Building units for N-backbone cyclic peptides. Part 4.[1] Synthesis of protected N^{α}-functionalized alkyl amino acids by reductive alkylation of natural amino acids

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A new method for the synthesis of protected N^{α}-(ω-Y-alkyl) amino acids (Y is a thio, amino or carboxy group) and related compounds by reductive alkylation of natural amino acids is reported. These new amino acids serve as building units for the synthesis of backbone-cyclic peptides. They are orthogonally protected at the α-amino position by butoxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc), using trimethylsilyl temporary protection, to allow for their incorporation into peptides by solid phase peptide synthesis.

Introduction

Backbone-Cyclization is a method developed in our laboratory for imposing long-range conformational constraint by cyclization of linear bioactive peptides, in order to enhance activity, stability to metabolic degradation, selectivity and bioavailability.[2,3] In the classical peptide cyclization methods the carboxyl or amino termini are often used to cyclize peptides. Alternatively, side-chain cyclization can be achieved by closing a lactam ring between the side-chains of lysine and aspartic or glutamic acid residues, or a disulfide ring between two cysteine residues. Unfortunately, these four natural amino acids offer quite a limited scope of cyclization possibilities. In order to overcome this circumscription several analogous amino acids, such as ornithine, penicillamine, *etc.* are quite often used. Nevertheless, the utilization of such natural or unnatural amino acids often requires their artificial insertion or substitution into the sequence. Consequently, crucial functional groups are replaced or altered or the peptide is subjected to conformational changes which frequently lead to loss in, or reduction of, the biological activity.[4] Thus, the development of new amino acids which will broaden the scope of cyclization possibilities and will enable minimal alteration of the native sequence is of considerable importance. In Backbone-Cyclization, ring closure is effected by bond formation between two functional groups which are linked to the backbone nitrogens by alkyl spacers. Cyclization can thus be accomplished without changing the original sequence or the chemical character of any amino acid residue required for bioactivity. This method also provides a convenient way to stabilize the ubiquitous turn motifs found in peptides, by replacing intramolecular hydrogen bonds with suitable covalent chains. If a particular N–H group in the peptide is required for biological activity, its replacement by an alkylated amide bond might reduce potency. However, this problem may usually be easily fixed by shifting the site of cyclization to the next amide bond.

In order to perform Backbone-Cyclization of peptides, unique, unnatural amino acids should be incorporated at the cyclization sites (Fig. 1). These 'building units' are protected N^{α}-(ω-Y-alkyl) amino acids—where Y is an orthogonally protected amino, carboxy or thiol group—which are analogous to the original amino acids at the sites chosen for cyclization. When the synthesis of the peptide is completed, the protecting groups of the cyclizing units are removed and cyclization is performed to give a lactam, a disulfide, a sulfide or a combination of these groups[5] (Fig. 2). Although other modes of cyclization are essentially possible, we decided to limit our studies to the same groups which are used for cyclization in nature.

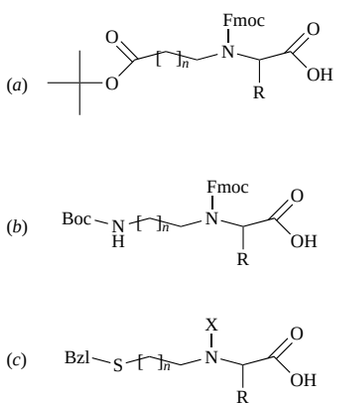


Fig. 1 Building units for N-Backbone-Cyclization; (*a*) ω-carboxy, (*b*) ω-amine, (*c*) ω-thiol. X = Boc, Fmoc; R = side-chain of α-amino acid.

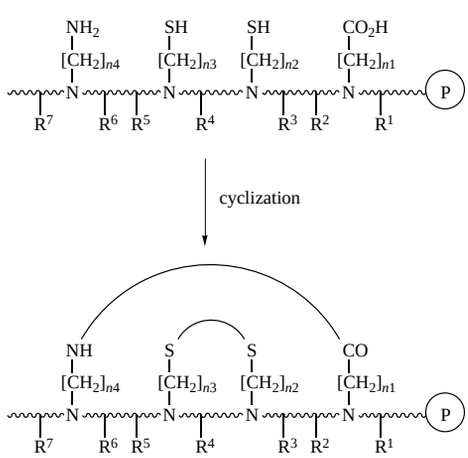


Fig. 2 Incorporation of building units into a peptide, and its cyclization

Building units for Backbone-Cyclization were previously synthesized by us and by others using several methods, which were all based on a nucleophilic substitution reaction as a key step.[1,6–8] Glycine derivatives were the easiest and hence the first to be prepared, by a nucleophilic attack of ω-substituted amines on bromoacetic acid or its esters. Other building units, based on chiral amino acids, could be prepared by the same method, albeit in low yield due to extensive side-reactions, using a large excess of the attacking amine with appropriate α-chloro acids as substrates.[7]

Reissmann *et al.* used the amino group of phenylalanine as a nucleophile to attack *tert*-butyl bromoacetate in the presence of Ag_2O and obtained a building unit based on phenylalanine with a carboxy group as the ω-functional group [see Fig. 1(*a*)] and a tether of one methylene group.[6] Unfortunately this method could not be generalized to longer alkyl tethers, because the *tert*-butyl esters of ω-halogeno carboxylic acids are not commercially available and their preparation is cumbersome.

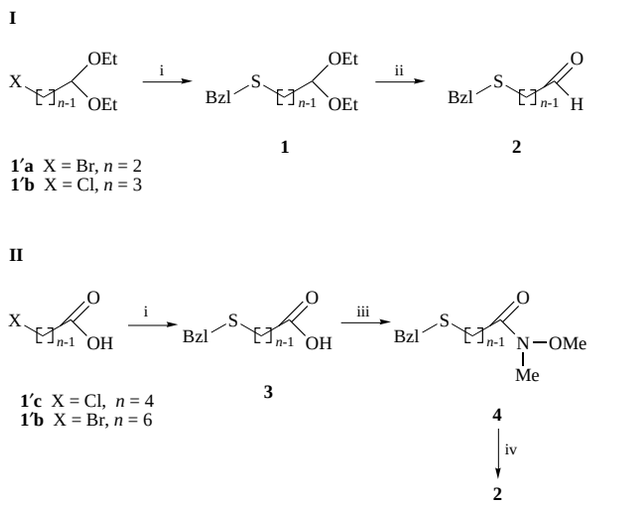
We have recently shown that several chiral building units with various tether lengths and functional groups could be obtained from α-triflate derivatives of D-hydroxy acids.[1,8] However, this approach suffered from several drawbacks: (1) the synthesis of one chiral building unit required 9–12 stages, beginning from costly D-amino acids; (2) triflates based on amino acids with sensitive side-chains are unstable (*e.g.* tryptophan[9]) and the synthesis of building units based on such amino acids is, accordingly, impractical; (3) in the case of ω-thiol building units there was a particular problem: in order to prevent a second substitution of the secondary amine formed, we had to employ semi-protection of the nucleophile with a benzyl group, which was removed after the substitution by catalytic hydrogenation.[1] This method was successful for the synthesis of ω-amino and ω-carbonyl building units, yet double substitution was unavoidable in the case of ω-thio building units. The semi-protection method is not suitable for the latter type of building units because catalytic hydrogenation fails due to poisoning of the catalyst by the sulfur. These problems inspired us to look for a new method for preparation of building units, which would be as general as possible and preferably reduce the cost, the number of synthetic steps, and be easy to execute. The other most common way for N-alkylation of amines and amino acids is reductive alkylation with aldehydes in the presence of a reducing agent.[10] This method was hence chosen to be examined for the preparation of building units for Backbone-Cyclization. Lately one example of the preparation of a building unit based on phenylalanine by reductive alkylation with Boc-glycinal was presented.[6] However, this was actually the preparation of a pseudo-dipeptide and until the present work no systematic investigation of this method for the preparation of building units was made, nor has any unit with a functionalized side-chain been prepared. The current research focused on ω-thiol building units, since this subgroup was the most problematic to prepare by the nucleophilic substitution method. The reductive alkylation method was, however, extended also for building units with ω-amino or ω-carboxy functional groups.

Results and discussion

Preparation of ω-functionalized aldehydes

Screening of the relevant literature revealed that essentially all of the previous work which employed reductive alkylation for derivatization of the α-amino group of amino acids used simple, and usually commercial, aldehydes. An exception to this generalization is found in syntheses of reduced pseudo-peptides, in which a Boc- or Fmoc-protected amino aldehyde is condensed with the amino group of an amino acid ester or a growing peptide. In our study of the synthesis of building units by reductive alkylation, we adopted the procedure developed by Fehrentz and Castro[11] as a general method for the preparation of ω-functionalized aldehydes with various chain lengths. In accordance with this method our synthetic root included the preparation of an ω-functionalized carboxylic acid—maintaining the ω-functional group suitably protected, conversion of the acid to the appropriate *N*,*O*-dimethyl hydroxamate and reduction to the aldehyde with $LiAlH_4$. In some cases, however, commercially available acetals could serve as more convenient starting materials for the preparation of the ω-functionalized aldehydes.

ω-(Benzylthio) aldehydes were prepared either from ω-halogeno acetals or from ω-halogeno carboxylic acids. In both cases the first step was substitution of the halogen with toluene-α-thiol. ω-(Benzylthio) acetals **1** were hydrolysed in 0.5 M H_2SO_4 to give the desired aldehydes **2** (Scheme 1-I). When ω-



Scheme 1 *Reagents*: i, Bzl-S^-, dry NMP; ii, 0.5 M H_2SO_4; iii, MeONHMe·HCl, BOP, TEA; iv, $LiAlH_4$, dry Et_2O

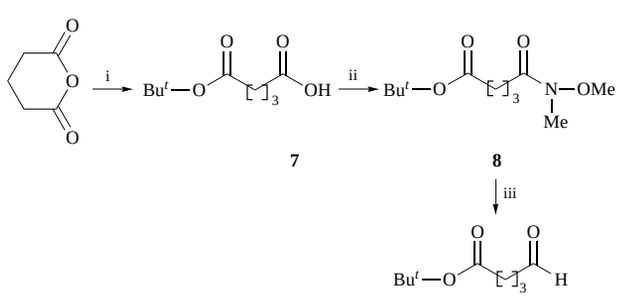
(benzylthio) carboxylic acids **3** were used then they were first converted to their *N*,*O*-dimethyl hydroxamates **4** and then reduced to the aldehydes with $LiAlH_4$ (Scheme 1-II). ω-Benzylthio-acet-, -propion-, -butyr- and -capro-aldehyde were obtained as liquids which could be preserved for long periods under argon at −5 °C.

By the same method ω-Boc-amino aldehydes **6** were prepared from ω-Boc-amino acids through reduction of the appropriate *N*,*O*-dimethyl hydroxamates **5** (the preparation of these aldehydes has been previously described[12,13]).

5a *n* = 2
5b *n* = 3

6a *n* = 2
6b *n* = 3

The preparation of ω-(*tert*-butoxycarbonyl) aldehydes was the most laborious of the three sub-groups prepared due to the necessity to obtain mono-*tert*-butyl esters of dicarboxylic acids. For the preparation of the mono-protected diacids we found that the opening of cyclic anhydrides with *tert*-butyl alcohol was most suitable (see also ref. 14). This method was demonstrated in the preparation of mono-*tert*-butyl glutarate **7** which was then converted to the *N*,*O*-dimethyl hydroxamate **8** and then reduced to the aldehyde **9** as described above (Scheme 2). This aldehyde is not known in the literature, yet similar aldehydes have been previously prepared.[15,16]



Scheme 2 *Reagents*: i, Bu^tOH, $ZnCl_2$; ii, MeONHMe·HCl, BOP, TEA; iii, $LiAlH_4$, dry Et_2O

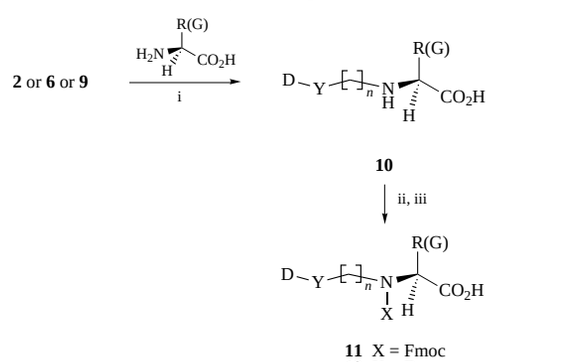
Table 1 Physical data of zwitterionic building units

Compound	Starting material	Y	*n*	Yield (%)	Mp (°C)
10a	γ-*o*-benzylglutamic acid	Bzl-S	6	56	150–151
10b	isoleucine	Bzl-S	2	63	241–243
10c	isoleucine	Bzl-S	3	56	215–217
10d	isoleucine	Boc-NH	3	50	204–206
10e	isoleucine	Bu^t-O_2C	4	19	229–231
10f	leucine	Bzl-S	2	55	210–211
10g	leucine	Bzl-S	3	59	191–192
10h	leucine	Boc-NH	3	46	212–214
10i	ε-Boc-lysine	Bzl-S	6	30	189–190
10j	methionine	Bzl-S	4	43	204–206
10k	methionine	Boc-NH	3	54	199–201
10l	phenylalanine	Bzl-S	2	61	220–223
10m	phenylalanine	Bzl-S	3	58	206–208
10n	phenylalanine	Boc-NH	3	69	206–209
10o	*O*-benzylserine	Boc-NH	2	29	65–68
10p	N^{In}-formyltryptophan	Bzl-S	4	19	167–170
10q	*O*-*tert*-butyltyrosine	Bzl-S	2	34	199–200
10r	valine	Boc-NH	3	59	202–204
10s	glycine	Bzl-S	2	30	181–182
10t	glycine	Bzl-S	3	42	173–175
10u	glycine	Bzl-S	4	41	
10v	glycine	Boc-NH	3	57	214–216
10w	glycine	Boc-NH	4	*a*	*a*

[a] Not isolated.

Preparation of chiral building units

Preparation of N^{α}-(ω-Y-alkyl) amino acids (other than glycine) by reductive alkylation was found to be a successful method and proved to be simpler and more economical in both time and money in comparison with the former method.[1,7] The reaction of various chiral, side-chain-protected (where appropriate) L-amino acids with aldehydes **2**, **6** or **9** was performed with slight modifications according to the procedure of Ohfune *et al.*[17] Thus, γ-benzylglutamic acid, isoleucine, leucine, ε-Boc-lysine, methionine, phenylalanine, *O*-benzylserine, N^{in}-formyltryptophan, *O*-*tert*-butyltyrosine and valine were N-alkylated with ω-Y-aldehydes to give the N^{α}-(ω-Y-alkyl) amino acids **10** with different functional groups and alkyl chains in 20–60% yield (Scheme 3). The yield depended mainly on the solubility of the product under the reaction conditions. In accord with the results observed by Ohfune *et al.*[17] the N-alkylated amino acids were formed as partially insoluble products which were collected and washed with methanol. In most cases no further purifiction was required. If a product contained any unreduced imine, or other impurities, it was recrystallized from boiling ethanol. No attempt was made to purify any dissolved product, which may have remained in the reaction mixture. Physical data for the zwitterionic units are summarized in Table 1.



R = side-chains of natural amino acids
G = side-chain orthogonal protecting groups
BTSA = *N*,*O*-bis(trimethylsilyl)acetamide

Y	D	*n*	X
NH	Boc	2,3	Fmoc
CO_2	Bu^t	4	Fmoc
S	Bzl	2,3,4,6	Boc, Fmoc

Scheme 3 *Reagents*: i, $NaBH_3CN$, MeOH; ii, BTSA; iii, Fmoc-Cl or $(Boc)_2O$

Toward the end of this work an attempt to develop a method for multiple simultaneous reductive alkylation reactions was made. Since the N-alkylated amino acids produced by this reaction were partly immiscible in the reaction medium, it seemed worthwhile to try simultaneously to prepare several products, in spatially separated vessels, and to purify them together by filtration. Multi-well blocks could offer a simple arrangement for this purpose; however, the volume available in each well of commercial multi-well blocks used for solid-phase syntheses is relatively small. Instead we carried out a preliminary exploratory experiment, in which the reactions were performed in simple polypropylene vessels arranged in an array and shaken together on a shaker or a vortex. Amino acids were chosen randomly off the shelf and reacted with three aldehydes in the following manner: alanine, arginine hydrochloride, asparagine, homophenylalanine, isoleucine, methionine, norvaline, phenylglycine and valine with aldehyde **2b**; methionine and valine with aldehyde **6a**; alanine, isoleucine and methionine with aldehyde **9**.

As no internal stirrer was used in this arrangement, it was found to be extremely important to apply vigorous shaking throughout the reaction, otherwise the parent amino acids precipitate in the bottom of the vessel and little or no reaction takes place. We used a strong vortex suitable for multiple vessels or arranged the vessels horizontally on an adhesive stripes-type shaker. However, evidently neither of these methods was sufficient and in all of the cases some unchanged material was detected by TLC. The yields were accordingly low, ranging between 7 and 35%.

Two of the amino acids, alanine (with both aldehydes **2b** and **6a**) and arginine hydrochloride, gave soluble products which were qualitatively observed by TLC, but which were not isolated. The asparagine product was identified as the dialkylated amino acid, N^{α},N^{α}-bis[3-(benzylthio)propyl]asparagine. All of the other products were the desired N^{α}-(ω-Y-alkyl) amino acids. Although no obvious pattern could be deduced for the dependence of the yield upon hydrophobicity, it is likely that the method is not suitable for hydrophilic amino acids, such as alanine or unprotected arginine and asparagine, because the products are soluble in methanol. However, appropriate side-chain protection may provide the desired low solubility of the produced *N*-alkylated amino acid.

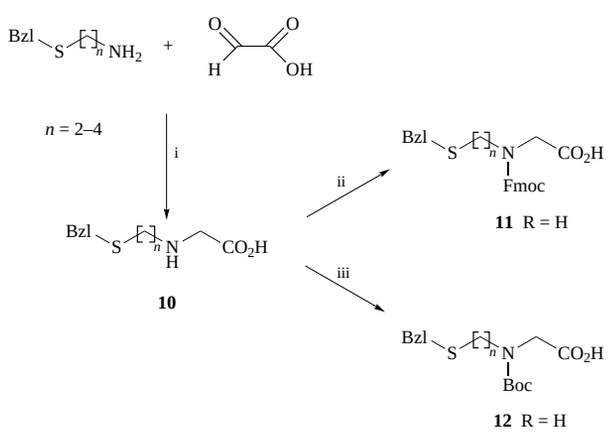
Table 2 Physical data of protected building unit

Compound	Yield (%)	Mp (°C)	$[a]_D^T$ (*c* 1, CH_2Cl_2)	Elemental analysis (%)
11b	42	115–117	−7.8 [18]	Calc: C, 71.54; H, 6.60; N, 2.78 Found: C, 71.88; H, 6.54; N, 2.82
11c	62	62–64	−9.3 [30]	Calc: C, 71.92; H, 6.81; N, 2.71 Found: C, 71.83; H, 6.82; N, 2.69
11d	48	56–58	−14.6 [25]	Calc: C, 68.21; H, 7.50; N, 5.49 Found: C, 67.95; H, 7.48; N, 5.47
11e	15	oil	+6.0 [25]	Calc: C, 70.70; H, 7.71; N, 2.75 Found: C, 70.22; H, 7.76; N, 2.78
11f	61	47–48	−17.0 [24]	Calc: C, 71.54; H, 6.60; N, 2.78 Found: C, 71.45; H, 6.71; N, 2.71
11g	70	oil	−21.6 [23]	Calc: C, 71.92; H, 6.81; N, 2.71 Found: C, 71.73; H, 6.82; N, 2.72
11h	20	45–47	n.d.[b]	Calc: C, 68.21; H, 7.50; N, 5.49 Found: C, 68.10; H, 7.50; N, 5.48
11i	53	semi-solid	−18.6 [24]	Calc: C, 69.41; H, 7.47; N, 4.15 Found: C, 69.26; H, 7.41; N, 4.08
11j	72	oil	−33.2 [25]	Calc: C, 67.73; H, 6.42; N, 2.55 Found: C, 67.53; H, 6.51; N, 2.46
11l	51	47–48	−86.9 [25]	Calc: C, 73.72; H, 5.81; N, 2.61 Found: C, 73.54; H, 5.92; N, 2.60
11m	48	53–54	−81.2 [23]	Calc: C, 74.02; H, 6.03; N, 2.54 Found: C, 74.10; H, 6.12; N, 2.58
11n	54	108–110	−87,0 [25,c]	Calc: C, 70.57; H, 6.66; N, 5.14 Found: C, 70.44; H, 6.60; N, 5.18
11o	30[a]	65–67	−23.5 [25]	Calc: C, 68.55; H, 6.47; N, 5.00 Found: C, 65.73; H, 6.39; N, 4.96
11p	53	60–61	−63.5 [23]	Calc: C, 69.78; H, 5.63; N, 3.13 Found: C, 69.65; H, 5.69; N, 3.24
11q	72	51–52 (decomp.)	−90.2 [23]	Calc: C, 72.88; H, 6.45; N, 2.30 Found: C, 72.62; H, 6.67; N, 2.19
11r	44	58–60	−14.7 [25]	Calc: C, 67.72; H, 7.31; N, 5.64 Found: C, 67.80; H, 7.29; N, 5.50
11s	42[a]	85–86		Calc: C, 69.78; H, 5.63; N, 3.13 Found: C, 69.61; H, 5.74; N, 3.23
12a	41	43–44	−36.3 [23]	Calc: C, 66.27; H, 7.60; N, 2.58 Found: C, 66.55; H, 7.52; N, 2.58
12f	42	oil	−26.2 [25]	Calc: C, 62.96; H, 8.19; N, 3.67 Found: C, 62.90; H, 8.18; N, 3.62
12s	88	71–72		Calc: C, 58.69; H, 7.70; N, 4.28 Found: C, 58.55; H, 7.72; N, 4.28
12t	85	oil		Calc: C, 60.15; H, 7.42; N, 4.13 Found: C, 59.98; H, 7.44; N, 4.11
12u	91	oil		Calc: C, 61.16; H, 7.70; N, 3.96 Found: C, 60.84; H, 7.68; N, 3.91
12v	78	124–125		*b*
12w	61	150–152		*b*

[a] Prepared by Method P. [b] Not determined. [c] *c* 1, MeOH.

This experiment demonstrated that although simultaneous synthesis of N^{α}-(ω-Y-alkyl) amino acids may be feasible and useful some further development is still required to overcome the technical problems which remain.

Most of the new building units were protected by *tert*-butoxycarbonyl (Boc) or fluoren-9-ylmethoxycarbonyl (Fmoc). Protection of the secondary α-amino group of N^{α}-(ω-Y-alkyl) amino acids was not possible by the common Boc- or Fmoc-introduction procedures, since these substances were all insoluble under the reaction conditions required for the introduction of both protecting groups. We have therefore adopted a method of temporary protection by the trimethylsilyl group,[6,18] using *N*,*O*-bis(trimethylsilyl)acetamide (BTSA) as the silylating agent, to introduce either of these protecting groups (Scheme 4). For ω-Boc-amino- or ω-*tert*-Butoxycarbonyl-containing units only the Fmoc group was used to protect the α-amine, whereas both Boc and Fmoc provided orthogonal protection in the case of ω-benzylthiol-containing units. The method proved to be very useful for introduction of Fmoc through Fmoc-Cl, yet the yield of Boc-N^{α}-[ω-(benzylthio)alkyl] amino acids was quite poor, probably because $(Boc)_2O$ is not reactive enough for this reaction. Prolonged reaction times did not increase the yield, but probably a more reactive agent such as Boc-Cl or Boc-N_3 would be preferable.



Scheme 4 *Reagents*: i, $NaBH_3CN$, MeOH; ii, Fmoc-Su; iii, $(Boc)_2O$

Physical data for units protected by Fmoc (**11**) or Boc (**12**) are summarized in Table 2. The integrity of the final products was verified by RP-HPLC and they were identified by their ^1H NMR spectra. Since all of the protected units existed as mixtures of isomers in solution, 2D NMR spectra were routinely employed for unambiguous peak assignment.

Preparation of glycine-based building units

An earlier attempt to force reaction between glycine and protected ω-amino aldehydes led to a mixture of products, from which the desired product could not be isolated.[19] In the current study it was found that N^{α}-[ω-Y-alkyl]glycine derivatives could be prepared by a variation of the method of Simon *et al*.[20] The first attempt to react ω-(Boc-amino)alkyl amines with glyoxylic acid and then to reduce the imine formed by catalytic hydrogenation, as in the original procedure, failed to yield the desired glycine building units. However, utilizing *in situ* reductive alkylation of ω-substituted primary amines of various lengths with glyoxylic acid in the presence of sodium cyanoborohydride gave the desired products as precipitates, when Y was a benzyl-protected thiol.

Since glycine building units bearing an ω-amino or ω-carboxy group were readily prepared by the nucleophilic substitution method,[1,7] no special effort was required for their preparation by the reductive alkylation method. Only in one case was a comparison of the two methods in the preparation of Fmoc-N^{α}-[3-(Boc-amino)propyl]glycine and N^{α}-Fmoc-[4-(Boc-amino)butyl]glycine made. The overall yield of the first unit was 45% in both methods. In this particular case the zwitterionic N^{α}-[3-(Boc-amino)propyl]glycine did not precipitate from the reaction medium during the reductive alkylation, and it was hence necessary to protect the crude product by Fmoc and to further purify it by column chromatography. On the other hand, the second unit, N^{α}-[4-(Boc-amino)butyl]glycine, did precipitate under the same conditions and consequently the overall yield of the Fmoc-protected unit was elevated to 61% when prepared by the reductive alkylation method. The overall yield of this unit was 47% when prepared by the nucleophilic substitution method. It was therefore concluded that the reductive alkylation of ω-protected amines with glyoxylic acid was equal to or better than the nucleophilic substitution of benzyl bromoacetate with the same amines for the preparation of building units based on glycine.

The reaction between ω-functionalized alkylamines and glyoxylic acid was particularly important for the preparation of N^{α}-[ω-(benzylthio)alkyl]glycine derivatives. As mentioned above, preparation of these compounds by the nucleophilic substitution method suffered from a high amount of double alkylation of the attacking amine. In the current study, however, ω-(benzylthio)alkylamines, prepared by previously reported procedures,[8] reacted with a slight excess of glyoxylic acid to yield the corresponding N^{α}-[ω-(benzylthio)alkyl]glycine with various alkyl chain lengths. In all cases the products were partly immiscible under the reaction conditions and could be filtered off and isolated in 30–40% yield before the next step. Although about the same amount of product still remained in solution, its purification was troublesome and we therefore preferred to use larger quantities of the cheap starting materials and did not try to increase the yield above that of the precipitated product. The crude products contained only small amounts of NaCN as the only impurity and could be unambiguously identified by their ^{1}H NMR spectra. The glycine derivatives were soluble in water at pH > 7 and the secondary α-amino group could therefore be protected by either Fmoc or Boc protecting groups to give the protected products without any difficulty.

The rapid development of efficient screening methods combined with combinatorial chemistry gives new powerful tools to chemists and sets new targets which could not have been achieved less than a decade ago.[21] Yet, with the ability to prepare large numbers of molecules in a short time and to 'fish out' only those which are biologically relevant comes the need for novel sophisticated building blocks which will augment the level of diversity and impart, through their unique chemistry, desirable pharmacological features to the molecules which are being produced. Several laboratories around the world offer a variety of substitutes for the natural building blocks of peptides and proteins, which are aimed at maintaining the basic structural and hence the biological properties of amino acids. Many of the makers of such building blocks, from *N*-alkylamino acids which form peptoids to the recently presented betides,[22] try to keep the side-chains of the amino acids unchanged while altering the construction which holds them together—the peptide backbone. In contrast to most of these building blocks, which usually focus on one aspect of chemical modification, the units described in this work offer three-dimensional diversity. The first dimension is the side-chains of 19 natural α-amino acids (excluding proline), to which many unnatural α-aminol acids with a primary amino group can be added. The second dimension is the ω-functional groups which may be used for their original function—cyclization—but also to connect other useful moieties like chelating agents and affinity, crosslinking or radioactive labels. We have limited ourselves in this work to three kinds of ω-functional groups, but other groups may be preferred for different particular cases. The third dimension is provided by the control of the spacer length, which we have been using to explore the conformational space available for bioactive peptides through backbone-cyclic analogue libraries.

Although cyclization of short bioactive peptides is a very popular manipulation, designated to bestow desirable pharmacological features, most laboratories are limited to classical cyclization methods. Backbone-Cyclization offers a smooth way to avoid sequence alteration and side-chain and/or termini modifications, usually required for cyclization. The lack of a general rigorous method for the preparation of building units was until recently one of the main obstacles which prevented Backbone-Cyclization from becoming more widely and commonly used. All the previous methods for the preparation of building units were limited to certain amino acids and/or certain tether lengths. This work, however, offers a simple synthetic pathway for the preparation of building units based on most (side-chain-protected) amino acids with various alkyl chain lengths, in 4–5 steps altogether. The first 2–3 steps (depending on the availability of starting compounds) are the preparation of ω-substituted aldehydes and the following two steps are reductive alkylation and protection of the α-amine.

Experimental

Materials and methods

Starting materials were purchased from either Merck, Darmstadt, Germany or Aldrich, Milwaukee, WI, USA and used without further purification. Analytical HPLC was performed on a Merck Hitachi 655A equipped with an L-6200A gradient pump and a UV–VIS detector with tunable wavelength set at 220 nm. The flow was fixed at 1 ml min^{-1} and the eluents were triply distilled water (TDW) and MeCN [containing 0.1% trifluoroacetic acid (TFA)] or MeOH. The column was Lichroprep RP-18, 250 × 4.2 mm i.d. from Merck. Mps were measured on a Mel-Temp II capillary equipment. Optical rotations were recorded on Perkin-Elmer 141 or 241 polarimeters in a 10-cm length cell and $[a]_{\mathbf{D}}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analysis was carried out at the microanalytical laboratory of the Hebrew University, Jerusalem. ^{1}H NMR spectra were recorded on Brucker WP-200, AMX-300, AMX-400 or DRX-400 spectrometers. 2D Chemical shift correlation (COSY) spectra of final products were routinely recorded and in some cases phase-sensitive 2-D total correlation spectrometry (TOCSY), nuclear Overhauser enhancement spectroscopy (NOESY), NOESY in a rotating frame (ROESY) and C–H correlation spectra were also used to assist with the proton assignment of highly overlapping 1D spectra. The numbering of methylene groups in the *N*-alkyl chain is always from the N^{α} of the amino acid to the ω-functional group. *J* Values are given in Hz.

Method A. Preparation of ω-(benzylthio) aldehyde diethyl acetals 1

0.51 Mol of toluene-α-thiol were added dropwise under dry N_2 to a stirred suspension of 0.51 mol of NaH in 300 ml of dry NMP at 0 °C. The resulting dark solution was stirred for an additional 30 min and then 0.5 mol of an ω-halogeno aldehyde diethyl acetal were slowly added. The mixture was stirred for 18 h at room temperature. Completion of the reaction was monitored by TLC. The mixture was then poured into 1 l of ice–water and the product was extracted with light petroleum (LP) (6 × 500 ml). The yellow organic solution was dried over $MgSO_4$, filtered and the solvent was evaporated off *in vacuo*. The remaining crude products were distilled *in vacuo* to give the pure title compounds as liquids.

Compound **1a** was prepared from 2-bromoacetaldehyde diethyl acetal **1′a** in 85% yield, bp 93 °C (0.04 mmHg) (Found: C, 65.31; H, 8.66. $C_{13}H_{20}O_2S$ requires C, 64.96; H, 8.39%); δ_H($CDCl_3$; 298 K) 7.33–7.22 (5 H, m, ArH), 4.54 (t, *J* 5.6, ^{1}CH), 3.79 (2 H, s, PhCH_2), 3.72–3.43 (4 H, m, MeCH_2), 2.59 (2 H, d, *J* 5.6, 2-H_2) and 1.21 (6 H, t, *J* 7.1, CH_3CH$_2$).

Compound **1b** was prepared from 3-chloropropionaldehyde diethyl acetal **1′b** in 61% yield, bp 105 °C (0.02 mmHg); δ_H-($CDCl_3$; 298 K) 7.33–7.23 (5 H, m, ArH), 4.56 (1 H, t, *J* 5.6, ^{1}CH), 3.71 (2 H, s, PhCH_2), 3.70–3.38 (4 H, m, MeCH_2), 2.47 (2 H, t, 7.4 ^{3}CH$_2$), 1.86 (2 H, dt, J_{21} 5.7, J_{23} 7.4, 2-CH$_2$) and 1.18 (6 H, t, *J* 7.0, CH_3CH$_2$).

Method B. Acid hydrolysis of ω-(benzylthio) aldehyde diethyl acetals 1 to aldehydes 2a,2b

0.4 Mol of an acetal **1** were stirred for 24 h with 300 ml of 1 M H_2SO_4 at 60 °C. The progress of the reaction was followed by TLC. The crude product was extracted with LP (6 × 300 ml) and the solution was dried over $MgSO_4$, filtered on active carbon and evaporated *in vacuo.* Aldehydes **2** were further distilled *in vacuo* and were collected as liquids.

Aldehyde **2a** (87%), bp 73 °C (0.06 mmHg); δ_H($CDCl_3$; 298 K) 9.40 (1 H, t, *J* 2.5, ^{1}CH), 7.34–7.24 (5 H, m, ArH), 3.63 (2 H, 5, PhCH_2) and 3.07 (2 H, d, *J* 1.4, ^{2}CH$_2$).

Aldehyde **2b** (80%), bp 101 °C (0.06 mmHg); δ_H($CDCl_3$; 298 K) 9.70 (1 H, t, *J* 1.1, 1-H), 7.34–7.24 (5 H, m, ArH), 3.73 (2 H, s, PhCH_2) and 2.72–2.65 (4 H, m, 2- and 3-H_2).

Method C. Preparation of ω-(benzylthio) carboxylic acids 3

0.51 Mol of toluene-α-thiol were added dropwise under dry N_2 to a mechanically stirred suspension of 1.01 mol of NaH in 1 l of dry NMP at 0 °C. The resulting solution was stirred for an additional 30 min and then 0.5 mol of an ω-halogeno carboxylic acid were slowly added. The mixture was stirred for 18 h at room temperature. Completion of the reaction was monitored by TLC. The mixture was diluted with 1 l of ice–water. The aqueous solution was washed three times with diethyl ether (300 ml), acidified with 0.5 M H_2SO_4 and extracted with 3 × 200 ml of diethyl ether. The yellow organic solution, which contained some residual toluenethiol, was dried over $MgSO_4$, filtered and the solvent was evaporated off *in vacuo.* The remaining crude product was distilled *in vacuo* to give the pure title compounds as liquids. Product **3d** crystallized on storage.

Compound **3c** (*n* = 4) was prepared from 4-chlorobutanoic acid **1′c** (81%), bp 141 °C (0.02 mmHg) [lit.,[23] 170 °C (0.01 mmHg)]; δ_H($CDCl_3$; 298 K) 7.31–7.23 (5 H, m, ArH), 3.69 (2 H, s, PhCH_2), 2.454 (2 H, t, *J* 7.1, 2-H_2), 2.447 (2 H, t, *J* 7.3, 4-H_2) and 1.95–1.80 (2 H, m, 3-H_2).

Compound **3d** (*n* = 6) was prepared from 6-bromohexanoic acid **1′d** (76%), bp 165 °C (0.08 mmHg); δ_H($CDCl_3$; 298 K) 7.32–7.23 (5 H, m, ArH), 3.70 (2 H, s, PhCH_2), 2.42 (2 H, t, *J* 7.1, 2-H_2), 2.33 (2 H, t, *J* 7.4, 6-H_2) 1.69–1.50 (4 H, m, 3- and 5-H_2) and 1.47–1.35 (2 H, m, 4-H_2).

Method D. Preparation of ω-(benzylthio) carboxylic acid *N*,*O*-dimethyl hydroxamates 4

0.1 Mol of diisopropylethylamine (DIEA) and 0.1 mol of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) were added to a suspension of 0.1 mol of an ω-(benzylthio) carboxylic acid in dichloromethane (DCM). The mixture was stirred for 5 min at room temperature by which time a clear solution was obtained. 0.11 Mol of *N*,*O*-dimethylhydroxylamine hydrochloride and 0.11 mol of DIEA were then added and the solution was stirred for 4.5 h at room temperature. The reaction was diluted with 200 ml of DCM, washed successively with 3 × 50 ml of 1 M H_2SO_4, 3 × 50 ml of saturated aq. $KHCO_3$ and 3 × 50 ml of saturated NaCl. The organic phase was dried over $MgSO_4$ and the solvent was evaporated off *in vacuo*. The crude product was further purified by column chromatography (silica; ethyl acetate–LP 40 : 60).

Compound **4c** (81%) (Found: C, 61.40; H, 7.60; N, 5.51. $C_{13}H_{19}NO_2S$ requires: C, 61.63; H, 7.56; N, 5.53%); δ_H($CDCl_3$; 298 K) 7.32–7.22 (5 H, m, ArH), 3.71 (2 H, s, PhCH_2) 3.67 (3 H, s, OCH_3), 3.17 (3 H, s, NCH_3), 2.52 (2 H, t, *J* 6.7, 2-H_2), 2.48 (2 H, t, *J* 6.9, 4-H_2) and 1.98–1.84 (2 H, m, 3-H_2).

Compound **4d** (81%), δ_H($CDCl_3$; 298 K) 7.32–7.22 (5 H, m, ArH), 3.70 (2 H, s, PhCH_2), 3.67 (3 H, s, OCH_3), 3.17 (3 H, s, NCH_3), 2.46–2.36 (4 H, m, 2- and 6-H_2), 1.70–1.52 (4 H, m, 3- and 5-H_2) and 1.47–1.35 (2 H, m, 4-H_2).

Method E. Reduction of *N*,*O*-dimethyl hydroxamates 4 to ω-(benzylthio) aldehydes 2c and 2d

5 Mol equiv. of $LiAlH_4$ were added in small portions to a solution of 80 mmol of an ω-(benzylthio) carboxylic acid *N*,*O*-dimethyl hydroxamate in 200 ml of dry diethyl ether stirred under N_2. The mixture was stirred for 60 min at room temperature, then was cooled with an ice–water-bath and hydrolysed with a solution of 19 g of $KHSO_4$ in 100 ml of water. Diethyl ether (50 ml) was added, the layers were separated and the aqueous layer was extracted with 3 × 50 ml diethyl ether. The combined ethereal layers were washed successively with 3 × 50 ml of 3 M HCl, 3 × 50 ml of saturated aq. $KHCO_3$ and 3 × 50 ml of saturated aq. NaCl. The organic phase was dried over $MgSO_4$ and the solvent was evaporated off *in vacuo*. The aldehydes were collected as colourless or pale yellow oils.

Compound **2c** (56%), δ_H($CDCl_3$; 298 K) 9.75 (1 H, t, *J* 1.2, 1-H), 7.34–7.22 (5 H, m, ArH), 3.70 (2 H, s, PhCH_2), 2.53 (2 H, dt, J_{21} 1.2, J_{23} 7.2, 2-H_2), 2.46 (2 H, t, *J* 7.0, 4-H_2) and 1.91–1.84 (2 H, m, 3-H_2) in agreement with the literature.[24]

Compound **2d** (67%), δ_H($CDCl_3$; 298 K) 9.72 (1 H, t, *J* 1.8, 1-H), 7.36–7.20 (5 H, m, ArH), 3.69 (2 H, s, PhCH_2), 2.40 (2 H, t, 7.2, 6-H_2), 2.38 (2 H, dt, J_{21} 1.7, J_{23} 7.4, 2-H_2), 1.60–1.52 (4 H, m, 3- and 5-H_2) and 1.39–1.35 (2 H, m, 4-H_2).

Method F. Preparation of ω-(Boc-amino) carboxylic acid *N*,*O*-dimethyl hydroxamates 5

The title compounds were prepared from Boc-glycine and Boc-β-alanine according to Method D.

Compound **5a** (*n* = 2) (62%), δ_H($CDCl_3$; 298 K) 5.27 (1 H, br s, NH), 4.09 (2 H, d, *J* 6.5, 2-H_2), 3.71 (3 H, s, OCH_3), 3.22 (3 H, s, NCH_3) and 1.39 (9 H, s, Bu^t).

Compound **5b** (*n* = 3) (93%), δ_H($CDCl_3$; 298 K) 5.23 (1 H, br s, NH), 3.63 (3 H, s, OCH_3), 3.37 (2 H, t, *J* 6.3, 3-H_2) 3.13 (3 H, s, NCH_3), 2.51 (2 H, t, *J* 6.7, 2-H_2) and 1.39 (9 H, s, Bu^t).

Method G. Reduction of *N*,*O*-dimethyl hydroxamates 5 to ω-(Boc-amino) aldehydes 6

The title compounds were prepared from hydroxamates **5** according to Method E.

Compound **6a** (36%), δ_H($CDCl_3$; 298 K) 9.64 (1 H, t, *J* 4.0, 1-H) 5.32 (1 H, br s, NH), 4.06 (2 H, d, *J* 4.1, 2-H_2) and 1.46 (9 H, s, Bu^t).

Compound **6b** (61%), δ_H($CDCl_3$; 298 K) 9.73 (1 H, t, *J* 3.9, 1-H), 5.15 (1 H, br s, NH), 3.35 (2 H, m, 3-H_2), 2.64 (2 H, t, *J* 6.0, 2-H_2) and 1.36 (9 H, s, Bu^t).

Method H. Preparation of mono-*tert*-butyl glutarate 7

Glutaric anhydride (5.7 g, 0.05 mol) was added to a mixture of 28.4 ml (0.3 mol) of dry *tert*-butyl alcohol and 0.1 g of zinc chloride. The mixture was stirred at 60 °C with exclusion of water for 3 days. 0.5 M Sodium hydroxide (40 ml) was added and after 20 min the product was extracted with diethyl ether (4 × 40 ml), washed with water (3 × 50 ml) and dried over $MgSO_4$. The solvent and excess of *tert*-butyl alcohol were removed *in vacuo*. The product was obtained as an oil (50%), δ_H($CDCl_3$; 298 K) 10.70 (1 H, br s, CO_2H), 2.42 (2 H, t, *J* 8.0, 2-H_2), 2.32 (2 H, t, *J* 8.0, 4-H_2), 1.92 (2 H, m, 3-H_2) and 1.45 (9 H, s, Bu^t) (for comparison see ref. 25).

Method I. Preparation of glutaric acid *tert*-butyl ester *N,O*-dimethyl hydroxamate 8

The title compound was prepared from semi-ester **7** according to Method D in 66% yield; δ_H($CDCl_3$; 298 K) 3.70 (3 H, s, OCH_3), 3.20 (3 H, s, NCH_3), 2.48 (2 H, t, *J* 8.2, 2-H_2), 2.30 (2 H, t, *J* 8.2, 4-H_2), 1.92 (2 H, m, 3-H_2) and 1.45 (9 H, s, Bu^t).

Method J. Reduction of *N,O*-dimethyl hydroxamate 8 to γ-(*tert*-butoxycarbonyl)glutaraldehyde 9

The title compound was prepared from hydroxamate **8** according to Method E in 81% yield; δ_H($CDCl_3$; 298 K) 9.75 (1 H, s, 1-H), 2.50 (2 H, t, *J* 7.0, 4-H_2), 2.25 (2 H, t, *J* 7.0, 2-H_2), 1.9 (2 H, m, 3-H_2) and 1.4 (9 H, s, Bu^t).

Method K. Preparation of chiral N^{α}-ω-Y-alkyl amino acids 10

A zwitterionic (suitably protected on the side-chain) amino acid (5–10 mmol) was dissolved or suspended in methanol (1.5 ml $mmol^{-1}$). Then 1.5 mol equiv. of aldehyde **2**, **6**, or **9** were added, followed by 1.1 mol equiv. of sodium cyanoborohydride, and the mixture was stirred at room temperature for 18 h. The precipitated product was collected by filtration on a glass sinter, washed with methanol, and dried *in vacuo*. Physical data for compounds **10** are summarized in Table 1. Their ^{1}H NMR data and assignments follow here.

***N*$^{\alpha}$-[6-(Benzylthio)hexyl]glutamic acid γ-benzyl ester 10a.** [$(CD_3)_2SO$; 310 K] 7.63–7.29 (8 H, m, ArH), 7.24–7.20 (2 H, m, ArH), 4.50 (2 H, s, OCH_2Ph), 4.16–4.13 (1 H, m, α-H), 3.71 (2 H, s, SCH_2Ph), 3.46 (1 H, m, 1-H_2), 2.82 (1 H, m, 1-H_2), 2.38 (2 H, t, *J* 7.3, 6-H_2), 2.27 (1 H, m, β-H_2), 2.24 (2 H, m, γ-H_2), 1.94 (1 H, m, β-H_2), 1.48 (2 H, m, 5-H_2), 1.29 (2 H, m, 4-H_2) and 1.18 (2 H, m, 3-H_2).

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]isoleucine 10b.** (D_2O; 350 K; as sodium salt) 7.86 (5 H, m, *Ph*CH_2), 4.26 (2 H, s, CH_2Ph), 3.33 (1 H, d, *J* 5.7, α-H), 3.21–3.02 (4 H, m, 1- and 2-H_2), 2.03–1.85 (2 H, m, γ-H_2), 1.67–1.50 (1 H, m, β-H) and 1.37–1.30 (6 H, m, γ- and δ-H_3).

***N*$^{\alpha}$-[3-(Benzylthio)propyl]isoleucine 10c.** (D_2O; 350 K; as sodium salt) 7.83 (5 H, m, *Ph*CH_2), 4.23 (2 H, s, CH_2Ph), 3.29 (1 H, d, *J* 5.8, α-H), 3.01–2.85 (4 H, m, 1- and 3-H_2), 2.22–2.06 (2 H, m, 2-H_2), 2.05–1.85 (2 H, m, γ-H_2), 1.60–1.47 (1 H, m, β-H) and 1.34–1.27 (6 H, m, γ- and δ-H_3).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]isoleucine 10-d.** (D_2O; 298 K; as sodium salt) 3.18–3.11 (2 H, m, 3-H_2), 3.01 (1 H, d, *J* 5.9, α-H), 2.68–2.49 (2 H, m, 1-H_2), 1.81–1.54 (3 H, m, 2-H_2 and β-H), 1.50 (9 H, s, Bu^t), 1.29–1.16 (2 H, m, γ-H_2), 1.00–0.97 (3 H, m, γ-H_3) and 0.96–0.93 (3 H, m, δ-H_3).

***N*$^{\alpha}$-[4-(*tert*-Butoxycarbonyl)butyl]isoleucine 10e.** (D_2O; 298 K; as potassium salt) 2.95 (1 H, d, *J* 5.9, α-H), 2.50 (2 H, m, 1-H_2), 2.30 (2 H, t, *J* 7.1, 4-H_2), 1.51 (5 H, m, 2- and 3-H_2 and β-H), 1.45 (9 H, s, Bu^t), 1.22–1.09 (2 H, m, γ-H_2) and 0.92–0.85 (6 H, m, γ-and δ-H_3).

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]leucine 10f.** (D_2O; 310 K; as sodium salt) 7.45–7.33 (5 H, m, *Ph*CH_2), 3.79 (2 H, s, CH_2Ph), 2.32 (1 H, dd, J_1 6.2, J_2 8.3, α-H), 2.71–2.55 (4 H, m, 1- and 2-H_2), 1.59–1.56 (1 H, m, γ-H), 1.44–1.33 (2 H, m, β-H_2), 0.91 (3 H, d, *J* 6.8, δ-H_3) and 0.89 (3 H, d, *J* 6.8, δ-H_3).

***N*$^{\alpha}$-[3-(Benzylthio)propyl]leucine 10g.** (D_2O; 350 K; as sodium salt) 7.91 (5 H, m, *Ph*CH_2), 4.32 (2 H, s, CH_2Ph), 3.54 (1 H, t, *J* 7.2, α-H), 3.07–2.92 (4 H, m, 1- and 3-H_2), 2.30–2.14 (2 H, m, 2-H_2), 2.14–2.01 (1 H, m, γ-H), 1.89 (2 H, dd, J_1 3.4, J_2 6.3, β-H_2) and 1.40 (6 H, m, δ-H_3).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]leucine 10h.** (D_2O; 298 K; as sodium salt) 3.38 (1 H, dd, J_1 6.0, J_2 8.2, α-H), 3.18–3.13 (2 H, m, 3-H_2), 2.63–2.48 (2 H, m, 1-H_2), 1.75–1.38 (5 H, m, 2- and β-H_2 and γ-H), 1.50 (9 H, s, Bu^t) and 1.00–0.95 (6 H, m, δ-H_3).

***N*$^{\alpha}$-[6-(Benzylthio)hexyl]-ε-Boc-lysine 10i.** Solubility was too low in all attempted systems.

***N*$^{\alpha}$-[4-(Benzylthio)butyl]methionine 10j.** (D_2O; 298 K; as sodium salt) 7.37 (5 H, m, *Ph*CH_2), 3.77 (2 H, s, PhCH_2), 3.10 (1 H, t, *J* 6.5, α-H), 2.53–2.41 (6 H, m, 1-, 4- and γ-H_2), 2.09 (3 H, s, ε-H_3), 1.86–1.78 (2 H, m, β-H_2) and 1.53 (4 H, m, 2- and 3-H_2).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]methionine 10k.** (D_2O; 298 K; as sodium salt) 3.41 (1 H, dd, J_1 5.5, J_2 7.4, α-H), 3.17–3.13 (2 H, m, 3-H_2), 2.65–2.48 (4 H, m, γ- and 1-H_2), 2.17 (3 H, s, ε-H_3), 1.96–1.86 (2 H, m, β-H_2), 1.76–1.61 (2 H, m, 2-H_2) and 1.49 (9 H, s, Bu^t).

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]phenylalanine 10l.** (D_2O; 298 K; as sodium salt) 7.55–7.36 (10 H, m, ArH), 3.84 (2 H, s, CH_2Ph), 3.41 (1 H, t, *J* 6.7, α-H), 2.99 (2 H, d, *J* 6.6, 1-H_2) and 2.81–2.65 (4 H, m, β- and 2-H_2).

***N*$^{\alpha}$-[3-(Benzylthio)propyl]phenylalanine 10m.** [$(CD_3)_2SO$; 350 K] 7.30–7.17 (10 H, m, ArH), 3.67 (2 H, s, CH_2Ph), 3.35 (1 H, t, *J* 6.7, α-H), 2.92 (1 H, dd, J_1 8.0, J_2 7.1, β-H_2), 2.77 (1 H, dd, J_1 9.6, J_2 8.4, β-H_2), 2.66–2.51 (2 H, m, 1-H_2), 2.40 (2 H, t, *J* 7.2, 3-H_2) and 1.70–1.55 (2 H, m, 2-H_2).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]phenylalanine 10n.** (D_2O; 298 K; as calcium salt) 7.34–7.22 (5 H, m, ArH), 3.29 (1 H, t, X of ABX, *J* 7.3, α-H), 3.08–2.97 (2 H, m, 3-H_2), 2.90 (2 H, dd, AB of ABX, $J_{\alpha\beta}$ 6.7, $J_{\beta\beta}$ 13.3, β-H_2), 2.59–2.48 (0.9 H, m, 1-H_2-*E*), 2.46–2.39 (1.1 H, m, 1-H_2-*Z*), 1.70–1.49 (2 H, m, 2-H_2) and 1.39 (9 H, s, Bu^t).

***N*$^{\alpha}$-[2-(Boc-amino)ethyl]-*O*-benzylserine 10o.** (D_2O; 298 K; as potassium salt) 7.77 (5 H, m, ArH), 4.92 (2 H, s, CH_2Ph), 4.05 (2 H, t, *J* 5.8, 2-H_2), 3.52 (1 H, t, *J* 4.7, α-H), 3.52 (2 H, t, *J* 6.0, 1-H_2), 3.12–2.87 (2 H, m, β-H_2) and 1.77 (9 H, s, Bu^t).

***N*$^{\alpha}$-[4-(Benzylthio)butyl]-*N*$^{\text{In}}$-formyltryptophan 10p.** (D_2O; 298 K; as sodium salt, isomer ratio due to formyl protection *E* : *Z* = 1 : 1.78) 7.76 (2 H, t, *J* 7.4, Fmoc 4- and 5-H), 7.66 [0.35 H, *J* 6.6, Fmoc 1-H (*E*)], 7.63–7.51 [1.65 H, m, Fmoc 1-H (*Z*)], 7.43–7.36 (2 H, m, Fmoc 3- and 6-H), 7.32 (2 H, t, *J* 8.1, Fmoc 2- and 7-H), 7.28–7.26 (2 H, m, SBzl *o*-H), 7.24–7.21 (2 H, m, SBzl *m*-H), 7.16–7.14 (1 H, m, SBzl *p*-H), 6.89–6.84 [2.6 H, m, Ph (*Z*)], 6.78 [0.7 H, d, *J* 8.3, Ph *o*-H (*E*)], 6.56 [0.7 H, d, *J* 8.2, Ph *m*-H (*E*)] 4.84 [0.35 H, dd, J_{12} 4.6, J_{22} 10.7, Fmoc CH_2 (*E*)], 4.70 [0.65 H, dd, J_{12} 5.6, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.58 [0.35 H, dd, J_{12} 4.8, J_{22}10.7, Fmoc CH_2 (*E*)]; 4.45 [0.65 H, dd, J_{12} 5.9, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.23–4.19 (1 H, m, Fmoc CH), 3.78 [0.65 H, m, α-H (*Z*)], 3.67 [0.35 H, m, α-H (*E*)], 3.59 [0.7 H, s, SCH_2Ph (*E*)], 3.47–3.39 (1.3 H, m, SCH_2Ph (*Z*)], 3.18–3.15 [1.3 H, m, β-H_2 (*Z*)], 3.11 [0.35 H, m, 1-H_2 (*E*)], 2.99–2.89 [0.65 H, m, 1-H_2 (*Z*)], 2.83–2.79 [0.35 H, m, 1-H_2 (*E*)], 2.58 [0.35 H, m, 2-H_2 (*E*)], 2.52–2.46 [0.65 H, m, 1-H_2 (*Z*)], 2.35–2.29 [0.35 H, m, 2-H_2 (*E*)], 2.23–2.19 [0.7 H, m, β-H_2 (*E*)], 2.07–2.00 [0.65 H, m, 2-H_2 (*Z*)], 1.97–1.94 [0.65 H, m, 2-H_2 (*Z*)], 1.33 [5.85 H, s, Bu^t (*Z*)] and 1.30 [3.15 H, s, Bu^t (*E*)].

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]-*O*-*tert*-butyltyrosine 10q.** (D_2O; 350 K; as sodium salt) 7.25–7.17 (5 H, m, SCH_2*Ph*), 7.09 (2 H, d, *J* 8.3, Ph *m*-H), 6.87 (2 H, d, *J* 8.0, Ph *o*-H), 3.55 (2 H, s, CH_2Ph), 3.1 (1 H, dd, α-H), 2.83 (1 H, dd, β-H_2), 2.62 (1 H, dd, $J_{\alpha\beta}$ 7.4, $J_{\beta\beta}$ 13.4, β-H_2), 2.56–2.54 (1 H, m, 1-H_2), 2.39 (3 H, m, 1-H and 2-H_2) and 1.18 (9 H, s, Bu^t).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]valine 10r.** (D_2O; 298 K; as sodium salt) 3.22–3.12 (2 H, m, 3-H_2), 2.92 (1 H, d, *J* 6.2, α-H), 2.69–2.49 (2 H, m, 1-H_2), 1.69–1.87 (1 H, m, β-H), 1.85–1.61 (2 H, m, 2-H_2), 1.51 (9 H, s, Bu^t), 1.02 (3 H, d, *J* 6.8, γ-H_3) and 0.98 (3 H, d, *J* 6.9, γ-H_3).

Method L. Simultaneous preparation of chiral N^{α}-ω-Y-alkyl amino acids 10

These compounds were prepared as in the above procedure (Method K); however, the reactions were performed in polypropylene vessels arranged in an array and shaken either by a Genie Vortex 2 from Scientific Industries, Bohemia, USA or by an adhesive stripes Labotron shaker from INFORS AG, Bottmingen, Germany. Since this experiment was carried out only once the results are given here separately and the products are not denoted by numbers and letters.

N,*N*-Bis-[3-(benzylthio)propyl]asparagine (35%), mp 167–168 °C; δ_H(D_2O; 298 K; as sodium salt) 7.20–7.12 (8 H, m, Ar 2-, 3-, 5-, 6-H), 7.07–7.04 (2 H, m, Ar 4-H), 3.57 (4 H, m, PhCH_2), 3.49 (1 H, t, *J* 7.4, α-H), 2.48–2.43 (5 H, m, 1- and β-H_2), 2.37–2.28 (5 H, m, 1- and β-H_2) and 1.57–1.39 (4 H, m, 2-H_2).

N-[3-(Benzylthio)propyl]homophenylalanine (29%), mp 212–213 °C; δ_H[$(CD_3)_2SO$; 340 K] 7.31–7.13 (10 H, m, ArH), 3.73 (2 H, s, PhCH_2), 3.07 (1 H, t, *J* 6.4, α-H) 2.76–2.59 (4 H, m, 1- and β-H_2), 2.51–2.47 (2 H, m, 3-H_2), 1.92–1.81 (2 H, m, γ-H_2) and 1.76–1.71 (2 H, m, 2-H_2).

N-[3-(Benzylthio)propyl]isoleucine (23%), mp 218 °C, NMR spectrum given above.

N-[4-(*tert*-Butoxycarbonyl)butyl]isoleucine (33%), mp 230–231 °C; δ_H(D_2O; 298 K; as potassium salt) 2.95 (1 H, d, *J* 5.9, α-H), 2.50 (2 H, m, 1-H_2), 2.30 (2 H, t, *J* 7.1, 4-H_2), 1.51 (5 H, s, 2- and 3-H_2 and β-H); 1.45 (9 H, s, Bu^t), 1.09–1.22 (2 H, m, γ-H_2) and 0.85–0.92 (6 H, m, γ-H_3 + δ-H_3).

N-[3-(Benzylthio)propyl]methionine (34%), mp 219–220 °C; δ_H(D_2O; 298 K; as sodium salt) 7.42–7.46 (5 H, m, PhH), 3.84 (2 H, s, PhCH_2), 3.15 (1 H, t, *J* 6.7, H and $^{\alpha}$CH), 2.48–2.63 (6 H, m, 1-, 3- and γ-H_2), 2.15 (3 H, s, γ-H_3) and 1.74–1.95 (4 H, m, 2- and β-H_2).

N-[2-(Boc-amino)ethyl]methionine (25%), mp 240–242 °C; δ_H(D_2O; 298 K; as sodium salt) 3.39 (1 H, t, *J* 6.4, α-H), 3.19 (2 H, m, 2-H_2), 2.71–2.51 (4 H, m, γ- and 1-H_2), 2.13 (3 H, s, ε-H_3), 2.00–1.82 (2 H, m, β-H_2) and 1.46 (9 H, s, Bu^t).

N-[4-(*tert*-Butoxycarbonyl)butyl]methionine (19%), mp 227–228 °C; δ_H(D_2O; 298 K; as potassium salt) 3.13 (1 H, t, *J* 6.0, α-H), 2.48–2.58 (4 H, m, 1- and γ-H_2), 2.30 (2 H, t, *J* 7.1, 4-H_2), 2.08 (3 H, s, SCH_3), 1.78–1.85 (2 H, m, β-H_2), 1.58 (4 H, s, γ-H_2), 0.85–0.92 (6 H, m, Bu^t).

N-[3-(Benzylthio)propyl]norvaline (7%), mp 224–225 °C; δ_H(D_2O; 298 K; as sodium salt) 7.43–7.22 (5 H, m, ArH), 3.81 (2 H, s, PhCH_2), 3.03 (1 H, dd, J_1 5.4, J_2 8.2, α-H), 2.56–2.48 (4 H, m, 1- and 3-H_2), 1.79–1.66 (2 H, m, 2-H_2), 1.64–1.43 (2 H, m, β-H_2), 1.355–1.24 (2 H, m, γ-H_2) and 0.91 (t, *J* 7.3, δ-H_3).

N-[3-(Benzylthio)propyl]phenylglycine (25%), mp 199–200 °C; δ_H[$(CD_3)_2SO$; 298 K] 7.40–7.22 (10 H, m, ArH), 4.21 (1 H, s, α-H), 3.67 (2 H, s, PhCH_2), 2.77–2.74 (1 H, m, 1-H_2) 2.63–2.60 (1 H, m, 1-H_2), 2.37 (2 H, t, *J* 7.1, 3-H_2) and 1.83–1.79 (2 H, m, 2-H_2).

N-[3-(Benzylthio)propyl]valine (25%), mp 218–219 °C; δ_H(D_2O; 298 K; as sodium salt) 7.43–7.22 (5 H, m, ArH), 3.81 (2 H, s, PhCH_2), 2.82 (1 H, t, *J* 6.0, α-H), 2.56–2.48 (4 H, m, 1- and 3-H_2), 1.83–1.80 (1 H, m, β-H), 1.77–1.74 (2 H, m, 2-H_2) and 0.96–0.86 (6 H, m, γ-H_3).

N-[2-(Boc-amino)ethyl]valine (26%), mp 256–258 °C; δ_H(D_2O; 298 K; as sodium salt) 3.19 (2 H, t, *J* 6.2, 2-H_2), 2.84 (1 H, d, *J* 6.1, α-H), 2.64–2.51 (2 H, m, 1-H_2), 1.83 (1 H, m, β-H), 1.43 (9 H, s, Bu^t) and 0.94–0.84 (6 H, m, γ-H_3).

Method M. Protection of the secondary α-amino unit of 10 with an Fmoc group by temporary trimethylsilyl (TMS) protection to give 11

Bis(trimethysilyl)acetamide (BTSA) (4.33 ml, 1.75 mol equiv.) and 1.74 ml (1 mol equiv.) of DIEA were added to 10 mmol of a substrate **10** suspended in 20 ml of DCM, with exclusion of water by a $CaCl_2$ drying tube. When the solution was nearly clear (5–10 min were usually required), 2.72 g (1.05 mol equiv.) of fluoren-9-ylmethoxycarbonyl chloride (Fmoc-Cl) were added and the mixture was stirred for 2 h. Methanol (2 ml) was carefully added, and the mixture was stirred for an additional 15 min, diluted with 80 ml of DCM, washed successively with 1 M HCl (3 × 50 ml) and saturated aq. NaCl (2 × 50 ml), and dried over $MgSO_4$, and the solvent was evaporated off *in vacuo*. The crude product was crystallized from diethyl ether–LP. If the product was not sufficiently pure it was further purified by chromatography.

Physical data for compounds **11** are summarized in Table 2. Their NMR data and interpretation follow here.

***N^{α}*-[2-(benzylthio)ethyl]-*N^{α}*-Fmoc-isoleucine 11b.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 6.14) 7.76 (2 H, d, *J* 7.5, Fmoc 4- and 5-H), 7.48 (2 H, d, *J* 7.5, Fmoc 1- and 8-H), 7.53 (2 H, dd, J_1 7.4, J_2 7.4, Fmoc 3- and 6-H), 7.33–7.20 (7 H, m, 2- and 7-H and *Ph*CH_2), 4.65–4.55 (2 H, m, Fmoc CH_2), 4.19 (1 H, t, *J* 5.1, Fmoc CH), 3.96 [0.14 H, d, *J* 10.0, α-H (*E*)], 3.79–3.73 [0.86 H, m, α-H (*Z*)], 3.52 (2 H, s, PhCH_2), 3.37 [0.27 H, m, 1-H_2 (*E*)], 3.25–3.18 [0.86 H, m, 1-H_2 (*Z*)], 2.96–2.88 [0.86 H, m, 1-H_2 (*Z*)], 2.62 [0.28 H, m, 3-H_2 (*E*)], 2.25 [1.73 H, t, *J* 8.0, 3-H_2 (*Z*)], 1.95 [0.86 H, m, β-H (*Z*)], 1.68 [0.14 H, m, β-H (*E*)], 1.23–1.18 (2 H, m, γ-H_2), 0.91–0.86 [2.59 H, m, γ-H_3 (*Z*)], 0.83 [0.41 H, m, γ-H_3 (*E*)], 0.79–0.76 [2.59 H, m, δ-H_3 (*Z*)] and 0.72 [0.41 H, m, δ-H_3 (*E*)].

***N^{α}*-[3-(benzylthio)propyl]-*N^{α}*-Fmoc-isoleucine 11c.** δ_H-[$(CD_3)_2SO$; 298 K; isomer ratio *E* : *Z* = 1 : 1.94] 7.88 [0.68 H, d, *J* 7.2, Fmoc 4- and 5-H (*E*)], 7.81 [1.32 H, d, *J* 7.3, Fmoc 4- and 5-H (*Z*)], 7.73 [0.68 H, m, Fmoc 1- and 8-H (*E*)], 7.62 [1.32 H, m, Fmoc 1- and 8-H (*Z*)], 7.38–7.23 (9 H, m, Fmoc 2-, 3-, 6- and 7-H and *Ph*CH_2), 4.60–4.56 (0.5 H, m, Fmoc CH), 4.44–4.40 (0.5 H, m, Fmoc CH), 4.35–4.31 [0.68 H, m, Fmoc CH_2 (*E*)], 4.24–4.21 [1.32 H, m, Fmoc CH_2 (*Z*)], 3.93 [0.34 H, d, *J* 10.4, α-H (*E*)], 3.85 [0.66 H, d, *J* 10.4, α-H (*Z*)], 3.70 [0.68 H, s, PhCH_2 (*E*)], 3.61 [1.32 H, s, PhCH_2 (*Z*)], 3.30 [0.34 H, m, 1-H_2 (*E*)], 3.16 [0.34 H, m, 1-H_2 (*E*)], 2.96 [0.66 H, m, 1-H_2 (*Z*)], 2.81 [0.66 H, m, 1-H_2 (*Z*)], 2.28 [0.68 H, m, 3-H_2 (*E*)], 1.96 [1.32 H, m, 3-H_2 (*Z*)], 1.62 (1 H, m, β-H), 1.42 [0.68 H, m, 2-H_2 (*E*)], 1.15 [3.32 , m, 2-H_2 (*Z*) and γ-H_2], 0.79 (3 H, m, γ-H_3) and 0.74 (3 H, m, δ-H_3).

***N^{α}*-[3-(Boc-amino)propyl]-*N^{α}*-Fmoc-isoleucine 11d.** δ_H($CDCl_3$; 298 K) 7.75 (2 H, d, *J* 7.4, Fmoc 4- and 5-H), 7.61–7.56 (2 H, m, Fmoc 1- and 8-H), 7.41–7.38 (2 H, m, Fmoc 3- and 6-H), 7.34–7.26 (2 H, m, Fmoc 2- and 7-H), 4.78 (0.75 H, dd, J_1 4.7, J_2 10.7, Fmoc CH_2), 4.69 (0.75 H, m, Fmoc CH_2), 4.56 (0.25 H, m, Fmoc CH_2), 4.40 (0.25 H, m, Fmoc CH_2), 4.21 (1 H, m, Fmoc CH), 4.10 (0.5 H, m, α-H), 3.80 (0.5 H, m, α-H), 3.27 (1 H, m, 1-H), 3.07 (2 H, m, 1- and 3-H), 2.76–2.71 (2 H, m, 3- and β-H), 2.17–1.71 (2 H, m, 2-H_2), 1.44 (9 H, s, Bu^t), 1.21 (2 H, m, γ-H_2), 0.89 (3 H, m, γ-H_3) and 0.80 (3 H, t, *J* 7.0, δ-H_3).

***N^{α}*-[4-(*tert*-Butoxycarbonyl)butyl]-*N^{α}*-Fmoc-isoleucine 11e.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.86) 7.66 (2 H, d, *J* 7.2, 4- and 5-H), 7.46 (2 H, d, *J* 7.2, Fmoc 1- and 8-H), 7.27–7.31 (2 H, m, Fmoc 3- and 6-H), 7.17–7.23 (2 H, m, Fmoc 2- and 7-H), 4.60 [0.65 H, s, Fmoc CH_2 (*Z*)], 4.54 [0.65 H, s, Fmoc CH_2 (*Z*)], 4.46 [0.35 H, s, Fmoc CH_2 (*E*)], 4.33 [0.35 H, s, Fmoc CH_2 (*E*)], 4.11 (1 H, s, Fmoc CH), 4.01 [0.31 H, d, *J* 10.5, α-H (*E*)], 3.87 [0.69 H, s, α-H (*Z*)], 3.14 [0.48 H, s, 1-H_2 (*E*)], 2.89 [0.73 H, s, 1-H_2 (*Z*)], 2.74 [0.79 H, s, 1-H_2 (*Z*)], 2.12 [0.7 H, s, 4-H_2 (*E*)], 1.93 [2.2 H, s, 4-H_2 (*E*) and β-H (*Z*)], 1.74 [0.25 H, s, β-H (*E*)], 1.47 [1.2 H, s, 2-H_2 (*E*) and 3-H_2 (*E*)], 1.36 (9 H, s, Bu^t), 1.17 [0.7 H, s, 3-H_2 (*Z*)], 1.13–1.15 [3.4 H, m, 2-H_2 (*Z*) and γ-H_2], 0.82–0.83 (3 H, m, γ-H_3) and 0.71–0.75 (3 H, m, δ-H_3).

***N^{α}*-[2-(Benzylthio)ethyl]-*N^{α}*-Fmoc-leucine 11f.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.86) 7.76 (2 H, m, Fmoc 4- and 5-H), 7.55 (2 H, m, Fmoc 1- and 8-H), 7.31 (2 H, m, Fmoc 3- and 6-H), 7.29 (2 H, m, Fmoc 2- and 7-H), 7.27–7.21 (5 H, m, *Ph*CH_2), 4.66–4.59 (2 H, m, Fmoc CH_2), 4.54–4.50 [0.65 H, m, α-H (*Z*)], 4.21 (1 H, t, *J* 5.7, Fmoc CH), 4.10 [0.35 H, m, α-H (*E*)], 3.73 [0.7 H, s, PhCH_2 (*E*)], 3.54 [1.3 H, s and 0.35 H, m, PhCH_2 (*Z*) and 1-H_2 (*E*)], 3.28–3.20 [0.65 H, m, 1-H_2 (*Z*)], 3.09

[0.35 H, m, 1-H_2 (*E*)], 2.95–2.87 [0.65 H, m, 1-H_2 (*Z*)], 2.69 [0.35 H, m, 2-H_2 (*E*)], 2.53 [0.35 H, m, 2-H_2 (*E*)], 2.36 [0.65 H, m, 2-H_2 (*Z*)], 2.29–2.33 [0.65, m, 2-H_2 (*Z*)], 1.73–1.66 [0.65 H, m, β-H_2 (*Z*)], 1.57–1.48 [0.65 H, m, β-H_2 (*Z*)], 1.45–1.36 (1 H, m, γ-H), 1.27–1.26 [0.7 H, m, β-H_2 (*E*)], 0.90–0.84 [3.9 H, m, δ-H_3 (*Z*)], 0.75 [1.05 H, d, *J* 6.1, δ-H_3 (*E*)] and 0.71 [1.05 H, d, *J* 6.2, δ-H_3 (*E*)].

***N*$^{\alpha}$-[3-(Benzylthio)propyl]-*N*$^{\alpha}$-Fmoc-leucine 11g.** δ_H($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:2.33) 9.79 (1 H, br s, CO_2H), 7.60 (2 H, d, *J* 7.4, Fmoc 4- and 5-H), 7.44 (2 H, d, *J* 3.6, Fmoc 1- and 8-H), 7.26–7.11 (9 H, m, *Ph*CH_2 and Fmoc 2-, 3-, 6- and 7-H), 4.53–4.49 (2 H, m, Fmoc, CH_2), 4.36 (1 H, dd, J_1 5.0, J_2 7.5, α-H), 4.10–4.06 (1 H, m, Fmoc CH), 3.56 [0.6 H, s, PhCH_2 (*E*)], 3.49 (1.4 H, s, PhCH_2 (*Z*)], 3.33 [0.6 H, m, 1-H_2 (*E*)], 2.98 [0.7 H, m, 1-H_2 (*Z*)], 2.73 [0.7 H, m, 1-H_2 (*Z*)], 2.26 [0.6 H, m, 3-H_2 (*E*)], 2.05–1.98 [1.4 H, m, 3-H_2 (*Z*)], 1.63 [0.6 H, m, 2-H_2 (*E*)], 1.55 (2 H, m, β-H_2), 1.41–1.35 [2.4 H, m, 2-H_2 (*Z*) and γ-H], 0.81 [2.1 H, d, *J* 6.1, δ-H_3 (*Z*)], 0.80 [2.1 H, d, *J* 6.4, δ-H_3 (*Z*)], 0.71 [0.9 H, d, *J* 5.6, δ-H_3 (*E*)] and 0.66 [0.9 H, d, *J* 5.6, δ-H_3 (*E*)].

***N*$^{\alpha}$-[3-(Boc-amino)propyl]-*N*$^{\alpha}$-Fmoc-leucine 11h.** δ_H($CDCl_3$; 298 K) 7.76 (2 H, m, Fmoc 4- and 5-H), 7.57 (2 H, d, *J* 7.4, Fmoc 1- and 8-H), 7.39 (2 H, m, Fmoc 3- and 6-H), 7.32 (2 H, m, Fmoc 2- and 7-H), 4.66 (2 H, m, Fmoc CH_2), 4.52 (0.5 H, m, Fmoc CH), 4.38 (0.5 H, m, Fmoc CH), 4.22 (1 H, m, α-H), 3.41 (1 H, m, 1-H_2), 3.03–2.89 (3 H, m, 1-H and 3-H_2), 2.77 (2 H, m, β-H_2), 1.62 (2 H, m, 2-H_2), 1.43 (9 H, s, Bu^t), 1.25 (1 H, m, γ-H) and 0.88–0.77 (6 H, m, δ-H_3).

***N*$^{\alpha}$-[6-(Benzylthio)hexyl]-ε-Boc-*N*$^{\alpha}$-Fmoc-lysine 11i.** δ_H($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:2.70) 7.67 (2 H, d, *J* 7.5, Fmoc 4- and 5-H), 7.49 (2 H, d, *J* 7.5, Fmoc 1- and 8-H), 7.33–7.29 (2 H, m, Fmoc 3- and 6-H), 7.24–7.19 (5 H, m, *Ph*CH_2), 7.18–7.16 (2 H, m, Fmoc 2- and 7-H), 4.59–4.53 (2 H, m, Fmoc CH_2), 4.15 (1 H, t, *J* 5.4, Fmoc CH), 4.11–4.05 [0.73 H, m, α-H (*Z*)], 3.98 [0.27 H, m, α-H (*E*)], 3.63 (2 H, s, PhCH_2), 3.25 [0.27 H, m, 1-H_2 (*E*)], 3.00 [0.73 H, m, 1-H_2 (*Z*)], 2.99 (2 H, t, *J* 6.8, ε-H_2), 2.89 [0.27 H, m, 1-H_2 (*E*)], 2.71 [0.73 H, m, 1-H_2 (*Z*)], 2.31 (2 H, t, *J* 7.3, 6-H_2), 1.90 [1.46 H, m, β-H_2 (*Z*)], 1.62 [0.54 H, m, β-H_2 (*E*)], 1.41 (2 H, m, 2-H_2), 1.39 [0.54 H, m, δ-H_2 (*E*)], 1.38 [1.46 H, m, δ-H_2 (*Z*)], 1.37 (9 H, s, Bu^t), 1.24 (2 H, m, 5-H_2), 1.22 [1.46 H, m, 4-H_2 (*Z*)], 1.20 [1.46 H, m, γ-H_2 (*Z*)], 1.19 [0.54 m, γ-H_2 (*E*)], 1.13 [0.54 H, m, 4-H_2 (*E*)] and 0.92 (2 H, m, 3-H_2).

***N*$^{\alpha}$-[4-(Benzylthio)butyl]-*N*$^{\alpha}$-Fmoc-methionine 11j.** δ_H($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:2.33) 9.54 (1 H, br s, CO_2H), 7.73 (2 H, d, *J* 7.4, Fmoc 4- and 5-H), 7.56 (2 H, d, *J* 7.2, Fmoc 1- and 8-H), 7.40–7.23 (9 H, m, ArH), 4.66–4.57 (2 H, m, Fmoc CH_2), 4.25 (1 H, dd, J_1 5.0, J_2 9.1, α-H), 4.21 [0.7 H, t, *J* 5.2, Fmoc CH (*Z*)], 4.17 [0.3 H, m, Fmoc CH (*E*)], 3.68 (2 H, s, PhCH_2), 3.47 [0.3 H, m, 1-H_2 (*E*)], 3.09–3.04 [1 H, m, 1-H_2 (*E*) and 1-H_2 (*Z*)], 2.84–2.80 [0.7 H, m, 1-H_2 (*Z*)], 2.53–2.48 [0.7 H, m, γ-H_2 (*Z*)], 2.41 [0.6 H, t, *J* 6.9, 4-H_2 (*E*)], 2.38–2.30 [2 H, m, γ-H_2 (*Z*) and β-H_2 (*E*)], 2.27 [1.4 H, t, *J* 6.8, 4-H_2 (*Z*)], 2.12–2.05 [1.4 H, m, β-H_2 (*Z*)], 2.07 [2.1 H, s, ε-H_3 (*Z*)], 2.02 [0.9 H, s, ε-H_3 (*E*)], 1.54 [1.4 H, m, 3-H_2 (*Z*)] and 1.34–1.27 [2.6 H, m, 3-H_2 (*E*) and 2-H_2].

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]-*N*$^{\alpha}$-Fmoc-phenylalanine 11l.** δ_H($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:4.00) 8.98 (1 H, br s, CO_2H), 7.64–7.61 (2 H, m, Fmoc 4- and 5-H), 7.40–7.38 (2 H, m, Fmoc 1- and 8-H), 7.27–7.03 (12.6 H, m, ArH), 6.92–6.90 (1 H, m, ArH), 6.58–6.58 (0.4 H, m, ArH), 4.72 [0.2 H, dd, J_{12} 4.4, J_{22} 10.6, Fmoc CH_2 (*E*)], 4.51 [0.8 H, dd, J_{12} 5.9, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.40 [0.2 H, dd, J_{12} 4.4, J_{22} 10.6, Fmoc CH_2 (*E*)], 4.30 [0.8 H, dd, J_{12} 6.1, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.09–4.04 (1 H, m, Fmoc CH), 3.90 [0.8 H, dd, J_1 4.6, J_2 10.5, α-H (*Z*)], 3.76 [0.21 H, m, α-H (*E*)], 3.470 [0.4 H, s, PhCH_2 (*E*)], 3.31 [1.6 H, s, PhCH_2 (*Z*)], 3.20–3.06 [1.6 H, m, β-H_2 (*Z*)], 2.91 [0.8 H, m, 1-H_2 (*Z*)], 2.78 [0.2 H, m, 1-H_2 (*E*)], 2.62 [0.4 H, m, β-H_2 (*E*)], 2.50 [0.8 H, m, 1-H_2 (*Z*)], 2.27 [0.2 H, m, 1-H_2 (*E*)], 2.16 [0.4 H, m, 2-H_2 (*E*)] and 2.00–1.90 [1.6 H, m, 2-H_2 (*Z*)].

***N*$^{\alpha}$-[3-(Benzylthio)propyl]-*N*$^{\alpha}$-Fmoc-phenylalanine 11m.** ($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:4.00) 9.00 (1 H, br s, CO_2H), 7.65–7.62 (2 H, m, Fmoc 4- and 5-H), 7.54–7.42 (2 H, m, Fmoc 1- and 8-H), 7.31–7.08 (12.6 H, m, ArH), 7.11–6.99 (1 H, m, ArH), 6.65–6.64 (0.4 H, m, ArH), 4.78 [0.2 H, dd, J_{12} 4.3, J_{22} 10.6, Fmoc CH_2 (*E*)], 4.56 [0.8 H, dd, J_{12} 5.7, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.47 [0.2 H, dd, J_{12} 4.3, J_{22} 10.6, Fmoc CH_2 (*E*)], 4.36 [0.8 H, dd, J_{12} 5.8, J_{22} 10.7, Fmoc CH_2 (*Z*)], 4.14–4.08 (1 H, m, Fmoc CH), 3.93 [0.8 H, d, J_1 5.4, J_2 9.9, α-H (*Z*)], 3.77 [0.2 H, m, α-H (*E*)], 3.47 [0.4 H, s, PhCH_2 (*E*)], 3.44 [1.6 H, s, PhCH_2 (*Z*)], 3.25–3.14 [1.6 H, m, β-H_2 (*Z*)], 3.03–3.00 [0.2 H, m, 1-H_2 (*E*)] 2.90–2.79 [0.8 H, m, 1-H_2 (*Z*)], 2.53–2.50 [0.2 H, m, 1-H_2 (*E*)], 2.46–2.39 [0.8 H, m, 1-H_2 (*Z*)], 2.31 [0.4 H, m, β-H_2 (*E*)], 2.17–2.12 [0.4 H, m, 3-H_2 (*E*)], 2.09–1.89 [1.6 H, m, 3-H_2 (*Z*)], 1.37–1.34 [0.4 H, m, 2-H_2 (*E*)] and 1.24–1.12 [1.6 H, m, 2-H_2 (*Z*)].

***N*$^{\alpha}$-[3-(Boc-amino)propyl]-*N*$^{\alpha}$-Fmoc-phenylalanine 11n.** The ^{1}H NMR spectrum was identical with that of the compound which was previously prepared by the nucleophilic substitution method.[26]

***O*-benzyl-*N*$^{\alpha}$-[3-(Boc-amino)propyl]-*N*$^{\alpha}$-Fmoc-serine 11o.** δ_H($CDCl_3$; 298 K; not enough resolution to determine the isomer ratio) 7.76–7.72 (2 H, m, Fmoc 4- and 5-H), 7.56–7.50 (2 H, m, Fmoc 1- and 8-H), 7.41–7.36 (2 H, m, Fmoc 3- and 6-H), 7.35–7.20 (7 H, m, Fmoc 2- and 7-H and *Ph*CH_2), 4.54–4.44 (~1 H, m, Fmoc CH_2), 4.51 (2 H, s, PhCH_2), 4.37–4.34 (~1.25 H, m, Fmoc CH_2 and Fmoc CH), 4.28 (~1 H, m, α-H), 4.25–4.22 (~0.25 H, m, Fmoc CH), 3.97–3.95 (~0.5 H, m, Fmoc CH), 3.77 (~0.5 H, m, 1-H_2), 3.64 (~1 H, m, β-H_2), 3.51 (~0.5 H, m, 1-H_2), 3.42 (~1 H, m, β-H_2), 3.37–3.27 (~2 H, m, 1-H and 2-H) and 3.06 (~1 H, m, 2-H_2).

***N*$^{\alpha}$-[4-(Benzylthio)butyl]-*N*$^{\alpha}$-Fmoc-*N*$^{\text{In}}$-formyltryptophan 11p.** δ_H($CDCl_3$; 298 K; isomer ratio *E*:*Z* = 1:1.86) 9.64 [0.35 H, s, CHO (*E*)], 9.24 [0.65 H, s, CHO (*Z*)], 8.24 [0.65 H, s, indole (*Z*)], 7.995 [0.35 H, s, indole (*E*)], 7.65 [2 H, m, Fmoc 4- and 5-H], 7.58 (1 H, m, indole), 7.44 (2 H, m, Fmoc 1- and 8-H), 7.44 (1 H, m, indole), 7.30 (2 H, m, *Ph*CH_2, *o*-H), 7.29 (2 H, m, Fmoc 3- and 6-H), 7.24 (2 H, m, Fmoc 2- and 7-H), 7.21 (1 H, m, *Ph*CH_2, *p*-H), 7.19–7.15 (2 H, m, *Ph*CH_2, *m*-H), 7.14 (1 H, m, indole), 6.92 [0.65 H, indole 8-H (*Z*)], 6.11 [0.35 H, m, indole 8-H (*E*)], 3.68 (2 H, s, PhCH_2), 3.50 (1 H, m, α-H), 3.19–3.09 (2 H, m, β-H_2), 2.77–2.69 (2 H, m, 1-H_2), 2.40–2.31 (2 H, m, 4-H_2) and 1.55–1.47 (4 H, m, 2- and 3-H_2).

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]-*O*-*tert*-butyl-*N*$^{\alpha}$-Fmoc-tyrosine 11q.** δ_H($CDCl_3$; 298 K; resolution too low to determine the isomer ratio) 7.76–7.71 (2 H, m, Fmoc 4- and 5-H), 7.56–7.50 (2 H, m, Fmoc 1-and 8-H), 7.41–7.63 (2 H, m, Fmoc 3-and 6-H), 7.34–7.20 (7 H, m, Fmoc 2- and 7-H and *Ph*CH_2), 4.54–4.44 (~1 H, m, Fmoc CH_2), 4.51 (2 H, s, PhCH_2), 4.37–4.33 (~1.25 H, m, Fmoc CH_2 and CH), 4.27 (1 H, m, ^{a}CH), 4.25–4.22 (~0.25 H, m, Fmoc CH), 3.97–3.95 (~0.5 H, m, Fmoc CH), 3.76 (0.5 H, m, ^{1}CH_2), 3.64 (1 H, m, ^{b}CH_2), 3.51 (0.5 H, m, ^{1}CH_2), 3.42 (1 H, m, ^{b}CH_2), 3.36–3.26 (2 H, ^{1}CH_2 and ^{2}CH_2), 3.06 (1 H, m, ^{2}CH_2) and 1.39 (9 H, s, Bu^t).

***N*$^{\alpha}$-[3-(Boc-amino)propyl]-*N*$^{\alpha}$-Fmoc-valine 11r.** δ_H($CDCl_3$; 298 K) 7.76 (2 H, d, *J* 7.4, Fmoc 4- and 5-H), 7.76–7.52 (2 H, m, Fmoc 1- and 8-H), 7.42–7.38 (2 H, m, Fmoc 3- and 6-H), 7.35–7.31 (2 H, m, Fmoc 2- and 7-H), 4.74 (1 H, m, Fmoc CH_2), 4.62 (0.5 H, m, Fmoc CH), 4.46 (0.5 H, m, Fmoc CH), 4.22 (1 H, m, Fmoc CH_2), 3.87 (0.35 H, m, α-H), 3.575 (0.65 H, m, α-H), 3.28 (0.7 H, m, 1-H_2), 3.06 (1.3 H, m, 1-H_2), 2.80–2.74 (2 H, m, 3-H_2), 2.32 (0.65 H, m, β-H), 2.055 (0.35 H, m, β-H), 1.73 (0.7 H, m, 2-H_2), 1.44 (9 H, s, Bu^t), 1.25 (1.3 H, m, ^{2}CH_2), 0.95 (1.95 H, d, *J* 6.4, γ-CH_3), 0.903 (1.05 H, m, γ-H_3), 0.724 (1.95 H, d, *J* 6.4, γ-H_3), 0.655 (1.05 H, m, γ-H_3).

Method N. Protection of the secondary α-amino group unit of 10 with the Boc group by temporary TMS protection to give 12

This procedure was identical with the latter (Method M) except for the addition of di-*tert*-butyl dicarbonate instead of Fmoc-

Cl and washing with saturated aq. $KHSO_4$ instead of HCl. Physical data for compounds **12** are summarized in Table 2. Their NMR data and interpretation follow here.

***N*$^{\alpha}$-[6-(Benzylthio)hexyl]-*N*$^{\alpha}$-Boc-glutamic acid γ-benzyl ester 12a.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.08) 7.29–7.19 (9 H, m, ArH), 7.19–7.16 (1 H, m, ArH), 5.06 (2 H, s, OCH_2Ph), 4.03 [0.52 H, m, α-H (*Z*)], 3.90 [0.48 H, m, α-H (*E*)], 3.63 (2 H, s, SCH_2Ph), 3.37 [0.52 H, m, 1-H_2 (*Z*)], 3.23 [0.52 H, m, 1-H_2 (*Z*)], 2.86 [0.96 H, m, 1-H_2 (*E*)], 2.40 (2 H, t, *J* 6.4, γ-H_2), 2.32 (2H, t, *J* 7.0, 6-H_2), 2.32 [0.96 H, m, β-H_2 (*E*)], 2.08 [1.04 H, m, β-H_2 (*Z*)], 1.56–1.43 (2 H, m, 5-H_2), 1.43 [1.04 H, m, 2-H_2 (*Z*)], 1.39 [0.96 H, m, 2-H_2 (*E*)], 1.38 (9 H, s, Bu^t), 1.30–1.22 (2 H, m, 4-H_2), 1.17–1.13 (2 H, m, 3-H_2).

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]-*N*$^{\alpha}$-Boc-leucine 12f.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.04) 7.97 (1 H, br s, CO_2H), 7.32–7.23 (5 H, m, *Ph*CH_2), 4.46 [0.51 H, m, α-H (*Z*)], 4.16 [0.49 H, m, α-H (*E*)], 3.75 [0.98 H, s, PhCH_2 (*E*)], 3.74 [1.02 H, s, PhCH_2 (*Z*)], 3.61 [0.49 H, m, 1-H_2 (*E*)], 3.40 [0.49 H, m, 1-H_2 (*E*)], 3.09 [1.02 H, m, 1-H_2 (*Z*)], 2.72 [1.02 H, m, 2-H_2 (*Z*)], 1.78–1.71 (1 H, m, β-H), 1.64–1.47 (2 H, m, β- and γ-H), 1.44 (9 H, s, Bu^t), 0.91 [1.47 H, d, *J* 4.7, δ-H_3 (*E*)] and 0.90 [1.53 H, d, *J* 4.7, δ-H_3 (*Z*)].

Method O. Preparation of *N*$^{\alpha}$-[ω-(benzylthio)alkyl]glycines 10s–10u

Glyoxylic acid (0.78 g, 10.5 mmol) was added to a stirred solution of 10 mmol of an ω-(benzylthio)alkylamine[26] and 63 mg (3.3 mmol, 1 mol equiv.) of sodium cyanoborohydride in 20 ml of methanol. The mixture was stirred overnight. The precipitated product was filtered off on a glass sinter, washed with methanol and dried *in vacuo*.

***N*$^{\alpha}$-[2-(Benzylthio)ethyl]glycine 10s.** δ_H(D_2O; 298 K) 7.413 (5 H, m, *Ph*CH_2), 3.835 (2 H, s, α-H_3), 3.521 (2 H, s, PhCH_2), 3.151 (2 H, t, *J* 6.7, 1-H_2) and 2.781 (2 H, t, *J* 6.8, 2-H_2).

***N*$^{\alpha}$-[3-(Benzylthio)propyl]glycine 10t.** δ_H(D_2O; 298 K) 7.392 (5 H, m, *Ph*CH_2), 3.800 (2 H, s, α-H_2), 3.540 (2 H, s, PhCH_2), 3.043 (2 H, t, *J* 7.7, 1-H_2), 2.564 (2 H, t, *J* 7.1, 3-H_2) and 1.986–1.840 (2 H, m, 2-H_2).

***N*$^{\alpha}$-[4-(Benzylthio)butyl]glycine 10u.** δ_H(D_2O; 298 K) 7.384 (5 H, m, *Ph*CH_2, 3.783 (2 H, s, α-H_2), 3.557 (2 H, s, PhCH_2), 2.980 (2 H, t, *J* 7.4, 1-H_2), 2.515 (2 H, t, *J* 6.8, 4-H_2) and 1.724–1.613 (4 H, m, 2- and 3-H_2).

Method P. Preparation of *N*$^{\alpha}$-[ω-(benzylthio)alkyl]-*N*$^{\alpha}$-Fmoc-glycines 11s–11u

Triethylamine (TEA) (5.6 ml, 40 mmol) and 6.42 g (20 mmol) of *N*-(fluoren-9-ylmethoxy)succinimide (Fmoc-OSu) in 120 ml of acetonitrile were added to a solution of 20 mmol of a substrate of **10s**–**10u** 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 successively with LP (3 × 100 ml) and with a mixture of 3:7 diethyl ether–LP (3 × 100 ml). The aqueous solution was acidified with 40 ml of 1 M HCl and extracted with ethyl acetate (4 × 100 ml). The combined organic solution was washed with 50 ml of saturated aq. NaCl, dried over $MgSO_4$ and evaporated *in vacuo*. The products were crystallized from diethyl ether–LP.

The spectrum of compound **11s**, prepared by the nucleophilic substitution method, has been published.[26]

***N*$^{\alpha}$-[3-(Benzylthio)propyl]-*N*$^{\alpha}$-Boc-glycine 11t.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.50) 7.31–7.23 (5 H, m, *Ph*CH_2), 3.92 [1.2 H, s, α-H_2 (*Z*)], 3.84 [0.8 H, s, α-H_2 (*E*)], 3.70 [2 H, s, PhCH_2], 3.32 (2 H, t, *J* 6.7, 1-CH_2), 2.44–2.41 (2 H, m, 3-H_3), 1.82–1.68 (2 H, m, 2-H_2), 1.45 [5.4 H, s, Bu^t (*Z*)] and 1.42 [3.6 H, s, Bu^t (*E*)].

Method Q. Preparation of *N*$^{\alpha}$-[ω-(benzylthio)alkyl]-*N*$^{\alpha}$-Boc-glycines 12s–12u

These products were prepared according to the procedure of Bodanszky and Bodanszky.[26] The spectra of compounds **12s** and **12t**, prepared by the nucleophlic substitution method, have been published.

***N*$^{\alpha}$-[4-(Benzylthio)butyl]-*N*$^{\alpha}$-Boc-glycine 12u.** δ_H($CDCl_3$; 298 K; isomer ratio *E* : *Z* = 1 : 1.50) 9.39 (1 H, br s, CO_2H), 7.35–7.20 (5 H, m, *Ph*CH_2), 3.95 [1.1 H, s, α-H_2 (*Z*)], 3.84 [0.9 H, s, α-H_2 (*E*)], 3.67 (2 H, s, PhCH_2), 3.23–3.20 (2 H, m, 1-H_2), 2.40 (2 H, t, *J* 6.5, 4-H_2), 1.53 (4 H, m, 2-and 3-H_2), 1.43 [4.1 H, s, Bu^t (*E*)] and 1.40 [4.9 H, s, Bu^t (*Z*)].

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