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NEW DIASTEREOSELECTIVE SYNTHESIS OF PROTECTED *MESO*-LANTHIONINE WITH DISCRIMINATION OF THE CHIRAL CENTERS

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Summary : A synthesis of meso-lanthionine with discrimination of the chiral centers is reported. Two cysteine residues of opposite configuration and with orthogonal protections are temporarily and reversibly linked in order to promote the formation of an intramolecular disulfide bridge thus avoiding symmetrization reactions during the sulfur extrusion step.

INTRODUCTION

Lanthionine 1 (Figure 1) is the particular aminoacid resulting from the connection of two alanine moieties through a sulfide bridge. Lanthionine was first isolated from wool[1], human hair or lactalbumine[2], and later feathers[3]. It presumably results from an intramolecular rearrangement of a cystine residue[4], which gives cysteine and dehydroalanine, affording lanthionine *via* a 1,4-addition reaction[5].

Both lanthionine and its 3-methyl homologue have received wide attention due to their biological importance. They are constituents of some polycyclic peptides named "lantibiotics"[6,7] including nisine[8-10] (an efficient food preservative), subtiline[11], epidermine[12] (active against staphylococcus and streptococcus), ancovenine[13] (an enzyme inhibitor) and new compounds having immunostimulant and antitumoral properties[14-16].

Two methods were considered for the preparation of lanthionine-containing peptides. The first one resulted from the oxidation of two cysteine residues, already incorporated in the appropriate peptide chain, thus affording the corresponding cystine peptide. This was followed by sulfur extrusion to give the thioether bridge[9,10,13,17]. The main difficulty, encountered when more than two cysteine residues were included in the peptide chain, is the lack of regioselectivity in the disulfide bridge formation. To avoid this difficulty, protection of each pair of cysteine residues with orthogonal protective groups was attempted. However, after formation of the first disulfide bridge, the oxidation of the second pair of thiols could presumably proceed with some disulfide exchange[18]. Also, it is not always convenient to control the final sulfide positions[19,20].

For instance, these difficulties have been previously encountered in the total synthesis of nisine. However, the synthesis was successfully achieved unequivocally[10] using fragment condensation of each separately prepared cyclic sulfide moiety.

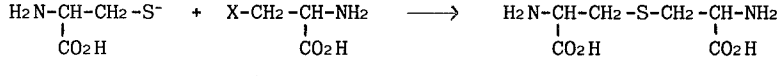
The second stategy involved lengthening the peptide chain by using a previously prepared *meso*-lanthionine derivative. This avoids regioselectivity problems. However, it is necessary to synthesize a *meso*-lanthionine synthon with suitable orthogonal protecting groups in order to differentiate the two opposite chiral centers.

There is currently no satisfactory enantioselective synthesis of such derivatives reported in the literature. Published syntheses of unprotected *meso*-lanthionine belong to two classes of reactions :

- nucleophilic substitution
- sulfur extrusion of cystine residues.

a) Nucleophilic substitution

The simplest procedure involves S-alkylation of L-cysteine with L- ß-chloroalanine[21] or serine[4] (Figure 1). However the required strongly basic conditions are responsible for a ß-elimination reaction followed by a 1,4-addition of the thiolate anion. As a result, a mixture of diastereoisomers, which is not easily separated, is formed in only moderate yield (10-30%)[14,22-24].



- Figure 1 -

Nevertheless, the method has been used in some cases to afford unsymmetrically substituted lanthionine derivatives from substituted starting compounds. However, the differentiation between the two chiral centers has been subject to criticism[23,24] because of further regioselective saponification of only one carboxylate function[25] (results which could not be reproduced by other workers[26]), or difficult regeneration of the carboxylic acid function from an amide group[24].

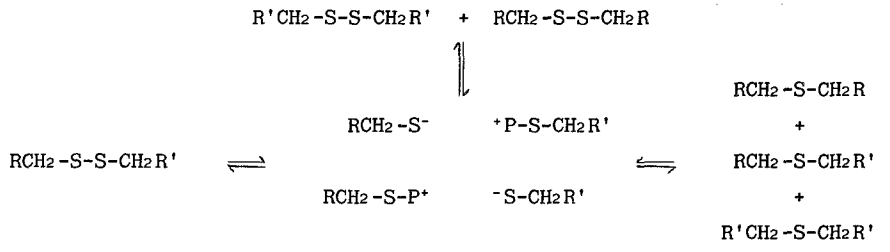
Low yields, added to problems of racemisation[27] and chiral center discrimination, make this approach unattractive for the desired synthesis of *meso*-lanthionine derivatives.

Symmetrical lanthionine derivatives were also prepared *via* the ring-opening of benzyl 1-benzyloxycarbonyl 2-aziridinecarboxylate with benzyl N-Z-cysteinate[28], but the overall yield is only 20% and, moreover, the starting optically active aziridine is not easily available.

Another new route to lanthionine is the ring-opening of the unprotected serine ß-lactone[29] with L-cysteine in water, the pH being kept at 5.0-5.5. Under these specific conditions, only a nucleophilic attack of sulfur on the ß-lactone methylene occurs[30]. This ring-opening reaction has been reported with other nucleophilic compounds[30] but part of these results were not confirmed by those of Le Goffic[31]. Be that as it may, the organic solvents necessary to dissolve the corresponding N-substituted lactones would preclude any thiolate anion formation[30] and the reaction would not occur. Therefore, this route is inappropriate for the synthesis of unsymmetrically substitued lanthionines.

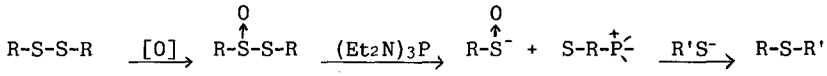
b) Sulfur extrusion of cystine residues

This approach appears the most advantageous because chiral centers are not involved in the reactions. On treatment with tris(diethylamino)phosphine, sulfur extrusion of symmetrical cystine derivatives takes place via a reversible reaction affording lanthionine derivatives mixed with the starting cystine[26,32]. On the other hand, similar treatment applied to unsymmetrical cystine derivatives affords a mixture of three lanthionines and three cystines arising the recombination of ionic intermediates due to phosphine-catalyzed equilibria[32,33] (Figure 2).



- Figure 2 -

To attain pure unsymmetrical lanthionines, Kini[26] suggested differentiation of the sulfur atoms of cystine by converting one of them into a sulfoxide. This should afford only one phosphonium ion which can then react with a cysteine sulfinate ion prepared separately.



- Figure 3 -

Unfortunately a mixture of cystines and lanthionines was again obtained due to the reversibility of the reactions. Moreover, carboxylic acid functions must be protected as they can react with aminophosphines[34,35].

RESULTS

With the aim of synthesizing a *meso*-lanthionine with discrimination of the chiral centers, we decided to use a cyclic disulfide. In this way, the equilibria shown in Figure 2 would be avoided and intramolecular reactions favored. Moreover, only one thioether will be obtained whatever the sulfur atom attacked by the phosphine molecule.

However, this new concept is not free of some challenges : it is necessary to synthesize for the first time a disymmetric cyclic derivative of *meso*-cystine allowing the further preparation of peptides with *meso*-cystine residues because of orthogonal protection of both amine functions.

In order to prepare such a cyclic derivative, we have connected two cysteine molecules with opposite configurations 2 and 3 via a bis-ester link (Figure 4).

Various groups have been considered for the thiol protection[36] (Trt, Acm, Bam, Bn, Nps, Npys); their use will be discussed later. The two amine functional groups have been protected by the usual orthogonal Boc and Z groups. The link between the carboxylic acid functions in 2 and 3 has to be totally stable during further reactions. It must also be easily removed without any racemization at the end of the synthesis. We have chosen, with success, ethyleneglycol as the linking group, the bond formed being short enough to favor intramolecular disulfide formation.

Ester 4 was obtained from S,N-diprotected (L)-cysteines according to two routes :

i- reaction with epoxyethane[37-39] occurring in high yield. However, the reaction must be catalyzed with triphenylphosphine which unfortunately precludes the use of S-protecting groups such as Nps or Npys[40]

ii- reaction with one equivalent of ethyleneglycol in the presence of coupling agents: DCC/DMAP[41], DCC/supported CCP[42], EEDQ[43,44], DPPA[45]. There is always formation of the diester derivative, the amount of which depends on the experimental conditions. Column chromatography separation is then necessary.

The highest yield of 4 (75%) was obtained with the S-Trt protecting group and epoxyethane as reagent.

The diester 5a was then prepared from 4a and N-Z, S-Trt (D) cysteine 3a in the presence of DCC/DMAP (Table 1).

The important second stage of the synthesis concerns the disulfide bridge formation[46], which can be carried out in either one or two steps.

In the two-step route, the S-protecting groups are first smoothly removed[40]. A further oxidation (Table 2) gives the disulfide bridge. When the S-protecting group is Nps, 6 is afforded in quantitative yield, but unfortunately the starting compound 5e is obtained in very low yield. For this reason the one step route was preferred.

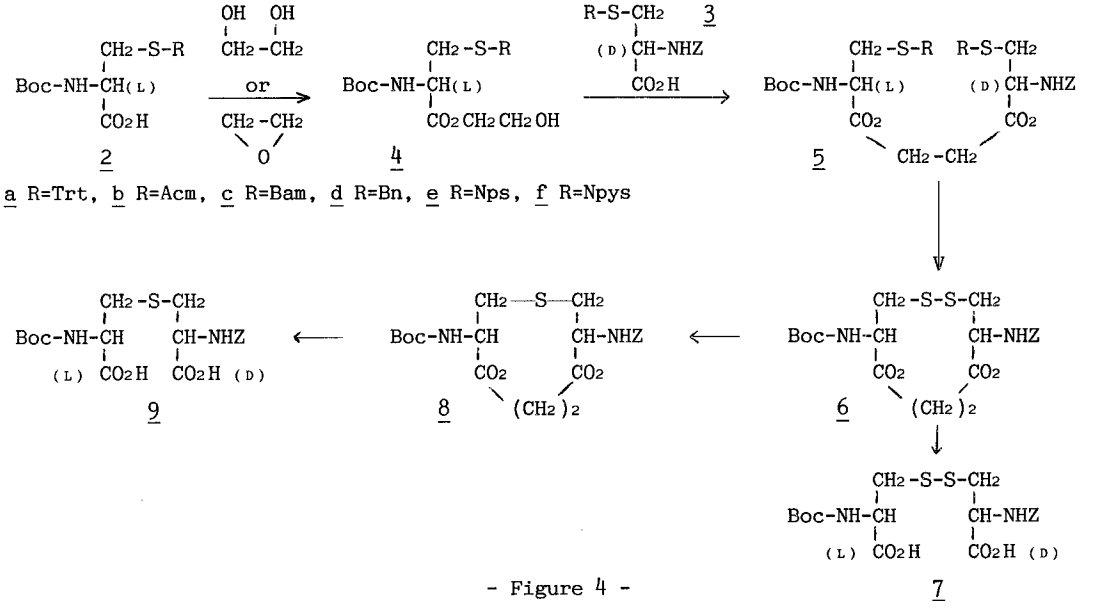
A well-known method in peptide synthesis is to directly oxidize, using iodine[47], S-protected cysteine residues to the corresponding cystines. Although high yields are obtained with residues such as Cys(Trt) or Cys(Acm) included in a peptide chain[47], we have found that this is not so in the case of an isolated cysteine derivative. For example, Boc-Cys(Trt)OCH_3 afforded di-Boc-cystine dimethyl ester in the low yield of 20%. Starting with Boc-Cys(Trt)OCH_2-CH_2OCys(Trt)-Z, we obtained under optimum experimental conditions (MeOH/I_2 100M) the cyclic cystine derivatives in 15% yield.

On the other hand, diacetoxyiodobenzene[48] afforded good yields of cystine derivatives (Table 2), better results being observed when using trityl protection of the thiol function.

In this way we prepared the N-Boc N'-Z cyclic cystine derivative 6 with a *meso*-configuration, in four steps and in 35% overall yield from the starting (D)-cysteine. The negative FAB mass spectrum showed the absence of any dimerization product resulting from an intermolecular reaction, no ion at higher masses was detected.

The diester link was smoothly saponified affording a novel cystine derivative 7 with *meso*-configuration and amine orthogonal protecting groups and allowing easy discrimination of both chiral centers.

The final difficulty in the synthesis was sulfur extrusion by reaction with a phosphine[32-34,49-51] in aprotic medium. In our case, regardless of the sulfur atom attacked by the phosphine, a single lanthionine derivative should be produced (Figure 4).



- Figure 4 -

Attempts with various phosphines (PBu_3, $P(C_6H_5)_3$, $P(O\text{-}C_6H_5)_3$, $P[N(CH_3)_2]_3$, $P[N(C_2H_5)_2]_3$) showed that only tris(diethylamino)-phosphine gave the desired cyclic lanthionine derivative 8. Due to the presence of the thiophosphine by-product, the final purification had to be carefully conducted and the yield was only 22%. Work is in progress using supported phosphines[52] in order to increase this yield.

No racemization took place during sulfur extrusion. NMR spectra of the L,D compound 8 alone and in mixture with the L,L isomer were recorded in $CDCl_3$ in presence of increasing amounts of $Eu(Fod)_3$; in the first case no splitting of the methylene signal of the ester moiety was observed, whereas it appeared $\Delta\Delta\delta$=0.06 ppm in the same conditions (0.3 eq. of $Eu(Fod)_3$) with the mixture of isomers.

Finally, the carboxylic acid groups were smoothly liberated by saponification of the glycolic diester.

The *meso*-lanthionine derivative 9 obtained in this way can be directly used in peptide synthesis. Because of the amine protecting groups which can be selectively cleaved, subsequent coupling reactions are regioselective.

Conclusion : Our new method involving use of a reversible link between two cysteine residues of opposite configurations, constitutes the first efficient synthesis of a new *meso*-lanthionine derivative with discrimination of the chiral centers.

EXPERIMENTAL PART

Uncorrected melting points were measured with a BÜCHI apparatus. Chromatographic eluents were : A = ether 7 - hexane 3; B = hexane 8 - acetone 2. ^1H NMR spectra were recorded on a BRUKER 250 MHz spectrometer with TMS as internal reference. Signal multiplicity is given as : d = doublet, dd = doublet of doublets, m = multiplet. Specific rotations were measured with a PERKIN-ELMER 241 polarimeter.

N-Boc S-Trt ethylene glycol mono ester of cysteine 4

Method A : Boc-Cys(Trt) 2 (7g, 15mmol) dissolved in anhydrous dichloromethane (100ml) was dropwise added to a solution of DCC (3.1g, 15mmol), DMAP (0.18g, 1.5mmol) and ethyleneglycol (0.95ml, 17mmol) in anhydrous dichloromethane (100ml). After stirring 3h at room temperature, some dicyclohexylurea was filtered off and the filtrate evaporated. The residue was dissolved in a small volume of ethyl acetate and left at 0°C for one night ; more dicyclohexylurea was removed by filtration. After evaporation of the filtrate, the residue was purified using column chromatography (silica, eluent A).

- The first fraction yielded a white solid identified as N'-Boc N-acylurea (0.3g, ca. 3%), R_f(A)=0.78;

^1H NMR ($CDCl_3$) δ ppm : 1.5 (31H, m, Boc and C_6H_{11}), 2.6 (2H, d, CH_2), 4.3 (1H, m, CH_α), 5.1 (1H, d, NH), 7.4 (15H, m, Trt).

Calculated for $C_{40}H_{51}N_3O_4S$: C 71.75, H 7.62, N 6.28; found C 71.69, H 7.71, N 6.10

- The second fraction yielded the ethyleneglycol bis ester of N-Boc S-Trt cysteine (2.8g, 20%), R_f(A)=0.48, mp=82-83°C.

^1H NMR ($CDCl_3$) δ ppm : 1.4 (18H, s, Boc), 2.7 (4H, d, $2CH_2$), 4.3 (6H, m, $2CH_\alpha$ and 2 OCH_2), 5.1 (2H, d, 2NH), 7.4 (30H, m, 2Trt).

Calculated for $C_{54}H_{60}N_2O_8S_2$: C 70.58, H 5.71, N 2.94; found C 70.66, H.5.68, N 2.89

- The third fraction was the required compound 4 (5.3g, 70%) R_f(A)=0.28, R_f(B)=0.42, mp=88-90°C.

^1H NMR ($CDCl_3$) δ ppm : 1.4 (9H, s, Boc), 2.7 (2H, d, CH_2), 3.8 (2H, m, O-CH_2), 4.3 (3H, m, CH_α and CO_2CH_2), 5.2 (1H, d, NH), 7.4 (15H, m, Trt).

Calculated for $C_{29}H_{33}NO_5S$: C 68.63, H 6.51, N 2.76; found C 68.54, H 6.60, N 2.96.

Method B : Epoxyethane (6.5g, 150mmol) was bubbled for 30 min through a stirred solution of Boc-Cys(Trt) 2 (2.3g, 5mmol) in chloroform (100ml) containing triphenylphosphine (0.005g), and cooled to -20°C. After being stirred for 24h at room temperature, the solution was evaporated and the ester was isolated as a white solid after purification on a silica column. Yield=75% (1.9g), mp=88-90°C, R_f(A)=0.27. The physical constants were identical to those of the third product isolated in method A.

Method C : To a solution of Boc-Cys(Trt) 2 (4.63g, 10mmol) in dichloromethane (60ml), were added at room temperature EEDQ (2.47g, 10mmol) and ethylene glycol (0.62, 11mmol). After the mixture had been stirred for 12h at the same temperature, the solvent and the quinoline formed were evaporated under reduced pressure to afford the product (2.8g) in 56% yield after chromatography on silica (eluent A).

Physical constants : see method A, third product.

Method D : To a solution of Boc-Cys(Trt) 2 (4.63g, 10mmol) in dichloromethane (60ml), were added at room temperature DPPA (4.3ml, 20mmol), triethylamine (2.8ml, 20mmol) and ethylene glycol (0.62ml, 11mmol). After the mixture had been stirred for 5h at room temperature, the solvent was evaporated under reduced pressure to afford the product (3.15g) in 62% yield after chromatography on silica column, eluent A.
Physical constants : see method A, third product.

Preparation of 5a (L,D)

To a stirred solution of 4 (3.04g, 6mmol), DCC (1.2g, 6mmol) and DMAP (0.073g, 0.6mol) in dichloromethane (60ml), was added dropwise a solution of (D)-Z-Cys(Trt) 3 (2.98g, 6mmol) in dichloromethane (40ml). After 3h at room temperature, the DCU was filtered and the solvent evaporated under reduced pressure. The residue was dissolved in 15ml of ethyl acetate and on cooling a further quantity of DCU precipitated. After filtration and evaporation, the residue was dissolved in 10ml of anhydrous ether which was evaporated and the foam-like residue was chromatographed on a silica column (eluent A).
The following products were successively isolated :
- N'-Z N-acyl urea, (0.16g) Yield 4%, R_f(A) = 0.65
- (L,D) Dissymmetrical ester 5a, (2.37g) Yield 40%, mp=88-89°C, R_f(A)=0.41, $[\alpha]_D$=-5 (c=1, CH_3OH)

^1H NMR ($CDCl_3$) δ ppm : 1.4 (9H, s, Boc), 2.6 (4H, d, $2CH_2$), 4.3 (6H, m, $2CH\alpha$ and 2 OCH_2), 5.1 (2H, s, CH_2-C_6H_5), 5.4 (1H, d, NH), 5.8 (1H, d, NH), 7.4 (35H, m, arom)
Calculated for $C_{59}H_{58}N_2O_8S_2$: C 71.80, H 5.88, N 2.84; found C 71.32, H 6.21, N 2.67
Other ethyleneglycol diesters are listed in Table 1.

esters obtained	yield	mp°C	$[\alpha]_D$
(L) (L) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	60	72-74	+ 25 (c=1,MeOH)
(L) (L) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Boc	99	80-82	- 28 (c=1,MeOH)
(L) (L) Z-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	99	106-108	- 27 (c=1,MeOH)
(L) (L) Boc-Cys(Acm)OCH_2CH_2OCys(Acm)-Boc	42	88-90	+ 17 (c=2,$CHCl_3$)
(L) (D) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	45	88-89	- 5 (c=1,MeOH)

- Table 1 : Glycolic esters 5 - DCC/DMAP method -

Disulfide bridge formation :

General procedure : to a solution of the starting material 5 in dichloromethane (1mmol/l) was added diacetoxyphenyliodide (1.1 eq.), and the solution was stirred at room temperature (for the stirring time, see Table 2). The solution was diluted with dichloromethane then extracted 4 times with water to remove most of the acetic acid formed. The organic layer was dried and evaporated under reduced pressure. The residue was then dissolved in ether and the reaction product precipitated by addition of hexane.
All products formed are listed in Table 2.

starting cysteine derivatives	mp°C of cystine derivat.	reaction time in mn	yield %
Boc-Cys-OH	142-145	5	88
Z-Cys-OH	120-122	5	90
Z-Cys(Trt)-OH	118-120	5	85
Boc-Cys(Trt)-OH	142-145	90	63
Boc-Cys(Acm)-OH	142-145	80	68
Boc-Cys(Bn)-OH	142-145	60	85
Boc-Ala-Cys(Trt)-OMe	oil	70	62
(L) (L) Z-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	84-86	50	65
(L) (L) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Boc	78-80	360	55
(L) (L) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	75-76	90	60
(L) (D) Boc-Cys(Trt)OCH_2CH_2OCys(Trt)-Z	72-74	60	70
(L) (L) Boc-Cys(Acm)OCH_2CH_2OCys(Acm)-Boc	78-80	150	58

- Table 2 : Oxidation of cysteine derivatives with $PhI(OAc)_2$ -

(L,D) Ethyleneglycol bis ester of N-Boc N'-Z cystine 6

According to the previous procedure, the product 6 was formed in 70% yield, $R_f(A)=0.21$, mp=72-74°C, $[\alpha]_D=-5$ (c=1, CH_3OH), FAB negative-ion spectrum : $[M-H]^-$: *m/z* 499
^{1}NMR ($CDCl_3$) δ ppm : 1.4 (9H, s ,Boc), 3.3 (4H, m, $2CH_2$: cystine), 4.2 (2H, m, $2CH\alpha$), 4.5 (4H, s, $O-CH_2CH_2-O$), 5.1 (2H, s, CH_2 : Z), 5.6 (1H, d, NH-Boc), 5.8 (1H, d, NH-Z), 7.5 (5H, s arom. : Z). L,L diastereoisomer : mp=75-76°C, $[\alpha]_D=-20$ (c=1, CH_3OH), identical NMR spectrum
Calculated for $C_{21}H_{28}N_2O_8S_2$: C 50.45, H 5.64, N 5.60; found C 50.21, H 5.56, N 5.42

(L,D) Ethyleneglycol bis ester of N-Boc N'-Z lanthionine 8

Tris(diethylamino)phosphine (25 eq.) was added to a solution of 6 in anhydrous DMF (1mmol/10ml). After 12h under nitrogen at room temperature, the solvent was eliminated under reduced pressure and the residue chromatographed on silica (eluent A) to afford the product in 22% yield. R_f(A)=0.48, $[\alpha]_D$=-6 (c=1, CH_3OH), FAB negative-ion spectrum $[M-H]^-$: *m/z* 467

^1H NMR ($CDCl_3$) δ ppm : 1.4 (9H, s ,Boc), 3.0 (2H, m, CH_2), 4.3 (6H, m, 2CHα and O-CH_2CH_2-O) 5.1 (2H, s CH_2 : Z), 5.7 (1H, d, NH-Boc), 6.0 (1H, d, NH-Z), 7.4 (5H, s, arom.).

Calculated for $C_{21}H_{28}N_2O_8S$: C 53.84, H 6.02, N 5.98; found C 53.78, H 6.14, N 55.87

(L,D) N-Boc N'-Z Lanthionine 9

A solution of 8 (5 mmoles, 2.35g) in isopropylic alcool (20ml) containing 5ml of a 0.1N NaOH solution was stirred 1h at room temperature. Water (10ml) was then added and the alcool evaporated under reduced pressure. The remaining aqueous solution was acidified to pH=3-4 with a 1N HCl solution then extracted with ethyl acetate. The organic layer was separated, washed two times with water, dried on Na_2SO_4 then concentrated under vacuum to afford a very hygroscopic foam-like product in quantitative yield.

$[\alpha]_D$=-5 (c=1, CH_3OH) ; FAB negative-ion spectrum $[M-H]^-$: *m/z* 441

^1H NMR ($CDCl_3$) δ ppm : 1.4 (9H, s, Boc), 3.0 (4H, m, $2CH_2$), 4.3 (2H, m, 2CHα), 5.1 (2H, s, CH_2 : Z), 5.7 (1H, d, NH-Boc), 6.0 (1H, d, NH-Z), 7.4 (5H, s, arom.),

Calculated for $C_{19}H_{26}N_2O_8S$: C 51.58, H 5.92, N 6.33; found C 51.41, H 5.79, N 6.48

(L,D) N-Boc N'Z Cystine 7

According to a similar procedure, the hygroscopic product was formed in quantitative yield.

$[\alpha]_D$=-4 (c=1, CH_3OH) ; FAB negative-ion spectrum $[M-H]^-$: *m/z* 473

^1H NMR ($CDCl_3$) δ ppm : 1.4 (9H, s, Boc), 3.3 (4H, m, $2CH_2$), 4.2 (2H, m, 2CHα), 5.1 (2H, s, CH_2 : Z), 5.6 (1H, d, NH-Boc), 5.8 (1H, d, NH-Z), 7.5 (5H, s, arom.),

Calculated for $C_{19}H_{26}N_2O_8S_2$: C 48.10, H 5.53, N 5.91; found C 47.97, H 5.47, N 6.15

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