sub>4</sub>H<sub>8</sub> – 2 CO<sub>2</sub>, 25%), 236 (MH<sub>2</sub><sup>+</sup> – 2 C<sub>4</sub>H<sub>8</sub> – 2 CO<sub>2</sub>, 30%); Anal. calcd for C<sub>18</sub>H<sub>32</sub>N<sub>2</sub>O<sub>8</sub>S: C, 53.44; H, 7.97; N, 6.92; S, 7.93. Found: C, 53.34; H, 7.56; N, 7.08; S, 7.59.

*(R)*-Benzyl-*N*-tert-butylloxycarbonyl-alanyl *(R)*-*N*-fluorenylmethylloxycarbonyl-alanyl sulfide **28**

Compound **19** (409 mg, 0.5 mmol) was stirred for 30 min at room temperature in a mixture TFA–dichloromethane 50–50 (20 mL). The solvent was evaporated under reduced pressure. The residue was resuspended in dichloromethane (10 mL) and the remaining acid was neutralized by a dropwise addition of triethylamine. Then, triethylamine (140  $\mu\text{L}$ , 2 equiv.) was added and the mixture was treated with di-tert-butyl dicarbonate (110 mg, 1 equiv.) overnight at room temperature. Standard work-up and purification by silica gel flash-chromatography (eluent: ethyl acetate–hexane–acetic acid 48–50–2) afforded **28** (292 mg, 94%):  $[\alpha]_{\text{D}}^{20} = -9.5$  ( $c=1.0$ , ethyl acetate);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.74–7.09 (m, 13H, CH Ar), 5.97 (bd) +5.90 (b) (2H, NH–Boc+NH–Fmoc); 5.17 (bm, 2H, CH<sub>2</sub> Bn), 4.8–4.5 (m, 2H, 2 CH–CO<sub>2</sub>), 4.5–4.0 (m, 3H, CH–CH<sub>2</sub> Fmoc), 3.5–2.9 (m, 4H, CH<sub>2</sub>–S–CH<sub>2</sub>),

1.42 (s, 9H, CH<sub>3</sub> *t*Bu); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.2 (CO<sub>2</sub>Bn), 156.2+155.9 (OCON Boc+Fmoc), 143.9+143.6+141.1+128.9–125.2 (complex) +119.8 (C+CH Ar), 82.0 (CH–CH<sub>2</sub> Fmoc), 80.4 (CMe<sub>3</sub>), 67.2 (CH<sub>2</sub> Bn), 54.4+53.7 (2 CH–CO<sub>2</sub>), 41.7 (CH–CH<sub>2</sub> Fmoc), 45.9 (CH<sub>2</sub>–S), 41.7 (S–CH<sub>2</sub>), 28.2 (CH<sub>3</sub> Boc); MS (DCI, NH<sub>3</sub>): *m/z* 638 (MNH<sub>4</sub><sup>+</sup>, 10%), 521 (MH<sub>2</sub><sup>+</sup>–C<sub>4</sub>H<sub>8</sub>–CO<sub>2</sub>, 5%), Anal. calcd for C<sub>33</sub>H<sub>36</sub>N<sub>2</sub>O<sub>8</sub>S: C, 63.85; H, 5.84; N, 4.51; S, 5.17. Found: C, 63.64; H, 6.21; N, 4.33; S, 4.92.

*(2R,6R)*-Benzyl-*N*-fluorenylmethylloxycarbonyl-cyclolanthionine **29**

Compound **19** (409 mg, 0.5 mmol) was stirred for 30 min at room temperature in a mixture TFA–dichloromethane 50–50 (20 mL). The solvent was evaporated under reduced pressure. The residue was resuspended in dry DMF (25 mL) and the remaining acid was neutralized by a dropwise addition of diisopropylethylamine. Then, diisopropylethylamine (140 μL, 2 equiv.) was added and the mixture was treated with carbonyldiimidazole (162 mg, 2 equiv.) overnight at room temperature. The reaction mixture was poured into 10% citric acid and the product was extracted with diisopropyl ether (3 times). The organic layer was washed twice with 10% citric acid and with brine and the solvent was evaporated *in vacuo*. Purification by silica gel flash-chromatography (eluent: ethyl acetate–hexane 30–70) gave compound **29** (176 mg, 72%): mp=185–186°C; [α]<sub>D</sub><sup>20</sup>=+13 (c=0.5, chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.76 (d, *J*=7.5, 2H, CH Ar), 7.60 (d, *J*=7.3, 2H, CH Ar), 7.43–7.26 (m, 9H, CH Ar), 6.74 (d, *J*=5.4, 1H, NH–CO–C), 6.29 (d, *J*=5.5, 1H, NH–Fmoc); 5.25 (s, 2H, CH<sub>2</sub> Bn), 4.67 (m, 1H, CH–CO<sub>2</sub>) +4.58 (dd, *J*=5.6, *J'*=9.2, 1H, CH–CO<sub>2</sub>), 4.39 (d, *J*=7.0, 2H, CH–CH<sub>2</sub> Fmoc), 4.21 (t, *J*=7.0, 1H, CH–CH<sub>2</sub> Fmoc), 3.03–2.65 (m, 4H, CH<sub>2</sub>–S–CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 171.7+168.9 (CO<sub>2</sub>Bn+CONH), 155.2 (OCON Fmoc), 143.7+141.2+134.1+129.2–127.0 (complex) +125.1+120.0 (C+CH Ar), 68.7+67.0 (CH<sub>2</sub> Bn+CH–CH<sub>2</sub> Fmoc), 58.9+57.5 (2 CH–CO<sub>2</sub>), 47.1 (CH–CH<sub>2</sub> Fmoc), 34.0+31.9 (CH<sub>2</sub>–S–CH<sub>2</sub>); MS (DCI, NH<sub>3</sub>): *m/z* 620 (MNH<sub>4</sub><sup>+</sup>, 100%); Anal. calcd for C<sub>28</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S: C, 66.91; H, 5.21; N, 5.57; S, 6.38. Found: C, 67.12; H, 5.65; N, 5.67; S, 5.96.

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- 16.

Nu <sup>-</sup>	Conditions	11 (%) <sup>a</sup> from 7	12 (%) <sup>a</sup> from 8	14 (%) <sup>a</sup> from 9
F <sup>-</sup>	Bu <sub>4</sub> NF (10 equiv.) / THF, 25°C, overnight (11) or reflux, 1 hr (12)	91	81	ND
N <sub>3</sub> <sup>-</sup>	NaN <sub>3</sub> (5 equiv.) / refluxing methanol, 30 min	95	97	ND
CN <sup>-</sup>	KCN (2 equiv.) / refluxing methanol, 15 min	96 <sup>b</sup>	99	95
Bn-NH <sub>2</sub>	Bn-NH <sub>2</sub> (2 equiv.) / refluxing methanol, 1 hr	92	93	ND
-SCH <sub>2</sub> -CH <sub>2</sub> S-	HSCH <sub>2</sub> -CH <sub>2</sub> SH (0.5 equiv.), Cs <sub>2</sub> CO <sub>3</sub> / methanol, 35°C (11) or reflux 1 hr (12 and 14)	75 + 16 (8)	91	90
I <sup>-</sup>	NaI (10 equiv.) / acetone, 35°C overnight (3) or reflux 1 hr (12 and 14)	0 + 10 (99.5) <sup>c</sup>	45	1 + 12 (31) + 13 (36)

<sup>a</sup> isolated yields; <sup>b</sup> ee = 100% (determined by derivatization of the deprotected aziridine and HPLC elution); <sup>14</sup> <sup>c</sup> carried out at the 50 mmol scale.

17. Coalescence of distinct signals, initiated above 35°C, could not be completed due to the simultaneous formation of aziridine over 50°C.

18. The existence of two distinct conformers has been clearly established for *N*-trityl-L-homoserine lactone: (a) Son, J. K.; Kalvin, D.; Woodard, R. W.; *Tetrahedron Lett.* **1988**, *29*, 4045–4048; <sup>1</sup>H NMR of sterically hindered  $\alpha$ -*t*-butyl esters of *N*-tritylglutamates were reported as single rotamers: (b) Baldwin, J.E.; North, M.; Flynn, A.; Moloney, M. G.; *Tetrahedron* **1989**, *45*, 1453–1464; (c) *ibid. Tetrahedron* **1989**, *45*, 1464–1474; more constrained *N*-phenylfluorenyl equivalents have been described: (d) Koskinen, A. M. P.; Rapoport, H.; *J. Org. Chem.* **1989**, *54*, 1859–1866.

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21.

Nu <sup>-</sup>	Conditions	# (%) <sup>a</sup>	11 (%) <sup>a</sup>
F <sup>-</sup>	Bu <sub>4</sub> NF (10 equiv.) / THF, 40°C, 6 hr	0	91
N <sub>3</sub> <sup>-</sup>	NaN <sub>3</sub> (5 equiv.) / methanol, reflux,	0	91
CN <sup>-</sup>	KCN (2 equiv.) / methanol, reflux 15 min	0	97 <sup>b</sup>
Bn-NH <sub>2</sub>	Bn-NH <sub>2</sub> (2 equiv.) / methanol, reflux	15 (20) <sup>c</sup>	70 <sup>b</sup>
-SCH <sub>2</sub> -CH <sub>2</sub> S-	HSCH <sub>2</sub> -CH <sub>2</sub> SH (0.5 equiv.), Cs <sub>2</sub> CO <sub>3</sub> / methanol, rt, 1 hr	16 (67)	6

<sup>a</sup> isolated yields; <sup>b</sup> ee = 100%; <sup>14</sup>c  $[\alpha]_D^{20} = -23$  (lit. -25, C = 5.0, 1N HCl)<sup>28</sup> after complete deprotection to (S)-2,3-diaminopropionate.

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min (>99%) compared with authentic samples of FLEC-(D,L)-Ala (D:  $rt=35.52$  min, 49%; L:  $rt=37.18$  min, 51%) and FLEC-(L)-Ala ( $rt=37.40$  min, 100%).

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