PhD Course:

"Metodologie per lo sviluppo di molecole d'interesse farmacologico"

(MDMP, *XXIII ciclo*)

Preparation of modified α-amino acids, useful building blocks for peptide synthesis

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Introduction

The discovery of the physiological role of a great number of peptides stimulated researchers all over the world towards design and synthesis of peptidomimetics (or peptide-like molecules). Peptidomimetics are compounds whose essential elements (pharmacophore) mimic a natural peptide or protein in 3D space and which retain the ability to interact with the biological target and produce the same biological effect.

Peptidomimetics are designed to circumvent some of the problems associated with the use of natural peptides as drugs like low absorption, rapid metabolism and low oral bioavailability. Advantages of peptidomimetics are various: conformationally restrained structures can minimize inding to non-target receptors and enhance the activity at the desired receptor; addition of hydrophobic residues and/or replacement of amide bonds results in better transport properties through cellular membranes; isosteres, retro-inverso peptides, cyclic peptides and non-peptidomimetics all reduce the rate of degradation by peptidases and other enzymes.

The demand for modified peptides with improved stability profiles and pharmacokinetic properties is driving extensive research effort in this field. Many structural modifications of

peptides guided by rational design and molecular modeling have been established to develop novel synthetic approaches.

In many successful approaches the chemical modifications involve the α-amino acid side chain. The selective modification of amino acid side chains in peptides and proteins is important for the irreversible inhibition of enzymes and the improvement of pharmacokinetic properties of peptide drugs.

Another appealing and broadly used technique lies in backbone amide replacement with amide bond look-alike surrogates, or isosteres (*Figure 1*)[1].

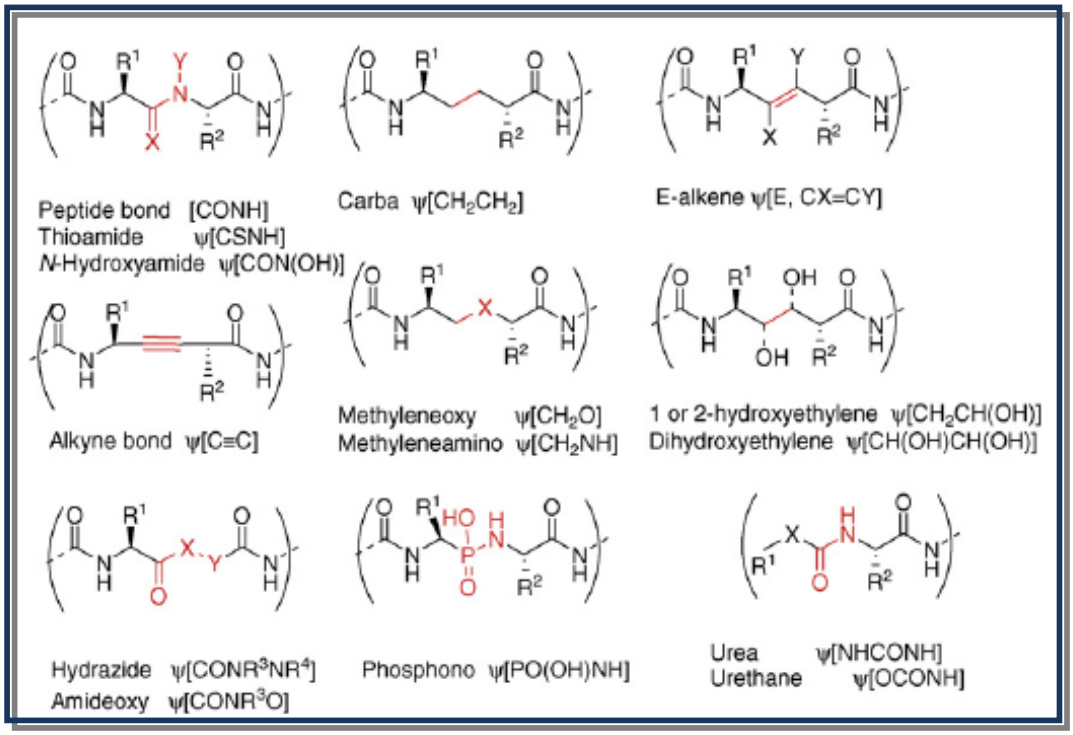


Figure 1. *Peptide Bond Isoster*

The –NH– group in a peptide bond can be replaced by alkylated –NR– or –O– groups.

N-Alkyl residues are important modifications which commonly occur in natural peptides. The N^{α}-alkylation induces significant structural effects: the occurrence of the *cis*- isomer in the secondary amide bond, steric effects, no H-bonding possibility, increased basicity, and decreased polarity of the adjacent carbonyl group. The incorporation of N^{α}-alkyl (mostly methyl) has resulted in many peptidomimetics with improved properties[2] and systematic N^{α}-alkylation is a powerful approach to improve biological activity.

In this contest, my research project was mainly based on the synthesis of modified amino acids and their introduction into small peptides. The aim was to have useful building blocks to be used in the peptides and peptidomimetics synthesis and simply involved during the chain elongation. In particular, I worked on new methodologies for the synthesis of *N*-alkylated amino acids, on the synthesis of side chain modified amino acids and on new methodologies for the protection and deprotection of amino and carboxy function of α-amino acids.

In a smaller part of my work my attenction was devoted to the reactivity study of two different substrates: hydrazides derivates

and the study of the acidity of their amino functions, using diazomethane. In this way we obtained *N*-Nosyl hydrazides of *N*-nosyl protected amino acids variously methylated on the nitrogen site; and hydroxyl steroids, in particular their reactivity in acidic media. The acid catalyzed elimination of water molecules is one of the most important processes that transforms polyhydroxy steroids into their corresponding unsaturated derivatives. This reaction is of great importance because it can produce molecules that play a key role in the understanding of the natural metabolism of steroids.

Chapter 1

Synthesis of modified amino acids

1. N-Alkyl-α-amino acids

N-Alkyl-α-amino acids are constituents of a large number of naturally occurring peptides and proteins.[2].

The substitution of *N*-alkyl-α-amino acids into biologically active peptides and in particular the *N*-methylation has resulted in analogues with enhanced pharmacological properties as a consequence of conformational modifications.[3,4]

Enhanced potencies have been observed when higher *N*-alkyl substitutions are employed.[5]

N-alkylation increases lipophilicity, which has the effect of increasing solubility in nonaqueous solvents and improving membrane permeability. On balance this makes peptides more bioavailable and makes them better therapeutic candidates.

Furthermore, the alkyl group removes the possibility of hydrogen bonding and in this way may improve the efficacy of a peptide by increasing its proteolitic resistance. In fact, generally, the first event in an enzyme proteolytic event is recognition of the target amide bond by hydrogen bonding.

Thus, *N*-methylation and *N*-alkylation are accepted tools in peptide and peptidomimetics drug design.

This leads the requirement for methods to prepare the desired monomers in forms suitable for solution and solid-phase peptide synthesis.

1.1 Synthesis of N-Methyl amino acids

The interesting biological activity of *N*-methylated peptides encouraged the development of various synthetic procedures for the preparation of *N*-methyl-α-amino acids[6] and their subsequent insertion into natural peptide chains to enhance and improve their activity.

N-Fmoc-*N*-methyl amino acids can be prepared by a well established procedure[7] based on the formation of 5-oxazolidinone intermediates which are successively reduced with triethylsilane and trifluoroacetic acid to the corresponding *N*-methylated derivatives.
Other methods already reported consist of the Lewis acid catalyzed reduction of *N*-Fmoc-protected amino acid oxazolidinones[8] and the base mediated alkylation of *N*-sulfonyl- and *N*-carbamoyl-protected amino acids.[9]
Recently it was reported a successful synthesis of *N*-methyl-α-amino acid methyl esters by a novel methodology based on the use of diazomethane as methylating reagent and α-amino acids protected on the amino function with the *p*-nitrobenzensulfonyl (nosyl) goup.[10]

The developed procedure proved very effective also in the site-specific methylation of *N*-nosyl-peptide protected on the carboxyl function as methyl esters.[11]

An interesting target to achieve is the obtainment of *N*-methyl-*N*-nosyl-α-amino acids not protected on the carboxyl function. In fact, *N*-methyl-*N*-nosyl-α-amino acids are useful building blocks, like such that after conversion into the corresponding *N*-Fmoc-*N*-

methyl-α-amino acids, in solution and solid phase synthesis of *N*-methylated peptides.[12]

In solution phase synthesis of *N*-methyl-α-amino acids and *N*-methyl-peptides the protection of carboxyl function as methyl ester is advantageous as it is stable during the entire synthetic process. However, the regeneration of the free carboxyl function presents some difficulties. In peptide synthesis, the most used method for methyl esters cleavage is the base-catalyzed hydrolysis.[13] This procedure, even when it is carried out under strictly controlled conditions, could cause racemization and other side reactions.[14] Therefore it is essential to use a carboxyl protecting group that keeps stable during the *N*-methylation reaction and easy to remove after the methylation of the sulfonamide nitrogen atom. Furthermore the unblocking conditions of the carboxyl function should be compatible with the presence of protecting groups of side chain functionalized α-amino acids.

To this aim we thought to develop practical and general methodologies for the preparation of *N*-methylated amino acids *N*-nosyl and *N*-Fmoc protected using diazomethane as methylating reagent and protecting the carboxy function with appropriate and easily removable protecting groups.

1.1.1 Preparation of N-methyl-a-amino acids from N-nosyl -α-amino acid benzhydryl esters

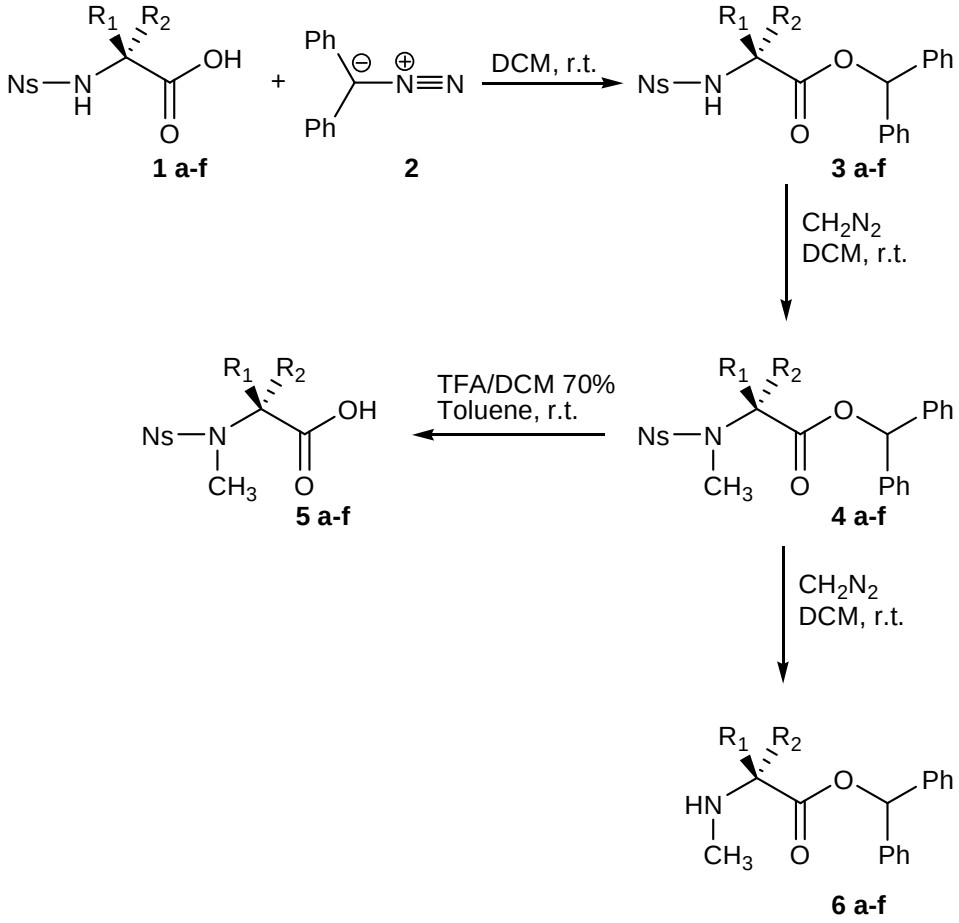
It seemed to be particularly advantageous to protect the carboxyl moiety as diphenylmethyl (benzhydryl) ester. [15] In fact, the benzhydryl derivatives are ready cleaved by hydrogenolysis[16]

or acidolysis[17] under conditions that usually do not affect the peptide skeleton. Moreover, these compounds can be prepared without difficulty by using diphenyldiazomethane upon neutral conditions[18]. Diphenyldiazomethane, a red crystalline solid, is obtained easily by the oxidation of benzophenone hydrazone with MagtrieveTM under mild and non-toxic reaction conditions[19]. Lipophilic α-amino acids were chosen as model systems in order to accurately study the synthetic strategy for the obtainment of the *N*-nosyl- and *N*-Fmoc-protected *N*-methylated amino acids. Subsequently the methodology was also extended to the *N*-nosyl-*S*-benzyl-L-cysteine. The *N*-nosyl-α-amino acid benzhydryl esters **3a–f** were prepared in quantitative yields by treating the corresponding *N*-nosyl-α-amino acids **1a–f**[10] with diphenyldiazomethane (**2**) (*Scheme 1*; *Table 1*).

The reaction of **3a–f** with diazomethane gave the corresponding *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** that were recovered in quantitative yields and high purity by evaporation of the solvent under reduced pressure. The reaction of diphenyldiazomethane with the α-amino acid carboxyl function is fast and during the reaction is possible to visually observe a rapid decoloration of the reaction mixture.

The *N*-methylated benzhydryl esters **4a–f** are the key precursors of both *N*-methyl-*N*-nosyl-amino acids and *N*-methyl-*N*-Fmoc-amino acids. The treatment of **4a–f** with 70% trifluoroacetic acid in dichloromethane afforded the corresponding *N*-methylated amino acids **5a–f** in high yields (94–98%) (*Scheme 1*; *Table 1*).

An alternative reaction path was developed to obtain from the *N*-methylated benzhydryl esters **4a–f**, the corresponding *N*-methyl-*N*-Fmoc-amino acids.



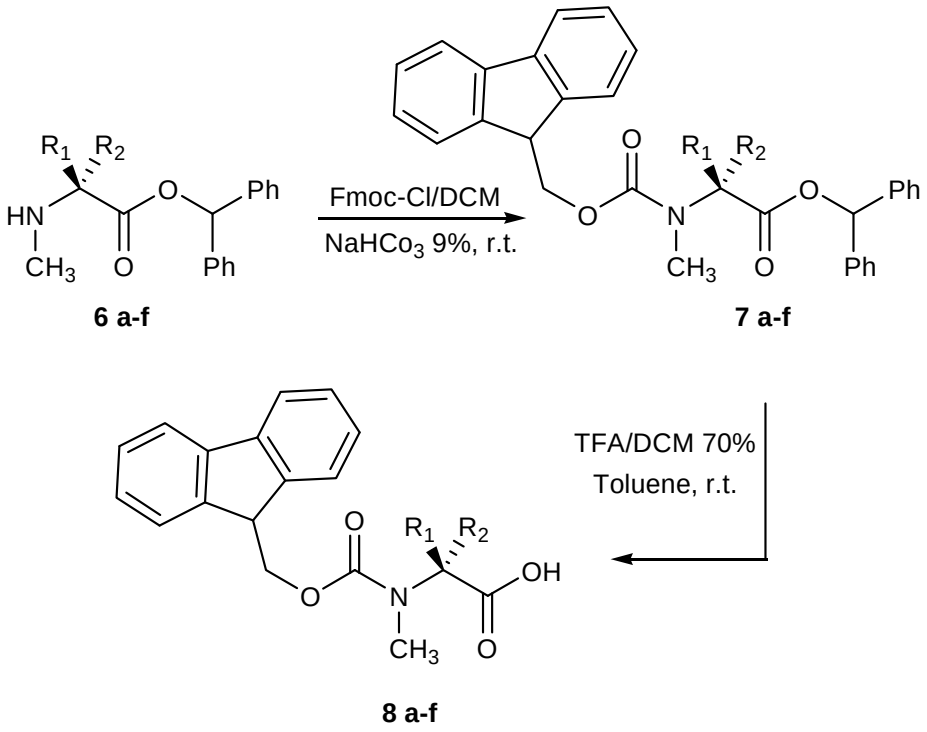
Scheme 1. ***Preparation of N-Methyl-N-nosyl-α-amino acid (5a–f) and N-methyl-α-amino acid benzhydryl esters (6a–f).***

Entry	R_1	R_2	5 (Yield%)[a]	6 (Yield%)[a]
a	$-CH(CH_3)_2$	-H	94	96
b	-H	$-CH(CH_3)_2$	94	96
c	$-CH_2CH(CH_3)_2$	-H	94	95
d	$-CH_2S(Bzl)$	-H	95	92
e	$-CH(CH_3)CH_2CH_3$	-H	98	95
f	$-CH_3$	-H	98	90

[a] *Isolated Yield*

Table 1. ***Results of the synthesis of N-Methyl-N-nosyl-α-amino acid (5a–f) and N-methyl-α-amino acid benzhydryl esters (6a–f).***

The main goal was to achieve the obtainment of *N*-Fmoc-*N*-methyl-α-amino acids to employ directly as building blocks for the construction of peptide chains based on Fmoc strategy. To this purpose, the synthetic intermediates **6a–f**, obtained from **4a–f** by deprotection of the amino function with the reagent system mercaptoacetic acid/sodium methoxide, were treated with Fmoc-chloride in aqueous 9% $NaHCO_3$ and dichloromethane (*Scheme 2*). After complete conversion of the starting reactants, the work-up of the reaction mixture allowed the recovery of the corresponding *N*-methyl-*N*-Fmoc-amino acid benzhydryl esters **7a–f** in high overall yields (88–95%) and with high purity grade (*Table 2*). The subsequent deprotection of the carboxyl function of **7a–f** by acidolysis with 70% trifluoroacetic acid in dichloromethane afforded the corresponding *N*-methyl-*N*-Fmoc-amino acids **8a–f** in excellent yields (94–98%) (*Scheme 2*; *Table 2*).



Scheme 2. ***Preparation of N-Methyl-N-Fmoc-α-amino acid (8a–f)***

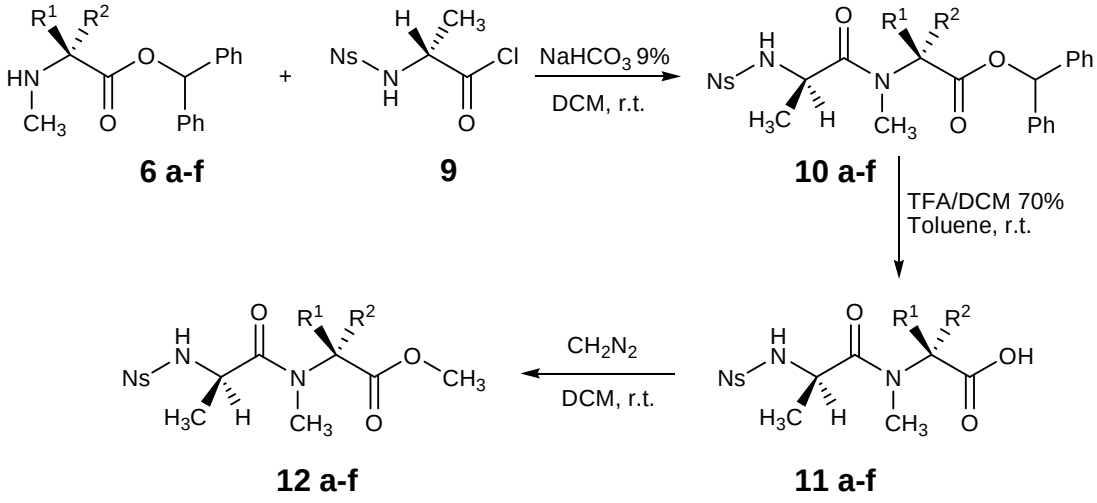
Entry	R^1	R^2	7 (Yield%)[a]	8 (Yield%)[a]
a	$-CH(CH_3)_2$	-H	92	96
b	-H	$-CH(CH_3)_2$	92	96
c	$-CH_2CH(CH_3)_2$	-H	90	98
d	$-CH_2S(Bzl)$	-H	95	94
e	$-CH(CH_3)CH_2CH_3$	-H	94	98
f	$-CH_3$	-H	88	96

[a] Isolated yield

Table 2. ***Results of the synthesis of N-Methyl-N-Fmoc-α-amino acid (8a–f)***

The obtained results demonstrate that the synthesis of *N*-methylated *N*-Fmoc- and *N*-nosyl-protected α-amino acids occurs in a simple way and in high yields.

The stereochemical integrity of the *N*-methylated products **6a–f** was investigated by converting **6a–b** into the corresponding diastereomeric dipeptides **10a–b**. The dipeptides **10a–b** were easily synthesized by coupling **6a–b** with *N*-nosyl-D-alanine chloride **9** under Schotten–Baumann reaction conditions[20]. The products **10a–b** were recovered in good overall yield (respectively, 89 and 91%) and isolated in high purity grade, without the need for chromatographic purification.



Scheme 3. ***Synthesis of the dipeptide 12 a-f***

^{1}H-NMR spectra of **10a** and **10b** were different for some signals and showed the presence of a single diastereomer in both samples. The presence of only one diastereoisomeric dipeptide in both crude products distinctly proved that the stereochemistry of the chiral centers is retained throughout the methylation process and the subsequent deprotection of the amino function. Furthermore, in order to exclude any detectable

racemization process the dipeptides **10a** and **10b** were also analyzed by GC/MS, after conversion into the corresponding more volatile methylated dipeptides **12a–b** (*Scheme 3*). GC/MS analysis performed on an appropriately prepared mixture containing 28 mg of the crude dipeptide **12a** and 70 mg of the crude dipeptide **12b** was compared with those obtained from the single products **12a** and **12b** (*Figure 2*). GC/MS analyses of the single products **12a** and **12b** showed the presence of only one diastereomer in both samples while the two diastereomeric dipeptides **12a** and **12b** appeared readily resolved in the GC/MS analysis of the mixture (*Figure 2*). Hence every step of the adopted procedure, the *N*-methylation of the *N*-nosyl-α-amino acid benzhydryl esters up to the deprotection of the amino function does not cause any loss of the chiral integrity of the asymmetric α-carbon atoms of the precursors.

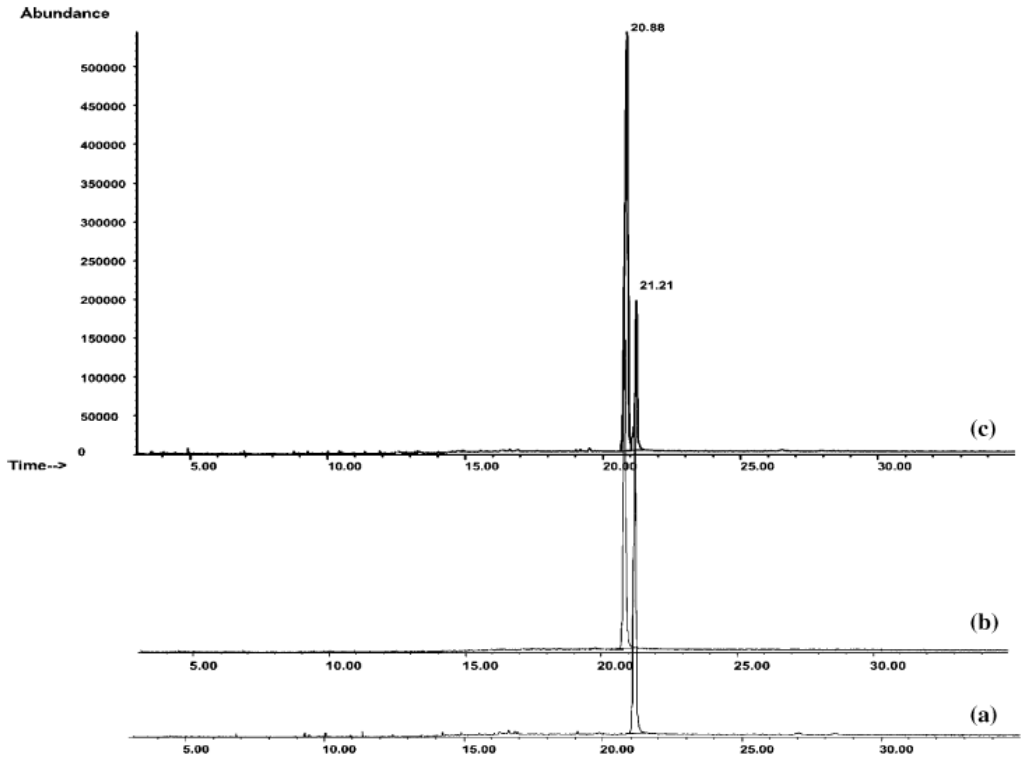


Figure 2. ***GC/MS analysis of 12a (a), 12b (b) and a mixture of the two products (c)***

The fully protected compound **4a–f** represent the real key intermediates of the developed procedure. They are obtained through the use of a couple of diazoalkanes that react in a rapid, clean and quantitative way at room temperature, and no by-products are observed. Furthermore, **4a–f** can be recovered by a mere evaporation of the reaction solvent without need for chromatography. An additional advantage is the possibility to use highly concentrated diazomethane solutions making the procedure suitable also for gram scale preparation. The *N*-methylated intermediates **4a–f** can also be converted at room temperature into the corresponding *N*-nosyl-α-amino acids in almost quantitative yields. The rapid kinetics of the changing of the amino protecting group and the deprotection of carboxyl

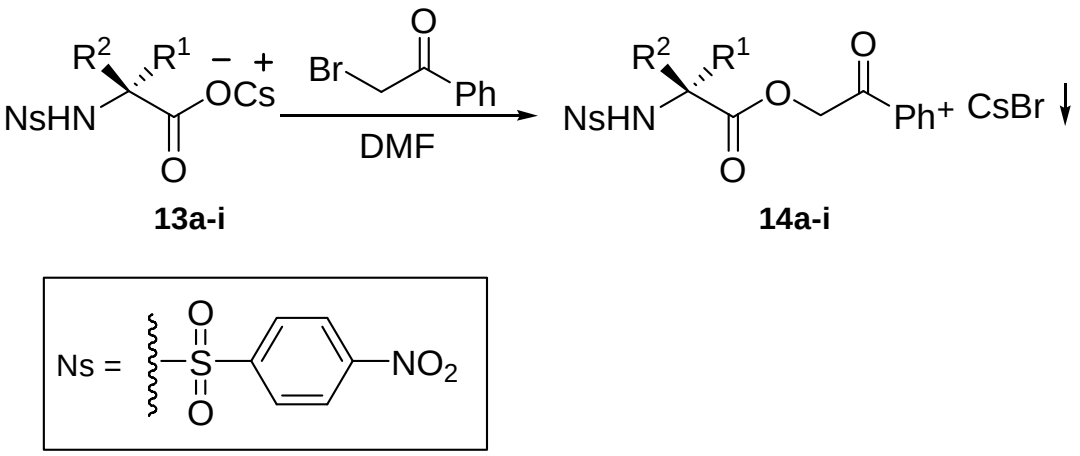
function of **4a–f** ensure the recovery of the final *N*-Fmoc-*N*-methylated amino acids in extraordinary high total yield.

1.1.2 Preparation of N-methyl-a-amino acids from N-nosyl -α-amino acid phenacyl esters

Another synthetic strategy developed during my PhD is based on the use, as starting materials, of *N*-nosyl-α-amino acids protected on the carboxyl function as phenacyl esters.

The choice of phenacyl group meets the need to obtain, after the formation of *N*-methyl-*N*-nosyl-α-amino acids, *N*-methyl-α-amino acids exploiting the same reagent to deprotect both the carboxyl and amino function. Moreover the phenacyl group is easily introduced on the carboxyl function and requires for its removal the treatment of the protected product with a sulphur nucleophile that could also be used to remove the nosyl group from the amino function.[6] The cleavage of the phenacyl ester is performed through the nucleophilic attack at the carbinol carbon atom under relatively mild conditions.

N-nosyl-α-amino acid phenacyl esters **14a-i** were prepared in high yields by treatment of cesium salts of *N*-nosyl-α-amino acids **13a-i**, obtained from the reaction of the corresponding *N*-nosyl-α-amino acids[2,7] with cesium carbonate, with phenacyl bromide[8] in *N*,*N*-dimethylformamide (DMF) (*Scheme 4, Table 3*).



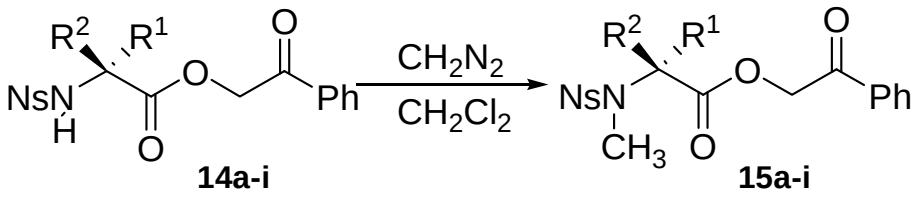
Scheme 4. ***Synthesis of N-nosyl-α-amino acid phenacyl esters 14a-i***

Entry	R^1	R^2	Yield (%)[a]
14a	H	$CH(CH_3)CH_2CH_3$	95
14b	H	$CH(CH_3)_2$	98
14c	H	$CH_2CH(CH_3)_2$	95
14d	H	CH_3	99
14e	H	$CH_2(C_6H_5)$	92
14f	H	CH_2S-(Bzl)	89
14g	H	$(CH_2)_4NH$-(Boc)	86
14h	H	CH_2CONH-(Trt)	98
14i	H	$CH_2C_6H_4O$-(*t*-Bu)	79

[a] Isolated yield

Table 3. ***Results of the synthesis of N-nosyl-α-amino acid phenacyl esters 14a-i***

Subsequently, *N*-methylation of **14a-i**, performed using a dichloromethane solution of diazomethane, provides the corresponding *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **15a-i** in quantitative yields (*Scheme 5*).



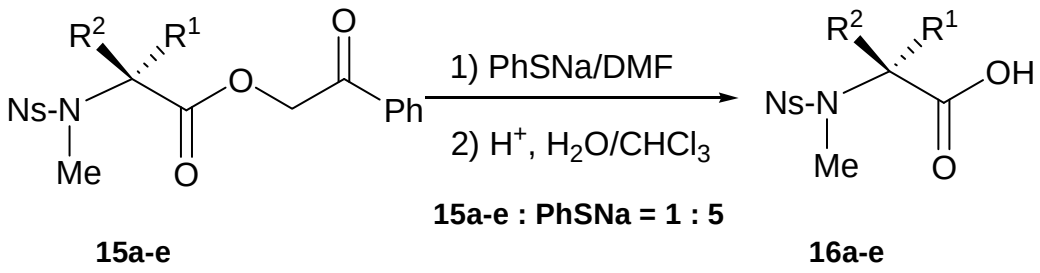
Scheme 5. ***Synthesis of N-Ns-N-Me-α-amino acid phenacyl esters 15 a-i***

Sodium thiophenoxide (PhSNa) was used as sulphur nucleophile to deprotect the carboxyl function of phenacyl esters **15a-i**.[9]

The deprotection reaction of the carboxyl function was investigated using as model systems lipophilic *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **15a-e**.

In a preliminary experiment the deprotection reaction was performed using *N*-methyl-*N*-nosyl-L-isoleucine phenacyl ester (**15a**) as starting material. A DMF solution of **15a** was treated with PhSNa in the molar ratio 1:5 respectively (*Scheme 6, Table 4*). After 30 minutes the TLC analysis (diethyl ether/petroleum ether 70:30 v/v) of the reaction mixture showed the disappearance of the starting material **15a**. The GC/MS analysis of the organic extracts obtained by the extraction of the basified reaction mixture revealed the formation of the deprotection adduct 1-phenyl-2-phenylsulfanyl-ethanone produced by the interaction of thiophenoxide with the carbinol carbon atom of the phenacyl ester function. The aqueous phase was then

acidified and extracted to give the *N*-methyl-*N*-nosyl-L-isoleucine (**16a**) in 70 % yield.



Scheme 6. ***Synthesis of N-Ns-N-Me-α-amino acids 16a-e***

Entry	R^1	R^2	Yield (%)[a]
16a	H	$CH(CH_3)CH_2CH_3$	70
16b	H	$CH(CH_3)_2$	70
16c	H	$CH_2CH(CH_3)_2$	87
16d	H	CH_3	76
16e	H	$CH_2(C_6H_5)$	70

[a] Isolated yield

Table 4. ***Results of the synthesis of N-methyl-N-nosyl-α-amino acids 16a-e***

The detailed examination of the spectroscopic data of the crude product *N*-methyl-*N*-nosyl-L-isoleucine (**16a**) allowed to investigate the stereochemical aspect of the reaction. The ^{1}H-NMR spectrum of **16a** shows resonances relative to a single reaction product and then to a single diastereoisomer.

Also the GC/MS analysis of **16a**, after treatment with diazomethane, showed the presence of a single chromatographic

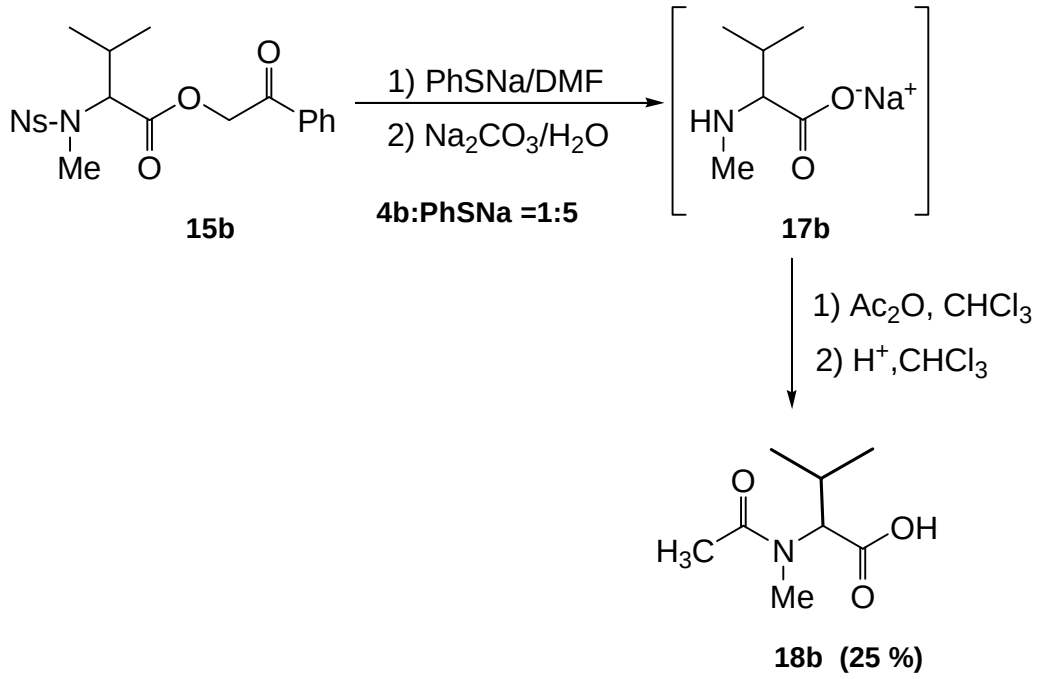
peak and then of a single diastereoisomer whose mass spectrum corresponds to that of *N*-methyl-*N*-nosyl-L-isoleucine methyl ester.

Therefore, in the deprotection reaction of the *N*-methyl-*N*-nosyl-L-isoleucine phenacyl ester **15a** with sodium thiophenoxide, epimerization, assessed on the base of sensitivity levels of the used analytical methodologies, is not observed.

N-Methyl-*N*-nosyl-α-amino acids **16b-e** were also obtained in good yields and high purity (*Scheme 6, Table 4*) as previously described for **16a**.

Therefore sodium thiophenoxide used in 5-fold molar excess allows to deprotect selectively and rapidly the carboxyl function of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **15a-e** providing the corresponding *N*-methyl-*N*-nosyl-α-amino acids **16a-e**.

Later the possibility of obtaining, from **15a-i**, the corresponding *N*-methyl-α-amino acids deprotected both on the amino and carboxyl function was also investigated using the same deprotecting reagent. Therefore, in an additional experiment the *N*-methyl-*N*-nosyl-L-Valine phenacyl ester (**15b**) was treated in DMF with PhSNa in the molar ratio 1:5 respectively (*Scheme 7*).



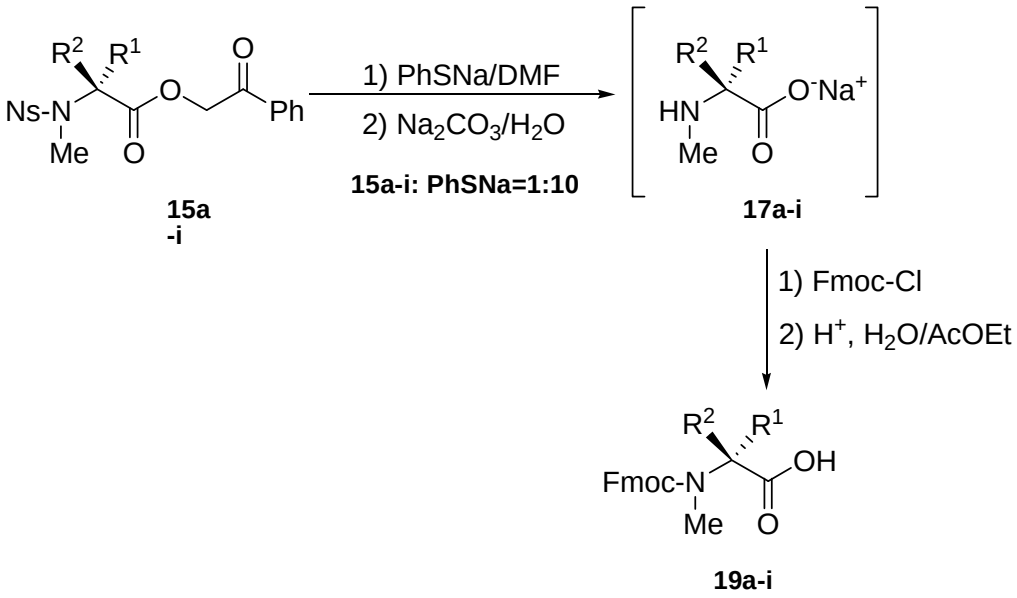
Scheme 7. ***Simultaneous deprotection of amino and carboxy function of 15b***

After about 1 hour the TLC analysis (diethyl ether / light petroleum 7:3 v/v) of the reaction mixture showed the formation of a second product that gave a typical coloration at the ninhydrin test proving the deprotection of the amino function.

After 12 hours the reaction mixture was acidified with a solution of HCl 1N and extracted with chloroform. The GC/MS analysis of the organic extract, after treatment with diazomethane, revealed the presence of the *N*-methyl-*N*-nosyl-L-Valine methyl ester. The aqueous solution was then basified and treated with an excess of acetic anhydride to convert the completely deprotected *N*-methyl-L-valine (**17b**) into the corresponding *N*-acetyl derivative **18b** (*Scheme 7*) that was characterized by GC/MS as methyl ester derivative.

It was evident that the adopted conditions for the obtainment of *N*-methyl-L-valine starting from the *N*-methyl-*N*-nosyl-L-valine phenacyl ester (**15b**) led to a partial deprotection of the amino function. In fact two products corresponding to the *N*-methyl-*N*-Nosyl-L-valine (**16b**) (40%) and the *N*-acetyl-*N*-methyl-L-valine (**18b**) (25%) were recovered.

On the base of these results the deprotection reaction was performed using a large excess of sodium thiophenoxide. *N*-methyl-*N*-nosyl-L-valine phenacyl ester (**15b**) (1 mmole) was then treated in DMF with sodium thiophenoxide in the molar ratio 1:10 respectively (*Scheme 8, Table 5*).



Scheme 8. ***Synthesis of N-Fmoc-N-methyl-α-amino acids 19a-i***

Entry	R^1	R^2	Yield (%)[a]
19a	H	$CH(CH_3)CH_2CH_3$	70
19b	H	$CH(CH_3)_2$	72
19c	H	$CH_2CH(CH_3)_2$	75
19d	H	CH_3	70
19e	H	$CH_2(C_6H_5)$	70
19f	H	CH_2S-(Bzl)	78
19g	H	$(CH_2)_4NH$-(Boc)	70
19h	H	CH_2CONH-(Trt)	70
19i	H	$CH_2C_6H_4O$-(*t*-Bu)	78

[a] Isolated yield

Table 5. ***Results of the synthesis of N-Fmoc-N-methyl-α-amino acids 19a-i***

The deprotection of amino function went to completion in 3 hours. In fact the GC/MS analysis of the organic extract of the acidified reaction mixture, after treatment with diazomethane, did not reveal the presence of the *N*-methyl-*N*-nosyl-L-valine methyl ester confirming that the amino function is completely deprotected. The aqueous phase, containing the completely deprotected *N*-methyl-L-valine (**17b**), was then basified and treated with 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) in order to obtain directly the *N*-Fmoc-*N*-methyl-L-valine (**19b**) that was recovered in 72 % yield (*Scheme 8, Table 5*).

N-Methyl-*N*-nosyl-α-amino acid phenacyl esters (**15a, 15c-i**) treated under the same conditions of **15b** produced the corresponding *N*-Fmoc-*N*-methyl-α-amino acids (**19a, 19c-i**) in 70–78% yield of isolated product (*Scheme 8, Table 5*).

The obtained results demonstrate that using a large excess of sodium thiophenoxide both the amino and carboxyl function of the *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters are effectively deprotected.

Furthermore the treatment of the deprotected products **17a-i** with Fmoc-Cl afforded the corresponding *N*-Fmoc-*N*-methyl-α-amino acids (**19a-i**) with good yields and high purities.

The adopted procedure for obtaining *N*-Fmoc-*N*-methyl-α-amino acids was applied with success also to *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **15f-i** with functional groups in their side chain and protected by acid labile protecting groups.

The recovery of *N*-Fmoc-*N*-methyl-α-amino acids **19f-i** required a special attention for the presence of the acid sensitive side chain protecting group. In fact the work-up was performed with a 5% aqueous solution of $KHSO_4$ to prevent the undesired deprotection of side chain functionalities. *N*-Fmoc-*N*-methyl-α-amino acids **19f-i** were recovered in good yields keeping unchanged the protecting group on the side chains.

Finally the analysis of the *N*-Fmoc-*N*-methyl-L-Ile-OH (**19a**) by ^{1}H-NMR and ^{13}C-NMR showed the absence of epimerization products in fact were observed only signals corresponding to one diastereoisomer.

The methods studied allows for an easy insertion of the Fmoc protecting group at the end of the entire procedure. The inconvenience related to the possible removal of the urethane masking group during the methylation step involved in the base

mediated *N*-alkylation methods can be now avoided using the nosyl protecting group which is very stable under acidic and basic environments. Nosyl group represents a valuable improvement for obtaining *N*-Fmoc-*N*-methylated amino acids with respect to the previously appeared works where only N-protecting group not sensible to basic conditions were used. Moreover, since diazomethane in our methodologies works under mild and neutral conditions, all the *N*-methylation reactions proceed without racemization. An additional advantage of the methods consists in the possibility to work with products that, when protected on the amino function with the nosyl group, are easily analyzed by GC/MS.

1.2 Synthesis of N-Ethyl amino acids

N-Ethyl amino acids can be widely applied as building blocks for the synthesis of *N*-ethylated peptides. A substitution of *N*-methyl-leucine of cyclosporin A by various *N*-ethyl amino acids was performed with the aim of blocking the main metabolic degradation pathways. The corresponding *N*-ethyl derivative resulted in analogues of cyclosporine A exhibiting nonimmunosuppressive and anti-HIV activity[21]. Various protocols have been developed for the synthesis of *N*-methyl amino acids. At the present time, however, only a few methods for the synthesis of *N*-ethylated amino acids and their derivatives are available in the literature[22]. Furthermore, the general *N*-alkylation of amino acids with nonmethyl alkylating agent seems tricky mostly due to the steric hindrance of longer carbon chains[23]. Papaioannou and co-workers have described a

Mitsunobu-type *N*-ethylation of tosylamino esters with excess ethanol[24]. However, the detosylation procedure is a drawback of this method. The *N*-ethylation of amino acids has been generally achieved by transforming the N–H function of amino acids into iminic or aminoacetalic systems. In recent years, the reductive amination, by using sodium cyanoborohydride, of imines derived from amino acids and acetaldehyde has constituted an efficient approach for the *N*-ethylation of amino acids[25], but over alkylation readily occurs and is of major concern[26]. In addition, the *N*-ethylation can be achieved smoothly by starting from α-amino acids with hexafluoroacetone as the protecting and activating agent; the subsequent reaction with a cuprate prepared from copper(I) cyanide and one equivalent of methyllithium gave the corresponding *N*-ethyl compounds[27]. Nevertheless, traces of the *N*-methyl analogues were also detected. In the attempt to obtain protected *N*-ethyl amino acids, an important development has been the reduction of the *N*-Fmoc-oxazolidinones (Fmoc = fluorenylmethyloxycarbonyl) of various amino acids to their corresponding *N*-ethyl *N*-Fmoc derivatives[28]. An alternative route for the synthesis of *N*-ethyl amino acids was provided by the reduction of *O*-alkyl acetohydroxamate obtained from amino acids[29]. Another approach describes the *N*-ethylation of carbobenzyloxy (Z)- and *tert*-butoxycarbonyl (Boc)-protected amino acids by starting from the generation of a dianion derivative at the oxygen and nitrogen atoms that is subsequently alkylated with ethyl iodide[30]. However, Hansen and co-workers[31] suggest that this procedure affords only a trace of ethylated product because a β-elimination reaction of the alkylating agent preferentially occurs. Moreover, the necessity of maintaining the reaction temperature at –78 °C

may make the reaction inconvenient. Thus, they posit that for a successful reaction the dianion had to be initially generated by using *tert*-butyllithium as a strong non-nucleophilic base and then treated with the powerful alkylating agent triethyloxonium tetrafluoroborate.

1.2.1 Preparation of N-Ethyl-α-amino acids from N-nosyl -α-amino acid methyl esters

Our attention was devoted to the development of a synthetic procedure that had to respond to the following requirements: (1) A one-pot process without the need of a low reaction temperature would be preferred. (2) Any possible side reactions responsible for a decrease in yield should be avoided. (3) The use of a weak or dilute base is preferred to avoid racemization. (4) A proper protecting group for the aminic function had to be selected to suppress the formation of diethylated product and to enhance the acidity of the N–H proton. (5) A synthetic method compatible with widely used Fmoc-based chemistry[32] would complement this approach, especially for amino acids containing functionalities incompatible with Boc chemistry. In this regard, the 4-nitrophenylsulfonyl (nosyl) protecting group, firstly described by Fukuyama[33], seemed to be the most promising group for our purpose. Thanks to its strong electron-withdrawing character, the nosyl group acts as both an activating and protecting group and enhance the reactivity of the N–H function towards various alkylating agents[34]. In addition, the compatibility of the nosyl group with the more practical Fmoc protecting group, commonly used in peptide and amino acid synthesis has been well documented[35].

On the basis of the above considerations, we initially subjected the *N*-nosyl-alanine methyl ester, (**20a**) chosen as a model system, to treatment with 2.5 equivalents of triethyloxonium tetrafluoroborate and 3.5 equivalents of N,N-diisopropylethylamine (DIPEA) in dichloromethane at room temperature (*Scheme 9, Table 6*). To our delight, the reaction was complete in only ten minutes and TLC analysis clearly showed the total conversion of **20a** into a single product; subsequently, a simple workup afforded the corresponding *N*-ethylated product **21a** in excellent yield. No additional chromatographic purification procedure was required.

20 a-l **21 a-l**

Scheme 9. ***Synthesis of N-Nosyl-N-ethyl-α-amino acid methyl esters 21a-l***

Entry	R^1	R^2	(Yield%)
21a	$-CH_3$	-H	96
21b	$-CH(CH_3)_2$	-H	89
21c	$-CHCH_2(CH_3)_2$	-H	97
21d	$-CH(CH_3)CH_2CH_3$	-H	95
21e	-H	$-CH(CH_3)CH_2CH_3$	96
21f	$-CH_2Ph$	-H	99
21g	$-CH_2CH_2COOtBu$	-H	85
21h	$-CH_2S(Bzl)$	-H	87
21i	$-CH_2C_6H_4O(Bzl)$	-H	91
21l	$-(CH_2)_4NH(Boc)$	-H	94

Table 6. ***Results of the synthesis of N-Nosyl-N-ethyl-α-amino acid methyl esters 21a-l***

Encouraged by this promising result, we tested the *N*-ethylation reaction with other amino acids. Compounds **20b–f** were ubjected to the above described reaction conditions to afford the *N*-ethylated derivatives in almost quantitative yields (*Scheme 9, Table 6*). It is worth noting that the triethyloxonium tetrafluoroborate could react with the functional groups of side-chain functionalized amino acids. For this purpose, we next investigated the scope of the reaction with respect to amino acids containing functionalized side chains with acid-labile protecting groups (e.g. Boc, *t*Bu, benzyl) to make the adopted procedure more general. The *N*-nosyl-glutamic acid methyl ester protected on the side-chain carboxylic function with a *tert*-butyl group (**20g**), was chosen as a model system. It was subjected to the ethylation reaction as described above and gave the *N*-ethylated derivative **21g** in 85% yield. The method works well also for preparing *N*-ethyl derivatives of other amino acids with functionalized side chains as indicated in *Table 6* by entries **21h–l**. It was observed that the use of a stoichiometric quantity of base could halve the reaction yield. To demonstrate this assumption, one equivalent of *N*-nosyl-alanine methyl ester **20a** was treated with 2.5 equivalents of triethyloxonium tetrafluoroborate in the presence of an equivalent amount of base. The starting compound **20a** was still detected in the reaction mixture even after 3 h. Thus, the mixture was washed with water at first and then with a NaOH aqueous solution. Final evaporation of the solvent gave the *N*-ethyl-*N*-nosyl-alanine

methyl ester **21a** in 50% yield. The reaction conducted on the starting compounds **20b–f** proceeded analogously affording the *N*-ethylated derivatives **21b–f** in 50% yields. The necessity of conducting the ethylation reaction by adding an excess of base is justified by the presence of BF_3 and F– arising from the decomposition of the tetrafluoroborated anion[36]. The fluoride ion represents the counteranion of the diisopropylammonium species, whereas BF_3 coordinates the aminic function of the nosyl-protected amino acid restraining its ability to interact with the electrophile.

Our next effort was devoted to the demonstration that the chiral integrity of the starting α-amino acids had been maintained throughout the synthetic process. To address this issue, the alkylating reaction described above was repeated with both *N*-nosyl-isoleucine methyl ester (**20d**) and *N*-nosyl-α-alloisoleucine methyl ester (**20e**). The resulting *N*-ethylated derivatives **21d** and **21e** were obtained in 95 and 96% yields, respectively. The two crude reaction products were analyzed by GCMS analysis.

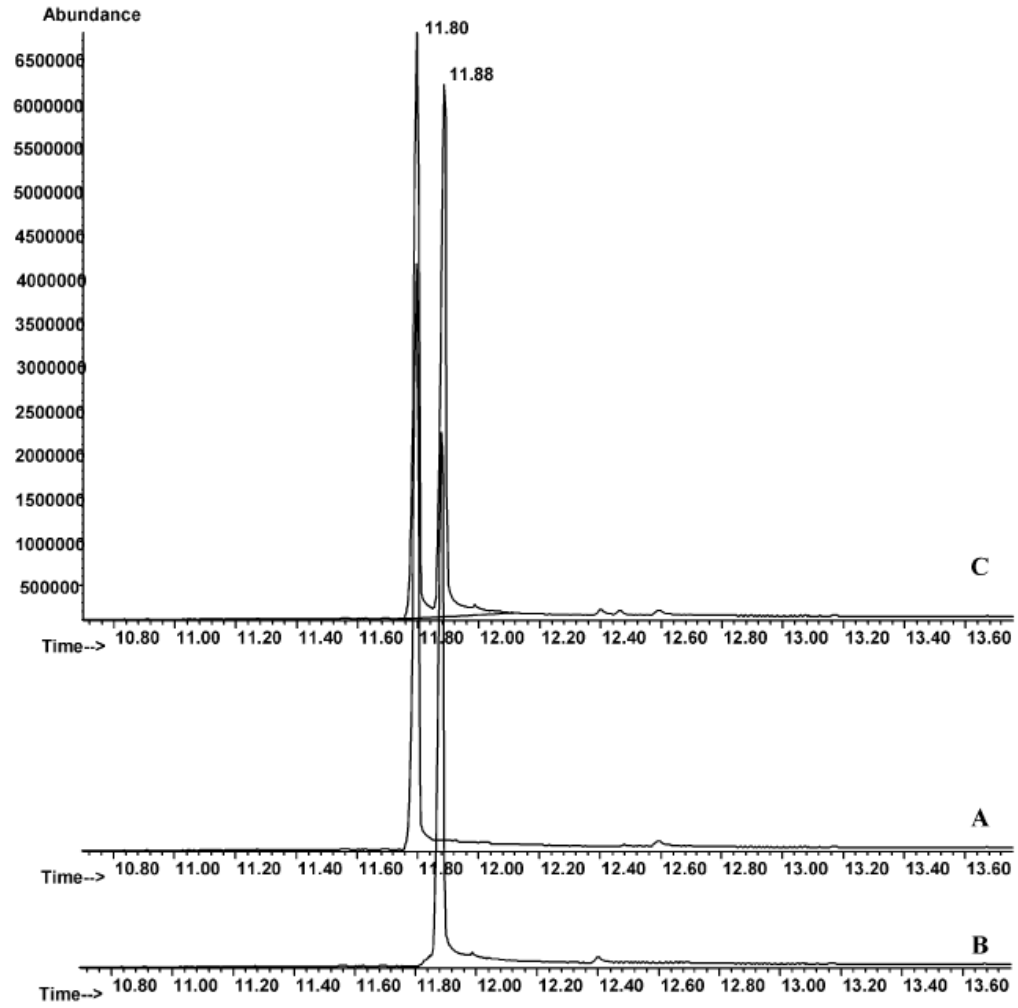
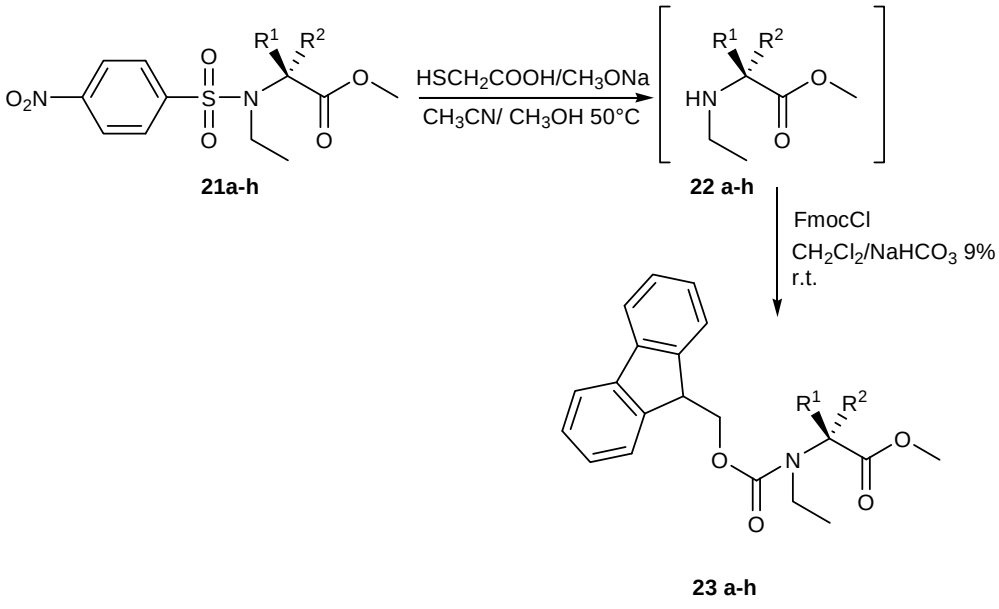


Figure 3. ***GC/MS analysis of 21d (A), 21e (B) and a mixture of the two products (C).***

The GC/MS analysis (*Figure 3*) revealed the detection of only one diastereoisomers in each of the gas chromatograms, thus confirming that the alkylation reaction occurred with retention of configuration of the original stereocenters. The complete separation of **21d** and **21e** was confirmed by performing the GC/MS analysis (*Figure 3*) of an opportunely prepared mixture of **21d** (25 mg) and **21e** (25 mg).

The final test of this methodology required the demonstration of the compatibility of our developed procedure with standard Fmoc chemistry. Thus, we planned the removal of the nosyl protective group and the subsequent introduction of the Fmoc group to obtain the desired *N*-ethyl- *N*-Fmoc-amino acid methyl esters. The removal of the nosyl group from the intermediate *N*-ethylated sulfonamide **21a–h** was accomplished by an aromatic nucleophilic substitution (SNAr) by treatment with the reagent system mercaptoacetic acid/sodium methoxide in acetonitrile /methanol (*Scheme 10*) by following a procedure already described in our previous paper for *N*-methylated amino acids.[37] Lastly, the amino function was reprotected with the Fmoc group by treatment with Fmocn chloride. The reaction was complete in 90 min and the Fmoc-*N*-ethyl- amino acid methyl esters **23a–h** were afforded in 71– 94% overall yields (*Table 7*). At this point methyl ester cleavage was required to make these compounds available as building blocks for Fmoc based chemistry.



Scheme 10. ***Synthesis of N-Fmoc-N-ethyl-α–amino acid methyl esters 23 a-h***

Entry	R^1	R^2	(Yield%)
23a	$-CH_3$	-H	90
23b	$-CH(CH_3)_2$	-H	91
23c	$-CHCH_2(CH_3)_2$	-H	80
23d	$-CH(CH_3)CH_2CH_3$	-H	94
23f	$-CH_2Ph$	-H	90
23g	$-CH_2CH_2COOtBu$	-H	76
23h	$-CH_2S(Bzl)$	-H	71

Table 7. ***Results of the synthesis of N-Fmoc-N-ethyl-α–amino acid methyl esters 23 a-h***

To avoid racemization during the saponification step,[38] methyl ester cleavage was easily realized by an SN_2 dealkylation according to the efficient procedure described by Biron and Kessler.[39] Treatment of *N*-ethyl-*N*-Fmoc-α-isoleucine methyl ester (**23d**), chosen as a model system, with lithium iodide in refluxing ethyl acetate, afforded after 20 h the corresponding *N*-ethyl-*N*-Fmoc-α-isoleucine (**24**) in 94% yield, which could be directly used without further purification. The cleavage reaction did not involve racemization as shown by ^{1}H NMR spectroscopic analysis of **24**. The *N*-ethyl-*N*-Fmoc amino acid **24** was now suitable for incorporation into a peptide chain. To demonstrate the application of this method, we prepared a dipeptide under the standard peptide coupling conditions by using *N*,*N*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt).[40] The *N*-ethyl-*N*-Fmoc-isoleucine **24** was coupled with the amino-free a-valine methyl ester hydrochloride by using DCC, HOBt, and *N*-methylmorpholine in THF for 1 h. The resulting dipeptide *N*-ethyl-*N*-Fmoc-α-ile-a-val-OMe (**25**) was isolated in 90% yield and only one diastereoisomers was observed by ^{1}H NMR spectroscopic analysis. Our methodology can readily be

accommodated to the standard Fmoc and nosyl-based peptide synthetic strategy. In an additional experiment, in fact, we successfully prepared a nosyl-protected dipeptide containing an *N*-ethyl amino acid. Thus, the *N*-ethyl-*N*-nosyl-α-isoleucine methyl ester (**21d**) was subjected to the above described SN_2 dealkylation reaction with LiI. Also in this case, the corresponding *N*-ethyl-*N*-nosyl-α-isoleucine (**26**) was afforded in excellent yield (96%) and without the need for chromatographic purification. The *N*-ethylated nosyl-protected amino acid **26** was finally activated and coupled with valine methyl ester hydrochloride by means of DCC/HOBt as the coupling reagents. The dipeptide *N*-ethyl-*N*-nosyl-α-ile-α-valOMe (**27**) was recovered after 1 h in high yield (92%) and without purification. Also in this case, no racemization was observed as shown by the ^{1}H NMR spectrum of the crude product of **27**.

2. Synthesis of (2S,3R)-3-methylglutamic acid

2.1 Structure of Daptomycine

Daptomycin is a cyclic non-ribosomal acidic lipopeptide derived from the fermentation of *Streptomyces roseosporus*. It is the first clinically approved antibiotic of this class, marketed under the trade name of Cubicin. It exerts its rapid bactericidal affect by perturbing the bacterial cell membrane and requires physiological levels of calcium ions for its activity.[41]

Non-ribosomal peptides often contain many unusual amino acid residues, as well as fatty acid and polyketide moieties and are often glycosylated.

Daptomycin is a depsipeptide containing 13 amino acids (Fig.1); as with the other calcium-dependent antibiotics (CDAs), it possesses an *N*-terminal fatty acid side chain and contains acidic residues, Asp, Glu and 3-methylglutamate, which are important for coordinating calcium ions.

Daptomycin is comprised of a decapeptide macrolactone ring derived from cyclization of the L-threonine side chain hydroxyl onto the *C*-terminal carboxyl group[42].

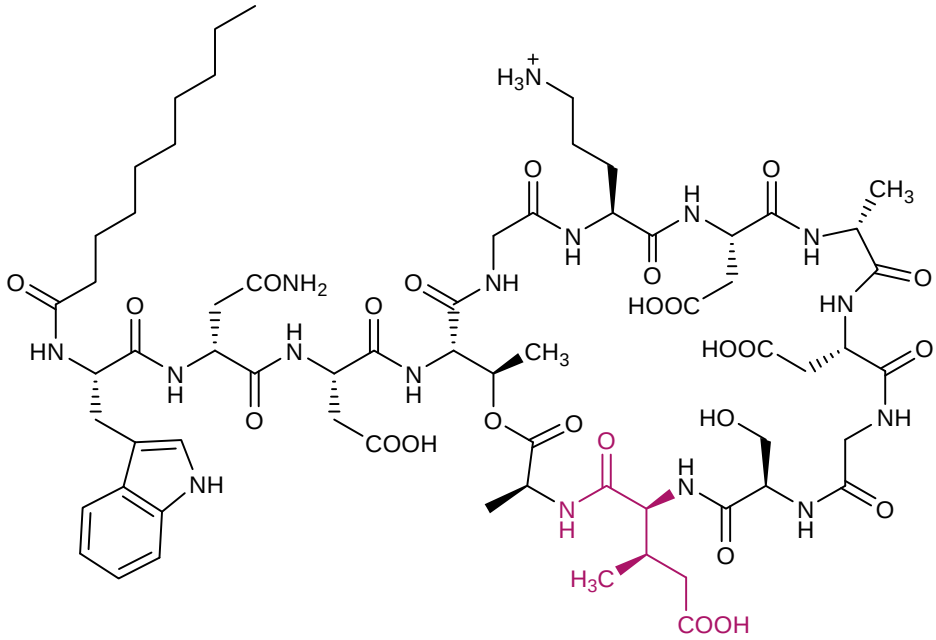


Figure 4. ***Daptomycin structure***

All CDAs have 3-methyl glutamate as their penultimate *C*-terminal residues.

Previous work demonstrated the 3-MeGlu in CDA was the L-isomer[43]; furthermore the stereochemistry of the 3-MeGlu

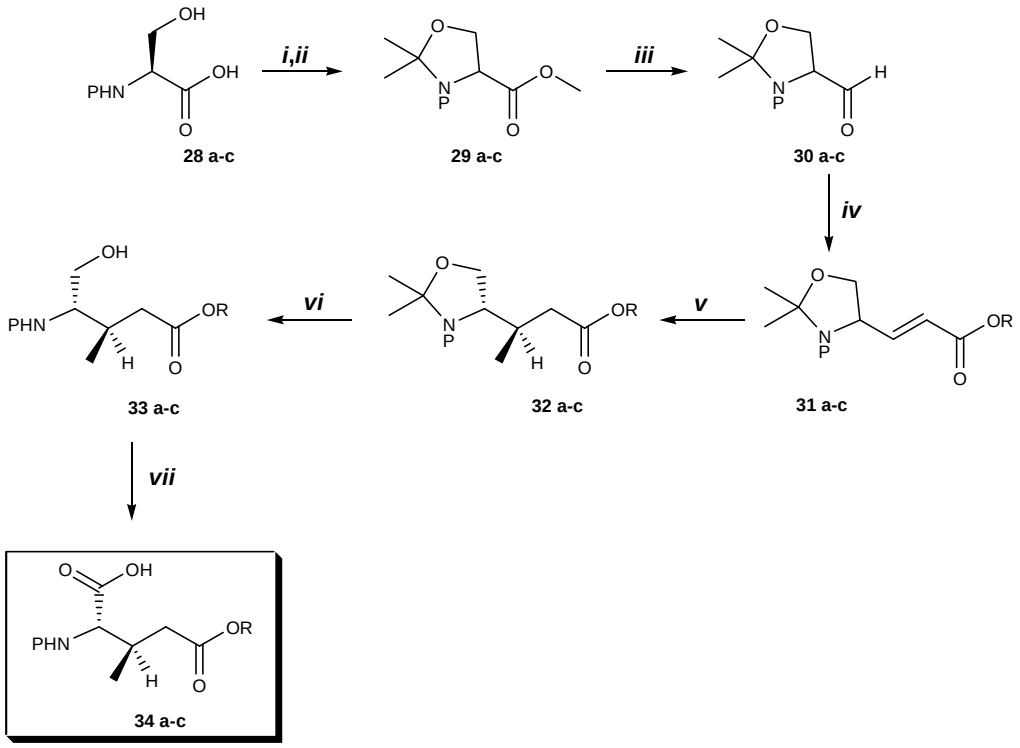
residue of daptomycin was inferred to be the L-*threo*- or (2*S*,3*R*)-stereoisomer as a consequence of the stereospecificity of two enzymes: glutamine synthase and glutamate decarboxylase.[44]

Asymmetric synthesis of each of the 3-MeGlu diastereoisomers have been reported[45,46,47]. A stereocontrolled synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-3-MeGlu has been developed for comparison with the natural 3-MeGlu derived from hydrolytic degradation of CDAs and daptomycin[48].

2.2 Strategies for the synthesis of (2S,3R)-3-methyl glutamic acid

Our goal was to develop a stereospecific synthesis of (2*S*,3*R*)-3-methylglutamic acid; an essential building block for the total solid phase synthesis of daptomycin.

Based on previous work[49], a synthetic strategy was proposed (*Scheme 11*).



Scheme 11. ***Strategies for the synthesis of (2S.3R)-3-methyl glutamic acid 34 a-c***

Entry	*P*	*R*
a	Boc	Me
b	CbZ	*t*-Bu
c	Boc	*t*-Bu

Table 8

In a first approach the synthesis was performed using as protecting group of the amino function the Boc group and protecting the carboxyl function as methyl ester.

The synthesis was successful and the product **34a** was obtained with high purity and stereoselectivity.

As previously discussed, methyl esters are not the ideal choice for the temporary protection of α-amino acid carboxy function.

For this reason, we decided to try the synthesis masking the carboxyl function as *t*-butyl ester and using as protecting group of the amino function the CbZ group, that could have been replaced at the end of the synthesis by the Fmoc-group.

In this case, we started from the *N*-CbZ-*L*-serine (**28b**). The amino acid was first converted into the corresponding methyl ester, using Cs_2CO_3 and iodomethane. The methyl ester was then treated with 2,2-dimethoxypropane at r.t. in presence of *p*-toluensulphonic acid and converted in the oxazolidine **29b** (enantiomeric mixture 2:1).

29b was converted into the Garner's aldehyde **30b** using DIBAL-H in dry toluene at -78°C for 2 h. **30b**, in enantiomeric mixture 2:1, was recovered after flash chromatographic column in good yields (80%).

The product **31b** was simply obtained by treating **30b** with Ph_3=CHCOO*t*Bu in dry toluene at room temperature for 2 h.

Unfortunately, the alkylation step to convert **31b** into **32b** wasn't successful. The attempt of methylation was performed using MeLi and CuI in presence of TMS-Cl in dry THF at-78°C and gave back as reaction product just the starting material.

Finally we decided to perform the synthesis protecting the amino function with the Boc-group and the carboxyl function as *t*-butyl ester. The selective removal of Boc group in absence of the t-butyl ester cleavage was reported in previous works[50].

We started with the oxazolidine *S*-**29c** and treated it with DIBAL-H to perform the reduction under the same condition of the previous attempts. The aldehyde *S*-**30c** was obtained in very good yields and converted into **31c** via the Wittig reaction, using Ph_3P=CHCOOtBu in dry toluene at room temperature.

The methylation of **31c** was successful and the (2*R*,3*S*)-diastereoisomer **32 c** was obtained selectively and in good yields.

The following steps of ring opening and oxidation of the alcoholic function produced the (2*R*,3*S*)-*N*-Boc-3-methyl-glutamic acid *t*-butyl ester (**34c**) in good yields.

Our future goal is to find the removal conditions of Boc group and introduction of Fmoc group without cleavage of the *t*-butyl ester. The *N*-Fmoc-3-methyl-glutamic acid *t*-butyl ester will be used for the solid phase synthesis of the daptomycin.

3. Sulfamoylation of L-Ornitine

3.1 Arginine and thrombine inhibitors

Arginine is a natural amino acid containing a strongly basic guanidine residue which is protonated at physiological pH values. Either alone or as a constituent of peptide structures, arginine plays a leading role in establishing noncovalent interactions with the active sites of a large number of enzymes involved in biologically important processes[51]and of the eukaryotic 26S proteasome[52]. Arginine-rich peptide motifs bound to RNA and DNA strands are also involved in the natural fate of nucleic

acids[53]. However, the requirement for arginine as the major determinant for selective recognition in biological systems is not rigorous. In fact, protonation of the guanidine residue determines lack of oral bioavailability and selectivity, and increases the toxicity of many arginine containing substrates and inhibitors. Replacement of arginine by any other amino acid in homologous peptide sequences, modification of its side-chain or guanidine moiety[54] by introducing non-polar and/or neutral groups of reduced basicity, and elimination of the cationic site from the sidechain[55] represent valuable approaches to afford a series of pharmaceutically relevant synthetic peptides[56] of greater protease specificity, bioavailability, inhibition potency, and stability against proteolysis together with improved binding affinities for many biologically relevant receptors[57]. The growing demand for an ideal protease inhibitor has increased the need for assorted libraries of synthetic compounds. In this context, non-proteinogenic amino acids are of considerable interest.

Motivated by pharmacological interest, and by the plethora of biological aspects related to the role of Arg and of its modified isosteres, we have exploited a facile synthetic access to a new L-ornithine derivative with the aim to develop a new class of human thrombin inhibitors. We report the synthesis of the polar and uncharged Arginine like derivative **40** (*Scheme 12*), a compound featuring a masked sulfamoyl group on the d carbon atom of the side-chain in substitution of the highly basic guanidine residue of natural arginine. The selected group is a constituent of biologically active sulfonamides[58] and sulfamoyl carbamates[59], but among the different moieties proposed for the chemical functionalization of the L-ornithine side-chain[60] no

mention can be found in the literature about the employment of the Sulfamoyl group. The NHSO2NH2 moiety in the L-ornithine skeleton could define a particular tetrahedral pharmacophore, surrogate of the guanidine group of arginine, because it is an efficient H-bonding acceptor[61] and should confer to compound **40** a weakly acidic character, due to the possible loss of the SO2NHR proton[62].

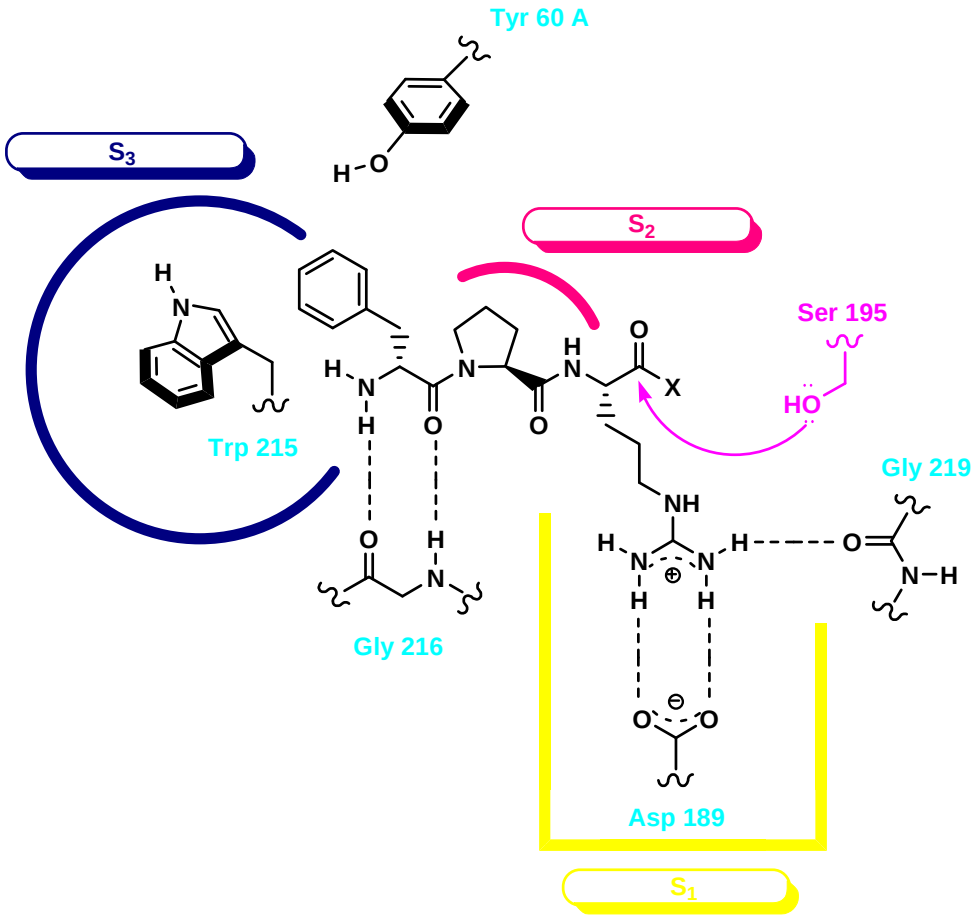
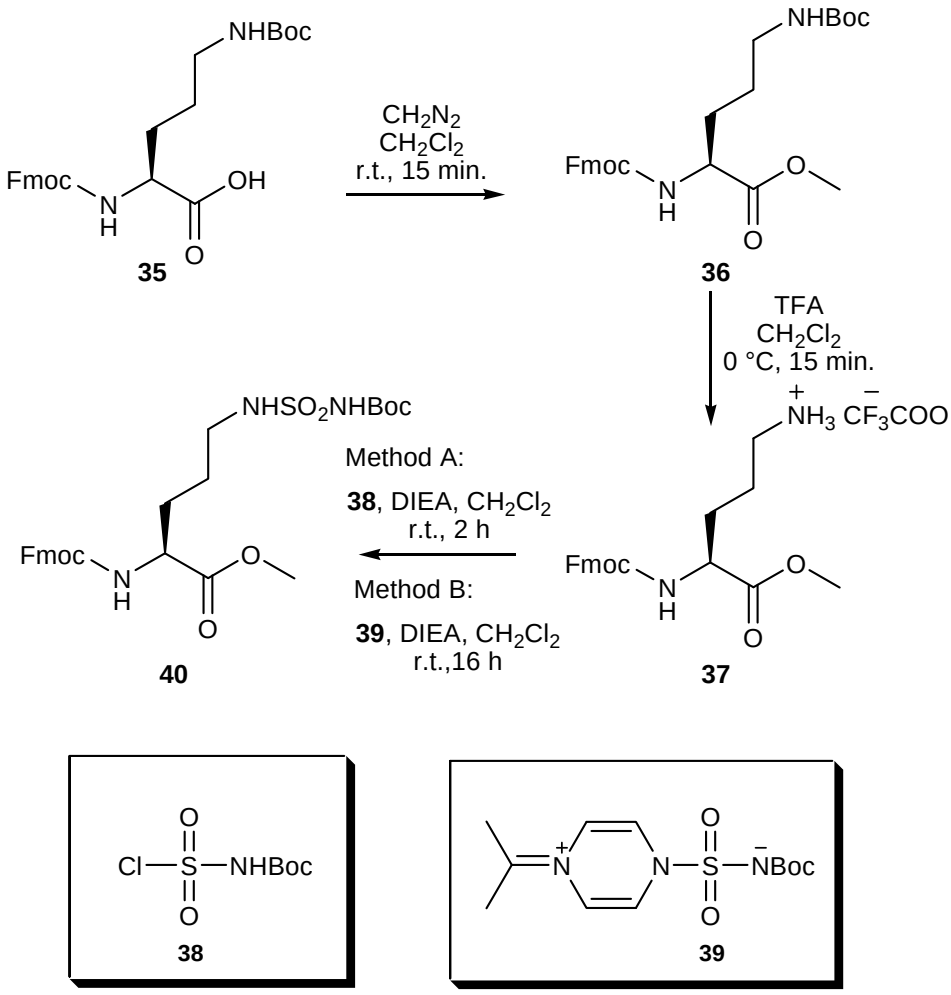


Figure 5. ***Interaction of the tripeptide sequence D-Phe-Pro-Arg with the active site of thrombine***

3.2 Synthesis of sulfamoylated L-ornithine, bioisostere of arginine

The preparation of **40** started from N^{α}-Fmoc-N^{δ}-Boc-L-ornithine (**35**), and involved a three-step procedure (*Scheme 12*).

Methyl ester **36** was obtained by treatment of the starting precursor **35** with a dichloromethane solution of diazomethane, at room temperature.

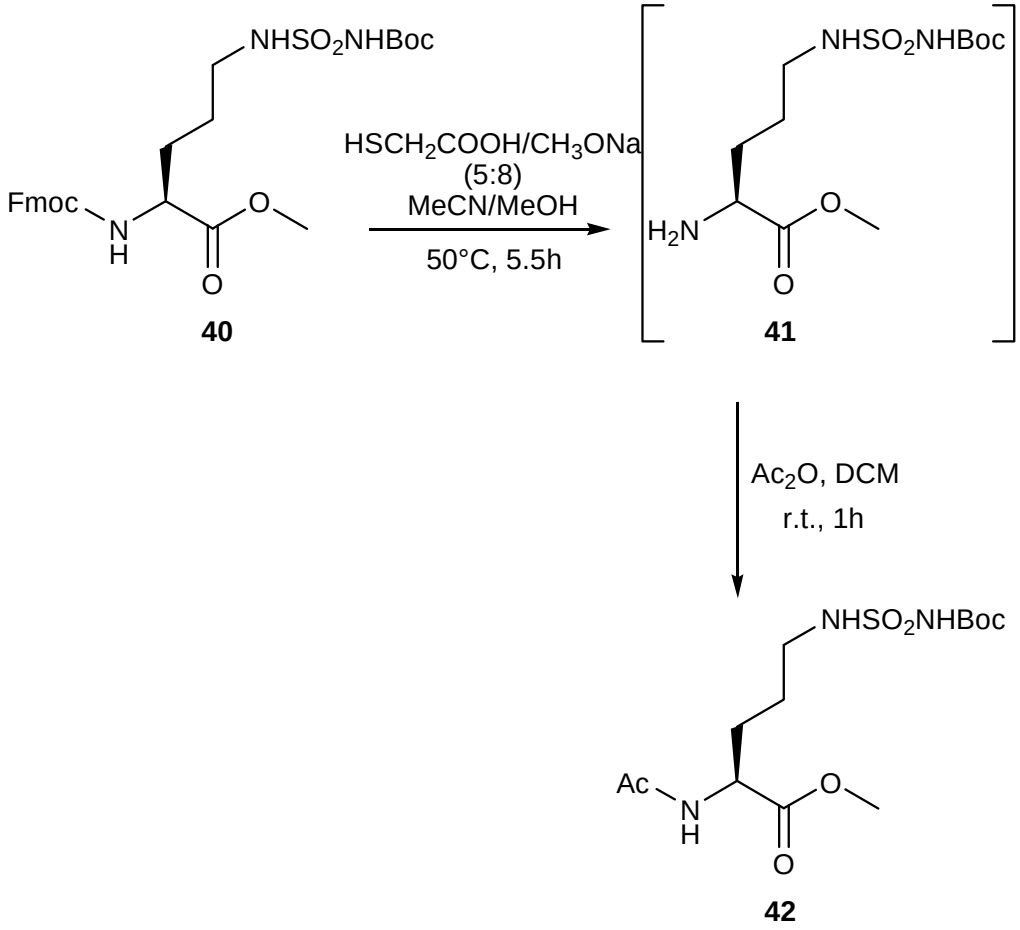


Scheme 12. ***Synthesis of the amino acid derivative 40***

Methylation afforded **36** in yields of 96%, and pure enough to be subjected to the next synthetic step without need for chromatography. Removal of the Boc group from **36** was performed by acidolysis at 0 °C, using TFA. Conversion of **36** was complete and the resulting salt **37**, not isolated, was immediately subjected to sulfamoylation. The α-amino function in the side-chain of **37** was finally functionalized using the sulfamoylating reagent **38** (*Scheme 12*, Method A), in the presence of DIEA. Reagent **38** was efficiently prepared from commercially available chlorosulfonyl isocyanate (CSI) and *tert*-butanol in dichloromethane, under the experimental conditions already described for conventional routes to this reagent[63]. Although **38** is rapidly formed in a quantitative yield by this approach, its use in the derivatization of the salt **37** proved to be troublesome. The chloride**38** is very unstable, and it cannot be stored for a long period. This aspect limits the possibility of an exact stoichiometric dosage of **38** imposing that the reagent must be prepared immediately before any sulfamoylation step. Moreover, under the experimental conditions adopted for the experiment in which **38** is used, the methyl ester **40** can be recovered as a pure product only after chromatography, and in yields not exceeding 65%. We found the zwitterionic azanide **39** to be the optimal reagent for the sulfamoylation of the trifluoroacetate **37** (*Sheme 12*, Method B). It can be obtained in very high yields and purity by a known procedure[64]. Since **39** is a stable crystalline solid, it can also be stored for prolonged periods and easily dosed for any purpose. The reaction of trifluoroacetate **37** with **39** was performed in the presence of DIEA to generate the free δ-amino group; and a simple work-up of the reaction mixture allowed the recovery of the methyl ester *38*

40 in yields of 92%, with no need for chromatography. The use of a base, DIEA, is strongly recommended: without the base, compound **40** can be recovered pure only after column chromatography and in yields not higher than 65%. DMAP produced during the sulfamoylation did not provoke removal of the base-labile protecting group Fmoc. The methyl ester **40** was fully characterized by NMR analysis, and both 1D- and 2D-homonuclear techniques confirmed the structure proposed for the new arginine-like derivative **40**. The Fmoc group was removed from the α-amino function of **40** avoiding the use of nitrogenated species. This was done in order to facilitate the separation of the unprotected methyl ester from the crude reaction mixture. As reported elsewhere[65], the Fmoc group can alternatively be removed from the corresponding methyl esters by reagent systems composed of $AlCl_3$ and toluene or *N*,*N*-dimethylaminoaniline, but acid-labile protecting groups, Boc, are not compatible with the experimental conditions adopted for the Lewis acid-assisted treatment.

Fmoc group was straightforwardly removed by treating **40** with the reagent system composed of mercaptoacetic acid and sodium methoxide, upon conditions similar to those adopted for the removal of the nosyl group from *N*-methylated amino acid derivatives.

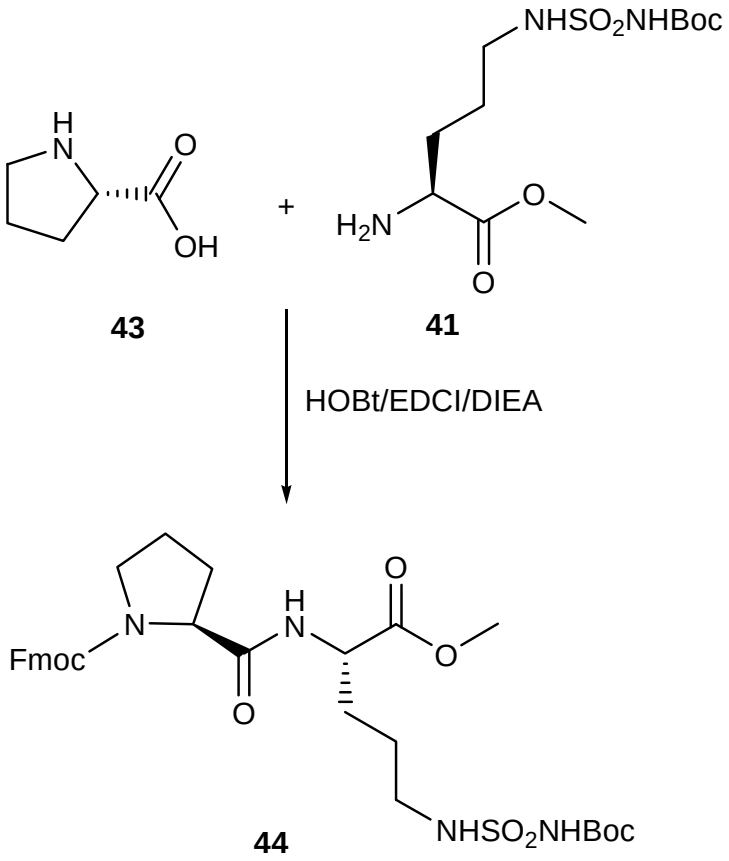


Scheme 13. ***Amino function deprotection of 40***

The appropriate stoichiometric ratio between the two components of the reagent system, referred to one equivalent of **40**, was found to be 5:8. Upon these conditions, treatment of **40** at 50 °C for 5.5 h afforded **41** (*Scheme 13*), which was not separated and immediately transformed into the corresponding acetyl derivative **42** by treating the crude material obtained from the unblocking step with an excess of acetic anhydride. Compound **42** was obtained in yields of 90%, which was reliable with the complete conversion of the precursor **40** into **41**. Removal of the Fmoc masking group was chemospecific, since *40*

both the Boc-protected Sulfamoyl moiety and the methyl ester functionality were not affected by the treatment.

Since proline occupies a special place among the natural amino acids, we coupled **41** with *N*-Fmoc-L-proline. The sequence Pro-Arg, in fact, is often selected as an ideal chemical probe in conformational studies, and in determining the absolute specificity and bioavailability of peptide inhibitors of proteolytic enzymes[66]. In order to evaluate the structural and conformational role of **41** in short peptide sequences, we prepared the dipeptide *N*-Fmoc-L-Pro-L-Orn-(N^{δ}-SO_2NHBoc)-OMe (**44**) (*Scheme 14*) by adopting a Fmoc-chemistry protocol usually used for the solution synthesis of peptides, upon conditions that can avoid the racemisation of the reaction partners[67].



Scheme 14. ***Synthesis of the dipeptide 44***

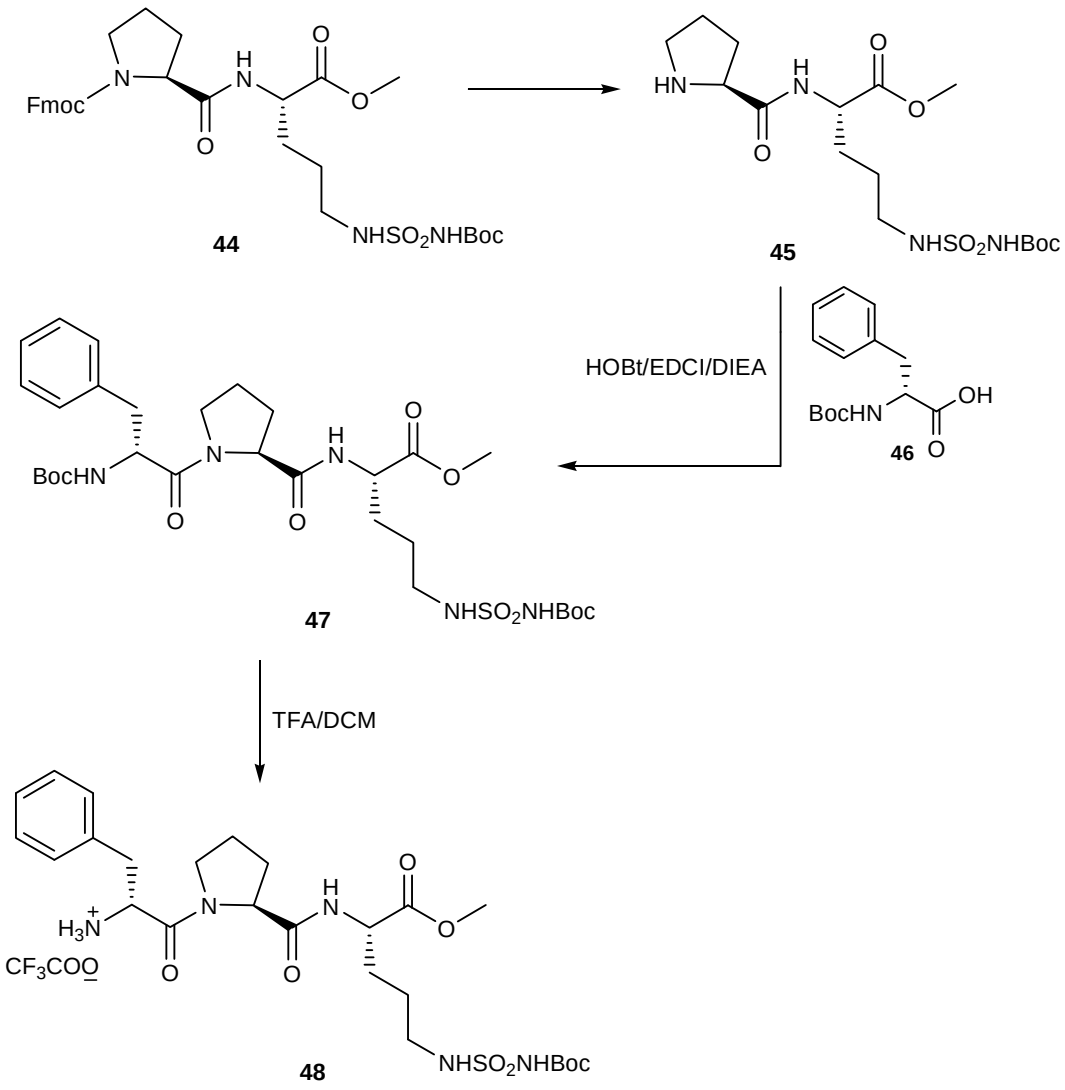
N-Fmoc-L-proline (**43**) was activated with the system HOBt/EDCI/DIEA and allowed to react with **41**, obtained from **40** after removal of the Fmoc group by treatment with a 5:8 mixture of mercaptoacetic acid and sodium methoxide as previously described. The only product obtained from the reaction was dipeptide **44**, which was recovered in yields of 88% after flash column chromatography (FCC). 1D- and 2D-homonuclear NMR analysis of **44** showed resonances which were consistent with the proposed structure. The 1D ^{1}H spectrum, recorded in $CDCl_3$ at 298 K, showed the presence of two sets of rotamers arising from the slow trans/cis isomerization around the CO–N linkage

between the Fmoc group and the proline ring. Based on the integration of non-overlapping signals, those appearing at 6.75 and 6.57 ppm, attributable to the α-NH proton of the ornithine skeleton included in the trans form and the same proton in the cis rotamer, respectively, we calculated a trans/cis ratio of 75:25 for the rotamers. The precise ratio was determined by the integration of the signals of both the α-NH protons which were assigned to the respective rotamer in analogy with the results reported in the literature for small dipeptides containing proline as the *N*-terminal residue[68]. 2D-homonuclear NMR showed resonances clearly attributable to the protons belonging to the ornithine skeletons, respectively. In particular, the cross-signals appearing in the contour plot of the TOCSY analysis performed in $CDCl_3$ at 298 K confirmed the presence of the trans/cis rotamer mixture and showed correlation patterns typical of the peptide sequence Pro-Orn. No other signals relative to protons attributable to any possible diastereomer of **44** appeared in the 1D and 2D proton spectra. On the other hand, the 1D- and 2D-NMR analysis of a sample of the crude dipeptide **44**, revealed resonances attributable only to the given structure, confirming that, limited to the sensitivity of NMR techniques, no racemisation of the α-carbon atom present in **40** occurred during the coupling. Thus, the stereochemistry of the protected arginine-like derivative **40** is retained both during its synthetic process and the Fmoc removal, as well as in the final coupling step. In an effort to evaluate the biological role of the Sulfamoyl group placed in the L-ornithine side-chain, **40** was finally used as building block in the synthesis of serine protease inhibitors. In particular, compound **40** was coupled with the dipeptide Boc-D-Phe-Pro-OH to prepare tripeptide analogues of the C-terminal

subsequence of fibrinogen, the natural thrombin substrate. Thrombin, the blood-clotting enzyme, is a serine protease with trypsin-like specificity and is able to cleave Arg-Xaa peptide bonds but only in a very limited number of substrates[69]. This enzyme has a critical position in the blood coagulation cascade and thus a central role in the regulation of haemostasis[70]. Moreover, for the prevention and treatment of thrombosis the control of thrombin activity is a key target[71] and the discovery of new classes of inhibitors of this enzyme could lead to useful drugs for treating thrombotic disorders, which constitute a serious source of mortality and morbidity in patients worldwide. Removal of the Fmoc group from **44** was performed as previously described for **40** using the system composed by mercaptoacetic acid and sodium methoxide, in the 5:8 stoichiometric ratio. Boc-D-Phe-OH was then coupled to dipeptide **45**, after activation of the free carboxylic group by the system HOBt/EDCI in the presence of DIEA. The fully protected tripeptide N-Boc-D-Phe-L-Pro-L-Orn-(N^{δ}-SO_2NHBoc)-OMe (**47**) was recovered pure by FCC in yields of 88%, and ^{1}H and ^{13}C NMR analysis confirmed the expected structure. In particular, the proton spectrum registered in $CDCl_3$ at 298 K showed the presence of a mixture of rotamers with a trans/cis ratio of 70:30, due to the different geometries of the peptide bond between the D-Phe and Pro residues. Acidolysis of **47** by using a solution of TFA in CH_2Cl_2 afforded D-Phe-L-Pro-L-Orn-(N^{δ}-SO_2NH_2)-OMe-CF_3COOH (**48**) which was obtained by precipitation of the crude product from a 1:2 MTBE/n-pentane mixture and further lyophilisation in order to remove all the residues of *tert*-butanol and any trace of possible co-products generated during the unblocking step. The ^{1}H-NMR spectrum of the trifluoroacetate

salt **48** registered at 298 Kin DMSO-d6/D_2O showed exchange of all protons on the N atoms, except for those of the SO_2NH_2 moiety, but did not display well resolved resonances for the other protons. Structure of **48** was definitively attributed by correlation with the corresponding resonances observed in the proton spectrum obtained for **47** and the molecular weight of the salt was confirmed by MALDI mass spectrometry. Both tripeptides **47** and **48** were then subjected to in vitro coagulation assays in order to evaluate, in a set of preliminary experiments, their inhibitory effects against human thrombin. The TT (Thrombin Time), a measure of the thrombin-fibrinogen reaction in vitro[72], and the activated partial thromboplastin time (APTT), the parameter used to evaluate the anticoagulant effect on the thrombin produced by the intrinsic pathway of the coagulation cascade[73], were determined using pooled human plasma treated with samples of **47** and **48** at different concentrations. Among the two tripeptides, **47** did not show appreciable inhibitory potency in all the performed experiments, while **48** exhibited a good and dose-dependent activity as determined by the clotting time of human plasma in TT and APTT tests. In particular, a sample containing 8.4 lmol of trifluoroacetate **48** prolonged the clotting time of pooled human plasma to 217.2 s (mean value, SD = ±10.2; n = 3), about three times the control value fibrin clot time in TT determination, and to 65.4 s (mean value, SD = ±2.5; n = 3), twice the corresponding control value in APTT measurements. With a more concentrated sample of **48** (25.3 lmol) a TT value of 493.7 s was recorded, while APTT was 123.8 s. Another sample containing 33.7 lmol of **48** showed a not detectable TT, with a value exceeding the coagulometer time limit (500 s), and an APTT value of 296.8 s was recorded. A not

detectable ([500 s) APTT value was also obtained when a sample of the potential thrombin inhibitor was prepared using 42.1 lmol of tripeptide **48**.



Scheme 15. ***Synthesis of the tripeptides 47 and 48***

Chapter 2

New methodologies for protection and deprotection of amino acids functionalities in peptide synthesis

1. Carboxyl function deprotection of α-amino acid methyl esters using sulphur ylides

1.1 Methodologies for cleavage of methyl esters

In solution-phase peptide synthesis is required the semipermanent protection of carboxyl terminal function; the protecting groups should be kept intact through the chain-building process and are removed at its completion.

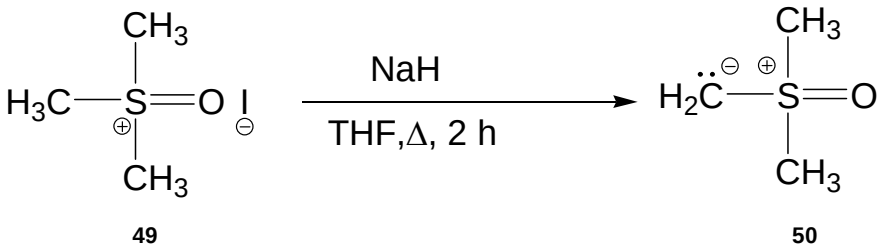
Methyl esters are normally removed with difficulty under basic conditions that could cause racemization of the amino acid chiral centres[74].

Alternatively amino acid methyl esters can be removed using a nucleophile that attacks the carbonyl function of methyl esters[75].

In this context, we developed a novel methodology for the cleavage of methyl ester function under mild conditions.

1.2 Cleavage of amino acid methyl esters using sulphur ylides

For this purpose, we thought sulphur ylides could act as nucleophiles on the carbonate carbon atom of methyl ester function, involving a sulphur catalytic action, and produce carboxyl function deprotection.



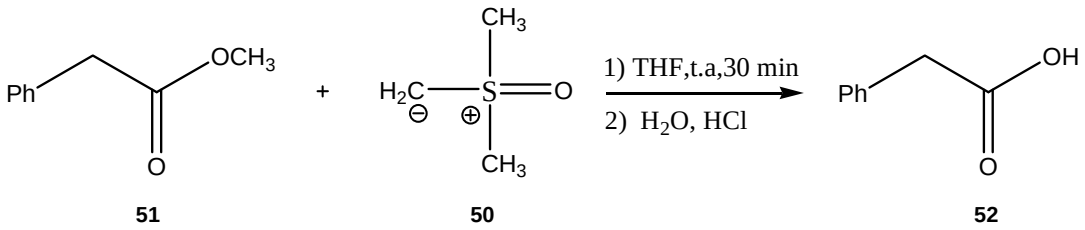
49 **50**

Scheme 16. ***Preparation of dimethylsulfoxonium methylide 50***

Dimethylsulfoxonium methylide **50** was prepared starting with trimethylsulfoxonium iodide **49** and treating it with sodium hydride in dry THF for 2 hours (*Scheme 16*).

Methyl phenylacetate was chosen as model system to test the reaction. The ester (1 mmol) was dissolved in tetrahydrofuran and treated with dimethylsulfoxonium methylide **50** (2 mmol) at room temperature (*Scheme 17*).

After a simple work-up, phenylacetic acid (**52**) was obtained in excellent yield without any further purification.

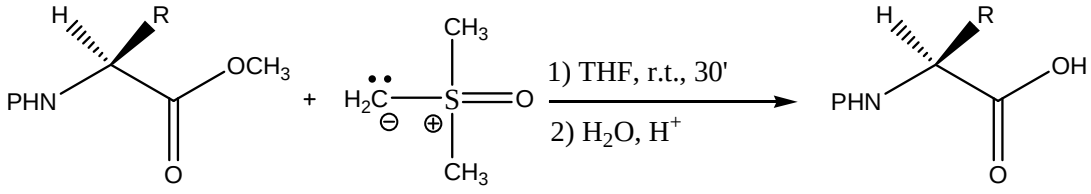


51 **50** **52**

Scheme 17. ***Ester cleavage of methyl phenylacetate 51***

In the light of the excellent result obtained with the aliphatic ester, the reaction was applied to α-amino acid methyl esters (**53 a-e**) variously protected on amino function.

The deprotection was performed in about 30 minutes and *N*-protected-α-amino acids (**54 a-e**) were recovered in excellent yields and high purity (*Scheme 18)*.



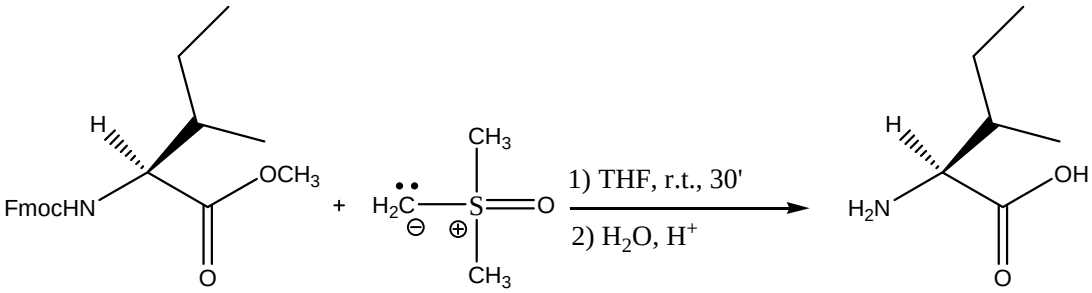
53 a-e **50** **54 a-e**

Scheme 18.*Carboxy function deprotection of N-protected-α-amino acids 53a-e*

Entry	R	P	Yield (%)
54 a	CH_3	Ns	**99**
54 b	CH_3	Cbz	**88**
54 c	$PhCH_2$	Boc	**98**
54 d	$(PhCH_2)OC_6H_4CH_2$	Ns	**70**
54 e	$CH_3CH_2(CH_3)CH$	Ns	**82**

[a] Total Yields

Table 9. ***Results of deprotection of N-protected-α-amino acids***



55 **50** **56**

Scheme 19. ***Deprotection of N-Fmoc-isoleucine methyl ester 55***

The reaction of deprotection was also tested with the methyl ester of an α- amino acid protected on the amino function with a base-labile urethane protecting group. The methyl ester of *N*-Fmoc-L-isoleucine (**55**) was chosen as model system. The reaction, carried out by treating the ester **55** (1 mmol) in THF with the ylide **50** (2 mmol), had a different trend compared to those made using the amino acids *N*-Nosyl, *N*-CbZ and *N*-Boc protected protected (*Scheme 19*).

In this case, after 30 minutes of reaction, the isoleucine **56** both deprotected on amino function and on carboxyl function was recovered in high yield. The reaction product was recovered after conversion into the correspondent *N*-acetylated derivative and analyzed by GC/MS.

50

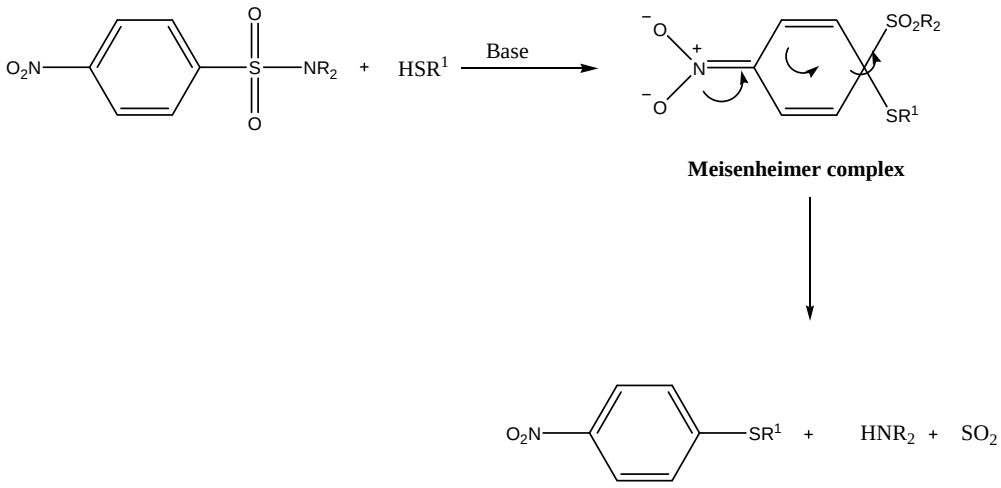
The simultaneous deprotection of the carboxyl function and the amino function of *N*-Fmoc-protected amino acid methyl esters is a very interesting result regarding the applicability of the procedure in solution phase peptide synthesis based on Fmoc strategy. Indeed, using the methodology described, at the end of the construction of a peptide Fmoc-protected on amino function and masked as methyl ester on carboxy terminal, is possible to get the deprotection of the two functions in a single reaction step and in a short time.

In this context, our future goal is to determinate the reaction mechanism and to apply the successful deprotection methodology to other ester substates.

2. Synthesis of resin-bound mercaptoacetic acid for the removal of Nosyl group in peptide synthesis

2.1 Removal of Nosyl group in peptide synthesis

The deprotection of amino function of *N*-Nosyl-α-amino acids is generally performed using sulfur nucleophiles via a nucleophilic aromatic substitution (*Scheme 20*).



Scheme 20. ***Mechanism of the Nosyl group removal***

The sulphur nucleophiles that has been used for this purpose are the tiophenol[76], the 2-mercaptoethanol[77] and the mercaptoacetic acid[78].

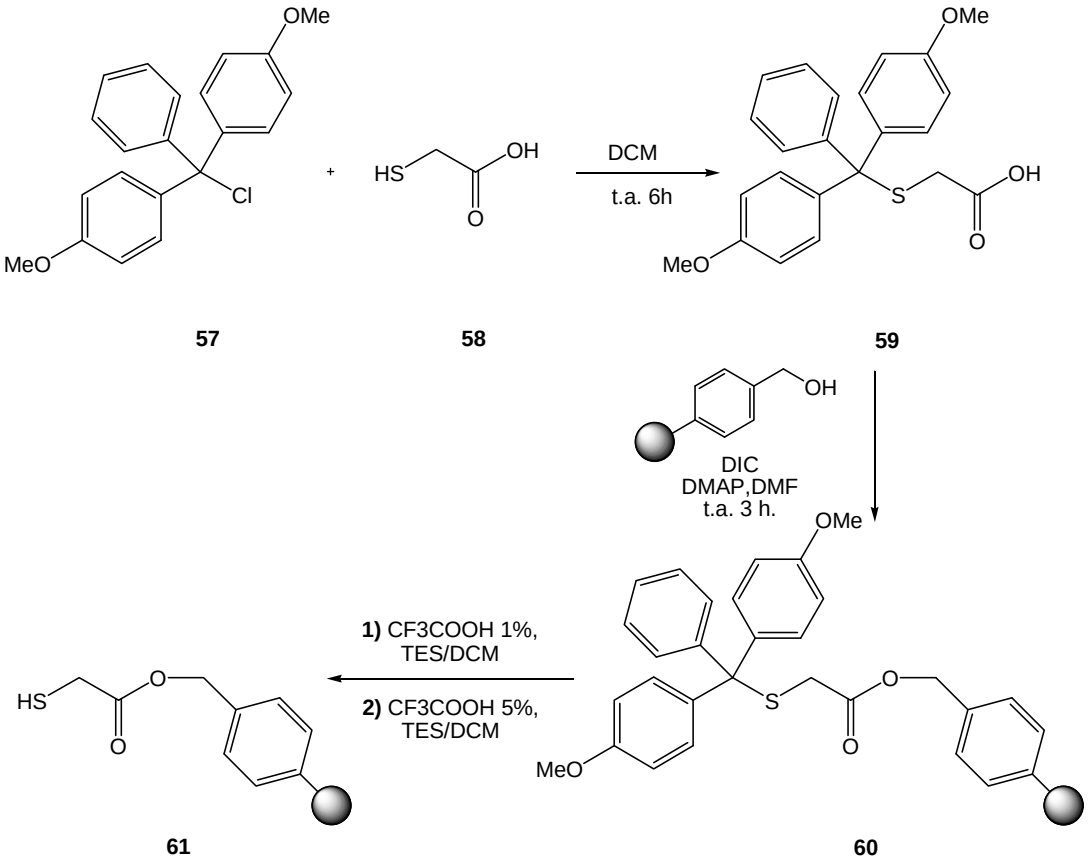
The reaction is often complicated because of the difficult separation of the deprotected products from the adduct formed by the nucleophile and the aromatic derivative.

For this reason the use of solid supported thiols results important in the deprotection of amino function of *N*-Nosyl protected amino acids.

2.2 Synthesis and use of solid-supported mercaptoacetic acid

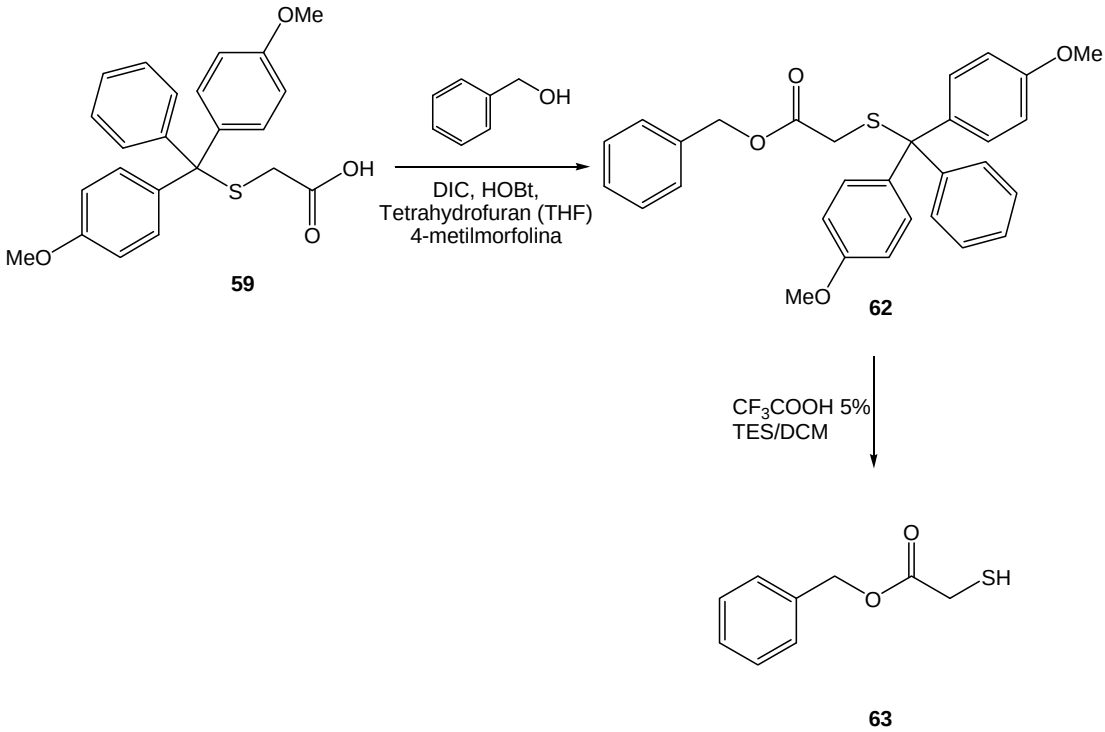
To attach mercaptoacetic acid to a solid support, the former was loaded onto the Wang resin through an ester bond. The procedure required appropriate protection of the thiol function, which was realized by using the 4,4'-dimethoxytrityl protecting group. 4,4'-Dimethoxytrityl chloride (**57**) dissolved in dichloromethane was treated with mercaptoacetic acid (**58**;

Scheme 19). After 6 h, the reaction mixture provided the corresponding protected product **59** in 75% yield. Attachment of *S*-(4,4'-dimethoxytrityl)mercaptoacetic acid (**59**) through its carboxy group onto the Wang resin was performed in dimethylformamide (DMF) in the presence of a large molar excess of **59** (10:1 with respect to the free hydroxyl groups). The formation of the ester bond between **59** and the resin was greatly facilitated through the activation of the carboxy function by *N*,*N*'-diisopropylcarbodiimide (DIC) in the presence of 4-(dimethylamino)pyridine (DMAP) (*Scheme 21*). The subsequent use of Wang-resin-bound *S*-(dimethoxytrityl) mercaptoacetic acid **60** required the unmasking of the thiol groups.



Scheme 21. ***Synthesis of supported mercaptoacetic acid 61***

To determine the appropriate conditions for the deprotection of the SH groups without affecting the ester bond, a model system was designed to mimic the removal of the trityl group in solution. For this purpose the *S*-protected mercaptoacetic acid **59** was treated with benzyl alcohol to afford benzyl *S*-(dimethoxytrityl)mercaptoacetate **62** (*Scheme 22*).



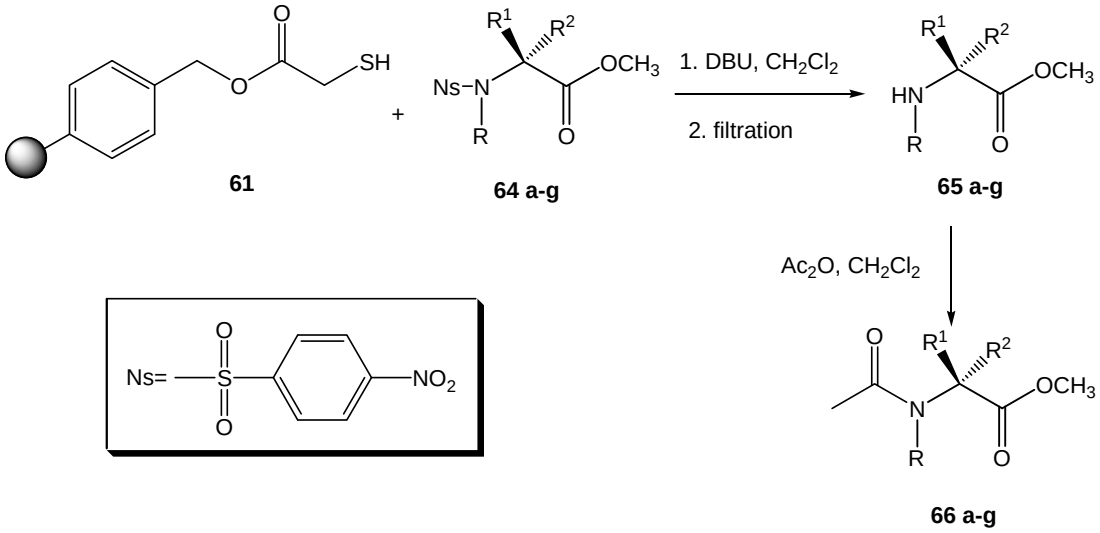
Scheme 22. ***Solution phase model system***

Removal of the trityl protecting group from the model system **62** was then tested by using trifluoroacetic acid in the presence of triethylsilane (TES) added as a scavenger for the stable trityl cation. Complete deprotection of the thiol-function was effected within 1 h by treatment with 5% trifluoroacetic acid (TFA) in the

presence of TES. This reaction enabled the recovery of benzyl mercaptoacetate (**63**) without affecting the ester function. In a similar fashion, the thiol resin **60** was treated with trifluoroacetic acid in dry dichloromethane in the presence of triethylsilane (*Scheme 21*). In this case a first cycle of 1 h with 1% TFA and a second cycle of 30 min with 5% trifluoroacetic acid resulted in the unmasking of the thiol function. The success of this step was shown by the GC-MS analysis of the filtrate solution obtained by subjecting an aliquot of deprotected resin to 50% TFA to cleave the mercaptoacetic acid from the solid support: the chromatogram, in fact, revealed the total absence of *S*-(dimethoxytrityl)mercaptoacetic acid. This result confirms the complete deprotection of the thiol functions under mild acidic conditions (1 and 5% TFA).

To estimate the concentration of thiol groups supported on the Wang resin we used the thiol resin to deprotect the amino function of nosyl-protected amino acid methyl esters. Thus, the utility of resin-bound mercaptoacetic acid **61** was investigated in a typical experiment carried out to deprotect the α-amino function of *N*-nosyl-*N*-methylalanine methyl ester (**64a**). The reaction was performed in the presence of DBU in dichloromethane at room temperature (*Scheme 23*). The first experiment was performed by using *N*-nosyl-*N*-methylalanine methyl ester (**64a**) in a 1:1 molar ratio with respect to the total active groups of the resin, assuming that the loading of the Wang resin with mercaptoacetic acid was quantitative. In this case the deprotection reaction of **64a** was not complete. A second reaction was then attempted by using a 0.8:1 ratio of **64a** with respect to the resin **61**. Again, in this experiment the reaction,

monitored by TLC analysis, was not complete after 2 h. However, by using a 0.7:1 ratio of **64a**, complete deprotection of the starting substrate **64a** had occurred after 1 h with the formation of *N*-methylalanine methyl ester **65a**. Finally, the *N*-methylalanine methyl ester was recovered after acetylation of the free amino function. Treatment of the α-amino acid methyl ester with acetic anhydride afforded the *N*-acetylated derivative in quantitative yield (*Scheme 23*). On the basis of the stoichiometry of the deprotection reaction, the equivalents of acetylated amino acid obtained correspond to the equivalents of thiol groups on the resin. Therefore it was possible to quantify the loading of resinbound free thiol groups, which was at least 0.77 mmol/g. This solid-supported solution-phase reaction adopted for the removal of the nosyl protecting group is advantageous in comparison with the reaction performed in solution. In fact, the excess reagents and byproducts trapped on the solid support are readily separated from the reaction mixture by filtration without need of conventional chromatographic purification of any intermediates. In addition, it is possible to monitor the reaction in real time by using conventional techniques, such as TLC and GC-MS analyses. Subsequently, we sought to extend this methodology to other *N*-nosyl-protected amino acids. *N*-Nosyl-α-amino acid methyl esters **64b–g** were treated with the thiol resin **61** in 0.7:1 molar ratios under the same reaction conditions as adopted for **64a** (*Table 10*, *Scheme 23*).



Scheme 23. ***Deprotection of N-Nosyl protected amino acids using the resin 61***

Entry	R^1	R	R^2
a	CH_3	CH_3	H
b	$CH_2CH(CH_3)_2$	H	H
c	$CH(CH_3)_2$	H	H
d	CH_2Ph	H	H
e	CH_3	H	H
f	$CH(CH_2)CH_2CH_3$	H	H
g	H	H	$CH(CH_2)CH_2CH_3$

Table 10. ***Results of the deprotection of N-Nosyl protected amino acids***

The *N*-acetylated products **66b–g** were obtained in quantitative yields. Furthermore, the GC-MS and ^{1}H NMR analyses of the single crude products **66f** and **66g** revealed the total absence of optically inverted amino acid derivatives, the diastereoisomers **66g** and **66f**, respectively, thus confirming the retention of chiral integrity of the C-α stereocentres during the deprotection of the α-amino function of the *N*-nosyl amino acid methyl esters. The

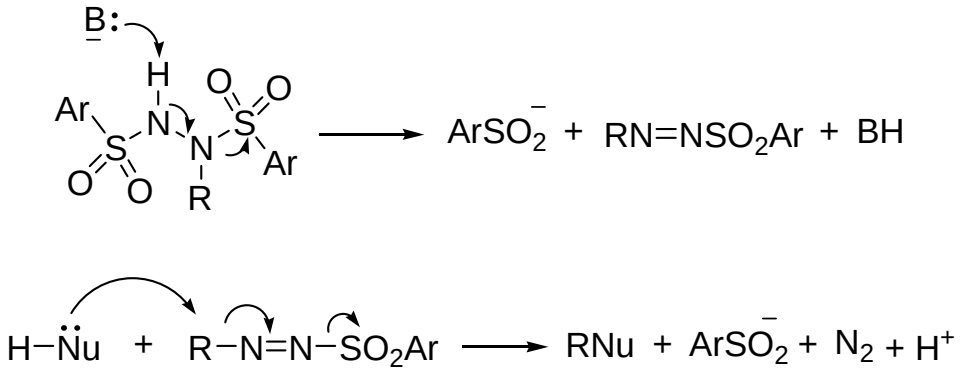
feasibility of the complete separation of **66g** and **66f** was confirmed by GC-MS analysis of an opportunely prepared mixture of **66g** (20 mg) and **66f** (20 mg).

Chapter 3

Reactivity studies

1. Site-Selective Methylation of N^{β}-Nosyl hydrazides of N-Nosyl protected α-amino acids

Hydrazine derivatives are useful reaction intermediates in organic synthesis.[79] Furthermore, some biologically active molecules or intermediates involved in their synthesis are characterized by the presence of the hydrazine functional group.[80] *N*-Alkyl-*N,N'*-bis(arylsulfonyl)hydrazines display significant antineoplastic activity against a variety of tumor cells.[81] Their tumor-inhibitory properties can be attributed to their capacity to generate, under physiological conditions, alkylating species that act by modifying directly the DNA.[82] Acyl hydrazine substrates substituted on the β nitrogen atom with an arenesulfonyl leaving group are of great interest also in organic chemistry.[83] Oxidizing agents convert *N*-acyl-*N'*-(arylsulfonyl)hydrazines to the corresponding acyl radicals,[84] which are useful reaction intermediates in carbon-carbon bond formation and in particular in the synthesis of carbocycles.[85]



Scheme 24

Generally, the activity of these hydrazine derivatives takes place through the generation of a reactive diimide intermediate. The formation and the nature of this species determine the biological activity of the *N*-alkyl-*N,N'*-bis- (arylsulfonyl)hydrazine derivatives and the chemical reactivity of *N*-acyl-*N'*-(arylsulfonyl)hydrazines. In the first case, the diimide intermediate formation depends on the acidity of the hydrazide proton and the leaving group ability of the arenesulfinate ion ($ArSO_2$-) (*Scheme 24*). After nucleophilic attack on the alkyl group, the diimide intermediate decomposes with loss of a molecule of nitrogen and expulsion of the second arenesulfinate ion and gives rise to the alkylated nucleophile (*Scheme 23*). The presence of the alkyl substituent is essential for the generation of reactive species involved in the antineoplastic activity. In fact *N,N'*-bis(arylsulfonyl)-hydrazines not alkylated on the hydrazine moiety do not show antitumor properties. The evolution of the *N*-acyl-*N'*-(arylsulfonyl)hydrazine systems is also associated with the formation of diimide species. The anion resulting from the removal of the sulfonamide proton, undergoes a 1,2-hydride shift, expulsion of sulfinate ion, and migration of an electron pair to form the N=N double bond. The acyl diimide thus obtained

decomposes spontaneously into the corresponding aldehyde. This reaction represents an alternative to the use of hydride reducing agents in the conversion of carboxylic acid derivatives to aldehydes; differently, the acyl alkyl diimides tautomerize readily to the corresponding hydrazones.[86] Furthermore, an acyl diimide derivative is also involved in the formation of the acyl radical that is the key intermediate responsible for the antitubercolar activity of isoniazid.[87] Several unalkylated *N*-acyl-*N'*-(arylsulfonyl)hydrazines show inhibitory activity against a series of serine proteases involved in the pathogenesis or in the control of some human diseases. In particular, a series of *N*-acyl-*N'*-(arylsulfonyl) substituted cyclic hydrazide derivatives are selective inhibitors of dipeptidyl peptidase IV.[88] Furthermore, tripeptides containing a C-terminal sulfonyl hydrazide functionality[89] have proven to be potent and selective inhibitors of a serine protease that is essential for the replication of the hepatitis C virus[90] and involved in viral persistence in organisms. Also, many types of sulfonamide compounds show biological activity[91] and are widely used in therapy as antibacterial,[92] hypoglycemic,[93] diuretic,[94] anticarbonic anhydrase,[95] and antithyroid[96] drugs.

With the aim to obtain new and potentially active substrates we have undertaken the synthesis of molecules having both sulfonyl and sulfonamide functionalities. For this purpose, the N^{β}-4-nitrobenzenesulfonyl hydrazides (N^{β}-nosyl hydrazides) of *N*-nosyl protected R-amino acids were chosen as starting model systems. The selective alkylation of these substrates was performed using diazomethane as methylating agent,[97] exploiting the different acidity of sulfonamide and amide protons.[98] Diazomethane acts

as a base and removes an acidic proton of the reacting molecule. The methyl diazonium ion obtained is capable of alkylating the nucleophile species generated from the initial acid-base reaction.[99] As reported in the literature, the acidity in DMSO of both sulfonamide and sulfonyl hydrazide protons is higher than that of the acyl hydrazine protons (pKa $PhSO_2NH_2$=16.1; pKa $PhSO_2NH_2NH_2$=17.1; pKa $PhCONH_2NH_2$=18.9); instead, the pKa values, measured in DMSO, of sulfonamides and sulfonyl hydrazides are rather similar. These data suggest that the protons of the sulfonamide and sulfonyl hydrazide functions present in the same molecule of the *N'*-(*N*-nosyl-R-aminoacyl)-*N''*-nosyl hydrazines could have comparable acidity, with both more acidic than the acyl hydrazine proton. With the aim to estimate the acidic properties of this kind of compounds, density functional theory (DFT) computations have been performed on the synthesized species. The gas-phase acidities were calculated following the procedure described in the Experimental Section. The three considered anionic forms, named An(N_a), An(N_b), and An(N_c) with regard to the amino groups of different nature present in the **69a** molecule, are shown in *Figure 6*. From these calculations it is evident that the An(N_a) anionic species (ΔΔH = 0.0 kcal/mol) is the most stable one followed by An(N_b) (ΔΔH=12.4 kcal/mol) and An(N_c) (ΔΔH = 14.1 kcal/mol), respectively, hence with regard to acidic properties of the three sites in the 3a molecule, we propose the acidity order of N_a > N_b > N_c.

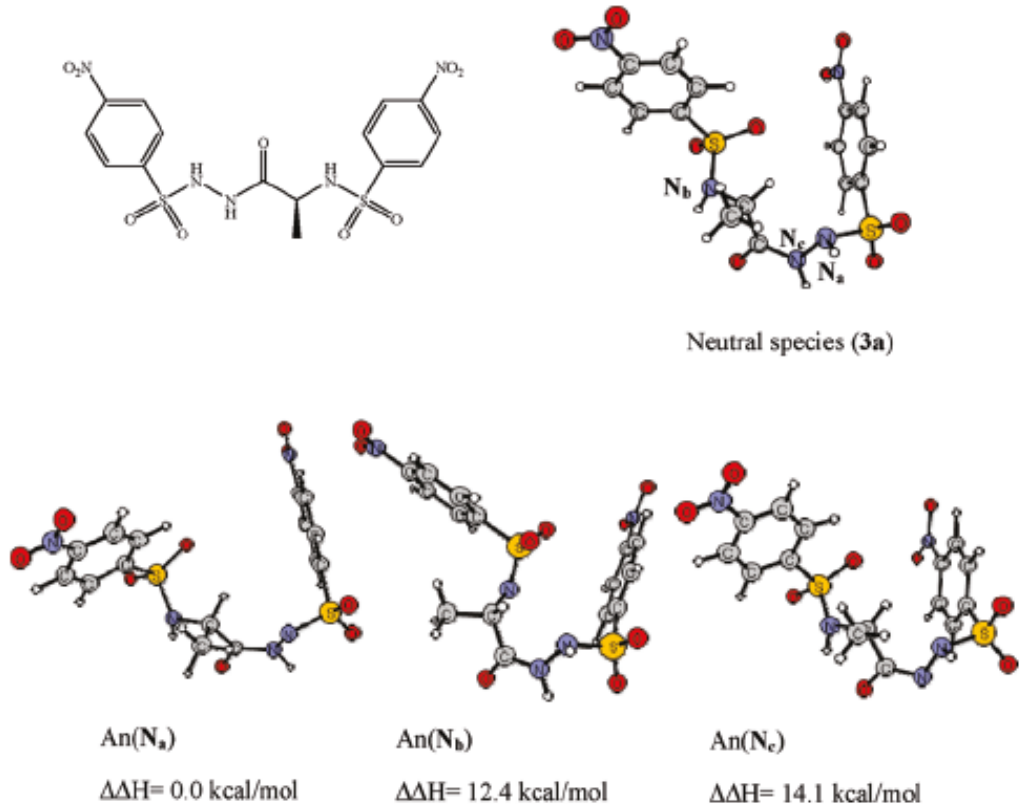
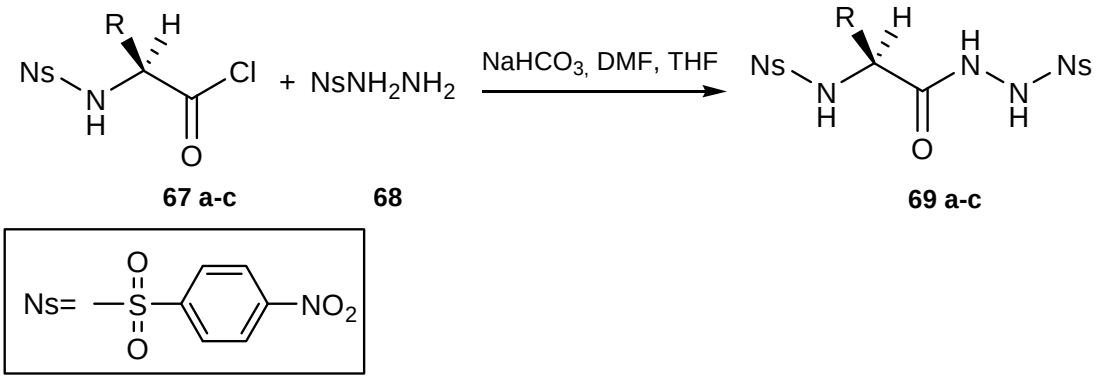


Figure 6. ***Anionic forms of 69a***

These theoretical findings also quantify the different acidic nature of the sulfonamide and sulfonyl hydrazide protons. On the basis of these predictions, we were interested in using the diazomethane as probe molecule to test the acidity of the N_a,N_b and N_c protons of the hydrazine substrates. The subsequent formation of the alkylation products, resulting from the reaction of the generated nucleophilic sites with the methyl diazonium ion, represents a measure of the base activity of diazomethane, depending on the acidity of the N_a, N_b, and N_c protons. The nosyl-substituted acyl hydrazines **69a-c** were easily prepared by

treatment of nosylhydrazide (**68**)[100] with the appropriate *N*-nosyl-R-aminoacyl chloride **67a-c** (*Scheme 25*, *Table 11*).



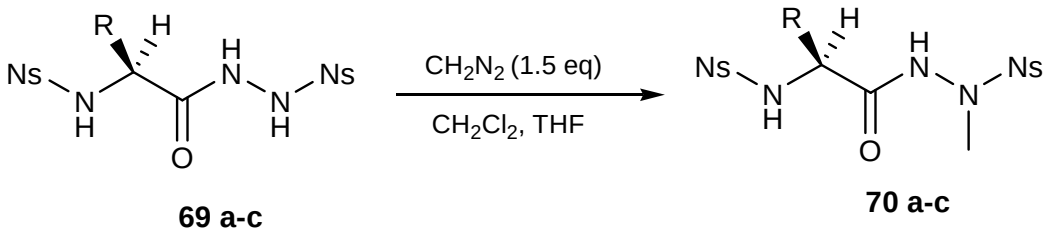
Scheme 25. ***Preparation of the nosyl-substituted acyl hydrazines 69a-c***

Entry	R	Yield (%)
69a	CH_3	**80**
69b	$CH_2CH(CH_3)_2$	**73**
69c	CH_2Ph	**70**

Table 11. ***Results of the Preparation of the nosyl-substituted acyl hydrazines 69a-c***

The *N'*-(*N*-nosyl-L-alanyl)-*N''*-nosylhydrazine (**69a**), chosen as model system, was dissolved in dry THF and treated with 1.5 equiv of a 0.66 M methylene chloride solution of diazomethane at room temperature. After 50 min, evaporation of the solvent

under vacuum afforded *N'*-(*N*-nosyl-L-alanyl)-*N''*-methyl-*N''*-nosylhydrazine (**70a**) in quantitative yield (*Scheme 26*).



69 a-c **70 a-c**

Scheme 26. ***Treatment of 69a with 1.5 eq of CH_2N_2***

Treatment of the hydrazines **69b,c** (*Scheme 26*) under the same conditions as **69a** led to the formation of the corresponding derivatives **70b,c**, selectively methylated on the sulfonyl hydrazide function. In all cases, the products were recovered in quantitative yields and high purity without need of chromatographic separation. The methylation reaction is chemospecific for the alkylation of the sulfonyl hydrazide function. Both small (1 mmol, 0.55 g of **69c**) and larger scale (14 mmol, 8.0 g of **69c**) reactions were performed successfully and with safety. To investigate the behavior of its acidic protons, B3LYP calculations were carried out also on **70a** species and its anionic forms. The optimized structures are depicted in *Figure 6*.

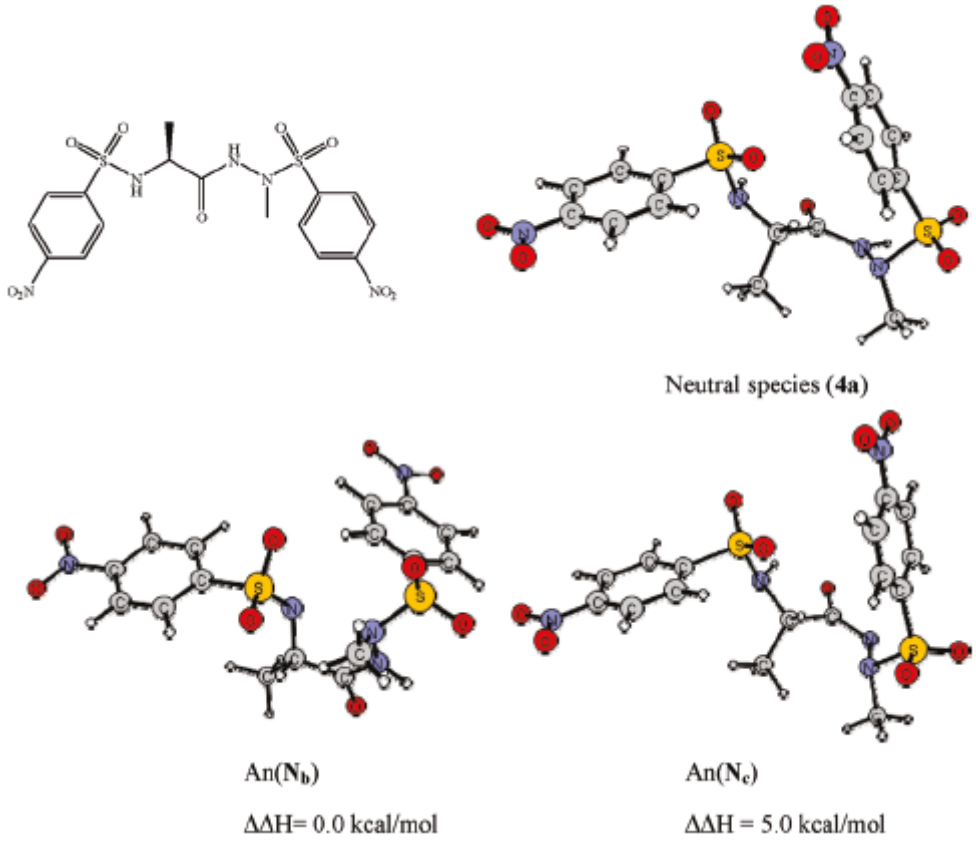
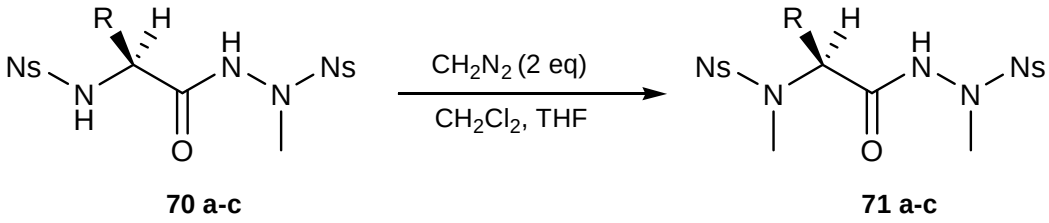


Figure 7. ***Anionic forms of 70a***

Also in this case, the acidities of N_b and N_c were calculated and showed the same trend as the **69a** species (see *Figures 6* and *7*). In particular the anion having the negative charge on the N_b site results more stable than that having the negative charge on the N_c one, by about 5 kcal/mol. The theoretical acidity order proposed for the monomethylated substrate **70a** was confirmed by experimental data. In fact, the treatment of hydrazines **70a-c** with 2 equiv of a 0.66M methylene chloride solution of diazomethane at room temperature led to the formation of the corresponding dimethylated products **71a-c** (*Scheme 27*), which are recovered in quantitative yields and high purity without need

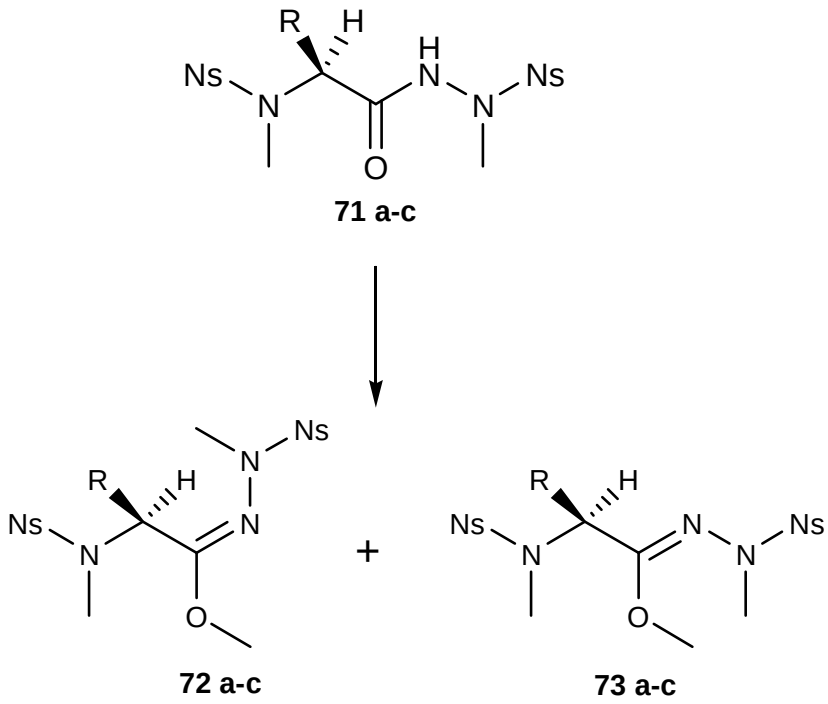
of chromatographic separation. The methodology was expanded to the large-scale synthesis (gram quantity).



70 a-c **71 a-c**

Scheme 27. ***Treatment of 70a with 2 eq of CH_2N_2***

Furthermore, in an additional experiment, the hydrazines **71a-c** were completely methylated by treating them with 5 equiv of a 0.66 M methylene chloride solution of diazomethane at room temperature (*Scheme 28*). The ^{1}H-NMR spectrum of each crude reaction product, obtained after evaporation of the solvent under vacuum, indicated the presence of resonance signals corresponding to a mixture of the two isomers **72a-c** and **73a-c** in an approximately 1:3 ratio, respectively (*Scheme 28*).



Scheme 28. ***Treatment of 71a with 5 eq of CH_2N_2***

Entry	R	Yield 72 (%)	Yield 73 (%)
a	CH_3	21	60
b	$CH_2CH(CH_3)_2$	24	72
c	CH_2Ph	27	77

Table 12. ***Results of the Treatment of 71a with 5 eq of CH_2N_2***

Chromatographic separation gave the trimethylated products **72a-c** and **73a-c** in 21-27% and 60-77% yield, respectively (*Table 12*). The methylation reaction was also performed successfully on a larger scale than 1 mmol. The formation of these compounds definitely confirms a chemospecific control of the methylation reaction of the sulfonyl hydrazide substrates by

using diazomethane. The hydrazines **69a-c** were then subjected to the reaction with different ratios of diazomethane: the treatment with 3.5 equiv of a 0.66M methylene chloride solution of diazomethane afforded the corresponding dimethylated derivatives **71a-c**. Also in this case, the methylation reaction shows chemospecificity, providing the corresponding products methylated on the nitrogen atoms directly bound to the nosyl groups. The same trimethylated products **72a-c** and **73a-c**, obtained from **71a-c** under the conditions previously described, were also obtained by treatment of the hydrazines **69a-c** with a large excess of diazomethane (10 equiv) for 5 h at room temperature. Therefore, the theoretical acidity order proposed above agrees well with the observed reactivity of the **69a** species with different stoichiometric ratios of diazomethane. Similar reasons can be advanced to account for the formation of the **69a** molecule starting from the **69a** one when an increased amount of diazomethane was considered. A further increase of the quantity of diazomethane added to the **69a** species yielded the **72a** and **73a** trimethylated products. These species show the peculiarity to have the third methyl group introduced on the carbonyl oxygen atom rather than on the N_c site. In this case the acidic properties do not represent the only factor to explain the results, but the charge distribution can contribute to rationalize the experimental evidence. For this purpose an NBO analysis on the anionic forms of the **69a** species was performed, and the obtained net charge values of the acidic sites and the carbonyl oxygen present in the **69a** species are collected in *Table 13*.

	N_a	N_b	N_c	O
67a				
Neutral species	-0.684	-0.904	-0.475	-0.619
An(Na)	-0.806	-0.914	-0.440	-0.708
An(Nb)	-0.716	-0.920	-0.542	-0.736
An(Nc)	-0.663	-1.007	-0.489	-0.636
69a				
Neutral species			-0.476	-0.627
An(Nc)			-0.541	-0.740

Table 13. ***NBO Charges in \|e\| for 67a and 69a species and their anionic forms***

At first glance on this table, it is possible to deduce that the N_c site is still the less favored one in the methylation process because its charge value is the least negative with respect to the other considered sites. These results support the presence of the *O*-methylated products in the course of the synthetic process. In fact the An(N_c) species shows an NBO charge value on the carbonyl oxygen (-0.636 e) of about 0.147 e more negative than that found in the Nc deprotonated site (-0.489 e). The NBO charges calculations were also extended to the **71a** neutral species and its relative anion and the obtained results are collected in *Table 13*. In the anionic species derived from **71a**, the charge value on the carbonyl oxygen (-0.740 e) is more negative than that present on the N_c site (-0.541 e, *Table 13*). These reasons can be advanced to explain the lack of the trimethylated form, having the third methyl group on the N_c position, as a possible product during the synthesis described in the present work.

2. Transformations of pregn-4-en-3β,17α,20β-triol with Lewis and anhydrous protic acids

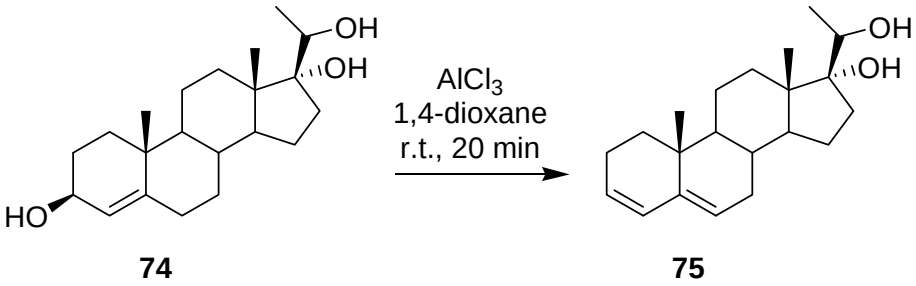
Hydroxy steroids play a pivotal role in the metabolic pathways of keto steroids. Biotransformation of this kind of compound strictly depends on the activity of carbonyl reducing enzymes/hydroxy steroids dehydrogenases.[101]Because these compounds are hormones which play vital roles in animal life, their chemical and biochemical molecular transformations have been extensively studied in recent years. The metabolic chain of hydroxy steroids is also regulated by acid-catalyzed processes which are able to produce profound modifications of the molecular structure, by generating $\Delta^{3,5}$-steradiene derivatives. These compounds can further evolve to more complex steroid derivatives through the intervention of carbocation species, furnishing stable intermediates of primary importance in the understanding of the mechanisms that regulate many chemical and biochemical reactions of steroids. A series of acid-catalyzed rearrangements are implicated in the enzymatic reactions involved in both the biosynthesis of animal hormones and in plant sterol biosynthesis.[102]These kinds of transformations have also been used in many chemical reactions carried out in the laboratory, with the aim of synthesizing suitable molecular probes useful in the investigation of the pathways related to the metabolic processes of steroid transformation that take place in nature.[103]Reactions assisted by aqueous acidic systems often lead to steroidal backbone rearrangement products.[104]For example, the acid treatment of 3-hydroxy-Δ^4-steroids affords the

corresponding $\Delta^{3,5}$-steradiene derivatives, probably via an allylic carbocation; dehydration of 3-hydroxy-Δ^4-steroids has been accomplished by treatment with 70% aqueous acetic acid at 100 °C to yield the corresponding $\Delta^{3,5}$-steradienes.[105]The conversion of 3-hydroxy-Δ^4-steroids (or 3-acetoxy-Δ^4-steroids) into $\Delta^{3,5}$-steradiene related structures has been used as a smart tool in determining gestagenic hormones by spectrophotometry,[106]and by liquid chromatography-mass spectrometry.[107]The role of $\Delta^{3,5}$-steradiene derivatives is essential in a number of applications, such as the analysis of human urine of cancer patients,[108] chemical and physical laboratory experiments for the study of the biotic chemistry of steroids,[109]the use of conjugated steroids as molecular markers for food control,[110]production of aromatic derivatives by gas-phase pyrolysis of plant sterols,[111]and the use as ideal precursors in the synthesis of polyhydroxy steroid derivatives of biological and pharmaceutical importance.[112] Thus, the clean and easy synthesis in large amounts and in excellent yields, and the knowledge of the behaviour of 3,5-steradienes obtained from naturally occurring steroids may offer the basis for the preparation of modified steroids that could be useful as standards in instrumental analyses of human matrices and other materials of biological importance.

All the reported conversions of hydroxy steroids into the corresponding $\Delta^{3,5}$-steradiene derivatives usually require harsh experimental conditions characterized by the use of concentrated aqueous acid solutions and high temperatures. Moreover, this kind of processes induces skeletal modifications of the steroids and produce the desired conjugated steradiene systems in low to moderate yields together with consistent

amounts of unknown compounds. Another class of acid-induced processes in which the hydroxyl groups of steroids are involved is the rearrangement of the D-ring in 17,20-dihydroxy steroids. The pinacol-pinacolone rearrangement of 17,20-glycols, for example, proceeds by the initial protonation of one of the two OH groups followed by migration of hydrogen or an alkyl residue.[113]The second hydroxyl group assists this rearrangement through the formation of a carbon-oxygen π-bond. Protonation of the hydroxyl group at C-17 determines a shift of the proton from C-20, while the possible formation of a carbocation at C-20 depends on the type of substituent,[114] and can drive the D-homoannulation of the steroid precursor.[115] The formation of D-homoannulated products can also be obtained by the acid treatment of 17β,20-dihydroxy-23-oxosteroids.[116]Unfortunately, many of these reactions take place in the presence of concentrated aqueous acid solutions and at high temperatures, affording complex mixtures of products that often contain variable amounts of unidentifiable species. Thus a more profound study of the transformation of steroids under different and milder acidic conditions could be of particular interest in the case of natural steroids containing a high number of hydroxy groups located in different parts of the backbone, namely the A- and D-ring. Polyhydroxy steroids often feature characteristics which are able to determine structural modifications via competitive chemical pathways regulating the formation of 3,5-steradiene simple and/or homoannulated derivatives.

The effects of non aqueous acid media in the competitive structural rearrangements possible for polyhydroxylated steroids were exploited by choosing pregn-4-en-3β,17α,20β-triol (**74**) as an ideal model characterized by the 4-en-3-ol allylic system and the 17,20-glycol function. Compound **74** was considered to be capable of generating highly reactive charged intermediates involving both the A- and D-ring. Thus we studied any possible competitive and/or simultaneous conversion of the A- and D-ring under milder experimental conditions than those already reported, performing all reactions in non-aqueous organic solvents and using Lewis acids or anhydrous protic acids. Pregn-4-en-3β,17α,20β-triol (**74**) was obtained by reduction of 17α-hydroxyprogesterone with $NaBH_4$ in methanol, according to a known synthetic route.[117]



74 **75**

Scheme 29. ***Treatment of 74 with $AlCl_3$***

Initially, the reaction of **74** and 3 molar equivalents of aluminum trichloride was analyzed. The process was carried out in dry 1,4-dioxane at room temperature; reaction times not exceeding 20 min were needed for the total consumption of the starting material (*Scheme 29*). After a simple work-up, the crude residue recovered was purified by chromatography to obtain only one product in an excellent yield (92%) based on the initial amount of

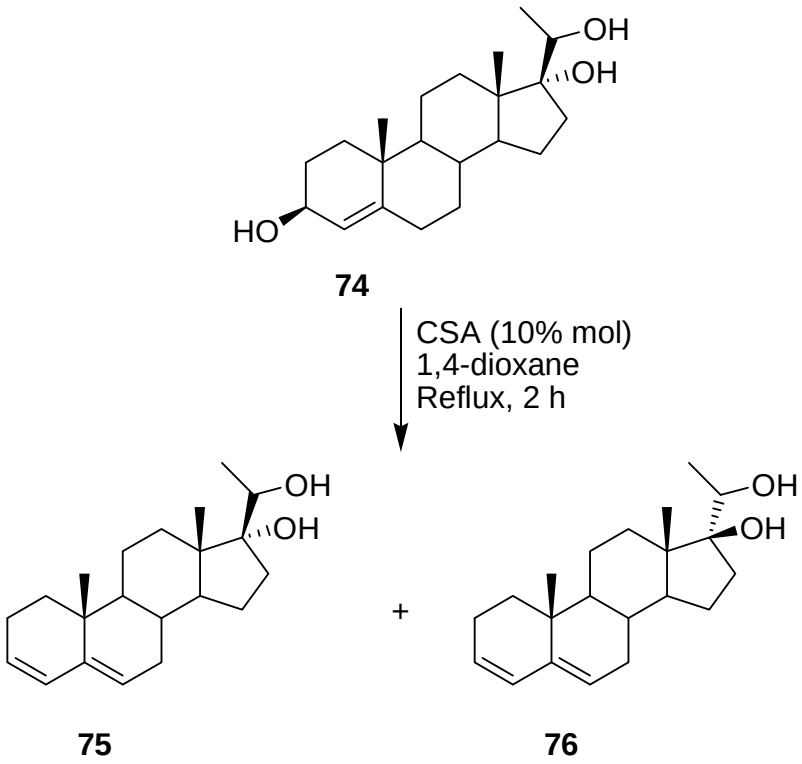
74. The compound thus obtained was recognized as pregn-3,5-dien-17α,20β-diol (**75**), and the expected molecular structure was unequivocally confirmed by one-dimensional ^{1}H and ^{13}C NMR spectroscopy, with the aid of extensive proton homo decoupling to establish the correlations between the most important proton spin systems of the molecule.

Clean ^{1}H and ^{13}C NMR spectra recorded in DMSO-d_6 at 25 °C were obtained for **75**, showing the typical signals attributable to the conjugated 3,5-diene system. In particular, the proton spectrum was characterized by a well defined doublet at 5.89 ppm and two multiplets centered at 5.56 and 5.34 ppm. These signals were indicative of the resonances of the 4-H, 6-H and 3-H protons, respectively. The multiplet at 3.75 ppm was attributed to the 20-H, and the protons of the C-17 and C-20 hydroxyl groups featured one singlet at 3.40 ppm and one doublet at 4.03 ppm**,** respectively. The assignment of the signals attributable to the hydroxyl protons was confirmed by isotopic exchange with D_2O. The data collected by one-dimensional proton NMR spectroscopy demonstrated that the functionalities of the C-17 side chain of the starting material **74** remained unaltered. Treatment of **74** with $AlCl_3$ determined only the loss of water from the allylic site of the A-ring with the consequent formation of the related 3,5-steradiene derivative. The driving force for this rapid dehydration was supposed to be the increased stability of the 3,5-steradiene structure, determined by the formation of the conjugated double bond system, with respect to the other possible isomers which could be produced from competitive pathways involving the transposition of the allylic carbocation. This charged species is most likely generated by the initial

coordination of the aluminum core of the Lewis species to the OH function at the C-3.

It is important to remark here that no evidence of D-ring rearrangements were detected by NMR spectroscopy. In particular, DEPT techniques were used to confirm the carbon backbone of **75** and to exploit the possible presence in the molecular structure of D-homoannulated moieties. The DEPT carbon analysis confirmed a five-membered structure for the D-ring, showing that no homo-annulation of the D-ring of compound **74** occurred under the experimental conditions adopted for the Lewis acid treatment. Therefore, we investigated whether it would be possible to induce the formation of D-homoannulated products using experimental conditions already reported in the literature for the Lewis acid assisted D-ring rearrangement of 17-hydroxy-20-keto steroids.[118]Following the previous protocol, 1 molar equivalent of pregn-4-en-3β,17α,20β-triol (**74**) was dissolved in dry 1,4-dioxane and treated with 3 equivalents of aluminum trichloride under reflux until the complete consumption of **74**. Hydrolytic work-up followed by column chromatography of the crude mixture afforded exclusively the product **75** in a 84% yield. Furthermore, in order to verify the reactivity of the 1,2-diol system under the acid conditions used, 1 molar equivalent of the $\Delta^{3,5}$-steradiene **75** was dissolved in dry 1,4-dioxane and treated with a molar excess (5 equivalents) of aluminum trichloride, under reflux for 3 h. Surprisingly, compound **75** was not reactive and recovered unchanged in a quantitative yield.

Successively, we exploited the reactivity of **74** towards protic acids. The trihydroxy steroid **74** was then allowed to react in the presence of catalytic amounts of anhydrous camphorsulfonic acid (CSA) in dry 1,4-dioxane (*Scheme 29*). The reaction was carried out in the presence of 10% molar ratio CSA, under reflux for 2 h. Under these conditions, we observed the formation of a mixture of the two epimers **75** and **76** (*Scheme 30*), recovered in a 89% total yield after column chromatography. The proton NMR spectra recorded on a dilute sample of the crude reaction product clearly indicated the presence of the two C-17 epimers **75** and **76** in an approximately 3:2 ratio. In fact, the one-dimensional proton NMR spectrum recorded in DMSO-d_6 at 25 °C revealed, for the obtained mixture of epimers, two sets of well distinguishable resonances attributable to the conjugated 3,5-diene system, the hydroxyl group at C-17, and the C-18, C-19, and C-20 methyl protons in both of the two epimers, together a series of overlapping signals generated by the protons of the other rings of both molecules.



Scheme 30. ***Treatment of 74 with CSA***

On the basis of the comparison with the spectroscopic data obtained for the pure epimer **75** the major isomer formed under the reaction conditions described above was recognized as the 17α epimer. The last experiment confirmed that organic protic acid catalysis converts the allylic alcoholic sub-skeleton of the A-ring into the corresponding 3,5-diene moiety by dehydration, but causes epimerization at the C-17 stereocentre. These results seem to indicate that compounds **75** and **76** are probably formed via a highly reactive intermediate carbocation located at the C-17 atom of the steroid structure. Furthermore, this probable charged species did not activate the other reaction channels already recognized in the case of steroid systems similar to **74** when they are treated with concentrated aqueous acid solutions to produce mixtures of unidentified products.

The reactivity of **74** with CSA as the catalyst was also tested by an NMR experiment, recording proton spectra in $CDCl_3$ at −10 °C at different times. A one-dimensional proton spectrum was initially obtained for the precursor **74**. The spectral window between 3.50 and 5.60 ppm clearly showed resonances at 5.28, 4.20, and 4.03 ppm attributable to the 4-H, 3-H, and 20-H protons, respectively. Catalytic amounts of CSA (10% molar ratio) was then added to the same sample and a new set of two one-dimensional proton spectra was recorded at −10 °C after 5, and 10 min. The spectrum recorded after 10 min showed the complete consumption of **74**. In particular, the signal at 4.20 ppm attributable to the 3-H proton disappeared and three new signals were visible at 5.93, 5.60, and 5.38 ppm for the 4-H, 3-H, and 6-H protons, respectively. These resonances were attributed to the 3,5-diene moiety, as confirmed by comparing the spectral data with those obtained for a sample of **75** prepared by reacting **74** with catalytic amounts of CSA in dry 1,4-dioxane. The spectrum recorded after 10 min, also showed the presence of two multiplets centered at 5.50 and 5.74 ppm, together a signal that partially overlaps the multiplet at 5.60 ppm. All these resonances were assigned to the protons of the 3,5-diene system of the second epimer **76**, according to the spectral data obtained for the mixture of epimers **75** and **76** prepared by treatment with CSA in 1,4-dioxane. Finally, no discernable resonances attributable to D-homoannulated compounds were observed in the proton spectra recorded.

The reaction performed using aluminum trichloride did not lead to epimerization at C-17 or modifications of the diol system on the D-ring. The different reaction course observed in this case

could be the result of the formation of a very stable coordination complex between the oxygen atoms of the two adjacent hydroxyl groups and the metallic core of the Lewis species. In order to confirm this hypothesis, we performed another experiment using $TiCl_4$. It is known that the Lewis acid $TiCl_4$ easily generates cyclic stable adducts involving oxygen and nitrogen atoms.[119]Treatment of one molar equivalent of **74** with three equivalents of $TiCl_4$, in dry DCM at room temperature for 20 min, gave **75** exclusively in a 89% yield, and with complete retention of the configuration at C-17. Proton NMR analysis confirmed that no epimeric mixtures were formed. The absence of any epimerization could reasonably be considered as direct proof of the stability of the supposed cyclic intermediate created by the coordination between the hydroxyl groups at C-17 and C-20 of **74** with the Lewis species. This assumption was further verified by performing the same reaction under reflux for longer periods (3-5 h). Under these new conditions, no modifications of the diol functionality on the D-ring was observed, as confirmed by proton NMR spectroscopy of a sample obtained from the hydrolytic work-up of the crude reaction mixture.

Experimental Section

General

Solvents were purified and dried by standard procedures and distilled prior to use. Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected.

Proton nuclear magnetic resonance (^{1}H NMR) spectra, and proton homodecoupling experiments were recorded at 300 MHz on a Bruker Avance 300 spectrometer. Carbon nuclear magnetic resonance (^{13}C NMR) spectra, and DEPT experiments were recorded at 75.5 MHz on the same instrument. All NMR spectra were recorded at 25 °C, using standard pulse sequence programs from the Bruker BioSpin firm. Samples were solubilized in DMSO-d_6 , $CDCl_3$, D_2O $_{or}$ CD_3OD. Chemical shift values (δ) are expressed in ppm relative to the residual proton of the solvent fixed at 2.50 ppm (central line of the quintet) for ^{1}H NMR spectra, and relative to the DMSO-d_6 resonance fixed at 39.5 ppm (central line of the septet) for ^{13}C NMR spectra. All coupling constants (J) are reported in Hertz (Hz).

GC/MS analyses were performed with an HP-5MS (30 m x 0.25 mm, PhMesiloxane 5%) capillary column. The mass detector was operated in the electron impact ionization mode (EI-MS) with an electron energy of 70 eV. GC-MS analyses were carried out in split mode, using helium as the carrier gas (1 ml/min flow rate). Elemental analysis was performed on a Perkin-Elmer Elemental Analyzer.

All reactions were monitored by thin-layer chromatography (TLC), using silica gel 60-F_{254} precoated glass plates. When required, the reactions were carried out under an inert atmosphere (N_2). The dichloromethane solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea with a classical procedure. The concentration of the diazomethane solution (0.66 M) was obtained by a backtitration performed with a standard benzoic acid solution. Caution: Diazomethane is highly toxic. Hence, this reagent must be handled carefully. Dichloromethane solutions of diazomethane are stable for long periods if stored on KOH pellets at -20 °C.

1. Preparation of N-methyl-α-amino acids from N-nosyl-α-amino acid benzhydryl esters

Preparation of Diphenyldiazomethane

Benzophenone hydrazone (1.05 mmol) in dry dichloromethane (10 mL) was treated with MagtrieveTM (15 mmol). The mixture was stirred at room temperature and immediately took on a purple color characteristic of diphenyldiazomethane. Oxidation was completed within 15 min, as checked by TLC analysis (EtOAc:hexane = 1:5) of the reaction mixture. The final mixture was used without further work-up.

N-Nosyl-α-amino acid benzhydryl esters 3a–f: general procedure

A solution of N-nosyl-α-amino acid **1a–f** (1 mmol) in dichloromethane was added to the purple solution of diphenyldiazomethane, prepared as previously described (1.05 mmol). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature. TLC analysis (solvent system A: EtOAc:hexane = 1:5; solvent system B: Et2O:petroleum ether = 1:1) showed complete conversion of the precursors **1a–f** after 40 min. After the reaction, MagtrieveTM was retrieved by filtration through a celite short pad and the solution was concentrated under reduced pressure to afford the corresponding benzhydryl esters **3a–f** in quantitative yields.

*N-Nosyl-L-valine benzhydryl ester (**3a**)*

Yellow solid, Mp 95–97 °C. Rf = 0.38 (solvent system A), 0.53 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.98–8.01 (m, 2 H, *o*-NO_2), 7.82–7.92 (m, 2 H, *m*-NO_2), 7.08–7.36 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.62 (s, 1 H, RCO_2CHPh_2), 5.85 (d, J = 9.2 Hz, 1 H, NH), 4.00 (m, 1 H, α-CH), 2.24 (m, 1 H, $(CH_3)2CH$), 0.98–1.02 (m, 3 H, $(CH_3)_2CH$), 0.82–0.88 (m, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.1, 149.8, 145.3, 138.9, 128.6, 128.4, 128.2, 127.8, 127.5, 126.9, 126.8, 126.8, 126.6, 124.1, 78.4, 61.3, 31.6, 19.2, 17.0 ppm. GC/MS (EI) m/z (%) 257 (100), 186 (20), 167 (54), 122 (35). Anal. calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16; N, 5.98; O, 20.49; S, 6.84. Found: C, 61.49; H, 5.17; N, 5.96.

*N-Nosyl-D-valine benzhydryl ester (**3b**)*

Yellow solid, Mp 96–98 °C. Rf = 0.38 (solvent system A), 0.53 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.1 Hz, 2 H, *o*-NO_2), 7.81 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.18–7.39 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.65 (s, 1 H, RCO_2CHPh_2), 5.88 (d, J = 9.2 Hz, 1 H, NH), 4.02 (m, 1 H, α-CH), 2.22 (m, 1 H, $(CH_3)_2CH$), 1.02 (m, J = 6.6 Hz, 3 H, $(CH_3)_2CH$), 0.82 (d, J = 6.6 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, CDCl3) δ 170.1, 149.8, 145.3, 138.9, 128.6, 128.4, 128.2, 127.8, 127.5, 126.9, 126.8, 126.6, 126.8, 124.1, 78.4, 61.3, 31.6, 19.2, 17.0 ppm. GC/MS (EI) m/z (%) 257 (100), 186 (20), 167 (54), 122 (35). Anal. calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16; N, 5.98; O, 20.49; S, 6.84. Found: C, 61.45; H, 5.15; N, 5.95.

*N-Nosyl-L-leucine benzhydryl ester (**3c**)*

Yellow solid, Mp 95–97 °C. Rf = 0.37 (solvent system A), 0.32 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.2 Hz, 2 H, *o*-NO2), 7.82 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.12–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.58 (s, 1 H, RCO_2CHPh_2), 5.52 (d, J = 9.3 Hz, 1 H, NH), 4.15 (m, 1 H, α-CH), 1.80 (m, 1 H, (CH_3)2CHCH2), 1.52–1.60 (m, 2 H, $(CH_3)_2CHCH_2$), 0.82–1.03 (m, 6 H, $(CH_3)_2$

$CHCH_2$) ppm. GC/MS (EI) m/z (%) 271 (60), 215 (22), 186 (28), 167 (100), 122 (21). Anal. calcd for $C_{25}H_{26}N_2O_8S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.33; H, 5.41; N, 5.79.

*N-Nosyl-S-benzyl-L-cysteine benzhydryl ester (**3d**)*

Yellow solid, Mp 91–93 °C. Rf = 0.53 (solvent system A), 0.42 (solvent system B). ^{1}H-NMR (300 MHz, CDCl3) δ 8.06 (d, J = 9.1

Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.08–7.42 (m, 15 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$), 6.70 (s, J = 9.3 Hz, 1 H, RCO_2CHPh_2), 6.06 (d, J = 9.1 Hz, 1 H, NH), 4.35 (m, 1 H, α-CH), 3.60–3.70 (m, 2 H, SCH_2Ph), 2.81–2.88 (m, 2 H, CH_2SBzl) ppm. Anal. calcd for $C_{29}H_{26}N_2O_6S_2$: C, 61.90; H, 4.66; N, 4.98; O, 17.06; S, 11.40. Found: C, 62.03; H, 4.65; N, 4.97.

*N-Nosyl-L-isoleucine benzhydryl ester (**3e**)*

Yellow solid, Mp 92–94 °C. Rf = 0.46 (solvent system A), 0.51 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.21–7.40 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.58 (s, 1 H, RCO_2CHPh_2), 5.60 (s, J = 9.3 Hz, 1 H, NH), 3.98 (d, J = 8.3 Hz, 1 H, α-CH), 1.90 (m, 1 H, $CH(CH_3)CH_2CH_3$), 1.50 (m, 1 H, $-CH(CH_3)CH_2CH_3$), 1.22 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.94 (d, J = 4.8 Hz, 3 H, $–CH(CH_3)CH_2CH_3$), 0.81 (t, J = 7.3 Hz, 3 H, $CH(CH_3)CH_2CH_3$) ppm. GC/MS (EI) m/z (%) 271 (60), 186 (28), 167 (100), 122 (21). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.18; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.23; H, 5.41; N, 5.82.

*N-Nosyl-L-alanine benzhydryl ester (**3f**)*

Yellow solid, Mp 100–102 °C. Rf = 0.26 (solvent system A), 0.34 (solvent system B). ^{1}H-NMR (300 MHz, CDCl3) δ 8.04 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.19–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.64 (s, 1 H, RCO_2CHPh_2), 5.76 (d, J = 9.1 Hz, 1 H, NH), 4.23 (m, 1 H, α-CH), 1.48 (d, J = 6.0 Hz, 3 H, CH_3) ppm. GC/MS (EI) m/z (%) 229 (44), 186 (18), 167 (100), 122 (15). Anal.

calcd for $C_{22}H_{20}N_2O_6S$: C, 59.99; H, 4.58; N, 6.36; O, 21.79; S, 7.28. Found C, 59.99; H, 4.59; N, 6.34.

N-Methyl-N-nosyl-α-amino acid benzhydryl esters 4a–f: general procedure

A 0.66 M solution of diazomethane in dry dichloromethane (8 mmol) was cautiously added dropwise to a magnetically stirred solution of the *N*-nosyl-α-amino acid benzhydryl esters **3a–f** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was stirred at room temperature. TLC analysis (solvent system A: EtOAc: hexane = 1:5; solvent system B: Et2O: petroleum ether = 1:1) showed the complete conversion of the precursors **3a–f** after 1.5 h. Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** in quantitative yields.

*N-Methyl-N-nosyl-L-valine benzhydryl ester (**4a**)*

Yellow solid, Mp 91–93 °C. Rf = 0.50 (solvent system A), 0.64 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 2 H, *o*-NO_2), 7.78 (d, J = 8.4 Hz, 2 H, *m*-NO_2), 7.16–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.62 (s, 1 H, RCO_2CHPh_2), 4.35 (d, J = 10.5 Hz, 1 H, α-CH), 2.92 (s, 3 H, NCH_3), 2.18 (m, 1 H, $(CH_3)_2CH$), 1.05 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$), 0.88 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.9, 149.8, 145.3, 138.9, 128.8, 128.5, 128.4, 128.3, 128.2, 127.0, 126.9, 124.3, 77.9, 65.2, 30.3, 28.2, 19.3 ppm. GC/MS (EI) m/z (%) 271 (100), 186 (20), 167 (62), 122 (35). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.15; H, 5.42; N, 5.79.

*N-Methyl-N-nosyl-D-valine benzhydryl ester (**4b**)*

Yellow solid, Mp 95–97 °C. Rf = 0.50 (solvent system A), 0.64 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 8.4 Hz, 2 H, *m*-NO_2), 7.14–7.35 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.67 (s, 1 H, RCO_2CHPh_2), 4.39 (d, J = 10.5 Hz, 1 H, α-CH), 2.94 (s, 3 H, NCH_3), 2.21 (m, 1 H, $(CH_3)_2CH$), 1.03 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$), 0.85 (d, J = 6.3 Hz, 3 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.9, 149.8, 145.3, 138.9, 128.8, 128.5, 128.4, 128.3, 128.2, 127.0, 126.9, 124.3, 77.9, 65.2, 30.3, 28.2, 19.3 ppm. GC/MS (EI) m/z (%) 271 (100), 186 (20), 167 (62), 122 (35). Anal. calcd for $C_{25}H_{26}N_2O_6S$: C, 62.23; H, 5.43; N, 5.81; O, 19.89; S, 6.64. Found: C, 62.41; H, 5.44; N, 5.79.

*N-Methyl-N-nosyl-L-leucine benzhydryl ester (**4c**)*

Yellow solid, Mp 93–95 °C. Rf = 0.57 (solvent system A), 0.58 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.0 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.0 Hz, 2 H, *m*-NO2), 7.18–7.38 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.60 (s, 1 H, RCO_2CHPh_2), 4.84 (m, 1 H, α-CH), 2.90 (s, 3 H, NCH_3), 1.69–1.78 (m, 3 H, $(CH_3)_2CHCH_2$ and $(CH_3)_2CHCH_2$), 1.03 (d, J = 6.0 Hz, 3 H, $(CH_3)_2CHCH_2$), 0.99 (d, J = 6.0 Hz, 3 H, $(CH_3)_2CHCH_2$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.8, 144.3, 139.1, 130.0, 128.6, 128.5, 126.7, 124.0, 78.0, 57.7, 38.2, 30.0, 24.5, 23.0, 21.0 ppm. GC/MS (EI) m/z (%) 285 (100), 186 (22), 167 (42), 122 (15). Anal. calcd for $C_{26}H_{28}N_2O_6S$: C, 62.89; H, 5.68; N, 5.64; O, 19.33; S, 6.46. Found: C, 63.01; H, 5.69; N, 5.63.

*N-Methyl-N-nosyl-S-benzyl-L-cysteine benzhydryl ester (**4d**)*

Amorphous yellow solid. Rf = 0.62 (solvent system A), 0.63 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 9.1 Hz, 2 H, *o*-NO_2), 7.88 (d, J = 9.1 Hz, 2 H, *m*-NO_2), 7.18–7.42 (m, 15 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$), 6.70 (s, 1 H, RCO_2CHPh_2), 4.90 (m, 1 H, α-CH), 3.76–3.82 (m, 2 H, SCH_2Ph), 2.98 (m, 1 H, CH_2SBzl), 2.81 (s, 3 H, NCH_3), 2.65 (m, 1 H, CH_2SBzl) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.1, 149.8, 139.1, 138.9, 137.3, 128.5, 128.4, 128.3, 128.0, 126.7, 124.1, 78.6, 58.6, 35.7, 30.2, 30.1 ppm. Anal. calcd for $C_{30}H_{28}N_2O_6S_2$: C, 62.48; H, 4.89; N, 4.86; O, 16.65; S, 11.12. Found: C, 62.22; H, 4.91; N, 4.84.

*N-Methyl-N-nosyl-L-isoleucine benzhydryl ester (**4e**)*

Yellow solid, Mp 100–102 °C. Rf = 0.59 (solvent system A), 0.70 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 9.0 Hz, 2 H, *o*-NO_2), 7.75 (d, J = 9.0 Hz, 2 H, *m*-NO_2), 7.20–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.60 (s, 1 H, RCO_2CHPh_2), 4.47 (d, J = 12.3 Hz, 1 H, α-CH), 2.94 (s, 3 H, NCH_3), 1.97 (m, 1 H, $CH(CH_3)CH_2CH_3$), 1.62 (m, 1 H, $CH(CH_3)CH_2CH_3$), 1.26 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.95 (t, J = 7.2 Hz, 3 H, $CH(CH_3)CH_2CH_3$), 0.82 (d, J = 6.9 Hz, 3 H, $CH(CH_3)CH_2CH_3$) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.0, 149.7, 144.3, 128.3, 128.2, 127.5, 127.0, 124.0, 77.8, 63.8, 34.2, 30.5, 25.3, 15.3, 10.4 ppm. GC/MS (EI) m/z (%) 285 (100), 229 (32), 186 (21), 167 (72). Anal. calcd for $C_{26}H_{28}N_2O_6S$: C, 62.89; H, 5.68; N, 5.64; O, 19.33; S, 6.46. Found: C, 62.96; H, 5.64; N, 5.67.

*N-Methyl-N-nosyl-L-alanine benzhydryl ester (**4f**)*

Yellow solid, Mp 102–105 °C. Rf = 0.44 (solvent system A), 0.57 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 9.3 Hz, 2 H, *o*-NO_2), 7.80 (d, J = 9.3 Hz, 2 H, *m*-NO_2), 7.10–7.42 (m, 10

H, $RCO_2CH(C_6H_5)_2$), 6.68 (s, 1 H, RCO_2CHPh_2), 4.93 (m, 1 H, α-CH), 2.85 (s, 3 H, NCH_3), 1.52 (d, J = 7.2 Hz, 3 H, CH_3) ppm. GC/MS (EI) m/z (%) 243 (100), 186 (26), 167 (64), 122 (20). Anal. calcd for $C_{23}H_{22}N_2O_6S$: C, 60.78; H, 4.88; N, 6.16; O, 21.12; S, 7.06. Found: C, 60.95; H, 4.87; N, 6.17.

Synthesis of N-methyl-N-nosyl-α-amino acid 5a–f: general procedure

To a solution of the *N*-methyl-*N*-nosyl-α-amino acid benzhydryl esters **4a–f** (1 mmol) in dichloromethane (2 mL), trifluoroacetic acid (5–7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3 x 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-*N*-nosyl-α-amino acid **5a–f** in 94–98% overall yields. Spectroscopic data of **5a–f** matched those obtained for the same compounds as reported elsewhere.

*N-Methyl-N-nosyl-L-valine (**5a**)*

Yield 94%. Anal. calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; O, 30.35; S, 10.14. Found: C, 45.46; H, 5.11; N, 8.85.

*N-Methyl-N-nosyl-D-valine (**5b**)*

Yield 94%. Anal. calcd for $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; O, 30.35; S, 10.14. Found: C, 45.62; H, 5.09; N, 8.87.

*N-Methyl-N-nosyl-L-leucine (**5c**)*

Yield 94%. Anal. calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; O, 29.06; S, 9.71. Found: C, 47.34; H, 5.50; N, 8.46.

*N-Methyl-N-nosyl-S-benzyl-L-cysteine (**5d**)*

Yield 95%. Anal. calcd for $C_{17}H_{18}N_2O_6S_2$: C, 49.74; H, 4.42; N, 6.82; O, 23.39; S, 15.62. Found: C, 49.91; H, 4.41; N, 6.83.

*N-Methyl-N-nosyl-L-isoleucine (**5e**)*

Yield 98%. Anal. calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; O, 29.06; S, 9.71. Found: C, 47.32; H, 5.50; N, 8.47.

*N-Methyl-N-nosyl-L-alanine (**5f**)*

Yield 98%. Anal. calcd for $C_{10}H_{12}N_2O_6S$: C, 41.66; H, 4.20; N, 9.72; O, 33.30; S, 11.12. Found: C, 41.52; H, 4.20; N, 9.73.

Removal of the nosyl group from N-methyl-N-nosyl-α-amino acid benzhydryl esters 4a–f: synthesis of 6a–f general procedure

To a solution of **4a–f** (1 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (3 mmol) was added and the mixture was maintained at 50 °C. Sodium methoxide (7 mmol) was then gradually added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The

resulting mixture was stirred for 40 min monitoring the conversion of the precursors **4a–f** by TLC (EtOAc:hexane = 1:5) and GC/MS analyses. Aqueous 1 N HCl was then added and the acidified solution (pH 2) was extracted with EtOAc (3 x 10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 and then extracted with EtOAc (39 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-α-amino acid benzhydryl esters **6a–f** in 90–96% overall yields. Compounds **6a–f** have been characterized only by GC/MS and immediately subjected to the next step.

*N-Methyl-L-valine benzhydryl ester (**6a**)*

Oil (96%). Rf = 0.55. GC/MS (EI) m/z (%) 167 (46), 86 (100).

*N-Methyl-D-valine benzhydryl ester (**6b**)*

Oil (96%). Rf = 0.55. GC/MS (EI) m/z (%) 167 (46), 86 (100).

*N-Methyl-L-leucine benzhydryl ester (**6c**)*

Oil (95%). Rf = 0.61. GC/MS (EI) m/z (%) 167 (42), 100 (100).

*N-Methyl-S-benzyl-L-cysteine benzhydryl ester (**6d**)*

Oil (92%). Rf = 0.46. MS (ESI-TOF) m/z calcd for $C_{24}H_{26}NO_2S$: 392.1684; found: 392.1697.

*N-Methyl-L-isoleucine benzhydryl ester (**6e**)*

Oil (95%). Rf = 0.63. GC/MS (EI) m/z (%) 167 (46), 86 (100).

*N-Methyl-L-alanine benzhydryl ester (**6f**)*

Oil (90%). Rf = 0.50. GC/MS (EI) m/z (%) 167 (64), 58 (100).

Synthesis of N-Fmoc-N-methyl-α-amino acid benzhydryl esters 7a–f: general procedure

The *N*-methyl-α-amino acid benzhydryl esters **6a–f** (1 mmol) were suspended in aqueous $NaHCO_3$ (6 mL, pH 8). A solution of FmocCl (1 mmol) in dichloromethane (6 mL) was added gradually and the resulting mixture was stirred at room temperature for 1 h monitoring the conversion of the precursors **6a–b** by TLC (solvent system A: EtOAc:hexane = 1:5; solvent system B: Et2O:petroleum ether = 1:1) and GC/MS analyses. After the reaction, the dichloromethane layer was separated and the aqueous phase was extracted with three additional portions of dichloromethane (3 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-Fmoc-α-amino acid benzhydryl esters **7a–f** in 85–94% overall yields.

*N-Fmoc-N-methyl-L-valine benzhydryl ester (**7a**)*

Yield 92%. Amorphous white solid. Rf = 0.65 (solvent system A), 0.81 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.65– 7.90 (m, 8 H), 7.25–7.48 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.99 (s, 1 H, A, RCO_2CHPh_2), 6.94 (s, 1 H, B, RCO_2CHPh_2), 4.76 (d, J = 10.3 Hz, 1 H, α-CH), 4.50–4.64 (m, 2 H), 4.29 (m, 1 H), 2.87 (s, 3 H, NCH_3), 2.30 (m, 1 H, A, $(CH_3)_2CH$), 2.18

(m, 1 H, B, $(CH_3)_2CH$), 1.02 (d, J = 6.5 Hz, 3 H, A, $(CH_3)_2CH$), 0.96 (d, J = 6.5 Hz, 3 H, B, $(CH_3)_2CH$), 0.90 (d, J = 6.5 Hz, 3 H, B, $(CH_3)_2CH$), 0.77 (d, J = 6.5 Hz, 3 H, A, $(CH_3)_2CH$) ppm. Anal. calcd for $C_{34}H_{33}NO_4$: C, 78.59; H, 6.40; N, 2.70; O, 12.32. Found: C, 78.70; H, 6.41; N, 2.69.

*N-Fmoc-N-methyl-D-valine benzhydryl ester (**7b**)*

Yield 92%. Amorphous white solid. Rf = 0.65 (solvent system A), 0.81 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.62– 7.88 (m, 8 H), 7.21–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 7.00 (s, 1 H, A, RCO_2CHPh_2), 6.94 (s, 1 H, B, RCO_2CHPh_2), 4.80 (d, J = 10.3 Hz, 1 H, α-CH), 4.52–4.62 (m, 2 H), 4.30 (m, 1 H), 2.88 (s, 3 H, NCH_3), 2.28 (m, 1 H, A, $(CH_3)_2CH$), 2.12 (m, 1 H, B, $(CH_3)_2CH$), 0.99 (d, J = 6.5 Hz, 3 H, A, $(CH_3)_2CH$), 0.95 (d, J = 6.5 Hz, 3 H, B, $(CH_3)_2CH$), 0.89 (d, J = 6.5 Hz, 3 H, A, $(CH_3)_2CH$), 0.75 (d, J = 6.5 Hz, 3 H, B, $(CH_3)_2CH$) ppm. Anal. calcd for $C_{34}H_{33}NO_4$: C, 78.50; H, 6.40; N, 2.70; O, 12.32. Found: C, 78.59; H, 6.41; N, 2.79.

*N-Fmoc-N-methyl-L-leucine benzhydryl ester (**7c**)*

Yield 90%. Amorphous white solid. Rf = 0.67 (solvent system A), 0.75 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B d 8.20 (d, J = 9.3 Hz, 2 H), 7.82–7.90 (m, 2 H), 7.59–7.68 (m, 4 H), 7.12–7.43 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.76 (s, 1 H, A, RCO_2CHPh_2), 6.61 (s, 1 H, B, RCO_2CHPh_2), 4.78–4.86 (m, 2 H, A+ B, α-CH), 4.22–4.65 (m, 3 H, Fmoc-CH and Fmoc-CH_2), 2.98 (s, 3 H, NCH_3), 1.71–1.75 (m, 3 H, $(CH_3)_2CHCH_2$ and

$(CH_3)_2CHCH_2$), 0.98–1.20 (m, 6 H,A+ B, $(CH_3)_2CHCH_2$) ppm. Anal. calcd for $C_{35}H_{35}NO_4$: C, 78.77; H, 6.61; N, 2.62.

*N-Fmoc-N-methyl-S-benzyl-L-cysteine benzhydryl ester (**7d**)*

Yield 95%. Amorphous yellow solid. Rf = 0.74 (solvent system A), 0.76 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 7.92– 8.02 (m, 4 H, Fmoc-Ar), 7.50–7.60 (m, 2 H, Fmoc-Ar), 7.22–7.45 (m, 17 H, $RCO_2CH(C_6H_5)_2$ and $SCH_2C_6H_5$ and Fmoc-Ar), 6.82 (s, 1 H, A, RCO_2CHPh_2), 6.72 (s, 1 H, B, RCO_2CHPh_2), 4.98 (m, 1 H, A, α-CH), 4.85 (m, 1 H, B, α-CH), 4.10–4.52 (m, 3 H, Fmoc-CH and Fmoc-CH_2), 3.64– 3.72 (m, 2 H, CH_2SBzl), 3.08 (m, 1 H, B, CH_2SBzl), 3.02 (m, 1 H, A, CH_2SBzl), 2.86 (s, 3 H, A, NCH_3), 2.81 (s, 3 H, B, NCH_3), 2.45–2.72 (m, 2 H, A+B, CH_2SBzl) ppm. Anal. calcd for $C_{39}H_{35}NO_4S$: C, 76.32; H, 5.75; N, 2.28; O, 10.43; S, 5.22. Found: C, 76.48; H, 5.73; N, 2.29.

*N-Fmoc-N-methyl-L-isoleucine benzhydryl ester (**7e**)*

Yield 94%. Amorphous white solid. Rf = 0.68 (solvent system A), 0.76 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 55:45 mixture of two rotamers A and B δ 8.08 (d, J = 9.3 Hz, 2 H), 7.70–7.78 (m, 2 H), 7.52–7.60 (m, 4 H), 7.20–7.48 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.84 (s, 1 H, A, RCO_2CHPh_2), 6.82 (s, 1 H, B, RCO_2CHPh_2), 4.75 (d, J = 12.3 Hz, 1 H, α-CH), 4.42–4.52 (m, 4 H, A+ B, Fmoc-CH_2), 4.15–4.30 (m, 1 H, Fmoc-CH), 2.78 (s, 3 H, NCH_3), 1.99 (m, 1 H, A, $CH(CH_3)CH_2CH_3$), 1.92 (m, 1 H, B, $CH(CH_3)CH_2CH_3$), 1.38 (m, 1 H, A $CH(CH_3)CH_2CH_3$), 1.24 (m, 1 H, B, $CH(CH_3)CH_2CH_3$), 1.06 (m, 1 H, $CH(CH_3)CH_2CH_3$), 0.85–0.90 (m, 3 H, $CH(CH_3)CH_2CH_3$), 0.73–0.79 (m, 3 H, $CH(CH_3)CH_2CH_3$) ppm. Anal. Calcd for $C_{35}H_{35}NO_4$: C,

78.77; H, 6.61; N, 2.62; O, 11.99. Found: C, 78.57; H, 6.62; N, 2.61.

*N-Fmoc-N-methyl-L-alanine benzhydryl ester (**7f**)*

Yield 88%. Amorphous white solid. Rf = 0.57 (solvent system A), 0.74 (solvent system B). ^{1}H-NMR (300 MHz, $CDCl_3$), 60:40 mixture of two rotamers A and B δ 7.96 (d, J = 9.3 Hz, 2 H), 7.64–7.71 (m, 2 H), 7.12–7.35 (m, 14 H, $RCO_2CH(C_6H_5)_2$ and Fmoc-Ar), 6.90 (s, 1 H, A, RCO_2CHPh_2), 6.88 (s, 1 H, B, RCO_2CHPh_2), 4.95 (m, 1 H, A, α-CH), 4.85 (m, 1 H, B, α-CH), 4.35–4.48 (m, 3 H, Fmoc-CH and Fmoc-CH_2), 2.88 (s, 3 H, B, NCH_3), 2.82 (s, 3 H, A, NCH_3), 1.38–1.41 (m, 3 H, CH_3) ppm. Anal. calcd for $C_{33}H_{31}NO_3$: C, 80.95; H, 6.38; N, 2.86; O, 9.80. Found: C, 81.13; H, 6.37; N, 2.84.

Synthesis of N-methyl-N-Fmoc-α-amino acid 8a–f: general procedure

To a solution of the appropriate *N*-methyl-*N*-Fmoc-α-amino acid benzhydryl esters **7a–f** in dichloromethane (2 mL) trifluoroacetic acid (5–7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3 x 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford the *N*-methyl-*N*-Fmoc-α- amino acid **8a–f** in 94–98% overall yields.

*N-Fmoc-N-methyl-L-valine (**8a**)*

Yield 96%. Anal. calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96; O, 18.11. Found: C, 71.40, H, 6.58, N, 3.98.

*N-Fmoc-N-methyl-D-valine (**8b**)*

Yield 96%. Anal. calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96; O, 18.11. Found: C, 71.12; H, 6.57; N, 3.97.

*N-Fmoc-N-methyl-L-leucine (**8c**)*

Yield 98%. Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81; O, 17.42. Found: C, 71.72; H, 6.87; N, 3.79.

*N-Fmoc-N-methyl-S-benzyl-L-cysteine (**8d**)*

Yield 94%. Anal. calcd for $C_{26}H_{25}NO_4S$: C, 69.78; H, 5.63; N, 3.13; O, 14.30; S, 7.16. Found: C, 69.59; H, 5.64; N, 3.11.

*N-Fmoc-N-methyl-L-isoleucine (**8e**)*

Yield 98%. Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81; O, 17.42. Found: C, 71.66; H, 6.88; N, 3.80.

*N-Fmoc-N-methyl-L-alanine (**8f**)*

Yield 96%. Anal. calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; O, 19.67. Found: C, 70.39; H, 5.86; N, 4.30.

Synthesis of N-nosyl-dipeptides benzhydryl esters 10a–b: general procedure

The appropriate *N*-methyl-α-amino acid benzhydryl esters **6a–b** (1 mmol) was suspended in an aqueous solution of $NaHCO_3$ (6 mL, pH 8). A solution of *N*-nosyl-D-alanine chloride (1 mmol) in dichloromethane (6 mL) was added gradually and the resulting mixture was stirred at room temperature for 1 h monitoring the conversion of the precursors **6a–b** by GC/MS analysis. After the reaction, the organic layer was separated and the aqueous phase was extracted with three additional portions of dichloromethane (3 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford **10a–b** in 89–91% yields.

*N-Nosyl-D-alanyl-N-methyl-L-valine benzhydryl ester (**10a**)*
Amorphous yellow solid (89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 8.12 (d, J = 9.3 Hz, 1 H, NH), 8.02 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.22–7.39 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.88 (s, 1 H, RCO_2CHPh_2), 4.85 (d, J = 10.8 Hz, 1 H, $CHCH(CH_3)_2$), 4.32 (m, 1 H, $CHCH_3$), 2.75 (s, 3 H, –NCH_3), 2.13 (m, 1 H, $CH(CH_3)_2$), 1.2 (d, J = 6.9 Hz, 3 H, CH_3), 0.87 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.46 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$) ppm. Anal. calcd for $C_{28}H_{31}N_3O_7S$: C, 60.74; H, 5.64; N, 7.59; O, 20.23; S, 5.79. Found: C, 60.68; H, 5.65; N, 7.58.

*N-Nosyl-D-alanyl-N-methyl-D-valine benzhydryl ester (**10b**)*

Amorphous yellow solid (91%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 9.2 Hz, 2 H, *o*-NO_2), 8.02 (d, J = 9.2 Hz, 2 H, *m*-NO_2), 7.95 (d, J = 9.3 Hz, 1 H, NH), 7.20–7.42 (m, 10 H, $RCO_2CH(C_6H_5)_2$), 6.85 (s, 1 H, RCO_2CHPh_2), 4.75 (d, J = 10.8 Hz, 1 H, $CHCH(CH_3)_2$), 4.30–

4.40 (m, 1 H, $CHCH_3$), 2.82 (s, 3 H, NCH_3), 2.20 (m, 1 H, $CH(CH_3)_2$), 1.30 (d, J = 6.9 Hz, 3 H, CH_3), 0.87 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$), 0.72 (d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$) ppm. Anal. calcd for $C_{28}H_{31}N_3O_7S$: C, 60.74; H, 5.64; N, 7.59; O, 20.23; S, 5.79. Found: C, 60.93; H, 5.62; N, 7.61.

Synthesis of N-nosyl-dipeptides 11a–b: general procedure

To a solution of the *N*-nosyl-dipeptide benzhydryl esters **10a–b** in dichloromethane (2 mL), trifluoroacetic acid (5– 7 mL) and toluene (2 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. After evaporation of the solvent under reduced pressure, saturated aqueous Na_2CO_3 was added and the aqueous solution was extracted with dichloromethane (3 x 20 mL). Aqueous 2 N HCl was then added and the acidified solution was extracted with EtOAc (39 20 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford **11–b** in 98% overall yields.

*N-Nosyl-D-alanyl-N-methyl-L-valine (**11a**)*

Yield 89%; Yellow solid; Mp 107–111 °C. ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.48 (d, J = 9.3 Hz, 1 H), 8.48 (d, J = 9.0 Hz, 2 H, *o*-NO_2), 8.05 (d, J = 9.0 Hz, 2 H, *m*-NO_2), 4.33–4.42 (m, 1 H, $CHCH(CH_3)_2$), 3.88 (m, 1 H, $CHCH_3$), 2.96 (s, 3 H, NCH_3), 2.02 (m, 1 H, $CH(CH_3)_2$), 1.14 (d, J = 7.4 Hz, 3 H, CH_3), 0.88 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.52 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$) ppm. Anal. calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; O, 28.91; S, 8.28. Found: C, 46.48; H, 5.45; N, 10.87.

*N-Nosyl-D-alanyl-N-methyl-D-valine (**11b**)*

Yield 98%; Yellow solid; Mp 105–108 °C. ^{1}H-NMR (300 MHz, DMSO-d6) δ 8.53 (d, J = 9.3 Hz, 1 H, *o*-NO_2), 8.48 (d, J = 9.0 Hz, 2 H, *m*-NO_2), 8.05 (d, J = 9.0 Hz, 2 H, NH), 4.52 (d, J = 10.8 Hz, 1 H, $CHCH(CH_3)_2$), 3.88 (m, 1 H, $CHCH_3$), 2.96 (s, 3 H, NCH_3), 2.02 (m, 1 H, -CH $(CH_3)_2$), 1.14 (d, J = 7.4 Hz, 3 H, CH_3), 0.88 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$), 0.52 (d, J = 7.0 Hz, 3 H, $CH(CH_3)_2$) ppm. Anal. calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; O, 28.91; S, 8.28. Found: C, 46.49; H, 5.43; N, 10.89.

Synthesis of N-nosyl-N-methyl-dipeptides 12a–b: general procedure

A 0.66 M solution of diazomethane in dry dichloromethane (8 mmol) was added cautiously dropwise to a suspension of the dipeptides **11a–b** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was magnetically stirred at room temperature under N_2. Evaporation of the solvent under reduced pressure afforded the *N*-methylated dipeptides **12a–b** in quantitative yields.

*N-Methyl-N-nosyl-D-alanyl-N-methyl-L-valine methyl ester (**12a**)*

Yield 98%. Yellow oil. GC/MS (EI) m/z (%) 243 (100), 229 (20), 186 (18), 122 (28), 56 (24). Anal. calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 48.98; H, 6.08; N, 10.09.

*N-Methyl-N-nosyl-D-alanyl-N-methyl-D-valine methyl ester (**12b**)*

Yield 98%. Yellow oil. GC/MS (EI) m/z (%) 243 (100), 229 (20), 186 (15), 122 (24), 56 (20). Anal. calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 49.23; H, 6.06; N, 10.12.

2. Preparation of N-methyl-α-amino acids from N-nosyl-α-amino acid phenacyl esters

N-Nosyl-α-amino Acid Phenacyl Esters 14a-i: general procedure

The *N*-nosyl-R-amino acids (1 mmol) were dissolved in ethanol and cooled to 0 °C. An aqueous solution of cesium carbonate (0.5 mmol) was added slowly and the reaction mixture was stirred for 1 h at room temperature. Then the solvent was evaporated under reduced pressure to afford the corresponding cesium salts of *N*-nosyl-R-amino acids **13a-i** as yellow solids in quantitative yields. To a solution of **12a-l** (1 mmol) in *N*,*N*-dimethylformamide (DMF) was added slowly a solution of phenacyl bromide (1 mmol) in DMF. During the reaction a white solid of cesium bromide was formed. The reaction mixture was stirred for about 1 h, monitoring the conversion of **13a-i** by TLC (diethyl ether/petroleum ether 70:30 v/v). The white solid was then separated by filtration and the solvent was evaporated under reduced pressure. The residue was treated with a 9% aqueous solution of sodium carbonate and extracted with chloroform

(3x10 mL). The combined organic extracts were washed with water and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the corresponding *N*-nosyl-R-amino acid phenacyl esters **14a-i** as pale yellow solids in 79-99% overall yields.

*N-Nosyl-L-isoleucine phenacyl ester (**14a**)*

yellow solid (95%); Mp 191-192 °C; $[\alpha]^{25}D$ +12.5 (*c* 0.50, $CHCl_3$); ^1H NMR (300 MHz,DMSO-d_6): δ 8.75 (d, 1H, J=9.3 Hz), 8.39 (d, 2H, J=9.0 Hz), 8.03 (d, 2H, J=9.0 Hz), 7.91-7.84 (m, 2H), 7.67 (m, 1H), 7.56-7.49 (m, 2H), 5.31 (s, 2H), 3.92 (dd, 1H, J=6.0 Hz, J=9.3 Hz), 1.85 (m, 1H), 1.48 (m, 1H), 1.17 (m, 1H), 0.92 (d, 3H, J = 6.9 Hz), 0.81 (t, 3H, J = 7.5 Hz) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 192.5, 170.4, 149.9, 146.9, 134.5, 134.1, 129.3, 128.5, 128.2, 124.8, 67.2, 60.7, 37.5, 24.6, 15.6, 11.4 ppm. MS(EI)m/z (rel intensity, %) 377 (0.4), 315 (0.8), 271 (26), 248 (2), 215 (17), 192 (21), 186 (9), 122 (15), 120 (55), 105 (100), 77 (24), 65 (3). nal. Calcd for $C_{20}H_{22}N_2O_7S$: C, 55.29; H, 5.10; N, 6.45; S, 7.38. Found: C, 55.11; H, 5.12; N, 6.48; S, 7.34.

N-Methyl-N-nosyl-α-amino acid Phenacyl Esters (15a-i): general procedure

A 0.66M solution of diazomethane in dichloromethane (6 mmol) was added cautiously dropwise to a stirred solution of *N*-nosyl-R-amino acid phenacyl esters **14a-l** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 1 h monitoring the conversion of the precursor by TLC

analysis (diethyl ether/petroleum ether 70:30 v/v). Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyl-R-amino acid phenacyl esters **15a-i** as oils in quantitative yields.

*N-Methyl-N-nosyl-L-isoleucine phenacyl ester (**15a**):*

yellow oil; $[\alpha]^{25}_{D}$ -33.0 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, 2H, J=9.0 Hz), 8.07 (d, 2H, J=9.0 Hz), 7.78-7.72 (m, 2H), 7.61 (m, 1H), 7.51-7.43 (m, 2H), 5.19 (d, 1H, J=16.2 Hz), 5.11 (d, 1H, J=16.2 Hz), 4.50 (d, 1H, J=10.5 Hz), 2.98 (s, 3H), 2.02 (m, 1H), 1.68 (m, 1H), 1.23 (m, 1H), 1.04 (d, 3H, J = 6.3 Hz), 0.98 (t, 3H, J = 7.5 Hz ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.5, 168.4, 149.9, 144.6, 134.2, 133.6, 129.1, 128.9, 127.5, 123.9, 65.9, 63.9, 33.6, 30.5, 24.9, 15.5, 10.7 ppm. MS (EI) m/z (rel intensity, %) 405 (0.1), 391 (3), 329 (0.9), 285 (100), 262 (13), 229 (26), 188 (13), 186 (12), 122 (20), 120 (23), 105 (44), 77 (20), 65 (3). Anal. Calcd for $C_{21}H_{24}N_2O_7S$: C, 56.24; H, 5.39; N, 6.25; S, 7.15. Found: C, 56.41; H, 5.41; N, 6.27; S, 7.18.

N-Methyl-N-nosyl-α-amino acid (16a-e): general procedure

Sodium benzenethiolate (5 mmol) was added cautiously to a stirred solution of *N*-methyl-*N*-nosyl-R-amino acid phenacyl esters **15a-e** (1 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 30 min monitoring the conversion of the precursor by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with an aqueous solution of 1N

NaOH and extracted with chloroform (3x10 mL). The resulting aqueous basic solution was then acidified with a solution of 1 N HCl and then extracted with chloroform (3x10 mL). The combined organic extracts were washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the corresponding *N*-methyl-*N*-nosyl-R-amino acids **16a-e** as oils in 70-87% overall yields. *N*-methyl- *N*-nosyl-R-amino acids **16a-e** (1 mmol) by treating with a diazomethane solution (3 mmol) were converted into the corresponding methyl esters and analyzed by GC/MS.

*N-Methyl-N-nosyl-L-isoleucine (**16a**)*:

yellow oil (70%); ^{1}H-NMR (300 MHz, CD_3OD): δ 8.40 (d, 2H, J = 9.0 Hz), 8.05 (d, 2H, J=9.0 Hz), 4.16 (d, 1H, J=10.5 Hz), 2.90 (s, 3H), 1.90 (m, 1H), 1.60 (m, 1H), 1.17 (m, 1H), 0.98-0.91 (m, 6H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ 171.2, 149.9, 143.9, 128.6, 123.8, 63.7, 33.2, 29.6, 24.8, 14.3, 9.3 ppm. Anal. Calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; S, 9.71. Found: C, 47.32; H, 5.47; N, 8.46; S, 9.68.

N-Methyl-N-nosyl-L-isoleucine methyl ester

(EI) m/z (rel intensity, %) 287 (32), 285 (100), 229 (31), 186 (23), 122 (20), 57 (3).

Synthesis of N-Acetyl-N-methyl-L-valine (18b).

Sodium benzenethiolate (2.37 mmol) was added cautiously to a stirred solution of *N*-methyl-*N*-nosyl-L-valine phenacyl esters

(**15b**) (0.47 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 12 h monitoring the conversion of the precursor by TLC analysis (diethyl ether/petroleum ether 70:30 v/v) and verifying the deprotection of the amino function by ninhydrin test. After evaporation of the solvent under reduced pressure the obtained residue was treated with an aqueous solution of 1 N NaOH and extracted with chloroform (3 x10 mL). The resulting aqueous basic solution was then acidified with a solution of 1 N HCl and then extracted with chloroform (3x10 mL). The evaporation of the organic solvent provided the *N*-methyl-L-valine (**17b**) (0.061 g) in 40% yields. The aqueous phase was made basic with a saturated Na_2CO_3 solution (pH 9.0) and treated with acetic anhydride (0.95 mmol) in chloroform at room temperature for 1 h. The mixture, after extraction with chloroform, was acidified with 1N HCl and again extracted with chloroform (3x8 mL). The combined organic extracts were dried and evaporated to dryness to afford the *N*-acetyl-*N*-methyl-L-valine (**18b**) (0.020 g) in 25% yield. The *N*-acetyl-*N*-methyl-L-valine (**18b**) 0.020 g, 0.12 mmol) by treating with a dichloromethane solution of diazomethane (0.36 mmol) was converted into the corresponding methyl esters and analyzed by GC/MS.

N-Acetyl-N-methyl-L-valine methyl ester

MS (EI) m/z (rel intensity, %) 187 (2), 144 (5), 128 (54), 102 (64), 86 (100), 43 (22).

N-Fmoc-N-methyl-α-amino Acid 19a-e: general procedure

Sodium benzenethiolate (10 mmol) was added to a stirred solution of *N*-methyl-*N*-nosyl-R-amino acid phenacyl esters **15a-e** (1 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 3 h monitoring the progress of the deprotection reaction by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with 1N HCl (pH 2) and the aqueous solution was extracted with ethyl acetate (3 x10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 (pH 9). To the basic liquors cooled at 0 °C, containing the corresponding completely deprotected *N*-methyl-R-amino acids **17a-e**, was added dropwise a solution of 9-fluorenylmethyloxycarbonyl chloride (1 mmol) in dioxane. The reaction mixture was stirred at 0 °C for 3 h with monitoring the complete conversion of the precursor **17a-e** by TLC analysis (chloroform/ methanol 90:10 v/v). After evaporation of the solvent under reduced pressure the basic aqueous solution was extracted with diethyl ether. The aqueous solution was then acidified with 1 N HCl (pH 2) and extracted with ethyl acetate (4x10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness to afford the *N*-Fmoc-*N*-methyl-R-amino acids **19a-e** (70-75% overall yields).

*N-Fmoc-N-methyl-L-isoleucine (**19a**)*

white solid (70%). Mp 182-183 °C; $[\alpha]^{25}D$ -51.4 (*c* 1.00, $CHCl_3$); ^1H NMR(300 MHz, DMSO-d_6) [mixture of two rotational isomers A and B (80:20)]: δ 7.94-7.24 (m, 8H), 4.48-3.92 (m, 4H), 2.71 (A) and 2.69 (B) (2s, 3H), 1.80 (m, 1H), 1.34-1.07 (m, 2H), 0.93-0.72

(m, 6H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) [two rotational isomers]: δ 172.5, 156.4, 144.3, 144.2, 141.3, 128.1, 127.5, 125.4, 120.5, 67.2, 62.7, 59.9, 47.2, 33.1, 30.4, 25.1, 22.1, 16.2, 10.8 ppm. Anal. Calcd for $C_{22}H_{25}NO_4C$, 71.91; H, 6.86; N, 3.81. Found: C, 71.69; H, 6.87; N, 3.82.

N-Fmoc-N-methyl-α-amino acid 19f-i: general procedure

Sodium benzenethiolate (10 mmol) was added to a stirred solution of *N*-methyl-*N*-nosyl-R-amino acid phenacyl esters **15f-i** (1 mmol) in dimethylformamide (DMF). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 3 h monitoring the progress of the deprotection reaction by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with a 5% aqueous solution of $KHSO_4$ (pH 3-4) and extracted with ethyl acetate (3x 10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 (pH 9). To the basic liquor kept at 0 °C by an ice bath, containing the corresponding completely deprotected *N*-methyl-R-amino acids **17f-i**, was added dropwise a solution of 9-fluorenylmethyloxycarbonyl chloride (1 mmol) in dioxane. The reaction mixture was stirred at 0 °C for 3 h monitoring the complete conversion of the precursor **17f-i** by TLC analysis (chloroform/methanol 90:10 v/v). After evaporation of the solvent under reduced pressure the basic aqueous solution was extracted with diethyl ether. The aqueous solution was then acidified with a 5% aqueous solution of $KHSO_4$ (pH 3-4) and extracted with ethyl acetate (4x10 mL). The combined organic

extracts were dried (Na_2SO_4) and evaporated to dryness to afford the*N*-Fmoc-*N*-methyl-R-amino acids **19f-i** (70-78% overall yields).

*N-Fmoc-N-methyl-O-tert-butyl-L-tyrosine (**19i**)*

(78%); $[\alpha]^{25}D$ -48.1 (*c* 1.00, DMF); ^1H NMR(300 MHz, DMSO-d_6) [mixture of two rotational isomers A and B (60:40)]: δ 12.85 (br s, 1H), 7.88-7.25 (m, 8H), 7.08 (d, 2H, J = 8.4 Hz), 6.80 (d, 2H, J=8.4 Hz), 4.71 (A) and 4.63 (B) (2 dd, 1H, J=4.8, 11.4 Hz), 4.31-4.10 (m, 3H), 3.19-2.89 (m, 2H), 2.68 (A) and 2.66 (B) (2s, 3H), 1.20 (B) and 1.15 (A) (2s, 9H) ppm. ^{13}C NMR(75 MHz, DMSO-d_6) [two rotational isomers]: δ 172.5, 172.3, 156.1, 153.8, 144.2, 144.1, 141.2, 133.0, 132.8, 129.7, 128.1, 127.5, 125.4, 123.9, 120.6, 78.0, 67.2, 60.9, 47.1, 46.9, 33.7, 32.2, 31.7, 28.9 ppm. Anal. Calcd for $C_{29}H_{31}NO_5$: C, 73.55; H, 6.60; N, 2.96. Found: C, 73.69; H, 6.62; N, 2.97.

3. Preparation of N-ethyl-α-amino acids from N-nosyl-α-amino acid methyl esters

Synthesis of N-Ethyl-N-nosyl Amino Acid Methyl Esters 21a–l: general procedure

DIPEA (3.5 mmol) and solid triethyloxonium tetrafluoroborate (2.5 mmol) were added to a solution of **20a–l** (150 mg, 1 mmol) in CH_2Cl_2 (20 mL) under an inert atmosphere. The reaction

mixture was stirred at room temperature for 10 min. The mixture was quenched with a 1N HCl solution (or a 10% citric acid solution for compounds **20g** and **l**) and then washed with a 1N NaOH solution. The organic layer was extracted with CH_2Cl_2 (2x10 mL) and dried with Na_2SO_4. Evaporation of the solvent afforded the corresponding *N*-ethyl-*N*-nosyl amino acid methyl esters **21a–l** as yellow oils in 85–99% overall yields.

*N-Ethyl-N-nosyl-L-alanine Methyl Ester (**21a**)*:

By following the general procedure, treatment of a solution of *N*-nosyl-alanine methyl ester (**20a**) (150 mg, 0.52 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.32 mL, 1.82 mmol) and triethyloxonium tetrafluoroborate (222 mg, 1.3 mmol) afforded **21a** (144 mg, 96% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.34 (d, *J* = 9Hz, 2 H, *o*-NO2), 8.01 (d, *J* = 9Hz, 2 H, *m*-NO2), 4.71 (q, *J* = 7.2 Hz, 1 H, α-C*H*), 3.54 (s, 3 H, OC*H*3), 3.39 (m, 1 H, NC*H*2CH3), 3.19 (m, 1 H, NC*H*2CH3), 1.51 (d, *J* = 7.5 Hz, 3 H, C*H*3), 1.25 (t, *J* = 7.1 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 171.2, 149.9, 145.7, 127.8, 123.9, 55.7, 52.3, 41.2, 17.1, 16.6 ppm. MS (EI) (rel. intensity %): *m*/*z* = 257 (100), 229 (10), 186 (25), 122 (17), 56 (14). $C_{12}H_{16}N_2O_6S$ (316.07): calcd. C 45.56, H 5.10, N 8.86, O 30.35, S 10.14; found C 45.47, H 5.08, N 8.88.

*N-Ethyl-N-nosyl-L-valine Methyl Ester (**21b**)*

By following the general procedure, treatment of a solution of *N*-nosyl-valine methyl ester (**20b**) (150 mg, 0.47 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.29 mL, 1.64 mmol) and triethyloxonium tetrafluoroborate (200 mg, 1.2 mmol) afforded

21b (134 mg, 89% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.33 (d, *J* = 8.7 Hz, 2 H, *o*-NO2), 8.03 (d, *J* = 8.7 Hz, 2 H, *m*-NO2), 4.13 (d, *J* = 10.5 Hz, 1 H, α-C*H*), 3.51 (m 1 H, NC*H*2CH3), 3.45 (s, 3 H, OC*H*3), 3.30 (m, 1 H, NC*H*2CH3), 2.10 [m, 1 H, C*H*(CH3)2], 1.23 (t, *J* = 7.7 Hz, 3 H, NCH2C*H*3), 1.07 [d, *J* = 6.6 Hz, 3 H, CH(C*H*3)2], 0.95 [d, *J* = 6.6 Hz, 3 H, CH(C*H*3)2] ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): δ = 170.6, 149.5, 145.9, 128.7, 123.9, 65.9, 51.6, 40.6, 28.6, 19.7, 16.2 ppm. MS (EI) (rel. intensity %): *m*/*z* = 301 (28), 285 (100), 186 (19), 158 (5), 122 (10), 56 (12). $C_{14}H_{20}N_2O_6S$ (344.10): calcd. C 48.83, H 5.85, N 8.13, O 27.87, S 9.31; found C 48.71, H 5.86, N 8.10.

*N-Ethyl-N-nosyl-L-leucine Methyl Ester (**21c**)*

Following the general procedure, treatment of a solution of *N*-Nosyl-leucine methyl ester **20c** (150 mg, 0.45 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.27 mL, 1.59 mmol) and triethyloxonium tetrafluoroborate (192 mg, 1.12 mmol) afforded **21c** (146 mg, 97% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.33 (d, *J* = 8.7 Hz, 2 H, *o*-NO2), 8.01 (d, *J* = 8.7 Hz, 2 H, *m*-NO2), 4.65 (m, 1 H, α- C*H*), 3.48 (s, 3 H, OC*H*3), 3.35 (m, 1 H, NC*H*2CH3), 3.20 (m, 1 H, NC*H*2CH3), 1.80–1.61 [m, 3 H, C*H*2CH(CH3)2 + CH2C*H*- (CH3)2], 1.26 (t, *J* = 7.7 Hz, 3 H, NCH2C*H*3), 1.01 [d, *J* = 6.3 Hz, 3 H, CH2CH(C*H*3)2], 0.98 [d, *J* = 6.3Hz, 3 H, CH2CH(C*H*3)2] ppm. ^{13}C NMR (CDCl3, 75 MHz, 25 °C): δ = 171.4, 149.9, 145.5, 128.7, 123.9, 58.5, 52.1, 42.2, 41.1, 39.2, 24.4, 22.9, 21.3, 16.6 ppm. MS (EI) (rel. intensity %): *m*/*z* = 299 (100), 243 (11), 186 (11), 122 (7), 56 (7). $C_{15}H_{22}N_2O_6S$ (358.12): calcd. C 50.27, H 6.19, N 7.82, O 26.78, S 8.95; found C 50.39, H 6.17, N 7.80.

*N-Ethyl-N-nosyl-L-isoleucine Methyl Ester (**21d**)*

By following the general procedure, treatment of a solution of *N*-nosyl-isoleucine methyl ester (**20d**) (150 mg, 0.45 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.28 mL, 1.57 mmol) and triethyloxonium tetrafluoroborate (192 mg, 1.12 mmol) afforded **21d** (153 mg, 95%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.33 (d, *J* = 9Hz, 2 H, *o*-NO2), 8.01 (d, *J* = 9Hz, 2 H, *m*-NO2), 4.21 (d, *J* = 10.5 Hz, 1 H, α-C*H*), 3.54 (m, 1 H, NC*H*2CH3), 3.43 (s, 3 H, OC*H*3), 3.30 (m, 1 H, NC*H*2CH3), 1.92–1.71 [m, 2 H, CH(CH3)C*H*2CH3], 1.28–1.12 [m, 4 H, NCH2C*H*3 + C*H*(CH3)CH2CH3], 0.86–0.98 [m, 6 H, CH(C*H*3)CH2CH3 + CH(CH3)CH2C*H*3] ppm. ^{13}C NMR (CDCl3, 75 MHz, 25 °C): δ = 170.7, 149.9, 145.5, 128.6, 123.9, 64.7, 51.5, 40.6, 34.8, 25.3, 16.4, 15.4, 10.8 ppm. MS (C.I.): *m*/*z* (%) = 387 (9) [M + C2H5]+, 359 (7) [M + H]+, 329 (2), 299 (100), 269 (4), 172 (3), 156 (3), 114 (2). $C_{16}H_{24}N_2O_6S$ (358.12): calcd. C 51.60, H 6.50, N 7.52, O 25.78, S 8.61; found C 51.72, H 6.48, N 7.54.

*N-Ethyl-N-nosyl-D-alloisoleucine Methyl Ester (**21e**)*

By following the general procedure, treatment of a solution of *N*-nosyl-D-alloisoleucine methyl ester (**20e**) (150 mg, 0.45 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.28 mL, 1.57 mmol) and triethyloxonium tetrafluoroborate (192 mg, 1.12 mmol) afforded **21e** (154 mg, 96%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.34 (d, *J* = 9Hz, 2 H, *o*-NO_2), 8.04 (d, *J* = 9Hz, 2 H, *m*-NO_2), 4.23 (d, *J* = 7.0 Hz, 1 H, α-C*H*), 3.53 (m, 1 H, NC*H*2CH3), 3.46 (s, 3 H, OC*H*3), 3.31 (m, 1 H, NC*H*2CH3), 2.03–1.90 [m, 2 H, CH(CH3)C*H*2CH3], 1.30–1.13 [m, 4 H, C*H*(CH3)CH2CH3 +

NCH2C*H*3], 0.99–0.83 [m, 6 H, CH(C*H*3)CH2CH3 + CH(CH3)CH2C*H*3] ppm. ^{13}C NMR (CDCl3, 75MHz, 25 °C): δ = 170.5, 149.7, 145.3, 128.7, 123.9, 63.7, 51.5, 40.2, 34.0, 25.7, 16.3, 15.2, 11.1 ppm. MS (C.I.): *m*/*z* (%) = 387 (8) [M + C2H5]+, 359 (6) [M + H]+, 329 (5), 299 (100), 269 (11), 172 (9), 156 (10), 114 (8). $C_{16}H_{24}N_2O_6S$ (358.12): calcd. C 51.60, H 6.50, N 7.52, O 25.78, S 8.61; found C 51.70, H 6.51, N 7.49.

*N-Ethyl-N-nosyl-L-phenylalanine Methyl Ester (**21f**)*

By following the general procedure, treatment of a solution of *N*-nosyl-phenylalanine methyl ester (**20f**) (150 mg, 0.41 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.25 mL, 1.44 mmol) and triethyloxonium tetrafluoroborate (175 mg, 1.02 mmol) afforded **21f** (148 mg, 99% yield) as yellow oil. ^{1}H NMR (CDCl3, 300 MHz, 25 °C): δ = 8.20 (d, *J* = 8.7 Hz, 2 H, *o*-NO2), 7.75 (d, *J* = 8.7 Hz, 2 H, *m*-NO2), 7.18–7.30 (m, 5 H, CH2C6*H*5), 4.88 (dd, *J* = 6.6, 8.7 Hz, 1 H, α-C*H*), 3.58 (s, 3 H, OC*H*3), 3.25–3.50 (m, 3 H, C*H*2Ph + NCH2C*H*3), 2.99 (m, 1 H, C*H*2Ph), 1.19 (t, *J* = 7.0 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR (CDCl3, 75 MHz, 25 °C): δ = 170.8, 149.7, 145.8, 136.4, 129.1, 128.8, 128.5, 127.1, 123.9, 61.4, 52.4, 40.9, 36.5, 15.6 ppm. MS (EI) (rel. intensity %): *m*/*z* = 333 (25), 301 (100), 186 (17), 122 (12), 91 (14), 56 (12). $C_{18}H_{20}N_2O_6S$ (392.10): calcd. C 55.09, H 5.14, N 7.14, O 24.46, S 8.17; found C 54.96, H 5.16, N 7.13.

*N-Ethyl-N-nosyl-L-glutamic acid γ-tert-Butyl Methyl Diester (**21g**):*

By following the general procedure, treatment of a solution of *N*-nosyl-glutamic acid(O*t*Bu) methyl ester (**20g**) (150 mg, 0.37 mmol) in dry CH_2Cl_2 (20 mL), with DIPEA (0.23 mL, 1.29 mmol) and triethyloxonium tetrafluoroborate (176 mg, 0.92 mmol) afforded **21g** (135 mg, 85% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.34 (d, *J* = 9Hz, 2 H, *o*-NO2), 8.02 (d, *J* = 9 Hz, 2 H, *m*-NO2), 4.66 (dd, *J* = 10.5, 4.8 Hz, 1 H, α-C*H*), 3.51 (s, 3 H, OC*H*3), 3.38 (m, 1 H, NC*H*2CH3), 3.14 (m, 1 H, NC*H*2CH3), 2.48–2.43 (m, 2 H, γ- C*H*2), 2.33 (m, 1 H, β-C*H*2), 1.92 (m, 1 H, β-C*H*2), 1.47 (s, 9 H, *t*Bu), 1.24 (t, *J* = 7.2 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 171.7, 170.6, 149.9, 145.5, 128.7, 123.9, 80.9, 59.6, 52.3, 42.2, 31.2, 28.1, 25.3, 14.1 ppm. $C_{18}H_{26}N_2O_8S$ (430.14): calcd. C 50.22, H 6.09, N 6.51, O 29.73, S 7.45; found C 50.32, H 6.07, N 6.49.

*N-Ethyl-N-nosyl-S-benzyl-L-cysteine Methyl Ester (**21h**)*

By following the general procedure, treatment of a solution of *N*-nosyl-cysteine(SBn) methyl ester (**20h**) (150 mg, 0.36 mmol) in dry CH_2Cl_2 (20 mL), with DIPEA (0.22 mL, 1.26 mmol) and triethyloxonium tetrafluoroborate (171 mg, 0.90 mmol) afforded **21h** (138 mg, 88% yield). ^{1}H NMR (CDCl3, 300 MHz, 25 °C): δ = 8.32 (d, *J* = 9.0 Hz, 2 H, *o*-NO2), 8.14 (d, *J* = 9.0 Hz, 2 H, *m*-NO2), 7.40–7.25 (m, 5 H, SCH2C6*H*5), 4.62 (t, *J* = 7.8 Hz, 1 H, α-C*H*), 3.76 (s, 2 H, SC*H*2Ph), 3.60 (s, 3 H, OC*H*3), 3.30 (m, 1 H, NC*H*2CH3), 3.18 (m, 1 H, NC*H*2CH3), 3.03 (dd, *J* = 13.8, 7.2 Hz, 1 H, C*H*2SBn), 2.71 (dd, *J* = 13.8, 8.2 Hz, 1 H, C*H*2SBn), 1.13 (t, *J* = 7.0 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR (CDCl3, 75MHz, 25 °C): δ = 169.8, 151.6, 145.4, 137.2, 129.0, 128.7, 128.6, 127.4, 124.0, 59.6, 52.5,

41.2, 36.4, 31.5, 15.7 ppm. $C_{19}H_{22}N_2O_6S_2$ (438.09): calcd. C 52.04, H 5.06, N 6.39, O 21.89, S 14.62; found C 51.98, H 5.08, N 6.38.

*N-Ethyl-N-nosyl-O-benzyl-L-tyrosine Methyl Ester (**21i**)*

By following the general procedure, treatment of a solution of *N*-nosyl-tyrosine(OBn) methyl ester (**20i**) (150 mg, 0.32 mmol) in dry CH_2Cl_2 (20 mL) with DIPEA (0.19 mL, 1.12 mmol) and triethyloxonium tetrafluoroborate (136 mg, 0.8 mmol) afforded **21i** (136 mg, 91% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 8.23 (d, *J* = 9 Hz, 2 H, *o*-NO2), 7.79 (d, *J* = 9 Hz, 2 H, *m*-NO2), 7.49– 7.32 (m, 5 H, OCH2C6*H*5), 7.10 (d, *J* = 8.7 Hz, 2 H, *m*-OBn), 6.88 (d, *J* = 8.7 Hz, 2 H, *o*-OBn), 5.03 (s, 2 H, OC*H*2Ph), 4.81 (dd, *J* = 7.2, 8.4 Hz, 1 H, α-C*H*), 3.56 (s, 3 H, OC*H*3), 3.50–3.25 (m, 3 H, C*H*2PhOBn + NCH2C*H*3), 2.93 (m, 1 H, C*H*2PhOBn), 1.20 (t, *J* = 7.2 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): δ = 170.8, 157.8, 149.6, 146.7, 130.2, 128.5, 128.3, 128.1, 127.6, 123.9, 115.1, 70.1, 61.6, 52.3, 40.9, 35.7, 15.8 ppm. MS (EI) (rel. intensity %): *m/z* = 281 (32), 225 (100), 207 (60), 91 (8). $C_{25}H_{26}N_2O_7S$ (498.15): calcd. C 60.23, H 5.26, N 5.62, O 22.46, S 6.43; found C 60.37, H 5.24, N 5.63.

*N^{α}-Ethyl-N^{α}-nosyl-N^{ε}-(tert-butyloxycarbonyl)-L-lysine methyl ester (**21l**)*

By following the general procedure, treatment of a solution of N^{α}-nosyl-lysine(Boc) methyl ester (**20l**) (150 mg, 0.34 mmol) in dry CH_2Cl_2 (20 mL), with DIPEA (0.21 mL, 1.19 mmol) and triethyloxonium tetrafluoroborate (161 mg, 0.85 mmol) afforded **21l** (150 mg, 93% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ =

8.34 (d, *J* = 9.0 Hz, 2 H, *o*-NO_2), 8.00 (d, *J* = 9.0 Hz, 2 H, *m*-NO_2), 4.54 (dd, *J* = 9.9, 5.4 Hz, 1 H, α-C*H*), 3.49 (s, 3 H, OC*H*3), 3.34 (m, 1 H, NC$H_2$$CH_3$), 3.22–3.09 (m, 3 H, NC$H_2$$CH_3$ + ε-CH_2), 1.98 (m, 1 H, β-CH_2), 1.78–1.65 (m, 2 H, δ-CH_2 + β-CH_2), 1.56– 1.48 (m, 3 H, γ-CH_2 + δ-CH_2), 1.44 (s, 9 H, *t*Bu), 1.23 (t, *J* = 7.2 Hz, 3 H, NCH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): *δ* = 170.9, 168.2, 156.0, 150.3, 128.6, 123.9, 80.0, 60.2, 52.2, 41.6, 34.0, 29.8, 29.4, 28.4, 23.2, 16.4 ppm. C20H31N3O8S (473.18): calcd. C 50.73, H 6.60, N 8.87, O 27.03, S 6.77; found C 50.88, H 6.59, N 8.89.

Synthesis of N-Ethyl-N-Fmoc Amino Acid Methyl Esters 23a–h: general procedure

Mercaptoacetic acid (3 mmol) was added to a solution of **21a–h** (1 mmol) in dry acetonitrile (10 mL) and the reaction mixture was maintained at 50 °C. Sodium methoxide (8 mmol) was then gradually added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for 30 min and the conversion of the precursors **21a–h** was monitored by TLC (Et_2O/petroleum ether = 6:4). A 1N HCl solution (or a 10% citric acid solution for compound **21g**) was then added and the mixture extracted with EtOAc (3x10 mL). The aqueous phase was basified with saturated aqueous $NaHCO_3$ (pH 8). The basic phase, containing the deprotected product, was then treated with a solution of Fmoc chloride (1 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1 h and then the organic layer was separated. The aqueous phase was extracted with three additional portions of CH_2Cl_2 (3x10 mL). The combined

organic extracts were dried with Na_2SO_4 and the solvents evaporated under vacuum to afford the corresponding *N*-ethyl-*N*-Fmoc-amino acid methyl esters as oils in 71–94% overall yields.

*N-Ethyl-N-Fmoc-L-alanine Methyl Ester (**23a**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-alanine methyl ester (**21a**) (200 mg, 0.63 mmol) with the reagent system mercaptoacetic acid (0.13 mL, 1.89 mmol)/sodium methoxide (272 mg, 5.04 mmol), and subsequently with Fmoc chloride (163 mg, 0.63 mmol), afforded **23a** as a colorless oil (200 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.81–7.28 (m, 8 H, Fmoc-Ar*H*), 4.61–4.10 (m, 4 H, Fmoc-C*H*, Fmoc-C*H*2, α-C*H*), 3.70 and 3.60 (2 s, 3 H, OC*H*3), 3.43–3.29, 3.24–3.05 (2 m, 2 H, NC*H*2), 1.44, 1.33 (2 d, *J* = 7.2 Hz, 3 H, CHC*H*3), 1.15, 1.06 (2 t, *J* = 7.2 Hz, 3 H, NCH2C*H*3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): (rotamers) δ 171.5, 155.2, 143.8, 141.4, 127.7, 127.0, 124.9, 119.9, 67.3, 54.9, 52.2, 47.3, 40.8, 15.4, 14.9 ppm. $C_{21}H_{23}NO_4$ (353.16): calcd. C 71.37, H 6.56, N 3.96, O 18.11; found C 71.56, H 6.54, N 3.97.

*N-Ethyl-N-Fmoc-L-valine Methyl Ester (**23b**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-valine methyl ester (**21b**) (200 mg, 0.58 mmol) with the reagent system mercaptoacetic acid (0.12 mL, 1.74 mmol)/sodium methoxide (250 mg, 4.64 mmol), and subsequently with Fmoc chloride (150 mg, 0.58 mmol), afforded **23b** as a colorless oil (201 mg, 91% yield). ^{1}H NMR ($CDCl_3$, 300

MHz, 25 °C): (rotamers) δ 7.78–7.30 (m, 8 H, Fmoc-Ar*H*), 4.59–4.40 (2 m, 2 H, Fmoc-CH_2), 4.34 (d, J = 10.5 Hz, 1 H, α-C*H*), 4.26 (t, J = 6 Hz, 1 H, Fmoc-C*H*), 3.71, 3.65 (2 s, 3 H, OCH_3), 3.40–3.07 (2 m, 2 H, NCH_2), 2.18, 2.06 [2 m, 1 H, C*H*(CH_3)$_2$], 1.10 (t, J = 7.0 Hz, 3 H, NCH_2CH_3), 0.99–0.83 [m, 6 H, CH(CH_3)$_2$] ppm. ^{13}C NMR (CDCl3, 75 MHz, 25 °C): (rotamers) δ 173.4, 157.2, 144.0, 142.7, 141.4, 128.2, 127.7, 127.3, 125.1, 124.8, 120.0, 119.9, 71.6, 64.0, 51.9, 47.4, 39.2, 27.7, 19.8, 18.8, 14.1, 13.5 ppm. $C_{23}H_{27}NO_4$ (381.19): calcd. C 72.42, H 7.13, N 3.67, O 16.78; found C 72.51, H 7.14, N 3.65.

*N-Ethyl-N-Fmoc-L-leucine Methyl Ester (**23c**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-leucine methyl ester 6 (**21c**) (200 mg, 0.56 mmol) with the reagent system mercaptoacetic acid (0.11 mL, 1.68 mmol)/sodium methoxide (242 mg, 4.48 mmol), and subsequently with Fmoc chloride (145 mg, 0.56 mmol), afforded **23c** as a colorless oil (176 mg, 80% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.80–7.27 (m, 8 H, Fmoc-Ar*H*), 4.74, 4.64 (2m, 1 H, α-C*H*), 4.58–4.42 (2m, 1 H, Fmoc-CH_2), 4.35–4.22 (2m, 1 H, Fmoc-C*H*), 3.70, 3.60 (2 s, 3 H, OCH_3), 3.48–3.25 (2 m, 1 H, NCH_2CH_3), 1.90–1.46 [2m, 3 H, $CH_2CH(CH_3)_2$ + $CH_2CH(CH_3)_2$], 1.24–1.12 (2m, 3H, NCH_2NCH_3), 1.08–0.94 [2m, 6H, $CH_2CH(CH_3)_2$] ppm. ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): (rotamers) δ 172.3, 156.2, 144.0, 141.3, 128.3, 128.0, 127.6, 127.4, 125.0, 124.8, 120.3, 120.1, 67.3, 67.0, 57.4, 52.1, 50.3, 47.2, 46.7, 40.9, 40.1, 38.4, 38.2, 24.7, 23.1, 21.8, 14.6, 13.9 ppm. $C_{24}H_{29}NO_4$ (395.21): calcd. C 72.89, H 7.39, N 3.54, O 16.18; found C 72.93, H 7.40, N 3.55.

*N-Ethyl-N-Fmoc-L-isoleucine Methyl Ester (**23d**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-isoleucine methyl ester (**21d**) (200 mg, 0.56 mmol) with the reagent system mercaptoacetic acid (0.11 mL, 1.68 mmol)/sodium methoxide (242 mg, 4.48 mmol), and subsequently with Fmoc chloride (145 mg, 0.56 mmol), afforded **23d** as a colorless oil (208 mg, 94% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.75–7.28 (m, 8 H, Fmoc-Ar*H*), 4.64–4.42 (m, 2 H, Fmoc-CH_2), 4.25 (t, J = 5.9 Hz, 1 H, Fmoc-C*H*), 4.15 (m, 1 H, α-C*H*), 3.68 (s, 3 H, OCH_3), 3.44– 3.11 (m, 2 H, NCH_2), 1.93 [m, 1 H, C*H*(CH_3)CH_2CH_3], 1.42–1.32 [m, 2 H, CH(CH_3)CH_2CH_3], 1.13 (t, J = 6.6 Hz, 3 H, NCH_2CH_3), 0.96–0.81 [m, 6 H, CH(CH_3)CH_2CH_3, CH(CH_3)CH_2CH_3] ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): (rotamers) δ 171.9, 156.9, 144.1, 141.5, 127.4, 127.3, 125.1, 119.9, 67.3, 63.1, 51.8, 47.5, 39.4, 33.8, 24.8, 15.9, 14.2, 10.9 ppm. $C_{24}H_{29}NO_4$ (395.21): calcd. C 72.89, H 7.39, N 3.54, O 16.18; found C 73.08, H 7.37, N 3.53.

*N-Ethyl-N-Fmoc-L-phenylalanine Methyl Ester (**23f**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-phenylalanine methyl ester (**21f**) (200 mg, 0.51 mmol) with the reagent system mercaptoacetic acid (0.11 mL, 1.53 mmol)/sodium methoxide (220 mg, 4.08 mmol), and subsequently with Fmoc chloride (132 mg, 0.51 mmol), afforded **23f** as a colorless oil (198 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.82–7.13 (m, 13 H, Fmoc-Ar*H* + C_6H_5), 4.81–3.99 (m, 4 H, Fmoc-C*H*, Fmoc-CH_2, α-C*H*), 3.74, 3.47 (2 s, 3

H, OC*H*$_3$), 3.36 (dd, *J* = 14.1, 5.4 Hz, 1 H, C*H*2Ph), 3.19 (2 m, 1 H, NC*H*$_2$CH$_3$), 2.97 (dd, *J* = 14.1, 5.4 Hz, 1 H, C*H*$_2$Ph), 2.79 (2 m, 1 H, NC*H*$_2$CH$_3$), 0.87–0.79 (2 m, 3 H, NCH$_2$C*H*$_3$) ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): (rotamers) *δ* 171.4, 155.8, 144.4, 141.5, 137.9, 129.2, 128.5, 127.6, 127.0, 126.6, 125.1, 120.1, 67.2, 65.2, 62.1, 61.6, 52.3, 50.3, 47.3, 43.3, 42.9, 35.6, 35.3, 13.7, 13.0 ppm. $C_{27}H_{27}NO_4$ (429.51): calcd. C 75.50, H 6.34, N 3.26, O 14.90; found C 75.36, H 6.35, N 3.24.

*N-Ethyl-N-Fmoc-L-glutamic Acid γ-tert-Butyl Methyl Diester (**23 g**):*

By following the general procedure, treatment of *N*-ethyl-*N*-nosylglutamic acid (O*t*Bu) methyl ester (**21g**) (200 mg, 0.46 mmol) with the reagent system mercaptoacetic acid (0.097 mL, 1.39 mmol)/sodium methoxide (198 mg, 3.68 mmol), and subsequently with Fmoc chloride (119 mg, 0.46 mmol), afforded **23g** as a colorless oil (164 mg, 76% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) *δ* 7.80–7.28 (m, 8 H, Fmoc-Ar*H*), 4.66–4.38 (2 m, 3 H, Fmoc-C*H*$_2$ + α-C*H*), 4.29–4.18 (2 m, 1 H, Fmoc-C*H*), 3.70, 3.52 (2 s, 3 H, OC*H*$_3$), 3.48–3.27 (2 m, 2 H, NC*H*$_2$CH$_3$), 3.15–3.01 (2 m, 2 H, NC*H*$_2$CH$_3$), 2.36–2.24 (m, 2 H, β-C*H*$_2$), 2.11–2.02 (m, 2 H, γ-C*H*$_2$), 1.45 (s, 9 H, *t*Bu), 1.12 (2 t, *J* = 7.1 Hz, 3 H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): (rotamers) *δ* 172.2, 168.0, 156.7, 143.9, 141.2, 136.2, 135.1, 127.9, 127.6, 127.3, 127.0, 125.2, 124.9, 120.0, 119.9, 80.6, 67.3, 65.1, 59.0, 58.5, 52.3, 52.2, 47.3, 41.7, 31.9, 31.7, 28.1, 24.9, 24.7, 14.2, 13.7 ppm. $C_{27}H_{33}NO_6$ (467.23): calcd. C 69.36, H 7.11, N 3.00, O 20.53; found C 69.17, H 7.13, N 2.98.

*N-Ethyl-N-Fmoc-(S)-benzyl-L-cysteine Methyl Ester (**23h**)*

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-cysteine(SBn) methyl ester (**21h**) (200 mg, 0.45 mmol) with the reagent system mercaptoacetic acid (0.095 mL, 1.37 mmol)/sodium methoxide (194 mg, 3.59 mmol), and subsequently with Fmoc chloride (116 mg, 0.45 mmol), afforded **23h** as a colorless oil (154 mg, 71% yield). *H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.82– 7.24 (m, 13 H, Fmoc-Ar*H* + $SCH_2C_6H_5$), 4.58–4.16 (m, 4 H, Fmoc-CH_2 + FmocC*H* + α-C*H*), 4.06, 4.03 (2 s, 2 H, SCH_2Ph), 3.96 (s, 3 H, OCH_3), 3.54–3.04 (m, 3 H, NCH_2CH$_3$ + CH_2SBn), 2.93 (m, 1 H, CH_2SBn), 0.91 (t, *J* = 6.9 Hz, 3 H, NCH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): (rotamers) δ 170.5, 165.8, 145.0, 144.5, 142.7, 141.5, 141.3, 128.5, 128.2, 127.3, 127.2, 125.1, 125.0, 123.8, 120.2, 120.0, 71.6, 65.1, 50.4, 44.7, 38.8, 36.7, 32.1, 13.3 ppm. $C_{28}H_{29}NO_4S$ (475.18): calcd. C 70.71, H 6.15, N 2.95, O 13.46, S 6.74; found C 70.58, H 6.13, N 2.96.

Synthesis of N-Ethyl-N-Fmoc-L-isoleucine (23d) and N-Ethyl-N-nosyl- L-isoleucine (21d): general procedure

The *N*-ethyl-*N*-Fmoc-isoleucine methyl ester (**23d**) or the *N*-ethyl-*N*-nosyl-L-isoleucine methyl ester (**21d**) (1 mmol) and LiI (5 mmol) were dissolved in ethyl acetate (5 mL). The reaction mixture was heated at reflux for 24 h and the conversion of the precursors **23d** and **21d** was monitored by TLC. A saturated aqueous Na_2CO_3 solution was then added and extracted with ethyl acetate. The aqueous phase was acidified with 1N HCl and

extracted with ethyl acetate (3x10 mL). The organic phase was washed with brine. The organic phase was dried with Na_2SO_4 and the solvents evaporated under vacuum to afford the corresponding amino acids as oils in 94 and 96% yields, respectively.

N-Ethyl-N-Fmoc-L-isoleucine

By following the general procedure, treatment of *N*-ethyl-*N*-Fmoc-isoleucine methyl ester (**23d**) (200 mg, 0.51 mmol) with LiI (339 mg, 2.53 mmol) afforded the correspondent amino acid as a colorless oil (182 mg, 94% yield). 1H NMR (CDCl3, 300 MHz, 25 °C): (rotamers) *δ* 7.82–7.27 (m, 8 H, Fmoc-Ar*H*), 4.57 (d, *J* = 6.0 Hz, 2 H, Fmoc-CH_2), 4.25 (t, *J* = 5.5 Hz, 1 H, Fmoc-C*H*), 4.10 (m, 1 H, α-C*H*), 3.31 (2 m, 1 H, NCH_2), 3.06 (m, 1 H, NCH_2), 2.15 [m, 1 H, C*H*(CH_3)CH_2CH_3], 1.45–1.31 [m, 2 H, CH(CH_3)-CH_2CH_3], 1.30–1.08 (2 m, 3 H, NCH_2CH_3), 1.01–0.82 [m, 6 H, CH(CH_3)CH_2CH_3, CH(CH_3)CH_2CH_3] ppm. ^{13}C NMR ($CDCl_3$ 75 MHz, 25 °C): (rotamers) *δ* 177.2, 155.8, 141.4, 134.3, 127.8, 127.1, 124.7, 120.0, 67.7, 65.1, 47.3, 43.3, 33.2, 25.2, 15.7, 13.8, 10.8 ppm. $C_{23}H_{27}NO_4$ (381,19): calcd. C 72.42, H 7.13, N 3.67, O 16.78; found C 72.23, H 7.12, N 3.66.

N-Ethyl-N-nosyl-L-isoleucine

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-isoleucine methyl ester (**21d**) (200 mg, 0.56 mmol) with LiI (374 mg, 2.79 mmol) afforded the correspondent amino acid as a colorless oil (186 mg, 96% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): *δ* 8.32 (d, *J* = 9 Hz, 2 H, *o*-NO_2), 8.00 (d, *J* = 9Hz, 2 H, *m*-NO_2), 4.14 (d, *J* = 10.2 Hz, 1 H, α-C*H*), 3.48–3.24 (m, 2 H, NCH_2CH_3), 1.84

[m, 1 H, $CH(CH_3)CH_2CH_3$], 165 [m, 1 H, $CH(CH_3)CH_2CH_3$], 1.23 (t, *J* = 7.05 Hz, 3 H, NCH_2CH_3) 1.11 [m, 1 H, $CH(CH_3)CH_2CH_3$], 0.97–0.86 [m, 6 H, $CH(CH_3)CH_2CH_3$ + $CH(CH_3)CH_2CH_3$] ppm. ^{13}C NMR ($CDCl_3$, 75MHz, 25 °C): δ 174.9, 149.9, 145.3, 128.6, 124.0, 64.7, 40.7, 34.6, 25.4, 16.2, 15.4, 10.7 ppm. $C_{14}H_{20}N_2O_6S$ (344,10): calcd. C 48.83, H 5.85, N 8.13, O 27.87, S 9.31; found C 48.75, H 5.86, N 8.10.

Synthesis of Dipeptides: general procedure

Valine methyl ester hydrochloride (1 mmol), HOBt (1.1 mmol), *N*-methylmorpholine (1 mmol), and the *N*-ethyl-*N*-Fmoc-isoleucine or *N*-ethyl-*N*-nosyl-isoleucine were dissolved in dry THF (20 mL). The solution was stirred and cooled in an ice/water bath while DCC (1.15 mmol) was added. Stirring was continued for 1 h at 0 °C and an additional hour at room temperature whilst monitoring by TLC. *N*,*N*'-Dicyclohexylurea was removed by filtration and the solvent evaporated in vacuo. A mixture of ethyl acetate (30 mL) and a saturated solution of $NaHCO_3$ in water (10 mL) was added to the residue and the organic phase extracted with a 10% solution of citric acid in water (10 mL), again with saturated $NaHCO_3$ (10 mL), and then brine. The organic layer was dried with Na_2SO_4, filtered, and the solvents evaporated to dryness in vacuo to afford the *N*-ethylated dipeptides as oils in 90 and 92% yields, respectively.

N-Ethyl-N-Fmoc-L-isoleucyl-L-valine(OMe)

By following the general procedure, treatment of *N*-ethyl-*N*-Fmoc-isoleucine (200 mg, 0.52 mmol) with valine methyl ester hydrochloride (88 mg, 0.52 mmol), HOBt (77 mg, 0.57 mmol), *N*-methylmorpholine (52 mg, 0.52 mmol), and DCC (124 mg, 0.60 mmol) afforded the dipeptide as a yellow oil (232 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): (rotamers) δ 7.87–7.28 (m, 9 H, Fmoc-Ar*H* + N*H*), 4.60– 4.03 (m, 5 H, Fmoc-CH_2 + FmocC*H* + α-C*H*Ile + α-C*H*Val), 3.78, 3.60 (2 s, 3 H, OCH_3), 3.43–3.09 (m, 2 H, NCH_2), 2.16 [m, 1 H, C*H*$(CH_3)_2$], 1.69–1.60 [m, 3 H, C*H*$(CH_3)CH_2CH_3$ + $CH(CH_3)CH_2CH_3$], 1.03–0.80 [m, 12 H, NCH_2CH_3 + $CH(CH_3)_2$ + $CH(CH_3)CH_2CH_3$] ppm. $C_{29}H_{38}N_2O_5$ (494.62): calcd. C 70.42, H 7.74, N 5.66, O 16.17; found C 70.59, H 7.72, N 5.67.

N-Ethyl-N-nosyl-L-isoleucyl-L-valineOMe

By following the general procedure, treatment of *N*-ethyl-*N*-nosyl-isoleucine (200 mg, 0.58 mmol) with valine methyl ester hydrochloride (97 mg, 0.58 mmol), HOBt (86 mg, 0.64 mmol), *N*-methylmorpholine (59 mg, 0.58 mmol), and DCC (137 mg, 0.67 mmol) afforded the dipeptide as a yellow oil (243 mg, 92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 8.35 (d, *J* = 9 Hz, 2 H, *o*-NO_2), 8.02 (d, *J* = 9Hz, 2 H, *m*-NO_2), 6.70 (d, *J* = 8.4 Hz, N*H*), 4.45 (dd, *J* = 8.4, 4.8 Hz, 1 H, NHC*H*), 3.85 (d, *J* = 10.8 Hz, C*H*CONH), 3.73 (s, 3 H, OCH_3), 3.55 (m, 1 H, NCH_2CH_3), 3.30 (m, 1 H, NCH_2CH_3), 2.18 [m, 1 H, C*H*$(CH_3)_2$], 1.91 [m, 1 H, C*H*$(CH_3)CH_2CH_3$], 1.29 (t, *J* = 7.2 Hz, 3 H, NCH_2CH_3), 1.06 [m, 1 H, $CH(CH_3)CH_2CH_3$], 0.97–0.90 [m, 6 H, $CH(CH_3)_2$], 0.83 [d, *J* = 6.3 Hz, 3 H, $CH(CH_3)CH_2CH_3$], 0.72 [t, *J* = 7.3 Hz, 3 H, $CH(CH_3)CH_2CH_3$], 0.45 [m, 1 H, $CH(CH_3)CH_2CH_3$] ppm. $C_{20}H_{31}N_3O_7S$ (457.54): calcd. C 52.50, H 6.83, N 9.18, O 24.48, S 7.01; found C 52.29, H 6.85, N 9.16.

4. Synthesis of (2S,3R)-3-methylglutamic acid

This work was undertaken at the "Shool of Pharmacy-University of London" during a period of six months as visiting postgraduate. It is part of a project not yet published. Here is reported just the unsuccessful strategy, that will be not part of the corresponding publication.

Synthesis of 3-benzyl-4-methyl(S)-2,2-dimethyloxazolidine-3,4-dicarboxylate (29b)

Preparation of CbZ-Serine methyl ester

N-CbZ- serine (**28b**) (1 mmol) was dissolved in dry methanol and stirred. A solution of cesium carbonate(0.5 mmol) in methanol and two drops of water was added dropwise at 0 °C over 10 minutes. The reaction was stirred at room temperature for 1 hour. The mixture was evaporated carefully to dryness. The resulted salt was than dissolved in dry DMF under nitrogen athmosphere. The methyl iodide (1.2 mmol) was addes dropwisw and the reaction left to stir overnight. The solvent was evaporated and the mixture dissolved in water, then extracted with ethyl acetate. The collected organic fractions were washed with a 5% solution of $NaHCO_3$ and brine, anidhryficated and evaporated to obtain the corresponding methyl ester in 88% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.63 (s, 1H, O*H*), 3.75 (s, 3H, $COOCH_3$), 3.92 (dd, 2H, C*H*$_2$OH), 4.41-4.46 (m, 1H, α-CH), 5.10 (s, 2H, CH_2Ph), 5.89 (d, J= 6.4, 1H, NH), 7.28- 7.36 (m, 5H, Ph).

*Synthesis of 3-benzyl-4-methyl-(S)-2,2-dimethyloxazolidine-3,4-dicarboxylate (**29b**)*

N-CbZ- serine methyl ester (1 mmol) was dissolved in 2,2 dimethoxypropane (10 mL). TsOH (0.12 mmol) was added and the reaction stirred at room temperature for 3 hours. The solvent was avaporated and the mixture redissolved in ethyl acetate, washed with a 5% solution of $NaHCO_3$ and brine. After anidhryfication and evaporation the crude product was purified by column chromatography (Hexane /ethyl acetate 9:1). **29b** was obtaine in 80% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) [2:1 mixture of enantiomers A and B]: δ 1.49 (s, 3H, CH_3, B), 1.56 (s, 3H, CH_3, A), 1.63 (s, 3H, CH_3, B), 1.70 (s, 3H, CH_3, A), 3.63 (s, 3H, COOCH_3, A), 3.76 (s, 3H, COOCH_3, B), 4.07-4.13 (m, 4H, CH_2, A+B), 4.47 (dd, J_1= 2.6, J_2= 6.8, 1H, CH_2, A), 4.55 (dd, J_1= 2.6, J_2= 6.8, 1H, CH_2, B), 5.03 (d, J= 12.4, 2H, CH_2Ph, B), 5.14-5.20 (m, 2H, CH_2Ph, A), 7.25-7.38 (m, 5H, Ph, A+B) ppm.

Synthesis of N-CbZ Garner's aldheyde 30b

To a stirred solution of 29b in dry toluene, at -78 °C under nitrogen athmosphere, was addesd dropwise DIBAL monitoring the internal temperature, that was maintained under -65 °C. The reaction was stirred at -78 °C for 2 hours. The reaction mixture was then quenched with CH_3OH and the mixture poured into ice cold 1N HCl solution and stirred for 30 minutes. The aqueous mixture was extracted with ethyl acetate; the organic layers were collected and washed with brine, dried and evaporated. The product was obtained after column chromatography (Hexane: Ethyl acetate 7:1) in 70% yield. ^{1}H-NMR (400 MHz,

$CDCl_3$) [2:1 mixture of enantiomers A and B]: δ 1.52 (s, 3H, CH_3, B), 1.58 (s, 3H, CH_3, A), 1.61 (s, 3H, CH_3, B), 1.68 (s, 3H, CH_3, A), 3.80-4.18 (m,2H, CH_2, A+B), 4.33 (m, 1H, CH, A), 4.43 (m, 1H CH, B), 7.30-7.48 (m, 5H, Ar-H, A+B), 9.57 (s, 1H, COH, A), 9.64 (s, 1H COH, B) ppm.

Synthesis of (E)-benzyl-4-(4-tert-butoxy-4-oxobut-1-enyl)-2,2-dimethyloxazolidine-3-carboxylate (31b)

Tetra butyl ammonium iodide was added to a solution of Garner's aldehyde **30b** in 3M potassium carbonate under nitrogene atmosphere to give an heterogeneous solution. After 15 minutes *tert*-butyl-diethl phosphonoacetate was added and the reaction mixture was stirred overnight. Water was added and the resultant mixture was extracted with ethyl acetate. The combined organic fractionswere combined, washed with brine, dried over magnesium sulfate, filtered and concentrated to give the crude product as yellow oil. Purification by column chromatography (ethyl acetate/hexane 1:9) gave the desired product **31b** in 76% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) [2:1 mixture of stereoisomers A and B]: δ 1.44 (s, 3H, CH_3, B), 1.47 (s, 9H, *tBu*, A+B), 1.49 (s, 3H, CH_3, B), 1.56 (s, 3H, CH_3, A), 1.67 (s, 3H, CH_3, A), 3.82 (d, J= 2.12, 2H, CH_2, B), 3.84 (d, J=2.16 , 2H, CH_2, A), 4.45 (m, 1H, CH, A), 4.46 (m, 1H, CH, B), 3.57 (s, 2H, CH_2Ph, A), 3.58 (s, 2H, CH_2Ph, B), 5.80 (d, J=15.5, 1H, CH=C*H*COO*tBu*, A), 5.91 (d, J=13.76, 1H, CH=C*H*COO*tBu*, B),6.75 (m, 1H, C*H*=CHCOO*tBu,*A+B), 7.30-7.42 (m, 5 H, Ar-*H*, A+B) ppm.

5. *Sulfamoylation of L-Ornithine*

Two-dimensional (2D) homonuclear $^{1}H,^{1}H$-TOCSY spectra were obtained using the MLEV pulse sequence for the isotropic mixing[120] with an 80-ms spin lock period. Coupling constant values were extracted[121] from the two-dimensional homonuclear $^{1}H,^{1}H$-DQF-COSY[122] and the mono-dimensional proton analysis. All mono- and two-dimensional spectra were recorded at 298 K, and the spectral results were consistent with the proposed structures. For MALDI MS analysis a 1-μL portion of a premixed solution of each sample and α-CHCA (0.3% in TFA) was spotted on the matrix target, dried at room temperature, and analyzed in the positive mode. For in vitro clotting assays an automatic coagulometer was used with a maximum coagulation time limit of 500 s for TT and APTT tests. TT and APTT determinations were performed with the kits Dade BC Thrombin Reagent and Dade Actin FSL, respectively.

*Synthesis of N^{α}-Fmoc-N^{δ}-Boc-L-ornithine methyl ester (**36**)*

A 0.66 M solution of diazomethane in CH_2Cl_2 (8.5 mL, 5.6 mmol) was added dropwise to a suspension of **35** (0.73 g, 1.6 mmol) in CH_2Cl_2 (5 mL). The resulting mixture was stirred at r.t. for 15 min, and the conversion of the precursor was monitored by TLC ($CHCl_3$/CH_3OH 95:5 v/v). The solvent was removed under reduced pressure condition and the residue was solubilised in AcOEt (5 mL), and washed with a 5% aqueous solution of

$NaHCO_3$ (2x5 mL) and once with brine (5 mL). The organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The solid residue was co-evaporated with a 1:1 Et_2O/*n*-hexane mixture and compound **36** was recovered as a white crystalline solid. Yield: 0.72 g, 96%. Mp 149–151°C. R_f = 0.69. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.4 Hz, 2 H), 7.58 (d, J = 7.4 Hz, 2 H), 7.38 (t, J = 7.4 Hz, 2 H), 7.30 (t, J = 7.4 Hz, 2 H), 5.43 (d, J = 8.0 Hz, 1 H), 4.55 (t, J = 6.9 Hz, 1 H), 4.31-4-42 (m, 3 H), 3.74 (s, 3 H), 3.12 (m, 2 H), 1.79-1.94 (m, 1 H), 1.46-1.74 (m; 3 H), 1.42 (s, 9 H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.8, 156.0, 143.8, 141.3, 128.9, 127.7, 125.1, 119.9, 79.3, 77.5, 67.0, 53.6, 52.5, 47.1, 40.0, 29.8, 28.4, 26.1 ppm. Anal. Calcd for $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.86; H, 6.89; N, 5.96.

*Synthesis of N^{α}-Fmoc-L-ornithine methyl ester trifluoroacetate (**37**)*

A solution of **36** (0.72 g, 1.54 mmol) in CH_2Cl_2 (3 mL) was treated with a solution of TFA in CH_2Cl_2 (9:1, 5 mL). The resulting mixture was stirred at 0°C for 15 min. After this time the consumption of **36** was complete as checked by TLC ($CHCl_3$/CH_3OH 95:5 v/v), and the volatile components of the mixture were removed under vacuum. The oily residue was co-evaporated with toluene (3x 5 mL) and finally with a 1:1 MTBE/*n*-hexane mixture. The obtained glassy solid was dried under vacuum and immediately subjected to the next reaction step. Rf = 0.11. MS (MALDI) *m/z* Calcd for $C_{21}H_{25}N_2O_4^+$ 369.1810; found: 369.1847 (Δ_m = +10 ppm).

N^{α}-Fmoc-N^{δ}-sulfamoyl(Boc)-L-ornithine methyl ester (40)

Method A

A solution of the sulfamoyl chloride **38** (0.95 g, 4.62 mmol), freshly prepared as reported in the literature[123], in dry ethanol-free CH_2Cl_2 (3 mL), was added dropwise to a solution of 3 (0.57 g, 1.54 mmol) in dry ethanol-free CH_2Cl_2 (3 mL) containing DIEA (1.18 mL, 6.78 mmol). The resulting mixture was stirred at r.t. and the progress of the reaction was monitored by TLC ($CHCl_3$/CH3OH 95:5 v/v). After 2 h, the solvent was removed under vacuum and the oily residue was solubilised in AcOEt (5 mL). The organic solution was washed with a 5% aqueous solution of $KHSO_4$ (3x5 mL), a 5% aqueous $NaHCO_3$ (3x5 mL) and once with brine (5 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum. The pale yellow solid residue was co-evaporated three times with a 1:1 Et2O/*n*-hexane mixture to give **40** as a pale yellow glassy solid. FCC showed pure **40** as a pale yellow powder. Yield: 0.55 g, 65%.

Method B

Compound **37**, obtained from the unblocking of **36** (0.46 g, 1 mmol), and DIEA (0.33 ml, 2 mmol) was added to a solution of the azanide **39** (0.3 g, 1 mmol) in dry ethanolfree CH_2Cl_2 (5 mL). The mixture was stirred at r.t. for 16 h, monitoring the conversion of **37** by TLC ($CHCl_3$/CH_3OH 95:5 v/v). The solvent was removed under vacuum and the solid residue was solubilised in EtOAc (10 mL). The organic solution was washed with a 5% aqueous solution of $KHSO_4$ (3x5 mL), a 5% aqueous solution of $NaHCO_3$ (3x5 mL), and once with brine (5 mL). The organic phase

was dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give pure **40** as pale yellow powder. Yield: 0.49 g, 92%. Mp 119–121 °C. Rf = 0.56. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.01 (s broad, 1 H), 7.74 (d, J = 7.4 Hz, 2 H), 7.58 (d, J = 7.4 Hz, 2 H), 7.38 (t, J = 7.4, 2 H), 7.30 (t, J = 7.4 Hz, 2 H), 5.44 (d, J = 8.3 Hz, 1 H), 5.35 (t, J = 6.16 Hz, 1 H), 4.20-4.43 (m, 3 H), 4.19 (t, J = 6.7 Hz, 1 H), 3.73 (s, 3 H), 3.08 (m, 2 H), 1.83-2.00 (m, 1 H), 1.54-1.78 (m, 3 H), 1.46 (s, 9 H) ppm. ^{13}C-NMR DEPT (75 MHz, CDCl3) δ 172.8 (CO), 156.1 (CO), 155.9 (CO), 143.8 (C), 141.2 (C), 128.0 (CH), 127.6 (CH), 125.1 (CH), 119.9 (CH), 79.3 (C), 67.0 (CH_2), 53.6 (CH), 52.5 (CH_3), 47.1 (CH), 40.0 (CH_2), 29.7 (CH_2), 28.4 (CH_3), 26.1 (CH_2) ppm. Anal. Calcd for $C_{26}H_{33}N_3O_8S$: C, 57.02; H, 6.07; N, 7.67. Found: C, 57.21; H, 6.09; N, 7.68.

N^{α}-Acetyl-N^{δ}-sulfamoyl(Boc)-L-ornithine methyl ester (42)

A solution of **40** (0.23 g, 0.42 mmol) in CH_3CN (10 mL) was reacted with mercaptoacetic acid (0.15 mL, 2.1 mmol) and sodium methoxide (0.18 g, 3.36 mmol) in CH_3OH (2 mL). TLC monitoring of the reaction mixture showed complete conversion of **40** after 5.5 h at 50 °C. The oily residue recovered from the work-up of the reaction mixture was immediately acetylated with an excess of freshly distilled acetic anhydride, in a 1:1 mixture of CH_2Cl_2 (5 mL) and a 10% aqueous solution of $NaHCO_3$ (5 mL). The biphasic system was vigorously stirred at r.t., and the reaction was monitored by TLC ($CHCl_3$/CH_3OH 95:5 v/v). After 1 h, the mixture was concentrated under vacuum and the aqueous residue was extracted with AcOEt (3x5 mL). The organic phase was washed with a 5% aqueous solution of $NaHCO_3$ (3x5 mL) and

once with brine (5 mL), then dried over Na_2SO_4, filtered, and evaporated to dryness under vacuum. The oily residue was chromatographed to give **42** as the only reaction product, as pale yellow foam, with the following yields: 0.14 g, 90%. R_f = 0.65. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.28 (s broad, 1 H), 6.54 (d, J = 7.9 Hz, 1 H), 5.74 (t, J = 6.8 Hz, 1 H), 4.56 (m, 1 H), 3.71 (s, 3 H), 3.05 (m, 2 H), 2.0 (s, 3 H), 1.52-1.96 (m, 4 H), 1.43 (s, 9 H) ppm. 13C-NMR (75 MHz, CDCl3) d 172.9, 170.6, 150.5, 83.5, 52.5, 51.6, 42.9, 29.5, 28.0, 25.1, 22.9 ppm. Anal. Calcd for $C_{13}H_{25}N_3O_7S$: C, 42.50; H, 6.86; N, 11.44. Found: C, 42.59; H, 6.88; N, 11.46.

Synthesis of dipeptide 44

A solution of Fmoc-Pro-OH (0.14 g; 0.42 mmol) in dry ethanol-free CH_2Cl_2 (2 mL) was treated with HOBt monohydrate (0.07 g, 0.46 mmol), EDCI (0.1 g, 0.52 mmol), DIEA (0.18 mL, 1.03 mmol), and the resulting mixture was stirred at r.t. for 12 h. Compound **41** (0.14 g, 0.42 mmol) solubilised in dry ethanol-free CH_2Cl_2 (2 mL) was then added, and the stirring was maintained for further 12 h. After this time, TLC ($CHCl_3$/CH_3OH 95:5 v/v) showed the complete consumption of **41**. The mixture was then evaporated to dryness under vacuum and the residue was solubilised with AcOEt (5 mL), washed with a 5% aqueous solution of $KHSO_4$ (3x5 mL), 5% aqueous solution of $NaHCO_3$ (3x5 mL), and once with brine (5 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give a solid residue which was triturated with a 1:4 MTBE/*n*-hexane mixture. The precipitate was collected and dried under vacuum. Dipeptide **44** was recovered pure as a yellowish powder. Yield: 0.24 g, 88%. ^{1}H-NMR (300 MHz, $CDCl_3$) [75:25 mixture of rotamers A and B]: δ

8.43 (s broad, 0.75 H, A), 7.74 (d, J = 7.4 Hz, 2 H), 7.60 (m, 2 H), 7.52 (s broad, 0.25 H, B), 7.38 (t, J = 7.4 Hz, 2 H), 7.29 (t, J = 7.4 Hz, 2 H), 6.75 (d, J = 8.1 Hz, 0.75 H, A), 6.57 (s broad, 0.25 H, B), 5.46 (t, J = 6.9 Hz, 0.75 H, A), 5.33 (s broad, 0.25 H, B), 4.58 (m, 1 H), 4.41 (m, 3 H), 4.13-4.30 (m, 2 H), 3.71 (s, 3 H), 3.31-3.65 (m, 2 H), 2.90-3.29 (m, 2 H), 2.11 (m, 2 H), 1.91 (m, 2 H), 1.56-1.78 (m, 4 H), 1.43 (s, 2.25 H, A+B), 1.39 (s, 6.75 H, A+ B) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) [75:25 mixture of the two rotamers A and B]: δ 172.3, 171.9, 155.7, 150.7, 144.0, 143.7, 141.3, 127.7, 127.1, 125.2, 120.0, 83.5, 67.9, 60.5, 52.5, 51.7, 47.1, 43.1, 31.6, 29.7, 29.5, 28.9, 27.9, 24.8, 24.7 ppm. MS (MALDI) m/z Calcd for $C_{31}H_{41}N_4O_9S^+$ 644.2523; found: 644,2452 (Δ_m = -11 ppm). Anal. Calcd for $C_{31}H_{40}N_4O_9S$: C, 57.75; H, 6.25; N, 8.69. Found: C, 57.94; H, 6.27; N, 8.67.

Synthesis of tripeptide 48

*Unblocking of **44***

A solution of **44** (0.32 g, 0.5 mmol) in CH_3CN (10 mL) was reacted with mercaptoacetic acid (0.18 mL, 2.5 mmol) and sodium methoxide (0.21 g, 4.0 mmol) in CH_3OH (2 mL). The progress of the reaction was monitored by TLC ($CHCl_3$/CH_3OH 95:5 v/v), showing the complete conversion of **44** after 5.5 h at 50 °C. The mixture was then made acidic (pH 5) by adding a 0.1 N aqueous solution of HCl, and extracted with Et_2O (3x10 mL). The ethereal extracts were discarded off and the aqueous phase was made basic (pH 8) with a 10% aqueous solution of $NaHCO_3$, and then extracted with AcOEt (3x10 mL). The collected organic layers

were dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give a glassy pale yellow solid which was used in the next step of coupling without further purification.

Coupling

A solution of Boc-D-Phe-OH (**46**) (0.13 g; 0.5 mmol) in freshly distilled DMF (2 mL) was treated with EDCI (0.31 g, 0.52 mmol), HOBt monohydrate (0.08 g, 0.55 mmol), DIEA (0.27 mL, 1.6 mmol), and the resulting mixture was stirred at r.t. for 2 h. The glassy solid obtained from the unblocking of **44** (0.21 g, 0.5 mmol) solubilised in freshly distilled DMF (2 mL) was then added dropwise to the mixture, and the stirring was maintained for further 24 h. After this time, TLC ($CHCl_3$/CH_3OH 95:5 v/v) showed the complete consumption of Boc-D-Phe-OH. The mixture was evaporated to dryness under vacuum and the solid residue was solubilised with AcOEt (5 mL), washed with a 5% aqueous solution of $KHSO_4$ (3x5 mL), 5% aqueous solution of $NaHCO_3$ (3x 5 mL), and once with brine (5 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give a solid residue which was triturated with a 1:4 MTBE/*n*-pentane mixture. The precipitate was collected and dried under vacuum. Tripeptide **47** was recovered pure as yellowish powder. Yield: 0.30 g, 89%. ^{1}H-NMR (300 MHz, $CDCl_3$) [70:30 mixture of rotamers A and B]: δ 7.18-7.41 (m, 6 H), 7.06 (d, J = 7.1 Hz, 1 H), 6.24 (m, 0.3 H, B), 6.02 (m, 1 H), 5.89 (d, J = 6.9 Hz, 0.7 H, A), 4.52-4.74 (m, 0.3 H, B), 4.50 (m, 0.7 H, A), 4.30-4.48 (m, 0.3 H, B), 4.28 (m, 0.7 H, A), 4.17 (m, 1 H), 3.70-3.90 (m, 1 H), 3.76 (s, 3 H), 3.52 (m, 0.3 H, B), 3.49 (m, 1 H), 3.28 (m, 0.7 H, A), 2.90-3.16 (m, 0.3 H, B), 2.67 (m, 0.7 H, A), 1.10-2.15 (m, 8 H), 1.52 (s, 3 H), 1.41

(s, 3 H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) [70:30 mixture of the two rotamers A and B]: δ 172.2, 172.0, 171.7, 170.9, 157.03, 155.3, 136.22, 134.7, 129.8, 129.6, 129.4, 129.1, 128.5, 128.0, 127.1, 126.8, 124.3, 120.4, 60.2, 55.7, 53.8, 52.44, 51.9, 51.4, 49.1, 47.2, 43.0, 41.7, 40.4, 39.4, 33.9, 33.3, 33.1, 32.7, 29.6, 28.3, 28.1, 25.6, 25.3, 25.0, 24.7. MS (MALDI) m/z Calcd for $C_{30}H_{48}N_5O_{10}S^+$ 669.3041; found: 669.3135 (Δ_m= +14 ppm). Anal. Calcd for $C_{30}H_{47}N_5O_{10}S$: C, 53.80; H, 7.07; N, 10.46. Found: C, 52.7; H, 7.33; N, 10.04.

Synthesis of tripeptide trifluoroacetate 48

A solution of **47** (0.30 g; 0.45 mmol) in CH_2Cl_2 (3 mL) was treated with a solution of TFA in CH_2Cl_2 (9:1, 5 mL). The resulting mixture was stirred at 0 °C for 30 min. After this time the consumption of **47** was complete as checked by TLC ($CHCl_3$/CH_3OH 95:5 v/v), and the volatile components of the mixture were removed under vacuum. The oily residue was co-evaporated with toluene (3x5 mL), absolute EtOH (3x5 mL), and triturated with a 1:2 MTBE/*n*-pentane mixture. The obtained precipitate was collected, dried under vacuum and finally lyophilized to give **48** as a pale yellow glassy solid. Yield: 0.25 g, 90%. Rf = 0.10. ^{1}H-NMR (300 MHz, DMSO-d_6/ D_2O) δ 7.11-7.40 (m, 5 H), 6.50 (s broad, 2 H), 4.02-4-41 (m, 3 H), 3.61 (s, 3 H), 3.4-3.6 (m, 2 H, partially overlapped by the water signal), 2.68-3.14 (m, 4 H), 1.34-1.90 (m, 8 H) ppm. 13C-NMR (75 MHz, DMSO-d6/D2O) d 172.9, 171.8, 166.8, 134, 94, 130.0, 129.0, 127.9, 59.7, 52.3, 52.1, 49.2, 47.2, 42.5, 37.2,

29.6, 28.6, 26.0, 24.2. MS (MALDI) m/z Calcd for $C_{20}H_{32}N_5O_6S^+$ 470.2070; found: 470.2014 (Δ_m = -12 ppm). Anal. Calcd for $C2_2H31N_5O_5S.CF_3CO_2H$: C, 45.28; H, 5.53; N, 12.00. Found: C, 44.02; H, 5.68; N, 11.65.

Clotting assays for tripeptides 47 and 48

Preparation of stock solution

Tripeptides 47 and 48 to be tested were dissolved in DMSO just prior to use and diluted with PBS buffer (pH 8) to yield a final 152 mM stock solution for tripeptide 47, and a 337 mM stock solution for tripeptide trifluoroacetate 48.

Clotting assays

In vitro coagulation assays were performed with pooled human plasma. Clotting time was measured using an automatic coagulometer according to the manufacturer's instructions. In order to exclude any influence of DMSO in clotting assays, a blank prepared by mixing the pooled human plasma (800 lL) and DMSO (200 lL) was subjected to TT and APTT tests. Control values of both the parameters were not modified with respect to those registered for a blank prepared by mixing the pooled human plasma (800 lL) and PBS (200 lL).

Determination of thrombin time (TT)

To each of the tubes containing plasma (800 lL), ncreasing amounts (25, 50, 75, 100, 125, and 150 lL; corresponding to 8.4, 16.8, 25.3, 33.7, 42.1, and 50.5 lmol, respectively) of the stock solutions of tripeptide **48** were added. The same protocol was

applied to prepare samples of tripeptide **47**. The resulting samples were diluted to a total volume of 1 mL by adding PBS, vortexed, and incubated at 37 °C for 1 minute, and then placed on the instrument sample wheel. The coagulometer automatically dispensed thrombin time reagent in the sample of each tube. The time for the appearance of a fibrin clot was measured and each value was obtained from a set of three separate experiments. Control value of clotting time for TT tests was determined for three experiments using a blank prepared by mixing the pooled human plasma (800 μL) and PBS (200 μL), and was 15.5 s (mean value; SD = ±0.6; *n* = 3). Clotting time values exceeding the coagulometer time limit (500 s) were not determined.

Determination of activated partial thromboplastin time (APTT)

A second series of samples prepared as described for TT determinations was treated with the activated partial thromboplastin time reagent. The coagulometer automatically dispensed the appropriate reagent in the sample of each tube. The time for the appearance of a fibrin clot was measured and each value was obtained from a set of three separate experiments. The same blank used for the control value of clotting time for TT tests was used to determine a control value of 31.2 sec (mean value; SD = ±1.2; *n* = 3) for clotting time in APTT tests. Clotting time values exceeding the coagulometer limit (500 s) were not determined.

6. Carboxyl function deprotection of α-amino acid methyl esters using sulphur ylides

Synthesis of dimethylsulfoxonium methylide (50)

Trimethylsulfoxonium iodide (3 mmol) was added to a solution of sodium hydride (1.1 mmol) in dry THF. The resulting mixture was stirred at reflux temperature for 2h. The dimethylsulfoxonium methylide so obtained was used for the deprotection reactions without any further purification steps.

Deprotection of methyl phenylacetate (51)

The methylphenyl acetate (**51**, 1 mmol) was added to the solution of dimethyl sulfoxonium methylide (**50**, 3 mmol), freshly prepared as previously described. The reaction was allowed to react at room temperature for 40 min. and monitored by TLC (chloroform/methanol 90:10 v/v). The solvent was removed under reduced pressure condition and the residue was solubilised in H_2O (5 mL). The solution was extracted with AcOEt (3x5 mL). The aqueous layer was then acidified with a 1N solution of HCl and extracted with AcOEt (3x5 mL). The organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to obtain the phenylacetic acid (**52**). Yield: 90% ^{1}H-NMR (300 MHz, DMSO): δ 3.55 (s, 2H, C*H*$_2$Ph); 7.29-7.41 (m, 5H, Ar*H*); 12.34 (s broad, 1H, O*H*) ppm. ^{13}C-NMR: δ : 40.21,

127.04, 129.84, 130.63, 135.48, 173.20 ppm. GC/MS (CI) m/z (%): 137 [$(M + H)^+$] (45), 119 (25), 91 (100).

Deprotection of N-protected amino acid methyl esters (53 a-e): general procedure

The *N*-protected amino acid methl ester (**53a-e**, 1 mmol) was added to the solution of dimethyl sulfoxonium methylide (**50**, 2 mmol). The reaction was stirred at room temperature and maintained under dry nitrogen atmosphere. The reaction was monitored by TLC (**54 b-d**: ethyl ether/petroleum ether 70:30 v/v; **54 a,e**: chloroform/methanol 90:10 v/v). The THF was removed under reduced pressure condition and the residue was solubilised in H_2O (5 mL). The solution was extracted with AcOEt (3x 5 mL). The aqueous layer was then acidified with a 5% solution of $KHSO_4$ and extracted with AcOEt (3x 5 mL). The organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to obtain the phenylacetic acid (**54 a-e**)

*N-Ns-Ala-OH (**54a**)*

Yield: 99%. Mp. 138-142 °C.^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.18 (d, 3H, CH_3, J = 7.2 Hz); 3.81-3.91 (m, 1H, CH COOH); 8.02 (d, 2H, ArHNs, J= 9.0 Hz), 8.38 (d, 2H, ArHNs, J= 9.0 Hz), 8.59 (d, 1 H, NH, J = 8.4 Hz); 12.72 (s broad, 1H, COOH) ppm.

*N-Z-Ala-OH (**54b**)*

Yield: 88%. Mp. 83-85 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.44 (d, 3H, C*H*$_3$, J= 7.2 Hz); 4.32-4.44 (m, 1H, C*H*-CH_3), 5.11 (s, 2H, C*H*$_2$),

5.48 (d, 1H, N*H*, J = 7.2 Hz); 7.31-7.39 (m, 5H, Ar*H*); 8.22 (s broad, 1H, O*H*) ppm.^{13}C-NMR: δ 18.47, 49.52, 67.05, 128.12, 128.23, 128.56, 136.20, 155.80, 176.81 ppm.

*N-Boc-Phe-OH (**54c**)*

Yield: 98%. Mp. 84-86 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, $(CH_3)_3C$-); 3.07 (dd, 1H, CH_2Ph, J= 6.0 Hz, J= 13.8 Hz); 3.21 (dd, 1H, CH_2Ph, J= 5.4 Hz, J= 13.8 Hz); 4.57-4.67 (m, 1H, CH-CH_2Ph); 5.02 (d, 1H, NH, J= 8.1 Hz); 7.15-7.33 (m, 5H, ArH), 8.89 (s broad, 1H, OH) ppm. ^{13}C-NMR: δ 28.29, 40.00, 54.30, 80.16, 127.02, 128.56, 129.44, 135.40, 155.05, 175.10 ppm.

*N-Ns-O-Benzyl-L-Tyr-OH (**54d**)*

Yield: 70%. ^{1}H-NMR (300 MHz, DMSO-d_6): δ (p.p.m.): 2.62 (dd, 1H, α-$CHCH_2$, J=10.5 Hz, J=13.8 Hz); 2.92 (dd, 1H, α-$CHCH_2$, J=2.4 Hz, J=13.8 Hz); 3.83-3.95 (m, 1H, α-CH), 4.94 (s, 2H, CH_2Ph) ; 6.71 (d, 2H, ArH, J=8.7 Hz); 6.99 (d, 2H, ArH, J=8.7 Hz); 7.25-7.48 (m, 5H, ArH); 7.71 (d, 2H, ArHNs, J=8.7 Hz); 8.19 (d, 2H, ArHNs, J=8.7 Hz) ; 8.69 (d, 1H, NH, J=9.0 Hz); 12.83 (s broad, 1H, OH) ppm. ^{13}C-NMR: δ 37.14, 58.39, 69.60, 114.67, 124.47, 128.07, 128.15, 128.24, 128.78, 129.17, 130.67, 137.49, 147.22, 149.39, 157.58, 172.75 ppm.

*N-Ns-Ile-OH (**54e**)*

Yield: 82%. Mp. 126-129 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.71-0.89 (m, 6 H, CH_2CH_3, $CHCH_3$); 1.03-1.15 (m, 1 H, CH_2CH_3); 1.30-1.40 (m, 1 H, CH_2CH_3); 1.78-1.91 (m, 1 H, $CHCH_3$); 3.89 (dd, 1 H, CHCOOH, J = 9.1 Hz, J = 6.3 Hz); 5.55 (d, 1H, NH, J= 9,1 Hz,); 7.61

(broad, 1 H, COOH), 8.05 (d, 2H, ArHNs; J = 8.7 Hz); 8.40 (d, 2 H, ArHNs; J = 8.7 Hz) ppm. ^{13}C-NMR: δ 11.31, 15.52, 24.43, 37.98, 60.33, 124.32, 128.50, 145.53, 151.60, 175.32 ppm.

Deprotection of N-Fmoc Isoleucine methyl esters (55)

The *N*-Fmoc isoleucine methyl ester (**55**, 1 mmol) was added to the solution of dimethyl sulfoxonium methylide (**50**, 2 mmol). The reaction was stirred at room temperature and maintained under dry nitrogen atmosphere for 10 minutes. The reaction was monitored by TLC (chloroform/methanol 90:10 v/v). The THF was removed under reduced pressure condition and the residue was solubilised in H_2O (5 mL). The solution was extracted with AcOEt (3x5 mL). The aqueous layer was then basified with an aqueous solution of Na_2CO_3 and treated with acetic anhydride in dry dichloromethane for 2h. The aqueous phase was separated from the organic layer and acidified with a 1N solution of HCl and extracted with dichloromethane (3x5 mL). The organic extracts were dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to obtain the *N*-acetyl-L-ile-OH in 80% yield, with was characterized by GC/MS after its conversion into the corresponding methyl ester, using a 0.66 M solution of CH_2N_2. GC/MS (EI) m/z : 131 (23%), 128 (96), 99 (65), 88 (63), 86 (100), 43 (85).

7. *Synthesis of resin-bound mercaptoacetic acid for the removal of Nosyl group in peptide synthesis*

Synthesis of S-dimethoxytrityl mercaptoacetic acid (59)

4,4'-Dimethoxytrityl chloride **57** (1.50 g, 4.4 mmol) was added to a solution of mercaptoacetic acid (**58**; 0.3 ml, 4.4 mmol) in dry methylene chloride (20 mL). The resulting reaction mixture was stirred at room temperature and maintained under dry nitrogen atmosphere for 6 h. The course of the reaction was followed by TLC (chloroform/methanol, 90:10). Evaporation of the solvent afforded the corresponding *S*-Dimethoxytrityl-mercaptoacetic acid **59** as an orange oil in 75 % yield.

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 9.50 (s broad, 1 H, COO*H*), 7.49-7.19 (m, 9H, Ar-*H*), 6.90-6.80 (m, 4H, Ar-*H*), 3.80 (s, 6H, OCH_3), 3.06 (s, 2H, 2-H_2) ppm. ^{13}C-NMR (300 MHz, $CDCl_3$, 25°C): δ 173.61, 158.29, 144.32, 136.23, 130.68, 129.26, 128.08, 126.87, 113.34, 66.01, 55.25, 34.56 ppm. Anal. Calcd for $C_{23}H_{22}O_4S$: C, 70.03; H, 5.82 %. Found: C, 70.16; H, 5.84 %.

Attachment of mercaptoacetic acid to Wang resin

Swelling of the resin

A sample of Wang resin (300 mg, 1.1 mmol/g) was placed in a dried 20 ml glass reaction vessel, shaken in 6 ml of dry DMF for 1 h, and drained.

*Attachment of S-dimethoxytrityl mercaptoacetic acid (**59**) to the Wang resin through an ester linkage.*

S-dimethoxytrityl mercaptoacetic acid **59** (1.30 g, 3.3 mmol) was dissolved in dry DCM and cooled in an ice bath. 3.3 mmol of diisopropylcarbodiimmide (DIC) dissolved in DCM was added and the reaction mixture was stirred for 20 minutes. The DCM was removed under reduced pressure; the residue was dissolved in DMF and added to the resin. 0.03 mmol of dimethylamino pyridine (DMAP) dissolved in DMF were added and the reaction was shaken for 3h at room temperature. The operation was repeated for a further two cycle after which the resin was washed with DCM (5× 2 min), then with isopropanol (IPA) (5 × 2 min), and washed again with DCM (5 × 6 min).

Removal of DMT group.

A total of 10 mL of DCM/TES (95:5) and 1% TFA was added to the thiol resin **60** and shaken for 1h. The cleavage mixture was filtered and the resin was treated with neat 10 mL of DCM/TES (95:5) and 5% TFA for 30 minutes. The resin was drained, washed with DCM (5 × 1 min), IPA (5 ×1 min), methanol (5 × 1 min), IPA (5 × 1 min) and DCM (5 × 1 min).

Synthesis of benzyl S-Dimethoxytrityl mercaptoacetate (62)

S-Dimethoxytrityl mercaptoacetic acid **59** (394 mg, 1 mmol) was added in one portion to a solution of benzyl alcohol (0.10 ml, 1 mmol), 1-hydroxybenzotriazole (148 mg, 1.1 mmol) and 4-

methylmorpholine (0.11 ml, 1 mmol) in dry THF (20 mL). This mixture was stirred under nitrogen and cooled in an ice bath. Then a slight excess of diisopropylcarbodiimide (0.18 ml, 1.15 mmol) was added. and the mixture was stirred for 1 h. The ice bath was removed, and the mixture was stirred for more 3 hours at room temperature monitoring the reaction by TLC (diethyl ether/petroleum ether, 60:40). The *N,N*-diisopropylurea was then filtered off and then the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL). After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure to give **62** as a white solid in 80% yield.

^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 7.42-7.18 (m, 14H, Ar-H), 6.85-6.75 (m, 4H, Ar-H), 5.01 (s, 2H, OCH_2Ph), 3.79 (s, 6 H, OCH_3), 3.03 (s, 2H, -SCH_2-) ppm. Anal. Calcd for $C_{30}H_{28}O_4S$: C, 74.35; H, 5.82 %. Found: C, 74.49; H, 5.84 %.

Deprotection of benzyl S-Dimethoxytrityl mercaptoacetate (62)

Benzyl *S*-dimethoxytrityl mercaptoacetate **62** (1 mmol) was dissolved a mixture of dry DCM/TES (95:5) and 5% TFA (1 mL). The solution was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was washed with methanol. Purification by column chromatography on silica gel (diethyl ether/petroleum ether, 60:40) afforded the benzyl mercaptoacetate **63** as an oil in 80% yield. GC/MS (EI) m/z (%): 182 (1, $M^{+\cdot}$), 123 (30), 105 (2), 91 (100), 77 (6), 65 (10), 47 (4). ^{1}H-

NMR (300 MHz, $CDCl_3$, 25°C): δ 7.23-7.15 (m, 5H, Ar-H), 5.18 (s, 2H, -OCH_2-), 3.30 (d, 2H, -SCH_2-), 2.02 (t, 1H, SH) ppm. Anal. Calcd for $C_9H_{10}O_2S$: C, 59.32; H, 5.53 %. Found: C, 59.43; H, 5.55 %.

Use of Wang resin bound mercaptoacetic acid for the removal of Nosyl protecting group.

*Deprotection of N-Nosyl-N-Methyl alanine methyl ester (**64a**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol, assuming that the loading of Wang resin with mercaptoacetic acid was quantitative) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-*N*-Methyl alanine methyl ester **64a** (0.100 g, 0.33 mmol) in dry DCM (20 mL). The reaction, monitored by TLC (diethyl ether/petroleum ether, 60:40), did not provide the complete conversion of **64a** in **65a** after 2 hours.

Another experiment was performed adding resin bound mercaptoacetic acid **61** and DBU (0.98 mL, 0.66 mmol) to a solution of 0.080 mg (0.26 mmol) of *N*-Nosyl-*N*-Methyl alanine methyl ester **64a** in dry DCM. Also in this case, after 2 hours the reaction was not complete.

An additional experiment was performed adding resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) to a solution of *N*-Nosyl-*N*-Methyl alanine methyl ester **64a** (0.070 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature providing the complete removal of Nosyl group. Then the resin was drained

and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates, containing the *N*-methyl alanine methyl ester **65a**, were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-N-Methyl alanine methyl ester (**66a**).*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65a** (0.23 mmol) in dry DCM and stirred for 4h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 x 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-*N*-methyl-alanine methyl ester (**66 a**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 159 (4, $M^{+\cdot}$), 128 (1), 116 (5), 100 (53), 58 (100), 43 (17). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 5.25 (q, J= 7.5 Hz, 1H, 2-H), 3.70 (s, 3H, OCH_3), 2.96 (s, 3H, N-CH_3), 2.12 (s, 3H, CH_3CO), 1.38 (d, J = 7.5 Hz, 3 H, 3-H_3) ppm. ^{13}C-NMR (300 MHz, $CDCl_3$, 25°C): δ 172.50, 171.30, 55.98, 51.69, 32.19, 21.88, 14.52 ppm. Anal. Calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80 %. Found: C, 52.92; H, 8.26; N, 8.78 %.

*Deprotection of N-Nosyl- leucine methyl ester (**64b**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl leucine methyl ester **64b** (0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by

TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5×1 min), isopropanol (5×1 min) and DCM (5×1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-leucine methyl ester (**66b**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65b** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3×10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-leucine methyl ester (**66 b**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 187 (2, $M^{+\cdot}$), 144 (4), 128 (76), 99 (16), 86 (100), 43 (46). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 6.20 (d, J= 8.1 Hz,1 H, NH), 4.60 (m, 1 H, 2-H), 3.69 (s, 3H, OCH_3), 2.02 (s, 3H, CH_3CO), 1.69-1.44 (m, 3H, $CH_2CH(CH_3)_2$) 0.92-0.89 (m, 6 H, $(CH_3)_2CH$) ppm. ^{13}C-NMR (300MHz, $CDCl_3$, 25°C) : δ 173.84, 170.05, 52.27, 50.69, 41.54, 24.82, 23.05, 22.80, 21.91 ppm. Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48 %. Found: C, 57.84; H, 9.18; N, 7.46 %.

*Deprotection of N-Nosyl-valine methyl ester (**64c**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-valine methyl ester **64 c** (0.073 g, 0.23 mmol) in dry DCM (20

mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-valine methyl ester (**66c**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65c** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-valine methyl ester (**66 c**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 173 (0.20 , $M^{+\cdot}$), 142 (0.45), 131 (4.5), 114 (77), 99 (23), 88 (55), 72 (100). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 6.05 (s broad, 1 H, NH), 4.57 (dd, J= 4.8, J= 8,7 Hz, 1 H, 2-H), 3.74 (s, 3H, OCH_3), 2.22- 2.06 (m, 1H, 3-H), 2.04 (s, 3H, CH_3CO), 0.93 (d, J= 6.9 Hz, 3H, -$CH(CH_3)_2$), 0.90 (d, J= 6.9 Hz, 3H, -$CH(CH_3)_2$) ppm. ^{13}C-NMR (300 MHz, $CDCl_3$, 25°C): $\delta = 173.21, 170.52, 57.02, 52.13, 31.22, 23.24, 18.87, 17.84$ ppm. Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09 %. Found: C, 55.57; H, 8.76; N, 8.07 %.

*Deprotection of N-Nosyl-phenylalanine methyl ester (**64d**).*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-phenilalanine methyl ester **64 d** (0.084 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-phenylalanine methyl ester (**66d**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65d** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-phenilalanine methyl ester (**66 d**) as a white solid in quantitative yield.

Mp: 85-88 °C. GC/MS (EI) m/z (%): 221 (0.5 , $M^{+\cdot}$), 162 (100), 131 (29), 120 (45), 91 (48), 88 (86), 43 (76). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 7.07-7.33 (m, 5 H, Ar-H), 6.01 (d, J= 7.12 Hz, 1 H, NH), 4.09 (m, 1H, 2-H), 3.73 (s, 3H, OCH_3), 3.18 (dd, J= 5.82 Hz, J= 13.82 Hz, 1H, 3-H_2), 3.07 (dd, J= 5.97 Hz, J= 13.82 Hz, 1H, 3-H_2), 1.99 (s, 3H, CH_3CO) ppm.^{13}C-NMR (300 MHz, $CDCl_3$, 25°C): δ = 172.14, 169.67, 135.81, 129.26, 128.61, 127.17, 53.11, 52.39,

37.82, 23.18 ppm. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33 %. Found: C, 65.22; H, 6.86; N, 6.31 %.

*Deprotection of N-Nosyl-alanine methyl ester (**66e**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-alanine methyl ester **64 e** (0.066 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-alanine methyl ester (**66e**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65e** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-alanine methyl ester (**66e**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 145 (5 , $M^{+\cdot}$), 102 (3), 86 (72), 59 (3), 44 (100). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 6.15 (s broad, 1 H, NH), 4.60 (m, 1 H, 2-H), 3.75 (s, 3H, OCH_3), 2.03 (s, 3H, CH_3CO), 1.40 (d, J= 7.2 Hz, 3H, 3-H_3) ppm. ^{13}C-NMR (300MHz, $CDCl_3$, 25°C) : δ 173.71, 171.62, 52.54, 48.02, 23.21, 18.60 ppm. Anal. Calcd for

$C_6H_{11}NO_3$: C, 49.65; H, 7.64; N, 9.65 %. Found: C, 49.73; H, 7.67; N, 9.63 %.

*Deprotection of N-Nosyl-isoleucine methyl ester (**64f**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-isoleucine methyl ester **64f** (0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-isoleucine methyl ester (**66f**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65f** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-isoleucine methyl ester (**66 f**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 187 (0.3 , $M^{+\cdot}$), 131 (25), 128 (96), 99 (69), 88 (60), 86 (100), 43 (85). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 6.4 (d, J= 9.14 Hz, 1H, NH), 4.66 (dd, 1 H, J= 4.25 Hz, J= 9,14 Hz, 2-H), 3.68 (s, 3H, OCH_3), 1.99 (s, 3, CH_3CO), 1.82 (m, 1H, 3-H), 1.30-

1.42 (m, 2H, 4-H_2), 0.90-1.05 (m, 6H, 3-CH_3, 5-H_3) ppm. Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48 %. Found: C, 57.82; H, 9.17; N, 7.46 %.

*Deprotection of N-Nosyl-D-alloisoleucine methyl ester (**64g**)*

Resin bound mercaptoacetic acid **61** (300 mg, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-Nosyl-D-alloisoleucine methyl ester **64g** (0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken for 1 h at room temperature and the removal of Nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), isopropanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were evaporated to dryness under reduced pressure.

*Synthesis of N-acetyl-D-alloisoleucine methyl ester (**66g**)*

0.1 ml (1.15 mmol) of acetic anhydride was added to a solution of **65g** (0.23 mmol) in dry DCM and stirred for 4 h at room temperature. The reaction mixture was then acidified with 1N hydrochloridric acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-D-alloisoleucine methyl ester (**66g**) as a colorless oil in quantitative yield.

GC/MS (EI) m/z (%): 131 (25), 128 (86), 99 (70), 88 (55), 86 (100), 43 (85). ^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): δ 6.4 (d, J= 8.71 Hz, 1H, NH), 4.55 (dd, 1 H, J= 5.24 Hz, J= 8,71 Hz, , 2-H), 3.68 (s, 3H, OCH_3), 1.97 (s, 3H, CH_3CO) 1.82 (m,1H, 3-H), 1.30-1.42 (m, 2H, 4-

H_2), 0.90-1.05 (m, 6H,5-H_3) ppm. Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48 %. Found: C, 57.82; H, 9.17; N, 7.46 %.

8. Site-Selective Methylation of N^{β}-Nosyl hydrazides of N-Nosyl protected α-amino acids

Synthesis of N'-(N-Nosyl-α-aminoacyl)-N''-nosyl hydrazines 69a-c: general procedure

Sodium bicarbonate (10mmol) and *N*,*N*-dimethylformamide (0.53 mmol) were added to a stirred solution of 4-nitrobenzenesulfonylhydrazide (1,1mmol) in dry tetrahydrofuran (10 mL); a solution of the appropriate *N*-nosyl-R-aminoacyl chloride **67 a-c** (1 mmol) in dry tetrahydrofuran (10 mL) was then added, and the resulting mixture was stirred at room temperature for 60-90 min, until TLC analysis (chloroform/ethylacetate 70:30 v/v) of the reaction mixture showed complete conversion of the precursor. Distilled water (15 mL) was added to the reaction mixture, and the solution was extracted with chloroform (3x10 mL). The combined organic extracts were washed once with 10% aqueous hydrochloric acid (10 mL) and once with brine (10 mL) and then dried overNa_2SO_4. The solvent was evaporated under vacuum to provide the corresponding nosyl-substituted acyl hydrazine **69a-c** in 70-80% yields.

*N'-(N-Nosyl-L-alanyl)-N''-nosylhydrazine (**69a**)*

Obtained as a pale yellow oil (80%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (d, J=2.4 Hz, 1H), 10.30 (d, J=2.4 Hz, 1H), 8.50 (d, J=8.4, 1H), 8.47-8.39 (m, 4H), 8.12-7.98 (m, 4H), 3.97-3.81 (m, 1H),1.02 (d, J=7.0 Hz, 3H); ^{13}CNMR(75 MHz, DMSO-d6) δ 169.8, 150.7, 150.3, 144.6, 144.3, 130.1, 129.0, 125.2, 125.0, 52.6, 15.5 ppm. Anal. Calcd for $C_{15}H_{15}N_5O_9S_2$: C, 38.05; H, 3.19; N, 14.79. Found: C, 38.19; H, 3.18; N, 14.74.

Synthesis of N'-(N-Nosyl-R-aminoacyl)-N''-methyl-N''-nosyl Hydrazines 70a-c: general procedure

A0.66 M methylene chloride solution of diazomethane (1.5 mmol) was added dropwise to a stirred solution of the appropriate N'-(*N*-nosyl-R-aminoacyl)-*N''*- nosyl hydrazine **69a-c** (1 mmol) in dry tetrahydrofuran at room temperature. The mixture was maintained under stirring for about 40-50 min, until TLC analysis (chloroform/ethyl acetate 60:40 v/v) of the reaction mixture showed complete conversion of the precursor. Evaporation of the solvent under vacuum provided the respective *N'*-(*N*-nosyl-R-aminoacyl)-*N''*-methyl-*N''*-nosyl hydrazine **70a-c** in quantitative yield. The methylation reaction was also performed successfully starting from 14 mmol (8.0 g) of the hydrazine **69c**.

*N'-(N-Nosyl-L-alanyl)-N''-methyl-N''-nosylhydrazine (**70a**)*

Obtained as a pale yellow oil (quantitative yield); ^{1}H NMR (300MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.57 (d, J=8.4 Hz, 1H), 8.41-8.32 (m, 4H), 8.04-7.92 (m, 4H), 3.85-3.73 (m, 1H), 2.89 (s, 3H),

1.05 (d, J=6.9 Hz, 3H) ppm. ^{13}C NMR (75MHz,DMSO-d_6) δ 170.3, 150.3, 149.7, 146.8, 142.0, 129.8, 128.1, 124.6, 124.5, 50.3, 38.0, 18.9 ppm. Anal. Calcd for $C_{16}H_{17}N_5O_9S_2$: C, 39.42; H, 3.52; N, 14.37. Found: C, 39.55; H, 3.50; N, 14.32.

Synthesis of N'-(N-Methyl-N-nosyl-α-aminoacyl)-N''-methyl-N''-nosyl Hydrazines 71a-c: general procedure

A 0.66M methylene chloride solution of diazomethane (2 mmol) was added dropwise to a stirred solution of the appropriate *N'*-(*N*-nosyl-α-aminoacyl)- *N''*-methyl-*N''*-nosyl hydrazine **70a-c** (1 mmol) in dry tetrahydrofuran at room temperature. The mixture was maintained under stirring for about 60-70 min, until TLC analysis (chloroform/ethyl acetate 60:40 v/v) of the reaction mixture showed complete conversion of the precursor. Evaporation of the solvent under vacuum provided the respective N'-(*N*-methyl- *N*-nosyl-α-aminoacyl)-*N''*-methyl-*N''*-nosyl hydrazine 5a-c in quantitative yield. The methylation reaction was also performed successfully starting from 13 mmol (7.5 g) of the hydrazine **70c**.

*N'-(N-Methyl-N-nosyl-L-alanyl)-N''-methyl-N''-nosyl hydrazine (**71a**).*

Obtained as a pale yellow oil (quantitative yield); ^{1}H-NMR (300MHz,DMSO-d_6) δ 10.35 (s, 1H), 8.48-8.38 (m, 4H), 8.01 (d, J=9.0 Hz, 4H), 4.49 (q, J=7.1 Hz, 1H),2.98 (s, 3H), 2.80 (s, 3H), 1.12 (d, J=7.1 Hz, 3H) ppm. ^{13}C-NMR(75MHz,DMSO-d_6) δ 169.8, 155.0, 150.7, 149.9, 144.0, 130.2, 128.9, 125.2, 124.9, 53.3, 38.47, 30.7,

15.5 ppm. Anal. Calcd for $C_{17}H_{19}N_5O_9S_2$: C, 40.71; H, 3.82; N, 13.97. Found: C, 40.69; H, 3.80; N, 14.04.

*Treatment of N'-(N-Methyl-N-nosyl-α-aminoacyl)-N''-methyl-N''-nosyl Hydrazines **71a-c** with Diazomethane.*

A 0.66 M methylene chloride solution of diazomethane (5 mmol) was added dropwise to a stirred solution of the appropriate *N'*-(*N*-methyl-*N*-nosyl-α-aminoacyl)-*N''*-methyl-*N''*-nosyl hydrazine **71a-c** (1 mmol) in dry tetrahydrofuran at room temperature. The mixture was maintained under stirring for about 80-90 min, until TLC analysis (diethyl ether/petroleum ether 70:30 v/v) of the reaction mixture showed complete conversion of the precursor. The organic solvent was removed under vacuum, and the oily residue was subjected to chromatography to provide compounds **72a-c** in 21-27% yields and compounds **73a-c** in 60-77% yields. Chromatographic purification of the crude reaction product obtained by the reaction performed starting from 5 mmol (3.0 g) of **71c** afforded the corresponding trimethylated products **72c** (0.64 g) and **73c** (2.2 g) in 22% and 75% yields, respectively.

*(E)-Methyl-N-Methyl-N-nosyl-2-(N-methyl-4-nitrophenyl sulfonamido) propanhydrazinoate (**72a**)*

Obtained as a pale yellow oil (21%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.30 (d, J=8.7 Hz, 4 H), 7.83 (d, J=8.7 Hz, 4 H), 5.45 (q, J=7.4 Hz, 1H), 3.50 (s, 3H), 2.92 (s, 3H), 2.74 (s, 3H), 1.32 (d, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.1, 166.0, 151.5, 150.0, 143.4, 132.7, 128.9, 125.3, 124.2, 56.4, 44.8, 38.5, 31.1, 15.7 ppm. Anal. Calcd for $C_{18}H_{21}N_5O_9S_2$: C, 41.94; H, 4.11; N, 13.59. Found: C, 42.09; H, 4.09; N, 13.53.

*(Z)-Methyl-N-Methyl-N-nosyl-2-(N-methyl-4-nitrophenyl sulfonamido)propanhydrazinoate (**73a**)*

Obtained as a pale yellow oil (60%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.40-8.52 (m, 4H), 8.17-8.00 (m, 4H), 5.12 (q, J=6.7 Hz, 1H), 4.04 (s, 3H), 2.74 (s, 3H), 2.71 (s, 3H), 1.03 (d, J=6.7 Hz, 3H) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ 161.2, 152.4, 148.7, 148.1, 143.2, 129.9, 128.1, 125.1, 123.3, 56.3, 51.5, 38.3, 30.9, 16.1 ppm. Anal. Calcd for $C_{18}H_{21}N_5O_9S_2$: C, 41.94; H, 4.11; N, 13.59. Found: C, 41.89; H, 4.12; N, 13.56.

Computational Details

Density Functional Theory calculations were performed on the **69a** and **70a** compounds and their relative neutral and anionic forms. The hybrid Becke three parameter exchange and Lee Yang and Parr correlation (B3LYP)[124] functional was used in both geometry optimization and frequency calculations as implemented in the Gaussian03 code.[125] All the calculations were carried out using the extended 6-311+G(2df,2p) basis set on all atoms. Vibrational frequency calculations performed to determine the nature of stationary point of all the investigated species and to take into account the zero-point frequencies and the enthalpy terms. This approach proved to be adequate to describe with reasonable accuracy both the gas-phase acidities and basicities of a wide variety of molecular systems. In this work the enthalpy variation for the deprotonation process ($\Delta_{ac}H$) at 298 K of the neutral compound AH

$$AH \rightleftharpoons A^- + H^+$$

is used as acidity of the AH neutral species in the gas phase and can be calculated as follows:

$$\Delta_{ac}H = \Delta E°_{elec} + \Delta ZPE + \Delta E^{298}_{vib} + 5/2\ RT$$

where $\Delta E°_{elec}$, ΔZPE, and ΔE^{298}_{vib} refer to the differences between the electronic energies at 0 K, the ZPE, and the thermal vibrational corrections of AH and A^-, respectively. The term 5/2RT, necessary to convert the energy in enthalpy, includes the PV work term and the differences between the translational and rotational energy contributions of the species involved in the deprotonation process. The relative acidity values ($\Delta\Delta_{ac}H$) were computed as differences between the value of the more acidic compound and the given ones. Natural bond orbital (NBO) analysis[126] was performed on all the neutral and charged species.

9. Transformations of pregn-4-en-3β,17α,20β-triol with Lewis and anhydrous protic acids

The experiment for the NMR study of the reaction of compound 1 with CSA as the catalyst was performed in $CDCl_3$ at −10 °C. In this case, chemical shift values are expressed in ppm relative to the residual proton of the solvent at 7.23 ppm for ^1H NMR spectra. When possible, resonances were correctly assigned by proton homodecoupling experiments. Attribution of the other signals is tentative.

Pregn-3,5-dien-17α,20β-diol (75)

Procedure A: Pregn-4-en-3β,17α,20β-triol (**74**) (1; 1.0 g, 3.0 mmol) was dissolved in dry 1,4-dioxane (50 mL) and aluminum trichloride (1.2 g, 9.0 mmol) was added to the resulting solution. After stirring for 20 minutes at room temperature, TLC analysis of the reaction mixture (chloroform/methanol, 20:1) showed complete conversion of the precursor into the corresponding $\Delta^{3,5}$-steradiene **75**. The organic solvent was removed under vacuum and the residue was treated with distilled water (30 mL), then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed once with brine (30 mL), dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The crude solid residue was purified by chromatography (chloroform/methanol 20:1 v/v) to afford pregn-3,5-dien-17α,20β-diol (**75**) as a solid (0.88 g, 92%). Mp = 139−141 °C. R_f = 0.37 (chloroform/methanol 20:1 v/v). ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.89 (d, , $^3J_{H,H}$ = 9.7 Hz, 1 H, 4-H), 5.56 (m, 1 H, 6-H), 5.34 (m, 1 H, 3-H), 4.03 (d, 1 H, $^3J_{H,H}$ = 6.8 Hz, 20-OH), 3.75 (m, 1 H, 20-H), 3.40 (s, 1 H, 17-OH), 1.98−2.21 (m, 3 H), 1.05−1.81 (m, 14 H), 1.01 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, 21-CH_3), 0.89 (s, 3 H, 19-CH_3), 0.75 (s, 3 H, 18-CH_3) ppm. ^{13}C NMR (DEPT, 75 MHz, DMSO-d_6, 25 °C): δ = 141.0 (C), 129.1 (CH), 124.7 (CH), 123.1 (CH), 84.7 (C), 69.5 (CH), 50.4 (CH), 47.6 (CH), 46.9 (C), 36.8 (C), 34.7 (CH_2), 33.8 (CH_2), 33.4 (CH_2), 31.8 (CH_2), 31.7 (CH), 31.6 (CH_2), 23.7 (CH_2), 22.7 (CH_2), 20.3 (CH_3), 18.9 (CH_3), 15.0 (CH_3) ppm. GC-MS (EI, 70 eV): m/z (%) = 316 (44) [$M^{+\cdot}$], 298 (7), 271 (44), 253 (100), 226 (10), 213 (61), 197 (18), 173 (8), 159 (15), 145 (20), 131 (14), 118

(12), 105 (31), 81 (33). $C_{21}H_{32}O_2$ (316.24): calcd. C 79.70, H, 10.19; found C 79.42, H 10.22.

Procedure B: Pregn-4-en-3β,17α,20β-triol (**74**) (1; 1.0 g, 3.0 mmol) was dissolved in dry 1,4-dioxane (120 ml) and aluminum trichloride (1.2 g, 9.0 mmol) was added. The resulting solution was refluxed under stirring until TLC analysis (chloroform/methanol, 20:1) showed the complete consumption of the precursor. The organic solvent was removed under vacuum and the residue was treated with distilled water (30 mL), then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed once with brine (30 mL), dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The crude solid residue was purified by chromatography (chloroform/methanol, 20:1) to afford pregn-3,5-dien-17α,20β-diol (**75**) as a solid (0.8 g, 84%). Compound **75** obtained by this procedure showed physicochemical properties and NMR spectra identical to those observed for a sample of the same compound prepared by applying the procedure A.

Treatment of Pregn-4-en-3β,17α,20β-triol (74) with Camphorsulfonic Acid (CSA)

To a solution of pregn-4-en-3β,17α,20β-triol (1; 1.0 g, 3.0 mmol) in dry 1,4-dioxane (50 mL), CSA (0.08 g, 0.32 mmol) was added. The resulting mixture was refluxed under stirring for 2 h. After this time, TLC analysis (chloroform/methanol, 20:1) showed complete consumption of the precursor. The organic solvent was removed under vacuum and the residue was treated with distilled water (30 mL), then extracted with ethyl acetate (3 × 30

mL). The combined organic layers were washed once with brine (30 mL), dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The crude solid residue was purified by chromatography (ethyl acetate/petroleum ether, 50:50) to afford a mixture of the two epimers **75** and **76** (0.85 g, 89% total yield). ^{1}H-NMR analysis performed on a sample of the product obtained after chromatography indicated an approximately 3:2 ratio of the C-17 α:β epimers. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 5.74 (m, 1 H, 4-H epimer 76) and 5.88 (d, $^3J_{H,H}$ = 9.9 Hz, 1 H, 4-H epimer 75), 5.52-5.65 (m, 2 H, 6-H epimer 75 and 6-H epimer 76), 5.34 (m, 1 H, 3-H epimer 75) and 5.45 (m, 1 H, 3-H epimer 76), 4.04 (d, $^3J_{H,H}$ = 7.2 Hz, 1 H, 20-OH epimer 76) and 4.06 (d, $^3J_{H,H}$ = 6.9 Hz, 1 H, 20-OH epimer 75), 3.68−3.80 (m, 2 H, 20-H epimer 75 and 20-H epimer 76), 3.41 (s, 1 H, 17-OH epimer 76) and 3.42 (s, 1 H, 17-OH epimer 75), 2.03−2.31 (m, 6 H, epimers 75 and 76), 1.03−1.81 (m, 28 H, epimers 75 and 76), 0.99 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, 21-CH_3 epimer 76) and 1.01 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, 21-CH_3 epimer 75), 0.86 (s, 3 H, 19-CH_3 epimer 76) and 0.89 (s, 3 H, 19-CH_3 epimer 75), 0.73 (s, 3 H, 18-CH_3 epimer 76) and 0.75 (s, 3 H, 18-CH_3 epimer 75) ppm.

Treatment of Pregn-4-en-3β,17α,20β-triol (74) with $TiCl_4$

A 1 M dichloromethane solution of titanium tetrachloride (5.4 mL, 5.4 mmol) was added to a suspension of pregn-4-en-3β,17α,20β-triol (1; 0.6 g, 1.8 mmol) in dry dichloromethane (40 mL). The mixture was maintained under magnetic stirring for 20 minutes at room temperature, until TLC analysis of the reaction mixture (chloroform/methanol 20:1 v/v) showed complete

consumption of the precursor. The organic solvent was removed under vacuum and the residue was treated with distilled water (25 mL), then extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed once with brine (25 mL), dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The crude solid residue was purified by chromatography (chloroform/methanol 20:1 v/v) to afford pregn-3,5-dien-17α,20β-diol (**75**) as a solid (0.52 g, 89%). ^{1}H and ^{13}C NMR spectra recorded for a sample of the pure product obtained after chromatography showed spectroscopic characteristics equal to those obtained for the same compound prepared by the procedure A, as previously described.

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