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Building Units for N-Backbone Cyclic Peptides. 2. Synthesis of Protected N-(ω-thioalkylene) Amino Acids and Their Incorporation into Dipeptide Units

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Abstract: A new family of amino acids which contain an ω-thioalkylene group on the N^{α}-amino nitrogen was synthesized by alkylation of ω-thioalkylamines with triflates of α-hydroxy acids. The reaction proceeded with inversion of configuration yielding optically pure products. The N^{α}-(ω-thioalkylene)amino acids were orthogonally protected to allow their incorporation into peptides by solid-phase peptide synthesis (SPPS) methodology. In addition some of these new protected N^{α}-(ω-thioalkylene)amino acids were incorporated into dipeptides by "solution" techniques.

INTRODUCTION

A few years ago we introduced a new method for conferring long-range conformational constraints on peptides called Backbone Cyclization [1]. This method overcomes one of the main drawbacks of the classical cyclization methods (which include side chain to side chain, side chain to ends and end to end cyclizations) that is, the loss of biological activity due to the use of crucial functional groups for cyclization.

The formation of a disulfide bond by the oxidation of sulfhydryl groups as well as the formation of a lactam is often used for the cyclization of linear peptides. If the linear peptide does not contain the appropriate ω-functional amino acids, various amino acids in the native sequence are replaced by Cys or other ω–thio amino acids. Alternatively, if a lactam ring is formed by the formation of an amide bond, various amino acids in the native sequence are replaced by Lys and Glu or other ω–amino and ω–carboxy amino acids. In addition, a lactam ring can be formed between the ω–amino side chain and the carboxyl terminus or the ω–carboxy side chain and the amino terminus.

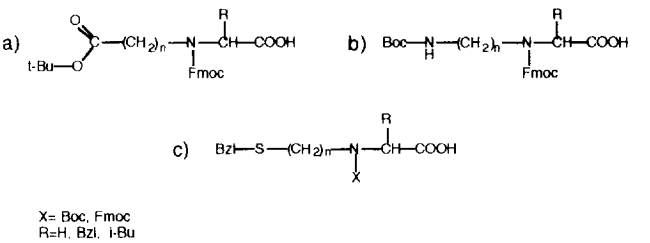


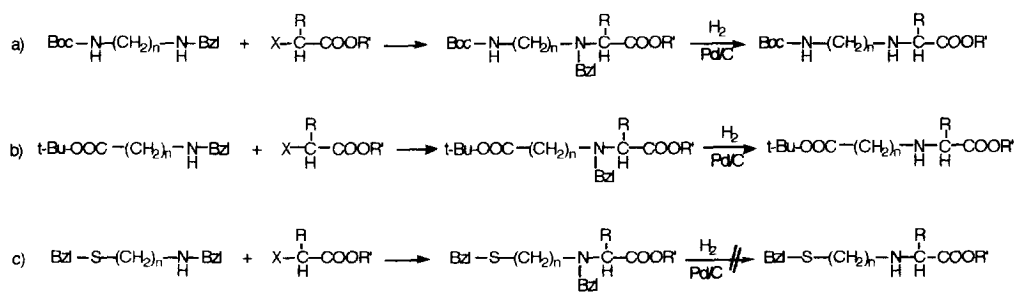
Figure 1. Building units for N-Backbone cyclization. a) ω-carboxyl. b) ω-amine. c) ω-thiol.

Despite the fact that active peptides were obtained using these methods, in many cases the replacement of residues in the native peptide by ω-functional amino acids and cyclization, either to other ω-functional amino acids or to the carboxy and amino ends, led to a strong decrease or even loss of the biological activity [2,3]. Backbone cyclization overcomes this encumbrance: ring closure is performed by the attachment of the same

ω-functional groups mentioned above, namely: amine, carboxyl or thiol, however, in backbone cyclization these functional groups are linked to the backbone nitrogens by an alkylene tether of changing length, retaining the side chains and termini which are essential for biological activity. Thus, the peptide bond N-H is replaced with N-R, where R is an ω-functional alkylene moiety (ω-amino, ω-carboxy or ω-thiol) bearing suitable orthogonal protecting groups (Figure 1).

These ring forming ω-functional groups are introduced into a peptide by replacement of the amino acid residue at the site of cyclization by a building unit, which is an N^{α}-[ω-(Y-alkylene)]amino acid, where Y is a protected amine, carboxyl or thiol group. The side-chain of the original amino acid at this site is retained, so none of the biologically active groups was altered. We have shown that properties like selectivity and metabolic stability could be achieved by this method, whereas the classical cyclization methods failed [4].

In the first article in this series we presented the synthesis of building units of the type N^{α}-[(ω-aminoalkylene)]amino acids (Figure 1b), which were prepared by a nucleophilic attack of mono protected alkylene diamines on α-chloro or α-bromo carboxylic acids. This method was efficient for the preparation of N^{α}-[(ω-aminoalkylene)]Gly, but gave low yields of side chain containing amino acids due to β–elimination and racemization [5]. Here we present an efficient synthesis of a new type of building units: N^{α}-[ω-(benzylthioalkylene)]amino acids (Figure 1c).



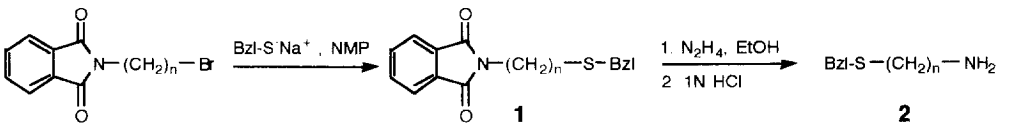
scheme 1

The synthesis of this new class of building units was based also on nucleophilic substitution, yet some significant features of both the nucleophile and the substrate were adjusted due to the change from the ω-amino group to ω-thiol, and in order to minimize the β–elimination and racemization side reactions. The first question was which S-protecting group to use. Although various protecting groups would have been superior for SPPS, we decided to use benzyl as the protecting group, because this facilitated the synthesis of the units. Unlike alkylene diamines, ω-mercaptoamines are not usually commercially available. Only in one case could we use cysteamine (2-mercaptoethylamine) hydrochloride as the starting material. Therefore, the easiest way to prepare the nucleophiles was by substitution of ω-bromoalkylphthalimides with benzyl mercaptane (scheme 2). Except for the case of glycine derivatives, the substrate for the nucleophilic substitution reaction was changed from α-halocarboxylic acids to α-hydroxy carboxylic ester triflates, since the latter avoid both β-elimination and racemization [6] which were side reactions with the former method [5]. Glycine derivatives could still be made from chloro or bromo acetate esters, as before. Double alkylation of the attacking amine was also a side reaction in the previous method [5] especially when the substrate was chloro or bromo acetate esters. When the

ω-functional groups used for cyclization were ω-amine and/or ω-carboxyl, this side reaction could have been avoided by semi-protection of the attacking amine with a benzyl group, and then deprotection of the tertiary amine formed by catalytic hydrogenation [7] (scheme 1a,b). However, When thiol was the ω-functional group used for cyclization, this possibility was denied, because the catalyst was poisoned by the sulfur (scheme 1c). Double alkylation was, hence, unavoidable, and was especially problematic with sterically unhindered substrates like haloacetate esters. In the case of bulkier substrates such as α–substituted α-hydroxy carboxylic ester triflates, only a small percentage of double alkylation was observed.

RESULTS AND DISCUSSION

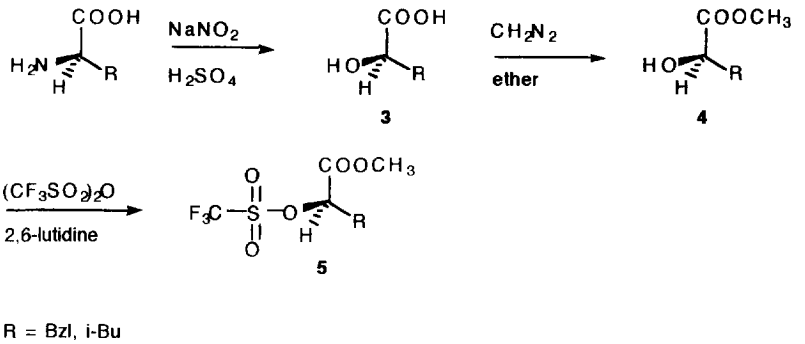
1. *Synthesis of protected N^{α}-(ω-thioalkylene)amino acids.* As in the case of the N^{α}-(ω-aminoalkylene)amino acids, the method for the preparation of their thiol analogs was nucleophilic substitution of an appropriate substrate derived from a D-amino acid, with S-protected ω-thioalkylamines. Except for the case of **2a** (n=2), which was prepared from cysteamine hydrochloride by reaction with benzyl bromide [8], the ω-benzylthioalkylamines were prepared from ω-bromoalkylphthalimides by reaction with benzyl mercaptane in the presence of sodium hydride in N-methylpyrrolidone. Hydrazinolysis of the phthalyl group from the crystalline products **1** gave amines **2** (scheme 2), which were then reacted with D-α-hydroxy acid triflates to form the building units. Data for compounds **1** and **2** is given in table 1.



scheme 2

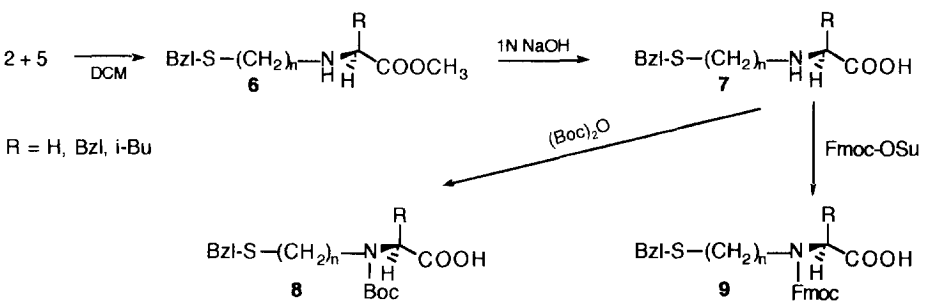
As we have previously reported, substituted α-chlorocarboxylic acids derived from natural amino acids undergo considerable racemization upon substitution with alkylamines [5]. We have found in accordance with the literature that the triflates of α-hydroxy, α–substituted D carboxylic acids give, when reacted with substituted amines **2**, the desired N^{α}-(ω-benzylthioalkylene)amino acids in high optical purity. Furthermore, using triflate as the leaving group also minimizes the undesired β-elimination reaction. Thus, except for glycine, for which bromoacetic acid could still be used as the starting material, D- amino acids were used as starting materials. The amino group was converted to hydroxyl by diazotization with $NaNO_2$ in 1N H_2SO_4 without inversion, giving D-α-hydroxy acids **3** [9]. Then the carboxyl was protected as a methyl ester by reaction with diazomethan in ether to give **4**, which in turn was reacted with trifluoromethanesulfonic anhydride to give triflates **5** [10] (scheme 3). Data for these compounds is given in table 2.

Reaction of **2** with **5** gave the desired N^{α}-(ω-thioalkylene)L-amino acid methyl esters **6** which could then be hydrolyzed with 1N NaOH and protected with Boc to give **8** without isolation of the zwiterion (scheme 4). Protection with Fmoc was successful for glycine derivatives, yet Fmoc protection of other amino acid derivatives was much more difficult, and gave poor yields due to a very low solubility of the zwiterionic N^{α}-(ω-thioalkylene)amino acids in the pH range required for Fmoc introduction. Data for building units is given in table 3.



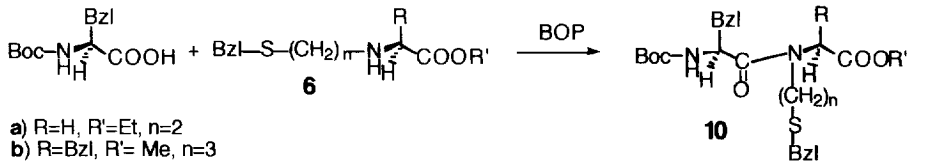
scheme 3

N,N double alkylation was always a side product, however its appearance could be reduced by careful control of the reaction conditions. The problem of double alkylation was most severe when the alkylating agents were haloacetic acid esters. We compared the percentage of double alkylation obtained when methyl chloroacetate, methyl bromoacetate and methyl glycolate O-triflate were used as alkylating agents. No significant difference in the percentage of double alkylation was observed. When the alkylating agent was, however, a bulkier molecule like the triflates derived from D-Phe or D-Leu, the amount of double alkylation diminished to 3-5% only.



scheme 4

2. *Synthesis of Protected Dipeptides Containing an N-(ω-thioalkylene) Groups on the Amide Bond.* To demonstrate the use of the protected N^{α}-(ω-thioalkylene)amino acids in peptide synthesis we attempted to couple one example of each parent amino acid, i.e. methyl esters of units derived from Gly, Leu and Phe, with Boc-Phe in solution, employing BOP as coupling reagent (scheme 5). These reactions gave moderate yields in the case of building units derived from Gly or Phe, while in the case of the building units derived from Leu (n=2,3) no coupling was observed with Boc-Phe, Boc-Gly or Fmoc-Gly using BOP, HBTU or PyBroP as coupling agents even at elevated reaction temperatures. Data for the prepared dipeptides is given in table 4 .



scheme 5

EXPERIMENTAL SECTION

Materials and methods. Starting materials were purchased from Merck or Aldrich and used without further purification. Diazomethane was prepared from Diazald® according to the known procedure [11]. Melting points were measured on a Mel-Temp II capillary equipment. Optical activity was recorded on a Perkin-Elmer-141 polarimeter with a sodium lamp in a 10-cm length cell at room temperature. Microanalysis was carried out at the Microanalytical Department of the Hebrew University, Jerusalem. ^{1}H NMR spectra were recorded on either Brucker WP-200 or AMX-400 spectrometers at 295K. COSY spectra of final products were routinely recorded to assist with the proton assignment of ambiguous spectra.

Table 1. Data for Compounds 1-2

	n	yield	m.p./b.p.
1b	3	96	57-8
1c	4	84	62-3
2a	2	86	85-87 (1 torr)
2b	3	50	102 (1 torr)
2c	4	51	110 (1 torr)

method A. Preparation of S-benzylcysteamine (2a). 0.1 mole cysteamine hydrochloride were S-benzylated according to the procedure of Nagakawa et al. [8]. The product was a colorless liquid. Yield: 85%.

method B. Preparation of N-[ω-(benzylthio)alkyl]phthalimides (1b,1c). 0.11 mole of NaH were suspended in 50 ml of dry NMP. 0.11 mole of benzyl mercaptane were added dropwise at 0°C with stirring under N_2. The resulting dark solution was stirred for additional 30 min. Then a solution of 0.1 mole ω-bromoalkylphthalimide dissolved in 50 ml of dry NMP was added dropwise. After the addition was complete the temperature was allowed to rise to ambient temperature and the mixture was stirred for 16 hours. The mixture was poured into ice-water with vigorous stirring. The product precipitated as small balls, and was collected and dried *in vacuo*.

NMR ($CDCl_3$):

1b - 7.87-7.70,m,4H (phthalyl); 7.30-7.14,m,5H (benzyl); 3.75,t,J=7.0Hz,2H ($^1CH_2$) [12]; 3.71,s,2H (benzyl); 2.43,t,J=7.4Hz,2H ($^3CH_2$); 2.00-1.86,m,2H ($^2CH_2$).

1c - 7.85-7.80,m,2H (phthalyl α); 7.75-7.69,m,2H (phthalyl β); 7.29-7.19,m,5H (benzyl); 3.69,s,2H (benzyl); 3.66,t,J=7.0Hz,2H ($^1CH_2$); 2.46,t,J=7.0Hz,2H ($^4CH_2$); 1.82-1.53,m,4H ($^2CH_2$+ $^3CH_2$).

Table 2. Physical Data for Compounds 3-5

	R	yield	m.p.	$[\alpha]_D$
3a	Bzl	75	126-6.5	$+20.8^{24}$ (C2, H_2O)
3b	i-Bu	75	78-9	$+27.0^{18}$ (C 1.5, 1N NaOH)
4a	Bzl	98	47-8	$+10.8^{24}$ (C7, benzene)
4b	i-Bu	74	oil	$+6.7^{18}$ (C 1, MeOH)
5a	Bzl	70	oil	$+29.2^{27}$ (C 1.13, DCM)
5b	i-Bu	56	oil	$+49.4^{25}$ (C1.77, DCM)
5c	H	71	25-5.5	-

method C. Hydrazinolysis of the phthalyl group. Hydrazinolysis was performed by refluxing 0.09 mole of N-[ω-(benzylthio)alkylene]phthalimide with 120 ml 1M solution of hydrazine hydrate in ethanol (diluted with additional 220 ml ethanol) for 2 hours. The formed precipitate was filtered and hydrolyzed with 180 ml of 2N HCl at 50°C for 0.5 hour. After evaporation to dryness the crude hydrochloride was dissolved in 50 ml of 25% ammonia solution. The free amine was extracted with DCM (4 × 100 ml) and the organic phase was washed with saturated NaCl (2 × 100 ml) and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The crude ω-(benzylthio)alkylamines were distilled under reduced pressure, and collected as colorless oils, which could be kept refrigerated under nitrogen for prolonged periods.

NMR ($CDCl_3$):

2a - 7.28-7.17,m,5H (benzyl); 3.66,s,2H (benzyl); 2.77,t,J=6.4Hz,2H ($^1CH_2$); 2.47,t,J=6.5Hz,2H ($^2CH_2$); 1.36,s,2H (NH_2).

2b - 7.32-7.23,m,5H (benzyl); 3.70,s,2H (benzyl); 2.74,t,J=7.0Hz,2H ($^1CH_2$); 2.46,t,J=7.1Hz,2H ($^3CH_2$); 1.75-1.61,m,2H ($^3CH_2$); 1.08,broad s,2H (NH_2).

2c - 7.33-7.25,m,5H (benzyl); 3.70,s,2H (benzyl); 2.66,t,J=6.9Hz,2H ($^1CH_2$); 2.42,t,J=7.1Hz,2H ($^4CH_2$); 1.66-1.40,m,4H ($^2CH_2$+ $^3CH_2$); 1.18,broad s,2H (NH_2).

method D. Preparation of (R)-α-hydroxy acids (3). 120 mmol $NaNO_2$ dissolved in 40 ml of water were added dropwise to a solution of a 80 mmol of D- amino acid in 120 ml 1N H_2SO_4 at 0°C. The mixture was stirred for 3 hours at 0°C and 2 additional hours at room temperature. The product was extracted with ether (3 × 40 ml). The organic phase was washed with saturated NaCl (2 × 100 ml) and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The resulting product was crystallized from petroleum-ether, filtrated and dried *in vacuo*.

NMR (D_2O):

3a - 7.38-7.23,m,5H (arom.); 4.51,dd (x of abx), J_1=7.2H, J_2=4.3Hz,1H ($^\alpha$CH); 3.21,dd (ab of abx),J_1=14.0Hz, J_2=4.3Hz, 1H ($^\beta$CH); 2.99,dd (ab of abx),J_1=14.0Hz,J_2=7.2Hz,1H ($^\beta$CH).

3b - 4.29,t (x of abx),J=6.7Hz,1H ($^\alpha$CH); 1.92,m,1H ($^\gamma$CH); 1.62,dd (ab of abx), J_1=7.4Hz,J_2=5.7Hz,2H ($^\beta CH_2$);

0.97,d,J=6.6Hz,6H ($^{\delta}CH_3$).

Table 3. Physical Data for Building Units

	R	n	yield	m.p.	Elemental analysis	$[\alpha]_D$
6a	Bzl	2	61	49-50	calc: C-69.27, H-7.04, N-4.25 fnd: C-69.55, H-7.21, N-4.08	-23.3^{14} (C 1, DCM)
6b	Bzl	3	71	38-39	calc: C-69.94, H-7.34, N-4.08 fnd: C-69.66, H-7.39, N-4.37	$+2.0^{26}$ (C 1, DCM)
6c	Bzl	4	81	oil	calc: C-70.55, H-7.61, N-3.92 fnd: C-70.51, H-7.69, N-4.22	$+4.9^{26}$ (C 1, DCM)
6d	i-Bu	2	57	oil	calc: C-65.05, H-8.53, N-4.74 fnd: C-66.29, H-9.03, N-4.49	-51.2^{14} (C 0.95, DCM)
6e	i-Bu	3	60	oil	calc: 65.98, H-8.79, N-4.53 fnd: 67.09, H-9.20, N-4.54	-17.4^{23} (C 1.44, DCM)
6f	H	2	19	oil	calc: C-61.16, H-7.70, N-3.96 fnd: C-61.45, H-8.03, N-3.49	-
6g	H	3	22	oil	not determined	-
8a	Bzl	2	78	82-83	calc: C-67.10, H-7.27, N-3.55 fnd: C-66.94, H-6.62, N-3.42	-105.9^{25} (C 1, DCM)
8b	Bzl	3	89	69-71	calc: C-67.69, H-7.50, N-3.16 fnd: C-66.85, H-7.23, N-3.27	-87.4^{25} (C1 ,DCM)
8f	H	2	88	71-72	calc: C-58.69, H-7.70, N-4.28 fnd: C-59.39, H-7.26, N-4.18	-
8g	H	3	78	oil	calc: C-60.15, H-7.42, N-4.13 fnd: C-58.92, H-7.48, N-4.01	-
9f	H	2	42	85-86	calc: C-69.78, H-5.63, N-3.13 fnd: C-69.52, H-5.82, N-3.43	-

method E. Esterification with diazomethane. An etheral solution of diazomethane was carefully poured into a solution of **3** in ether. Evaporation of the solvent gave clean colorless methyl esters **4**.

NMR ($CDCl_3$):

4a - 7.35-7.18,m,5H (arom.); 4.43,m (x of abx),1H ($^{\alpha}CH$); 3.76, s,3H (Me ester); 3.12,dd (ab of abx),J_1=13.9Hz, J_2=4.5Hz, 1H ($^{\beta}CH$); 2.95,dd (ab of abx),J_1=13.9Hz, J_2=6.8Hz, 1H ($^{\beta}CH$); 2.79,broad s,1H (OH).

4b - 4.15,t (x of abx),J=6.2Hz,1H ($^{\alpha}CH$); 3.72,s,3H (Me ester); 2.92, broad s,1H (OH); 1.84,m (x of abx),1H ($^{\gamma}CH$); 1.51,dd (ab of abx),J_1=6.8Hz,J_2=6.8Hz,2H ($^{\beta}CH_2$); 0.89,dd (ab of abx),J_1=6.4Hz,J_2=2.0Hz,6H ($^{\delta}CH_3$).

method F . Preparation of (R)-α-hydroxy acids methyl ester triflates (5) [13]. Trifluoromethanesulfonic anhydride (1.15 eq.) was added dropwise to a solution of 0.1 mole of **4** in dry DCM stirred at -78°C in the presence of 2,6-lutidine (1,30 eq.). After 30 min. the temperature was allowed to rise to ambient temperature and the mixture was stirred for additional 2 hours. The pink solution was partitioned between DCM (1 liter) and water (500 ml). The aqueous phase was further extracted with 3 × 20 ml DCM. The combined organic extracts were dried on $MgSO_4$ and evaporated *in vacuo*. The crude filtrate was dissolved in a 1:1 solution of DCM : petroleum-ether and filtered through silica, furnishing, after evaporation the pure title compounds as colorless

oils.

NMR ($CDCl_3$):

5a - 7.40-7.19,m,5H (arom.); 5.25,dd (x of abx),J_1=8.5H, J_2=4.3Hz,1H ($^{\alpha}$CH); 3.84,s, 3H (Me ester); 3.36, dd (ab of abx), J_1=14.6Hz,J_2=4.3Hz,1H ($^{\beta}$CH); 3.20, dd (ab of abx), J_1=14.6Hz,J_2=8.5Hz,1H ($^{\beta}$CH).

5b - 5.15,dd (x of abx),J_1=9.0Hz,J_2=1.8Hz,1H ($^{\alpha}$CH); 3.84,s,3H (Me ester); 2.00-1.69,m,3H ($^{\gamma}$CH)+ ($^{\beta}CH_2$); 0.98,d,J=6.1Hz,6H ($^{\delta}CH_3$).

5c - 4.92,s,2H ($^{\alpha}CH_2$); 3.87,s,3H (Me ester).

method G. Preparation of (L) N$^{\alpha}$-[ω-(benzylthio)alkylene]amino acid methyl esters (6). A solution of 15 mmol (R) α-hydroxy acid methyl ester triflate in 55 ml DCM was added dropwise to a solution of 15 mmol ω-(benzylthio)alkylamine and 15 mmol of DIEA in 55 ml DCM at 0°C. The reaction mixture was stirred at room temperature for 18 hours, diluted with 100 ml DCM and washed with water (3 × 100 ml). The crude product was cleaned on a silica-gel column with DCM:MeOH-99:1, and was further crystallized from ether : hexane.

In the case of glycine derivatives, chloroacetic and bromoacetic acid esters were the starting materials, as well as methyl glycolate triflate. Identical results were obtained when these substrates were reacted with ω-(benzylthio)alkylamines.

NMR ($CDCl_3$):

6a - 7.30-7.16,m,10H (arom.); 3.634,s,2H (Benzyl); 3.627,s,3H, (Me ester); 3.50,t,J=6.9Hz,1H ($^{\alpha}$CH); 2.94,t,J=6.5Hz,2H ($^{1}CH_2$); 2.75,m,1H ($^{\beta}$CH); 2.5767,m,1H ($^{\beta}$CH); 2.50,m,2H ($^{2}CH_2$); 1.83,broad s,1H (NH).

6b - 7.33-7.13,m,10H (arom.); 3.67,s,2H (Benzyl); 3.64,s,3H (Me ester); 3.48,t,J=6.9Hz,1H ($^{\alpha}$CH); 2.92,t,J=6.9Hz,2H ($^{1}CH_2$); 2.72-2.47,m,2H ($^{\beta}CH_2$); 2.3956,t,J=7.2Hz,2H ($^{3}CH_2$); 1.74-1.59,m,2H ($^{2}CH_2$); 1.51,broad s,1H (NH).

6c - 7.33-7.14,m,10H (arom.); 3.68,s,2H (Benzyl); 3.64,s,3H (Me ester); 3.49,t,J=6.9Hz,1H ($^{\alpha}$CH); 2.93,t,J=6.9Hz,2H ($^{1}CH_2$); 2.63-2.34,m,4H ($^{\beta}CH_2$+$^{4}CH_2$); 1.61-1.49,m,4H ($^{2}CH_3$ + $^{3}CH_3$).

6d - 7.32-7.21,m,5H (arom.); 3.715,s,3H (Me ester); 3.709,s,2H (Benzyl); 3.27,t (x of abx),J=7.2Hz,1H ($^{\alpha}$CH); 2.82-2.49,m,4H ($^{1}CH_2$+$^{2}CH_2$); 1.73,broad s,1H (NH); 1.71,m (x of abx),1H ($^{\gamma}$CH); 1.47,dd (ab of abx),J_1=6.8Hz,J_2=6.8Hz,2H ($^{\beta}CH_2$); 0.91,dd (ab of abx),J_1=6.8Hz,J_2=5.0Hz,6H ($^{\delta}CH_3$).

6e - 7.30-7.22,m,5H (arom.); 3.700,s,3H (Me ester); 3.698,s,2H (Benzyl); 3.23,t (x of abx),J=7.2Hz,1H ($^{\alpha}$CH); 2.67-2.62,m (ab of abx),1H ($^{\beta}$CH); 2.51-2.44,m (ab of abx),1H ($^{\beta}$CH); 2.47,t,J=8.0Hz,2H ($^{1}CH_2$); 1.72-1.67,m,3H ($^{3}CH_2$+ $^{\gamma}$CH); 1.47-1.41,m,3H (NH+ $^{2}CH_2$); 0.90,dd (ab of abx),J_1=6.8Hz,J_2=11.2Hz,6H ($^{\delta}CH_3$).

6f - 7.33-7.23,m,5H (arom.); 3.73,s,5H (Me ester+ $^{\alpha}CH_2$); 3.40,s,2H (Benzyl); 2.77,t,J=6.0Hz,2H ($^{1}CH_2$); 2.57,t,J=6.3Hz,2H ($^{2}CH_2$); 1.83,broad s,1H (NH).

6g - 7.34-7.23,m,5H (arom.); 3.73,s,3H (Me ester); 3.71,s,2H ($^{\alpha}CH_2$); 3.39,s,2H (Benzyl); 2.67,t,J=6.9Hz, 2H ($^{1}CH_2$); 2.48,t,J=7.2Hz,2H ($^{3}CH_2$); 1.81,m,3H ($^{2}CH_2$+ NH).

method H. Preparation of Boc-N-[ω-(benzylthio)alkylene]amino acids (8). 10 mmol of N-[ω-(benzylthio)alkylene] amino acid methyl ester were dissolved in 50 ml of 1,4-dioxane. 50 ml of 1N NaOH were added and the mixture was stirred at room temperature. The disappearance of the starting material was followed by TLC ($CHCl_3$:MeOH- 1:4). When all the ester was hydrolyzed, 50 ml of water were added, followed by 30 mmol of di tert-butyl bicarbonate. The mixture was stirred overnight. The dioxane was evaporated *in vacuo*, the mixture was cooled in an ice-water bath, covered with 100 ml of EtAc and acidified with saturated $KHSO_4$ to

pH 2-3. The layers were separated, and the aqueous layer was extracted with additional 2 × 100 ml EtAc. The organic layer was washed with water (2 × 100 ml) and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The crude product was cleaned on a silica-gel column with DCM:MeOH-99:1 or crystallized from ether:hexane.

NMR ($CDCl_3$):

8a - 7.34-7.25,m,8H (arom.); 7.10,t,J=3.2Hz,2H (arom); 4.00-3.96,m,0.55H ($^{\alpha}CH$ (E)); 3.78-3.75,m,0.45H ($^{\alpha}CH$ (Z)); 3.65,s,0.9H (Bzl (Z)); 3.61,s,1.1H (Bzl (E)); 3.36-3.25,m,≈2.2H ($^{1}CH_2$ (Z)+ $^{\beta}CH_2$ (E)); 3.16-3.08,m,≈1.3H($^{1}CH_2$ (E) + $^{\beta}CH_2$ (Z)); 2.68,m,≈0.5H ($^{1}CH_2$ (E)); 2.51-2.19,m,2H($^{2}CH_2$); 1.48,s,4.95H(t-Bu (Z)); 1.43,s,4.05H (t-Bu (E)).

8b - 7.31-7.17,m,10H (arom.); 3.99,dd (x of abx),J_1=5.1Hz,J_2=11.1Hz,0.7H ($^{\alpha}CH$ (E)); 3.52,dd (x of abx),J_1=5.0Hz,J_2=11.1Hz,0.3H ($^{\alpha}CH$ (Z)); 3.61,s,2H (benzyl); 3.43-3.27,m,1.4H ($^{\beta}CH_2$ (E)); 3.26-3.24,m,0.4H ($^{1}CH_2$ (Z)); 3.19-3.14,m,0.6H ($^{\beta}CH_2$ (Z)); 3.12-3.09,m,0.9H ($^{1}CH_2$ (E)); 2.69-2.64,m,0.5H ($^{1}CH_2$ (E)); 2.51-2.49,m,0.2H ($^{1}CH_2$ (Z)); 2.35-2.25,m,0.6H ($^{3}CH_2$ (Z)); 2.27-2.13,m,1.4H ($^{3}CH_2$ (E)); 1.57-1.52,m,2H ($^{2}CH_2$); 1.46,s,9H (t-Bu).

8f - 7.32-7.26, m,5H (arom.); 3.94,s,1.2H ($^{\alpha}CH_2$ (Z)); 3.89,s,0.8H ($^{\alpha}CH_2$ (E)); 3.73,s,2H (benzyl); 3.40,t,J=7.3Hz,0.8H ($^{1}CH_2$ (E)); 3.33,t,J=7.4Hz,1.2H ($^{1}CH_2$ (Z)); 2.60,m,2H ($^{2}CH_2$); 1.45,s,9H (t-Bu).

8g - 7.31-7.23,m,5H (arom.); 3.92,s,1.2H ($^{\alpha}CH_2$ (Z)); 3.84,s,0.8H ($^{\alpha}CH_2$ (E)); 3.71,s,2H (benzyl); 3.32,t,J=6.7Hz,2H ($^{1}CH_2$); 2.44-2.41,m,2H ($^{3}CH_3$); 1.83-1.68,m,2H ($^{2}CH_2$); 1.45,s,5.4H (t-Bu (Z)); 1.42,s,3.6H (t-Bu (E)).

method I. Preparation of N-Fmoc-N-[ω-(benzylthio)alkylene]amino acids (9). 5.6 ml (40 mmol) TEA and 6.42 gr (20 mmol) Fmoc-OSu in 120 ml of MeCN were added to a solution of 20 mmol of **6** in 60 ml of water. The reaction mixture was stirred at room temperature for 4 h. Then 180 ml of water were added and the solution was washed with petroleum-ether (3 × 100 ml) and with a mixture of 3:7 - ether : petroleum-ether (3 × 100 ml). The aqueous solution was acidified with 40 ml of 1N HCl and extracted with EtAc (4 × 100 ml). The organic solution was washed with 50 ml of saturated NaCl, dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The product was crystallized from ether : petroleum-ether.

NMR ($CDCl_3$):

9f - 7.75,d,J=7.5Hz,1.1H (fluorenyl 4 (Z)); 7.72,d,J=7.6Hz,0.9H (fluorenyl 4 (E)); 7.55,d,J=7.4Hz,1.1H (fluorenyl 1 (Z)); 7.51,d,J=7.4Hz,0.9H (fluorenyl 1 (E)); 7.39-7.20,m,9H (fluorenyl 2+3 and benzyl); 4.52,d,J=6.0Hz,1.1H, (CH_2 Fmoc (Z)); 4.43,d,J=6.4Hz,0.9H (CH_2 Fmoc (E)); 4.22,t,J=6.2Hz,0.55H (CH Fmoc (Z)); 4.18,t,J=6.3Hz,0.45H (CH Fmoc (E)); 3.94,s,1.1H ($^{\alpha}CH$ (Z)); 3.86,s,0.9H ($^{\alpha}CH$ (E)); 3.71,s,0.9H (benzyl (E)); 3.66,s,1.1H (benzyl (Z)); 3.39,td (aa of aabb),J_t=7.1Hz,J_d=3.7Hz,0.9H ($^{1}CH_2$ (E)); 3.18,t (aa of aabb),J=7.4Hz,1.1H ($^{1}CH_2$ (Z)); 2.86,t (bb of aabb),J=7.2Hz,0.9H ($^{2}CH_2$ (E)); 2.3417,t (bb of aabb),J=7.4Hz,1.1H ($^{2}CH_2$ (Z)).

method J. Preparation of the dipeptides (L,L)-Boc-Phenylalanyl-N-[ω-(benzylthio)alkylene] amino acid esters (10). A solution of 1.1 mmol Boc-L-Phenylalanine, 1 mmol N-[ω-(benzylthio)alkylene]amino acid ester, 1.1 mmol BOP and 3 mmol of DIEA in 10 ml DCM was stirred at room temperature for 2 hours. The mixture was then diluted with 40 ml of DCM, washed successively with saturated $KHSO_4$ (3 × 100 ml), saturated $KHCO_3$ (3 × 100 ml) and saturated NaCl (2 × 100 ml), dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was cleaned on a silica-gel column with DCM:MeOH-99:1 or crystallized from ether:hexane.

NMR ($CDCl_3$):

10a - 7.33-7.15,m,10H (arom.); 5.22,d,J=9.3Hz,0.4H (NH (Z)); 5.19,d,J=9.3Hz,0.6H (NH (E)); 4.80-4.75,m,0.6H ($^{\alpha_p}$CH (E)); 4.59-4.537,m,0.4H ($^{\alpha_p}$CH (Z)); 4.17,q,J=6.5Hz,2H (CH_2 of Et ester); 4.00-3.77,m,2H ($^{\alpha_g}CH_2$); 3.72,s,0.8H (benzyl (Z)); 3.67,s,1.2H (benzyl (E)); 3.60-3.53,m,0.8H ($^1CH_2$ (Z)); 3.27,t,J=7.5Hz,1.2H ($^1CH_2$ (E)); 3.07-2.87,m,2H, ($^{\beta_p}CH_2$); 2.57-2.26,m,2H ($^2CH_2$); 1.40,s,5.4H (t-Bu (E)); 1.37,s,3.6H (t-Bu (Z)); 1.26,t,J=7.1Hz,1.8H (CH_3 of Et ester (E)); 1.25,t,J=7.1Hz,1.2H (CH_3 of Et ester (Z)).

10b - 7.33-6.97,m,15H (arom.); 5.07,d,J=9.5Hz,1H (NH); 4.74,dt (x of abx),J_d=9.3Hz,J_t=7.5Hz,1H, $^{\alpha}CH_{p1}$; 3.97,dd (x of abx),J_1=9.6Hz,J_2=5.4Hz,1H ($^{\alpha}CH_{p2}$); 3.68,s,3H (Me ester); 3.65,3.64,2s,2H, Bzl; 3.37,dd (ab of abx),J_1=14.2Hz,J_2=5.1Hz,1H ($^{\beta}CH_{2_{p2}}$); 3.19-1.17,m,1H ($^1CH_2$); 3.07,dd (ab of abx),J_1=14.3Hz,J_2=9.5Hz,1H ($^{\beta}CH_{2_{p2}}$); 3.01,dd (ab of abx),J_1=13.6Hz,J_2=7.0Hz,1H ($^{\beta}CH_{2_{p1}}$); 2.84,dd,J_1=13.7Hz,J_2=7.4Hz,1H ($^{\beta}CH_{2_{p1}}$); 2.66,m,1H ($^1CH_2$); 2.29-2.25,m,2H ($^3CH_{2}$; 1.61-1.52,m,2H ($^2CH_2$); 1.37,s,9H (t-Bu).

Table 4. Physical Data for Dipeptides

	R	R'	n	yield	m.p.	Elemental analysis	$[\alpha]_D$
10a	H	Et	2	61	oil	calc: C-64.77, H-7.25, N-5.6 fnd: C-64.39, H-7.02, N-5.5	+4.5[16] (C 0.9, DCM)
10b	Bzl	Me	3	46	127-8	calc: C-69.12, H-7.17, N-4.7 fnd: C-69.44, H-7.42, N-4.7	-115.9[26] (C1, $CHCl_3$)

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