UNIVERSITÀ DELLA CALABRIA

UNIVERSITA' DELLA CALABRIA

Dipartimento di Farmacia e Scienze della Salute e della Nutrizione

Scuola di Dottorato

Scuola di Dottorato Scienza e Tecnica "Bernardino Telesio"

Indirizzo

Organic Molecules of Pharmacological Interest
(OMPI)

CICLO

XXVIII

NEW METHODOLOGIES FOR THE ELABORATION OF AMINOACIDS AND PEPTIDES

CHIM/06

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ALLEGATO B – EMANUELA ROMIO

La candidata ha usufruito di una borsa aggiuntiva di dottorato di ricerca MIUR – Bando Fondo Sostegno Giovani. Il Collegio dei Docenti ha valutato l'attività di ricerca della candidata che si è sviluppata nel campo della sintesi peptidica e della sintesi organica in generale, e ha preso in esame i risultati conseguiti, riportati in n° 8 lavori a stampa e n° 1 in corso di stampa su riviste internazionali con referee a buon IF medio, n° 3 lavori in preparazione, e n° 4 comunicazioni in congressi nazionali.

Il Collegio ha inoltre valutato:

l'attività svolta dalla candidata durante il periodo di stage condotto presso la University of Aberdeen (UK) dal 20 gennaio 2014 al 22 luglio 2014.

- l'attività formativa della candidata che si è realizzata attraverso la partecipazione a n° 2 Convegni nazionali
- l'attività formativa della candidata che si è realizzata a seguito della assidua frequenza all'attività didattica proposta dalla Scuola di Dottorato.

Con riferimento a quanto sopra richiamato, il Collegio dei Docenti del corso di Dottorato di Ricerca in *Scienza e Tecnica – Curriculum OMPI (Organic Materials of Pharmaceutical Interest)*, giudica l'attività della candidata EMANUELA ROMIO ampiamente positiva e la presenta con piena soddisfazione al giudizio della Commissione.

Il Coordinatore OMPI

(Prof. Bartolo GABRIELE)

"To strive, to seek,

to find,

and not to yield"

NEW METHODOLOGIES FOR THE ELABORATION OF AMINOACIDS AND PEPTIDES

Introduction

Peptides are naturally found in animals, plants, and microorganisms and are of great interest to medicine, pharmacology, and the food industry. Endogenous peptides exert antibiotic and antimicrobial activities, and possess enormous potential for the treatment of various pathologies.

This discovery has stimulated researcher towards the design and synthesis of new peptide drugs called also peptidomimetics. Peptidomimetics, are designed to mimic the structures of natural bioactive peptides presenting, at the same time, important modifications in some key points of peptide chain useful to improve their pharmacological properties.

Though, the widespread use of bioactive peptides as therapeutics is hardly hampered by their poor pharmacokinetic as well as physico-chemical properties and susceptibility to enzymatic degradation. The peptide backbone can be elaborated both at level of a single amino acid residue and by incorporation of non-natural amino acids.

The simplest and minimal modification of a single amino acid or peptide bonds is represented by *N*-methylation.

N-Methylation is an important approach for peptide backbone modifications. These peptides exhibit important biological activities such as antitumor, antibiotic or immunosuppressive activities. This modification can improve the pharmacokinetic properties of biologically active peptides as well as resulting in analogues that show specific biological activity such as enzyme inhibitors, receptor antagonists and agonists, building blocks in combinatorial chemistry for the screening of new potential drugs. Further, structural and conformational studies performed with *N*-methylated analogues of natural amino acids and peptides enabled to produce stable foldamers with different topology with respect to the helix of natural and endogenous peptides, confer to modified peptides high stability against proteases and enhance lipophilicity and bioavailability for pharmacological purposes.

Another important elaboration is about the amide bond.

Amide bonds are the main chemical linkages that join amino acids together to give peptides and proteins.

The synthesis of amides is of huge importance in organic, coordination, and medicinal chemistry. So, improved and innovative methods for the synthesis of amides are in great demand both by chemical and pharmaceutical industry. It is evident that not only the amide bond synthesis results significant but also its modification.

This bond is extremely polar and it is a natural target of many enzymes causing a rapid degradation of peptides. Among the modifications necessary for getting peptides more stable, the most important are cyclization, unnatural amino acids insertion, and backbone replacements by amide bond surrogates. In particular, the substitution of amide bond by an aminomethylene group, [–CONH– → –CH_2NH–] produces a reduction of polarity and strengthens resistance against protease degradation when compared to natural peptides.

In this contest, the first part of my research was mainly based on the elaboration of aminoacids.

To this aim, I worked on the identification of a new system for the protection of the α-amino functionality of amino acids. In my work, different strategies for the protection of

α-aminoacids have been developed. Considering its remarkable and innovative properties, an ionic liquid was selected as solvent for our protocol. In fact, the ionic liquids, as green solvents, have been studied widely due to their appealing properties such as negligible vapour pressure, large liquid range, high thermal stability, high ionic conductivity, large electrochemical window, and ability to solvate compounds of widely varying polarity.

Utilizing ionic liquids is one of the goals of green chemistry because they create a cleaner and more sustainable chemistry and are receiving increasing interest as environmental friendly solvents for many synthetic and catalytic processes.

Furthermore, my attention was focused on the synthesis of *N*-methyl-α-amino acids. The developed methodology involves the use of the reagent system $AlCl_3$/diazomethane. The removal of Fms protecting group is achieved under the same conditions to those used for Fmoc removal. The Fms group can be interchangeable with the Fmoc group in the synthesis of *N*-methylated peptides using standard Fmoc-based strategies.

Additionally, in the respect of elaboration of amino acids and considering the remarkable importance of amino function, a rapid and efficient method for synthesis of amino group in one single step has been developed. The nitro compounds were reduced by using reducing systems generated by the action of an excess of $LiAlH_4$ on $TiCl_4$.

The second part of my project, instead, was devoted to the elaboration of peptide bond. To this aim, direct procedures for the synthesis and reduction of amide bond have been developed.

The first method for peptide bond synthesis was carried out in the presence of silver acetate. The approach is successful in forming peptide bonds starting from *N*-(4-nitrobenzenesulfonyl)-amino acid chlorides and allows the formation of dipeptides also when *N*-methylated amino acid derivatives are used.

The second procedure concerns using of $SOCl_2$, as a chlorinating agent in the formation of acyl chlorides. It works also as coupling agent by activating in situ the carboxylic function of the acid through the formation of a

acyl chloride that reacts with the amine by generating the amide, so, the reaction proceeds under one-pot conditions.
Peptide modified covers an important role in pharmaceutical chemistry so an expeditious and practical method for the reduction of amides to amines was investigated in the present project. The procedure is based on the activation of amides with titanium tetrachloride followed by reduction with lithium aluminum hydride. The reducing system can be applied both to *N*-α-protected amino acid and dipeptides in order to increase the stability against protease degradation.

Finally part of my activity was dedicated to the food chemistry. In particular my study was about the evolution, during ripening, of the fatty acid profile and the fatty acid composition in acylglycerols of three different fermented sausages. Statistical analysis (ANOVA) was applied to the results obtained for the profiles to check all the differences between samples. The study comprised also an evaluation of the lipid oxidation level.
In additional, I focused my attention on the study of bergamot essential oil. It is generally used as a flavouring in

the pharmaceutical and cosmetics industries, it is also used widely in aromatherapy to reduce stress and anxiety despite limited scientific evidence.

My work proposes a new GC/MS methodology for the direct analysis of volatile compounds of bergamot essential oil.

Chapter 1
New methodologies for the elaboration of aminoacids

1.1 Strategies for the protection of amino function of α-amino acids by using IL

1.1.1 Deprotection and reprotection of amino group in α-amino acids by using [bmim][BF_4]

The presence of an amine functionality in a wide range of biologically active compounds makes protection of amines an important and frequently required exercise in synthetic organic chemistry.[1]

In particular, protection of the α-amino functionality of amino acids is one of the most important issues in peptide

synthesis and it is mandatory to prevent polymerization of the amino acid once it is activated.

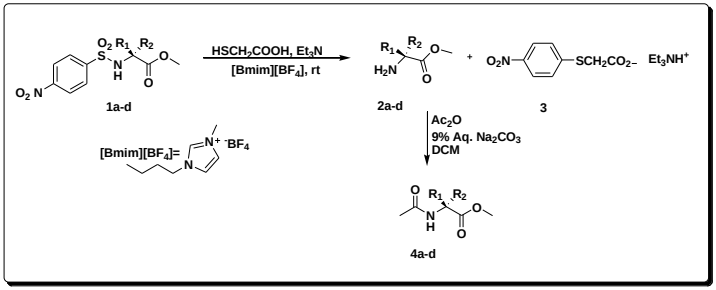
On the other hand, peptide synthesis, both in solution and on solid phase, is preferentially carried out in the *C* to *N* direction, and α-amino temporary protecting groups are removed several times during the synthesis; therefore, removal must be done in mild conditions that do not affect the remaining protecting groups or even the peptide chain.[2]

Recently, the use of ionic liquids (ILs) in organic synthesis, has received great attention due to their unusual properties as non-conventional solvents.[3] In fact, they are characterized by reasonable stability, low flammability, no miscibility with non polar solvents.[4]

In addition, ILs offer the possibility to carry out the organic reactions with excellent improvement in product yields and selectivity with respect to classical conditions and enable an easy recover of the reaction products by simple extraction procedures.[5] In particular, 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim][BF_4], have demonstrated to

serve as excellent catalysts for the *N*-tert-butyloxycarbonylation of various amines and amino acids.[6]

The study of this protocol began selecting *N*-nosyl-L-valine methyl ester (**1a**), prepared as reported in literature,[7] as a model substrate. Compound **1a** was treated with the reagent system mercaptoacetic acid/sodium methoxide.[8]
The deprotection reaction was conducted at room temperature and in [bmim][BF_4] as the ionic liquid medium.
TLC analysis of the reaction mixture showed that the starting material remained totally unchanged with respect to the standard used for the analysis over 24 h of treatment.
Upon the conditions adopted in the above described experiment the basic reagent sodium methoxide was not soluble, hampering the formation of the S-nucleophile required for the removal of the nosyl group.
These preliminary data indicated us the need for searching different basic media to successfully conduct the reaction.
The same experiment was thus repeated replacing sodium methoxide with triethylamine (Scheme 1).



Scheme 1. Removal of Nosyl Group from 1a-d in [Bmim][BF_4] and *N*-Acetylation of the Free Amino Function of 2a-d

A reduced amount of base (only 5 equiv) was used instead of the high excess of sodium methoxide required in the precedent protocol.

TLC revealing that starting material disappeared after only 5 min of stirring.

The complete removal of the nosyl group was definitively assessed by the ninhydrin test.

The free amino acid methyl ester **2a** was recovered through a simple extraction with diethyl ether from [bmim][BF_4].

In order to demonstrate the successful removal of the nosyl group in IL, **2a** was characterized after its conversion into the corresponding *N*-acetyl derivative **4a** by treatment with acetic anhydride under Schotten-Baumann conditions.

The reaction afforded the *N*-acetylated derivative **4a** which was obtained pure in 96% yield.

Compound **4a** was completely characterized by GC/MS and ^{1}H and ^{13}C NMR spectroscopy.

The practicability of the deprotection protocol was next demonstrated with a series of lipophilic nosyl protected α-amino acid methyl esters prepared as elsewhere reported[7] (Table 1).

Table 1. Preparation of 2a-d in [bmim][BF_4] and their *N*-acetilation

Entry	R^1	R^2	Time (min)[a]	Yields of 4 (%)[b]
a	$CH(CH_3)_2$	H	5	96
b	CH_2Ph	H	5	92
c	$CH_2CH(CH_3)_2$	H	4	89
d	$CH(CH_3)CH_2CH_3$	H	6	90

[a] Referred to the deprotection step in IL, [b] Isolated yield based on starting 1a-d

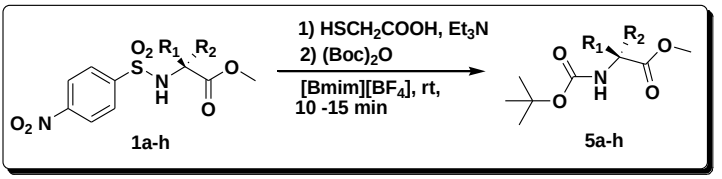
Thus, we exploited a one-pot nosyl deprotection and Boc reprotection of the α-NH_2 group of amino acid derivatives in [bmim][BF_4]. The first experiment was performed selecting again **1a** as case of study.

The removal of nosyl group was accomplished by treating **1a** with the reagent system mercaptoacetic acid/triethylamine using [bmim][BF_4] as the solvent. The

resulting *N*-deprotected intermediate was not isolated from the IL and directly treated with an equimolar amount of di-tert-butyl dicarbonate (Boc_2O) to reprotect the free α-amino function (Scheme 2).

The reprotection reaction was conducted at room temperature without further addition of base. TLC monitoring demonstrated that the entire process was complete within 10 min.

Extraction with diethyl ether and acidic washing of the organic layers afforded the corresponding *N*-Boc-valine methyl ester **5a** which was recovered in an excellent yield referred to the initial amount of the *N*-nosyl protected precursor (Table 2). As expected ^{1}H and ^{13}C NMR spectroscopy clearly confirmed the molecular structure of **5a**. The data obtained confirmed the feasibility of a one-pot procedure which can straightforwardly be used in a sequential deprotection/reprotection of the α-amino function in α-amino acid methyl esters.



Scheme 2. One pot deprotection/reprotection of the α-amino function of 1a-h in IL.

We thus investigated a larger number of *N*-nosyl protected α-amino acid methyl esters.

Therefore, the reactivity of other lipophilic as well as some selected polar amino acid systems was tested. Starting from the *N*-nosyl-α-amino acid methyl esters **1b-f** (Table 2), the corresponding *N*-Boc protected derivatives **5b-f** were uniquely formed.

Despite the methods already reported, the new protocol required only 10-15 min of stirring at room temperature for the complete removal of the nosyl group as checked by TLC and GC/MS analysis of the reaction mixtures.

Also the treatment with $(Boc)_2O$ for the subsequent Boc reprotection of the free amino acid methyl esters proceeded in very short times (Table 2).

In all cases, yields in recovered products were excellent ranging from 92% to 96%. Differently from other procedures already reported for the Boc protection of

amines in ILs,[9] no chromatography needed to recover the final products.

In fact, compounds **5a-f** were obtained in high purity as verified by ^{1}H and ^{13}C NMR spectroscopy. As expected, the spectroscopic characteristics of each compound matched those published for the same molecules produced by other methodologies.[10]

Due to the effectiveness of the method, we considered very attractive the investigation of its versatility by exploiting the reactivity of *N*-nosyl-α-amino acid methyl esters having side-chain functional groups protected with suitable masking moieties, which should survive the conditions of deprotection and reprotection of the "one-pot" protocol.

Thus, *N*-nosyl-Tyr(*O*Bn) and *N*-nosyl-Cys(*S*Bn) methyl esters, **1g** and **1h** respectively, were used (Table 2).

The choice of an *O*- and *S*-benzyl protection was motivated by the fact that the Bn (benzyl) group has largely been used for the solution and solid-phase synthesis of a number of relevant peptides using the so-called "Boc/Bn strategy".[11]

Table 2. Preparation of *N*-Boc-α-Amino Acid Methyl Esters 5a-h in IL

Entry	R^1	R^2	Time (min)[a]	Yields of 5 (%)[b]
a	$CH(CH_3)_2$	H	10	94
b	CH_2Ph	H	15	96
c	$CH_2CH(CH_3)_2$	H	12	95
d	$CH(CH_3)CH_2CH_3$	H	10	94
e	CH_3	H	10	94
f	H	CH_2Ph	15	96
g	$CH_2C_6H_4OBn$	H	15	93
h	CH_2SBn	H	15	92

[a] Referred to the deprotection step in IL, [b] Isolated yield based on starting 1a-h

In both cases the "one-pot" strategy afforded the corresponding *N*-Boc-protected derivatives **5g** and **5h** in almost quantitative yields referred to the initial amounts of the respective precursors.

Also in these cases, the expected molecular structure of **5g** and **5h** was confirmed by ^1H and ^{13}C NMR, demonstrating the full compatibility of Bn group with the adopted experimental conditions. Retention of configuration is a fundamental consideration in amino acid chemistry. It is well known, in fact, that basic media could induce racemization of the α-carbon atom. Our protocol uses a weak base, such as triethylamine; although the deprotection/reprotection steps are performed at room temperature in very short times, we decided to check the

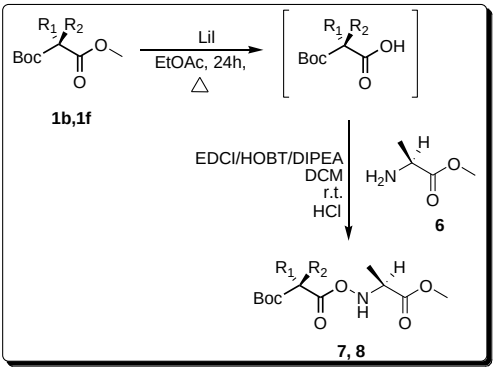
stability of the configuration of amino acid skeleton during the entire process. Firstly, we subjected to the protocol *N*-nosyl-isoleucine methyl ester **1d**. The proton and carbon spectra of the corresponding Boc-derivative **5d** showed only one set of resonances which was diagnostic for the absence of racemization, at least within the sensibility limits of the spectroscopic techniques. Thus, the retention of configuration was definitively assessed by preparing *N*-Boc-phenylalanine methyl ester **1b** and its enantiomer **1f** by the one-pot protocol in [bmim][BF_4]. Both compounds were then used as obtained to synthesize the diastereomeric pair of *N*-Boc-dipeptides **7** and **8** (Scheme 3).

To this aim, compounds **1b** and **1f** were demethylated[12] to afford the corresponding acids which in turn were immediately reacted with alanine methyl ester hydrochloride (**6**) under typical conditions of a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/1-hydroxybenzotriazole (EDCI/HOBt) assisted coupling for the peptide synthesis.[13] Samples of the crude dipeptides **7** and **8** were analyzed by ^{1}H and ^{13}C NMR.

The complete spectroscopic analysis of **7** did not exhibit residual signals attributable to the other diastereomer **8.** Furthermore, the proton as well as the carbon spectrum of **7** and **8** were both characterized by unique series of signals, demonstrating that the configurations of each single enantiomer **1b** and **1f** was preserved during the deprotection/reprotection process in [bmim][BF_4].

The chemical shift differences between the most significant signals of the two diasteromeric dipeptides resulted evident from the spectroscopic analysis recorded for a sample prepared by mixing approximately equimolar amounts of **7** and **8** (Figure 1).

In particular, the methyl protons of the alanine residue showed well distinguishable resonances in the spectra recorded for both diastereomers. Different chemical shifts were observed also for the amide NH and OCH_3 protons.



Scheme 3. Synthesis of the Diastereomeric Pair of *N*-Boc-Protected Dipeptide Methyl Esters 7 and 8 by EDCI/HOBt Mediated Coupling

Table 3. *N*-Boc-Protected Dipeptide Methyl Esters **7** and **8** by EDCI/HOBt Mediated Coupling

Entry	R^1	R^2	R^3	R^4	Yield (%)[a]
7	CH_2Ph	H	H	CH_3	92
8	H	CH_2Ph	H	CH_3	91

[a] Yields in recovered products referred to 1b and 1f

The validity of this method was further investigated by preparing *N*-Boc-*N*-methyl valine methyl ester. *N*-Methyl-α-amino acids, in fact, cover an important role in medicinal chemistry, and many efforts have been directed at optimizing synthetic methods for their preparation and use in peptide chemistry.[2] *N*-Nosyl-*N*-methyl valine methyl ester, prepared as previously reported,[7] was subjected to the one pot protocol in [bmim][BF_4]. The complete removal

of nosyl group was observed after 30 min at room temperature. The subsequent reprotection of the free amino group provided *N*-Boc-*N*-methyl-valine methyl ester in 5 min and in 90% yield.

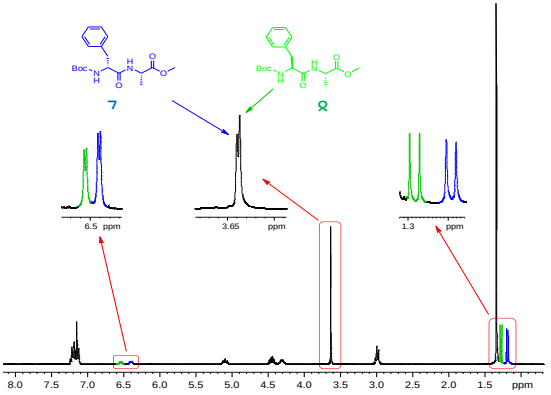


Figure 1. ^{1}H NMR Spectrum of a Mixture of **7** and **8** (Approx. 1:1). Spectral Windows: 6.50 ppm, Signals of BocNH Protons; 3.65 ppm, Signals of OCH_3 Protons; 1.20-1.30 ppm, signals of CH_3 Protons of the Ala Residue. (Colors: Blue, Resonances of **8**; Green: Resonances of **7**)

In summary, we have described here a one-pot procedure for the sequential deprotection/reprotection of the amino function of amino acid methyl esters in an IL. Mild conditions, room temperature, reduced amount of base, very short reaction time, the easy of extraction of products

from IL, excellent yields and purities, and the IL recyclability were the distinct advantages of the protocol. The process appears charming and highly versatile, representing a valuable tool for the easy removal of 4-nitrobenzenesulfonyl group as well as for the introduction of the Boc moiety.

(The work has been published: Di Gioia, M.L.; Barattucci, A.; Bonaccorsi, P.; Leggio, A.; Minuti, L.; Romio, E.; Temperini, A.; Siciliano, C. "Deprotection/Reprotection of the Amino Group in α-Amino Acids and Peptides. A One-Pot Procedure in [Bmim][BF_4] Ionic Liquid" RSC Advances, 2014, 4, 2678-2686)

1.1.2. N-Urethane protection of aminoacids in IL

It is well known that masking the α-amino function of amino acids with urethane protecting groups has become topic of fundamental importance in the preparation of building blocks both in solution as well as solid phase peptide synthesis.[14]

Consequently, several kinds of protecting groups have been successfully used in this regard.[15]

In this context, the carbamate derivatives occupy a prominent position in the ranks of commonly used amine protecting groups (PGs).[16]

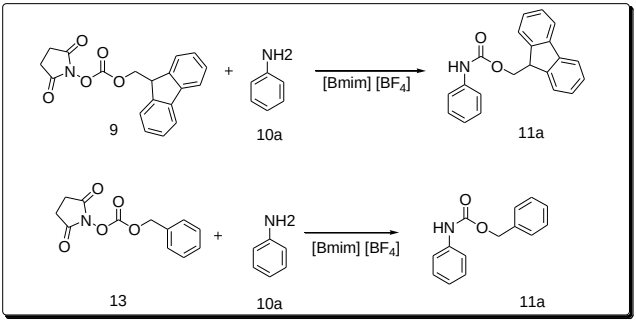
So, we have developed an efficient, mild, solvent-free protocol for the *N*-Fluorenylmethoxycarbonylation and *N*-benzyloxycarbonylation of aminoacids trought using IL.

In order to investigate the optimum model reaction, we selected, aniline (**10a**), an aromatic amine, as model substrate because of the low nucleophilicity of the amine nitrogen. Aniline (**10a**) (Scheme 4) was treated with 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) (**9**) (1:1 mmol).

The reaction conducted in [Bmim] [BF_4] (1 mL) at room temperature was completed in 5 minutes and the expected *N*-(9-fluorenylmethoxycarbonyl)aniline **(11a**) was obtained in 86% yield after a simple extraction procedure with diethyl ether from the ionic liquid and without the need for chromatographic purification.

Encouraged by these promising results, the developed protocol was also attempted to introduce the Cbz urethane group, by using *N*-(Benzyloxycarbonyloxy)succinimide (Cbz-Osu) (**13**). Aniline (**10a**) was treated with 1 equiv of CBz-Osu (**13**) in [Bmim] [BF_4] (1 mL). The reaction conducted at room temperature, was completed in 5 minutes and the protected aniline (**12a**) was recovered with 87% yield after

a simple extraction procedure with diethyl ether without the need for chromatographic purification.

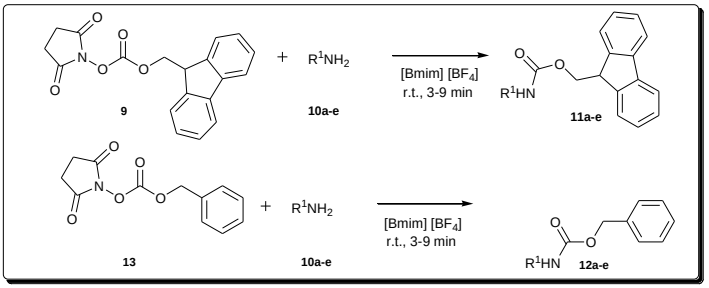


Scheme 4. *N*-Protection of Aniline with Fmoc-OSu and Bzl-OSu in IL

So, after the effective results in the *N*-Fmoc and *N*-Bzl protection of aniline we have further extended our study by applying the protocols to the α-amino group of amino acid methyl esters.

Nevertheless, our protocol was not applicable for the Fmoc protection of L-valine methyl ester hydrochloride (**10d**) due to the lack of solubility of the amino acid methyl ester as a HCl salt in the ionic medium. The best results were obtained after addition to the mixture of 0.5 mL of an aqueous solution of sodium bicarbonate therefore allowing the liberation of the amino group, which can then act as a good nucleophile for the reaction of protection. Under the

optimized reaction conditions, the Fmoc protection of valine methyl ester was thus achieved in 8 minutes and in 86% yield (Scheme 5; Table 4, **11d**).



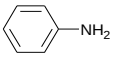
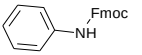
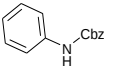
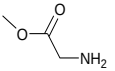
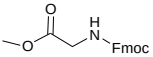
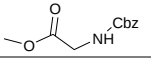
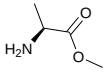
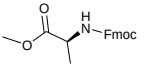
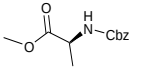
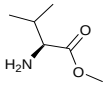
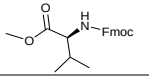
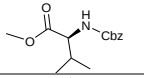
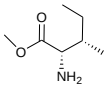
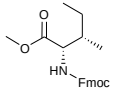
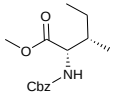
Scheme 5. *N*-Fmoc and *N*-Cbz protection of amines and -α-amino acid methyl esters in [bmim] [BF_4]

Similarly, the treatment of other α-amino acid methyl esters (**10b, 10c, 10e**) with FmocOsu (**9**) in [Bmim][BF_4] using $NaHCO_3$ as an additive provided the respective *N*-protected compounds in good to very good yields (Table 4, **11b, 11c, 11e**).

In addition, we have applied our protocol for the *N*-Bzl-protection of α-amino group of amino acid methyl esters.

Also in this case the use of 0.5 mL of an aqueous solution of sodium bicarbonate was needful for obtain a good nucleophile for the reaction of protection.

Table 4. Preparation of *N*-protected amines and -α-amino acid methyl esters in [bmim] [BF_4]

10		Product (11 and 12)		Time (min)	Yield[a] (%)
10a			**11a**	5	86
			**12a**	5	87
10b			**11b**	5	87
			**12b**	9	90
10c			**11c**	9	85
			**12c**	7	86
10d			**11d**	8	86
			**12d**	8	90
10e			**11e**	8	85
			**12e**	8	88

[a] Isolated yield

So, after adding 0.5 mL of an aqueous solution of sodium bicarbonate, amino methyl esters (**10b-e**) were treated with 1 equiv. of Bzl-Osu (**13**) in [Bmim] [BF_4] (1 mL). The reactions conducted at room temperature, were completed

in 7-9 minutes and the *N*-Bzl-protected amino methylesters (**12b-e**) were recovered in high yields after a simple extraction procedure with diethyl ether without the need for chromatographic purification.

In summary, we have reported a simple and convenient protocol based on the use of [Bmim][BF_4] for the preparation of Fmoc and Cbz-protected amino acid methyl esters. In particular, the absence of organic solvents or bases, the use of mild conditions, short reaction times, the high yields, the simple work-up procedure and finally the possibility to recycle the ionic liquid represent important features and advantages of the method providing a valuable contribution to the existing methodologies for the introduction of urethane amine protecting groups.

(The work has been published M. L. Di Gioia, A. Gagliardi, A. Leggio, V. Leotta, E. Romio, and A. Liguori, N-Urethane protection of amines and amino acids in ionic liquid. RSC Advances, Volume 5, Issue 78, 2015, Pages 63407-63420)

1.2 *N*-methylation of Fms-α-amino acid methyl esters

N-methyl amino acids are constituents of several biologically active natural peptides with antibiotic

(enniatine), antineoplastic (dolastatine) and immunosuppressive activity (cyclosporine).[17]

N-methylation represents an important modification of peptide bond.

The presence of a methyl group on the amide nitrogen atom of modified natural peptides can improve properties like enzymatic stability and bioavailability, as well as modify conformational preferences due to the decreased ability to form hydrogen bonds.[18]

N-methylation of synthetic peptides might also stabilize those conformations that are less prone to interact with protein binding sites.

The promising therapeutic profile of these compounds has prompted further research to develop new and more effective methods for their synthesis.

Various procedures have been developed over the years for the synthesis of optically active *N*-methyl amino acids, but few synthesis are effectively simple and general. In particular drastic reaction conditions, long synthetic sequences and formation of racemization products characterize several reported experimental procedures.[19]

The most commonly used synthetic methodologies involve the direct methylation, under basic conditions, of the suitably *N*-protected α-amino acids or α-amino acid esters by using methyl halides and other methylating agents, or the reductive alkylation of *N*-protected α-amino acids with formaldehyde.[20]

The use of *N*-methyl-*N*-nosyl-α-amino acids in the Fmoc-based synthesis of *N*-methylated peptides, requires deprotection of the amine function that is realized with difficulty through a nucleophilic aromatic substitution reaction by using a sulfur nucleophile, and the subsequent transformation of the deprotected product into the corresponding *N*-Fmoc-*N*-methyl derivative.

Hence it was necessary a sulfonamide-based protecting group enabling the methylation of nitrogen atom with diazomethane and compatible with Fmoc-chemistry.

A recently published paper[21] has reported a new sulfonamide-based protecting group, the (9H-fluoren-9-yl)methanesulfonyl (Fms) group, which can be employed in a similar way to the well-established Fmoc group.

This group in fact is removable under the identical conditions used for the Fmoc cleavage.

Moreover, the presence of the sulfonyl group on the nitrogen atom could promote the *N*-methylation with diazomethane as reported in the literature.[7] Thus the Fms protecting group could be interchangeable with the Fmoc group in Fmoc-based peptide synthesis when it is necessary to obtain *N*-methylated peptides.

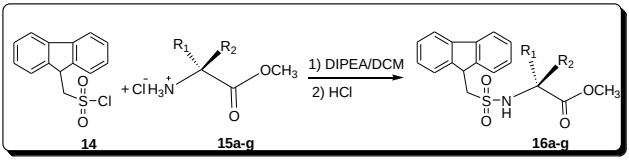
The *N*-Fms-*N*-methyl-α-amino acids could be used as an alternative to *N*-Fmoc-*N*-methyl-α-amino acids which are prepared with greater difficulty.

All these remarks led us to test this protecting group for the methylation of the amino function of α-amino acids.

The development of our methodology started synthesizing *N*-Fms-α-amino acid methyl esters. *N*-9-fluorenylmethanesulfonyl-α-amino acid methyl esters (**16a-f**) were prepared by treating lipophilic α-amino acid methyl esters (**15a-**f) with FmsCl (**14**) and *N,N*-diisopropylethylamine (DIPEA) in dichloromethane (DCM) at room temperature (Scheme 6). After about 2 h the reaction afforded *N*-9-fluorenylmethanesulfonyl-α-amino acid methyl esters (**16a-f**) in very good yields (Table 5) and high purity as verified by TLC and elemental analysis.

The protection reaction was subsequently tested with one side chain functionalized amino acid, L-tyrosine methyl ester protected on side chain with the benzyl group (**15g**).

The corresponding *N*-Fms-protected derivative **16g** was obtained in 72 % yields (Scheme 6, Table 5) and high purity as confirmed by ^{1}H and ^{13}C NMR spectroscopy



Scheme 6. Synthesis of *N*-9-fluorenylmethanesulfonyl-α-amino acid methyl esters **16a-g**

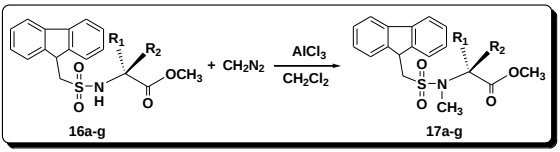
Table 5. Results of the Synthesis of **16a-g**

Entry	R_1	R_2	Yields 16 (%)[a]
a	H	CH_3	78
b	CH_3	H	66
c	H	$CH(CH_3)_2$	77
d	H	$CH(CH_3)CH_2CH_3$	82
e	H	CH_2Ph	75
f	H	H	84
g	H	$CH_2C_6H_4OCH_2Ph$	72

[a]Isolated yield based on starting **15a–g**.

Subsequently, we investigated the possibility of methylating the amino function of the *N*-Fms-α-amino acid methylesters **16a-g** using aluminum trichloride. The reaction was tested

first with *N*-Fms-L-phenylalanine methyl ester (**16e)**. **16e** (1 mmol) was dissolved in DCM and treated with $AlCl_3$ (1.3 mmol) and a dichloromethane solution of diazomethane (6 mmol, 0.66 N) (Scheme 7). The reaction was completed within 1 h and **17e** was recovered in 70 %(Scheme 7, Table 6).



Scheme 7. Synthesis of *N*-Fms-*N*-methyl-α-amino acid methyl esters 17a-g

Table 6. Results of the Synthesis of 17a-g

Entry	R_1	R_2	Yields 17 (%)[a]
a	H	CH_3	70
b	CH_3	H	73
c	H	$CH(CH_3)_2$	72
d	H	$CH(CH_3)CH_2CH_3$	69
e	H	CH_2Ph	70
f	H	H	68
g	H	$CH_2C_6H_4OCH_2Ph$	70

[a]Isolated yield based on starting 16a–g.

Encouraged by this promising result, we tested the developed methylation protocol with other lipophilic amino acid methyl esters. *N*-Fms-α-amino acid methyl esters **16a-d, 16f** (Scheme 7, Table 6) were subjected to the above

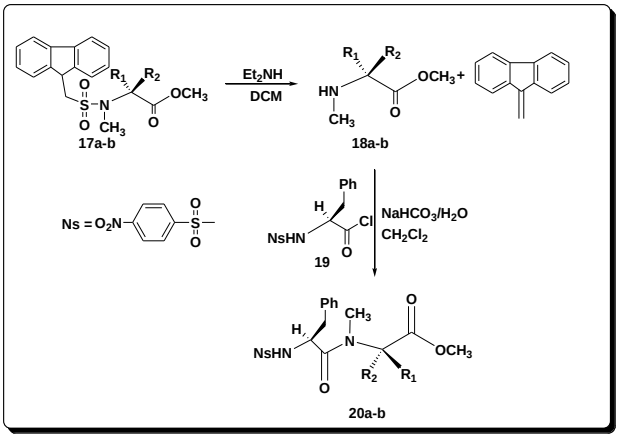
described treatment affording the corresponding *N*-methylated derivatives **17a-d, 17f** in very good overall yields (68-72 %). ^{1}H NMR and ^{13}C NMR spectroscopy clearly confirmed the molecular structure of **17a-d, 17f.**

The methylation methodology was extended successfully to the *N*-Fms-O-benzyl-L-tyrosine methyl ester (**16g**) having in the side chain an acid labile protecting group. The corresponding *N*-Fms-*N*-methyl-O-benzyl-L-tyrosine methyl ester (**17g**) was obtained (70 % yield) keeping unchanged the acid-sensitive side chain protecting group (Scheme 7, Table 6). In order to give to the developed methodology a greater applicability in peptide synthesis, it appeared interesting to investigate whether the configuration of the amino acid chiral centres was retained during the methylation reaction and the subsequent deprotection of the amino function, by synthesizing a couple of diastereoisomeric dipeptides: *N*-*p*-nitrobenzensolfonil-L-phenylalanine-*N*-methyl-L-alanine methyl ester (**20a**) and *N*-*p*-nitrobenzensolfonyl-L-phenylalanine-*N*-methyl-D-alanine methyl ester (**20b**).

To this aim, the methylation of *N*-Fms-L-alanine methyl ester (**16a**) and *N*-Fms-D-alanine methyl ester (**16b**) was

followed by the deprotection of the amine function of **17a** and **17b** with diethylamine[22] (Scheme 8). The deprotected products *N*-methyl-L-alanine methyl ester (**18a**) and *N*-methyl-D-alanine methyl ester (**18b**) were then coupled with *N*-*p*-nitrobenzensolfonyl-L-phenylalanine chloride (**19**) (*N*-nosyl-L-Phe-Cl)[23] for obtaining respectively the diastereomeric *N*-nosyl dipeptides **20a** and **20b** (Scheme 8, Table 7).

The choice of *N*-nosyl-L-phenylalanine (**19**) was related to the need to identify easily the two diastereomers by ^{1}H NMR analysis. In fact, the aromatic protons of nosyl group give rise, in the ^{1}H NMR spectrum, to simple and easily distinguishable signals that differ in the two diastereoisomers.



Scheme 8. Synthesis of *N*-Ns-L-Phenylalanine-*N*-methyl-L-alanine methyl ester **20a** and *N*-Ns-L-Phenylalanine-*N*-methyl-D-alanine methyl ester **20b**

Table 7. Results of the synthesis of **20a-b**

Product	R_1	R_2	Yield 20 (%)[a]
20a	H	CH_3	72
20b	CH_3	H	75

[a]Isolated yield based on starting **17a–b**.

The ^{1}H-NMR spectrum of the mixture showed that some signals of the two diastereomers were completely separated (Figure 2 B). In particular, well separated resonances for the aromatic protons of the nosyl group (7.78 and 8.18 ppm for 20a and 7.91 and 8.27 ppm for 20b), the methyl protons of ester function (3.70 ppm for 20a and

3.61 ppm for 20b), and the α-proton of alanine at 4.89 (20b) and 5.02 ppm (20a) were observed.

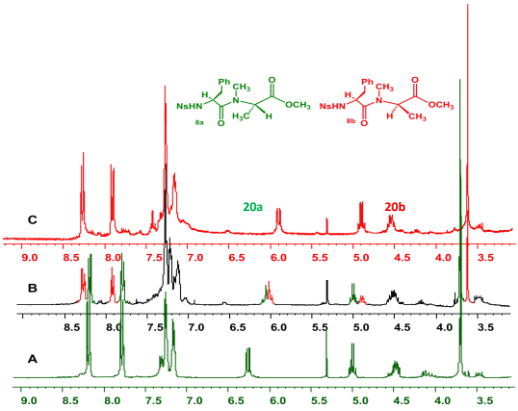


Figure 2 Overlapped spectral windows (9.0-3.5 ppm) of ^{1}H NMR spectra of crude *N*-Ns-L-Phenylalanine-*N*-methyl-L-alanine methyl ester (**20a**, **A**), crude *N*-Ns-L-Phenylalanine-*N*-methyl-D-alanine methyl ester (**20b**, **C**) and a mixture of crude **20a** and **20b** (approx. 65 % : 35 %, **B**).

The absence of racemization was also monitored by LC/MS analysis. The two diastereoisomers of a new prepared mixture of crude **20a** and **20b** (approx. 42 % : 58 %) were readily resolved by LC/MS: the presence of two chromatographic peaks with retention times of 20.379 and 21.392 minutes was observed (Figure 3 A).

Chromatograms of the single crude dipeptides **20a** (Figure 3 C) and **20b** (Figure 3 B) showed the presence of a unique

peak with retention times of 20.363 and 21.547 minutes respectively.

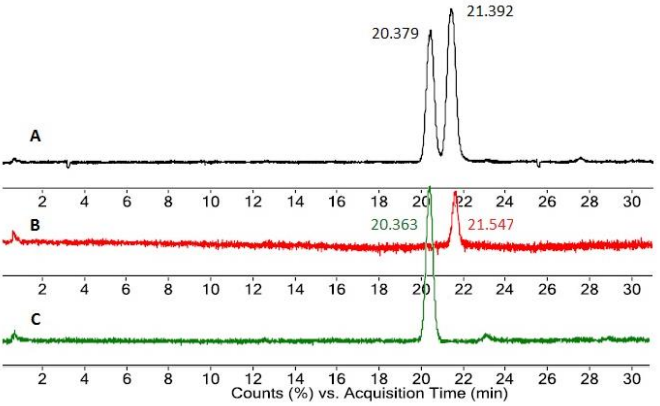


Figure 3 UHPLC/MS analysis of crude N-Nosyl dipeptides **20a** and **20b**: (A) a mixture of 20a and 20b (approx. 42 % : 58 %); (B) N-Nosyl-L-Phe-D-(Me)-Ala-OMe (20b); (C) *N*-Nosyl-L-Phe-L-(Me)-Ala-OMe (20a).

LC/MS analysis in combination with ^{1}H NMR data of the two diastereomeric dipeptides, **20a** and **20b,** demonstrated the absence of epimerization products proving that the stereochemistry of the original chiral carbon atoms is totally retained throughout the entire synthetic procedure.

All these results suggested that $AlCl_3$/diazomethane is an efficient reagent system to methylate in short time and very

good yields the amino function of *N*-Fms protected α-amino acid methyl esters.

N-Fmoc-protected α-amino acids do not give methylation reaction under these conditions. In fact the treatment of *N*-Fmoc-L-alanine methyl ester with a dichloromethane solution of diazomethane and $AlCl_3$ under the same reaction conditions of **16a-g** gave a mixture of dibenzofulvene and the starting material. The action of $AlCl_3$ on the substrate *N*-Fmoc-protected amino methyl ester causes the partial removal of the Fmoc group. The interaction between the Lewis acid $AlCl_3$ and the urethanic function of *N*-Fmoc-L-alanine methyl ester produces a highly reactive electrophilic species, which turns into dibenzofulvene.[24]

The results obtained showed the applicability and reliability of the methylation procedure of the *N*-Fms-α-amino acid methyl esters based on the reactivity of diazomethane, in the presence of aluminum trichloride, on the 9-fluorenylmethansulfonylamide function. This synthetic strategy provided the *N*-Fms-*N*-methyl-α-amino acids methyl esters in very good yields. It is particularly attractive because the adopted conditions did not cause any detectable loss of the optical integrity at the chiral centres

of the precursors.The adopted procedure for the *N*-methylation of *N*-Fms-α-amino acids methyl esters takes on an important synthetic and applicative validity since the obtained *N*-methylated *N*-Fms-α-amino acids methyl esters can be used as such in solution peptide synthesis of *N*-methylated peptides.

(The work has been published: Leggio, A. Alò, Danila; Belsito, E. L.; Di Gioia, M. L.; Romio, E.; Siciliano C.; Liguori, A. "Lewis acid catalysed methylation of N-(9H-fluoren-9-yl)methanesulfonyl (Fms) protected lipophilic α-amino acid methyl esters" Journal of Peptide Science, Volume 21, Issue 8, 2015, Pages 644-650)

1.3 A new methodology for synthesis amines from nitro nitro compounds

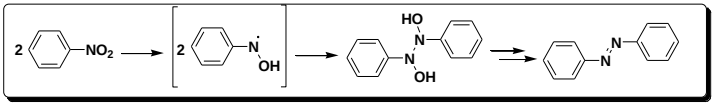
Aminoacids are characterized by the simultaneous present of carboxylic and amine group.

We focused our attention on amino group not only because it is responsable of chemical, phisical and biological properties of aminoacids but also because it represents one of the most important and valuable organic functional groups in naturally occurring molecules, pharmaceuticals, agrochemicals, and polymers.[25]

Improved and innovative methods for the synthesis of amines are in great demand both by chemical and pharmaceutical industry.

Therefore, a rapid and efficient method for synthesis of amines in one single step has been developed. The aromatic nitro compounds were reduced by using reducing system generated by the action of an excess of $LiAlH_4$ on $TiCl_4$.

Lithium aluminium hydride ($LiAlH_4$) represents a very versatile reducing agent that is extremely useful in synthetic organic chemistry. It is a more powerful reducing agent than $NaBH_4$ and reduces aromatic nitro compounds affording their corresponding azo compounds.[26] The reaction most likely proceeds, in analogy with the catalytic hydrogenation of nitro compounds[27], through electron transfer, in an aprotic solvent, from hydride to nitro group with the formation of a radical intermediate that dimerizes by providing the azo-derivative (Scheme 9).



Scheme 9. Probable formation mechanism of azo compounds.

It has been found a reducing system, obtained by using $LiAlH_4$ and $TiCl_4$ in the molar ratio 5:1 that is able to reduce efficiently nitro aromatic derivatives to amines.

It was prepared by adding $LiAlH_4$ (5 mmol) to a stirred suspension of $TiCl_4$ (1 mmol) in diethyl ether. The reaction mixture was stirred at room temperature for 15 minutes.

As a result of filtration of the obtained black suspension was recovered a black solid.

The so prepared reagent system was tested to reduce *N,N*-diethyl-4-nitrobenzensulfonamide (**21a**) chosen as model substrate. In a typical experiment **21a** (1 mmol), was added slowly to the obtained black reducing suspension in diethyl ether. The reaction was completed in 15 minutes and, after work up of the reaction mixture, 4-amino-*N,N*-diethylbenzenesulfonamide (**22a**) was recovered in 92% yield (Scheme 10, Table 8).

Also 3-amino-*N,N*-diethylbenzenesulfonamide (**21b**) and 2-amino-*N,N*-diethylbenzenesulfonamide (**21c**), treated under the same reaction conditions of **21a**, afforded the corresponding amines **22b** and **22c** in high yields (Scheme 10, Table 8).

In all cases the reduction reaction provided, at room temperature and within very short reaction times, the corresponding amines as unique reaction product.

Subsequently we investigated the reaction of other nitrobenzenes substituted with electron-withdrawing substituents under the optimized conditions above.

The reduction of halogen substituted nitrobenzenes proceeded without dehalogenation.

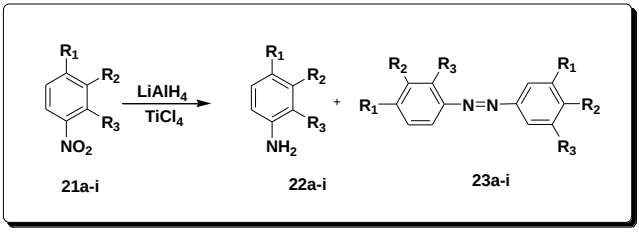
The reduction of 1-fluoro-4-nitrobenzene **(21d**), 1-chloro-4-nitrobenzene **(21e),** and 1-bromo-4-nitrobenzene **(21f),** was, in all cases, completed in 45 minutes and provided the corresponding aniline derivatives **22d-f** in excellent yields (Scheme 10, Table 8).

All the recovered products did not require further purification and their structures were assigned based on ^{1}H and ^{13}C NMR spectra.

On the contrary, the reaction of nitrobenzene (**21g**) with the same reducing system went to completion in 15 hours at room temperature as detected by TLC.

After this time, aniline **(22g,** 48% yield), 1,2-diphenyldiazene (**23g**, 25% yield) were recovered after short column chromatography (diethyl ether/petroleum ether 60:40 v/v). Long reaction times were also required for the reduction of 1-methyl-4-nitrobenzene (**21h**), that, after 26 hours, afforded *p*-toluidine (**22h**, 45% yield), 4,4'-dimethylazobenzene (**23h,** 28% yield).

The treatment of 1-methoxy-4-nitrobenzene (**21i**) provided, after 29 hours and separation of reaction mixture by short column chromatography (diethyl ether/petroleum ether, 60:40 v/v), 4-methoxyaniline (**22i**, 43% yield) and 1,2-bis(4-methoxyphenyl)diazene (**23i** 20% yield).



Scheme 10. Reduction of substituted nitrobenzenes with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 5:1.

Table 8. Results of reduction of substituted nitrobenzenes with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 5:1

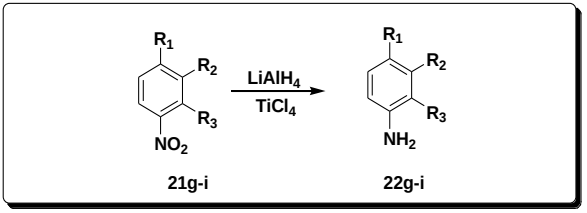
Entry	R_1	R_2	R_3	Yield [a] 22 (%)	Yield [a] 23 (%)	R.t.
a	SO_2NEt_2	H	H	92	-	15 min
b	H	SO_2NEt_2	H	89	-	25 min
c	H	H	SO_2NEt_2	90	-	25 min
d	F	H	H	98	-	45 min
e	Cl	H	H	91	-	45 min
f	Br	H	H	94	-	45 min
g	H	H	H	48	25	15 h
h	CH_3	H	H	45	28	26 h
i	OCH_3	H	H	43	20	29 h

[a]Isolated yield

The reduction of nitrobenzenes bearing electron-donor substituents in the 4-position **21h-i** gave a mixture of azobenzene and aniline derivatives and reaction times became significantly longer (Scheme 10, Table 8).

Additional experiments were performed in order to explore the reaction progress for synthesis of aromatic amino compounds under modified reaction conditions. Nitrobenzene (**21g**, 1 mmol), 1-methyl-4-nitrobenzene (**21h**, 1 mmol) and 1-methoxy-4-nitrobenzene (**21i,** 1 mmol) were treated with a different reducing system prepared by

adding $LiAlH_4$ (10 mmol) to a suspension of $TiCl_4$ (1 mmol) in diethyl ether at room temperature.



Scheme 11. Reduction of nitrobenzene and nitrobenzenes bearing electron donor substituents with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 10:1.

Table 9. Results of reduction of nitrobenzenes **21g-i** with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 10:1.

Entry	R_1	R_2	R_3	Yield[a] 22(%)	R.t.
g	H	H	H	90	25 min
h	CH_3	H	H	88	30 min
i	OCH_3	H	H	86	30 min

[a]Isolated yield

The reaction was monitored by thin layer chromatography (diethyl ether/petroleum ether 6:4 v/v) and when completed (after about 30 minutes of stirring), a NaOH aqueous solution was added and the phases were separated.

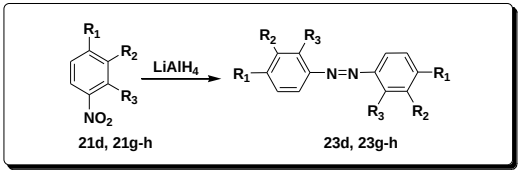
The resulting organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness under

reduced pressure to give the corresponding substituted anilines 22g-22i in very high yields (86-90%) (Scheme 11, Table 9), reaction by-products were not observed.

The results of reduction reactions of substrates 21g-21i suggested that azobenzenes could be key intermediates of the reduction process.

In order to gain a better understanding of the reduction reaction progress, symmetrical azobenzenes **23d, 23g** and **23h** were prepared by using a classical procedure.[28] To this aim 1-fluoro-4-nitrobenzene (**21d**, 1 mmol), nitrobenzene **(21g**, 1 mmol) and 1-methyl-4-nitrobenzene (**21h**, 1 mmol), were treated separately with 5 mmol of $LiAlH_4$.

In all cases, after about 15 minutes, the reaction was completed with the formation of a single product, the corresponding substituted azobenzene **23d, g, h** (Scheme 12, table 10).



Scheme 12. Reduction of substituted nitrobenzenes with $LiAlH_4$

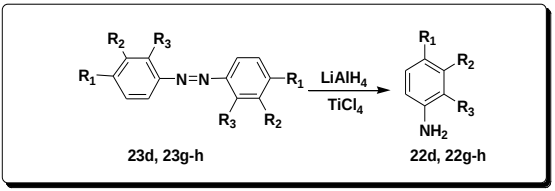
Table 10. Results of reduction of substituted nitrobenzenes with $LiAlH_4$

Entry	R_1	R_2	R_3	Yield [a] 23 (%)	R.t.
d	F	H	H	88	10 min
g	H	H	H	89	15 min
h	CH_3	H	H	92	15 min

[a] Isolated yield

The formation of azobenzenes was fast and did not require the presence of $TiCl_4$, furthermore it was also verified that even in very long times (24 hours) the obtained azobenzenes **23d** and **23g-h** are not converted into the corresponding amines by treatment at room temperature with just $LiAlH_4$ in the molar ratio 1:5.

However, when the azobenzenes **23d, 23g-h** (1 mmol) were treated with the different reducing system prepared by adding $LiAlH_4$ (10 mmol) to a stirred suspension of $TiCl_4$ (1 mmol) in diethyl ether, they were converted rapidly and in high yields (87-98%) into the corresponding amines **22d and 22g-h** (Scheme 13, Table 11).



Scheme 13. Reduction of substituted azobenzenes with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 10:1.

Table 11. Results of reduction of substituted azobenzenes with the reducing system $LiAlH_4$:$TiCl_4$ in molar ratio 10:1

Entry	R_1	R_2	R_3	Yield[a] 22 (%)	R.t.
d	F	H	H	98	5 min
g	H	H	H	87	10 min
h	CH_3	H	H	92	10 min

[a] Isolated yield

These results indicated that nitrobenzene and nitrobenzenes with electron-donor substituents in the 4-position, only in the presence of a large excess of lithium aluminum hydride and equimolar amounts of titanium tetrachloride can be converted rapidly, through the formation of azobenzenes **23** into the corresponding anilines **22**.

Therefore, in all the cases we examined the azobenzenes are reaction intermediates for the formation of anilines from the corresponding nitrobenzenes.

In conclusion, a rapid and practical method employing $LiAlH_4$ and $TiCl_4$ to reduce aromatic nitro compounds into their corresponding substituted anilines is reported.

Efficient reducing systems are generated by treating $LiAlH_4$ with a suspension of $TiCl_4$ in diethyl ether in different molar ratios.

In a “one-pot” reaction, $LiAlH_4$ can reduce the nitro compound into symmetrical azobenzene derivative. Then, the azobenzene intermediate is converted into the corresponding amine by the reducing species of titanium and aluminum generated by the action of $LiAlH_4$ on $TiCl_4$.

The conversion with $LiAlH_4$ of azobenzenes into aniline derivatives is achieved only in the presence of $TiCl_4$. The proposed procedure works well for both "electron poor" and “electron rich" substrates.

Substrates with electron donor substituents however, require a larger excess of lithium aluminum hydride. The reducing power of the different prepared reagent systems for reducing substituted nitrobenzenes depends on the respective molar ratio of $LiAlH_4$ and $TiCl_4$.

(The work has been published: Maria Luisa Di Gioia, Antonella Leggio, Isabella Federica Guarino Vanessa Leotta, Emanuela Romio and Angelo Liguori. Simple formation of anilines by $LiAlH_4/TiCl_4$ reduction of aromatic nitro compounds. Tetrahedron Letters, Volume 56, Issue 39, 2015, Pages 5341-5344)

Chapter 2

New strategies for the elaboration of simple peptide

2.1 Direct formation of amide bond using innovative systems

The amide group plays a relevant role in biological and pharmaceutical compounds, and is commonly present in a large number of synthetic or natural molecules, bioconjugates, bioactive macrocyclic drugs, multifunctional nanostructures.[29] Amide bonds are also the main chemical linkages that join amino acids together to give peptides and proteins. So, amide bond formation is a fundamental process in organic synthesis.[30]

The direct condensation of an amine with a carboxylic acid in order to give an amide proceeds only at high temperature, conditions which are incompatible with the presence of other labile functionalities.

A plethora of synthetic strategies for making amides from various carboxylic acid derivatives have been developed.[31] Mild reagents are generally employed to prepare apart the acylating agents, and the carboxy components are transformed into the corresponding chlorides, mixed anhydrides, active esters, or isocyanates[32] then subjected to aminolysis. In all cases, it is necessary to find the optimal working conditions for the method. Steric hindrance of the reaction partners, racemization, optimization of yields, minimizing of by-products, facile final isolation, exploitation of economical reagents are all aspects that should be evaluated in designing new strategies for amide preparation.

In this second part dedicated to peptide chemistry, I was attracted by the need for a highly efficient, robust and helpful method for the realization of the peptide bond.

2.1.1 Silver acetate-assisted synthesis of amides

We investigated the use of weak bases acting in the presence of metal species for the aminolysis of isolable activated carboxylic acid derivative, acyl chlorides. From the

data reported in the literature, it is evident that the use of organic bases such as tertiary amines should be avoided in the formation of amide bond. In fact, this kind of bases does not preserve the configurations of chiral atoms of substrates, and racemization takes place through the base-assisted formation of ketene intermediates.[33] On the other hand, the use of inorganic bases in water environments is causative of not complete reactions, hydrolysis of reagents and products. Weak inorganic bases have been used to reduce the formation of side-products in the acylation of primary amines with carboxylic acid chlorides operating under heterogeneous phase conditions.[34] Nevertheless, in many cases the conversion of starting materials occurs in long reaction times, limiting the efficiency of the process.

We argued that the use of a weak base containing Lewis species should be striking in assisting the acylation of amines. Thus, the effectiveness of silver acetate in the reaction of primary and secondary amines with available or easily affordable acyl chlorides was investigated. The silver cation enhances the reactivity of the carbonyl group in acyl chlorides, especially in electrophilic aromatic substitutions.[35] Moreover, this metal species generates

silver chloride which precipitates in the most part of solvents currently used in organic synthesis, allowing the complete and rapid removal of silver particles at the end of the reaction. Finally, the acetate anion shows a basic strength similar to those of many tertiary amines used as bases and it can efficiently be quenched by forming acetic acid in the reaction environment.

The feasibility of a silver acetate-assisted aminolysis of acyl chlorides (Scheme 14) was exploited by reacting benzoyl chloride with *N,N*-diethylamine.

The aminolysis was performed in diethyl ether at room temperature using almost equimolar amounts of the acyl chloride and secondary amine. In this first experiment, a diethyl ether solution of benzoyl chloride (one equivalent) was added to a stoichiometric amount of *N,N*-diethylamine dissolved in the same ethereal medium.

After adding silver acetate (three equivalents), reaction went to completion in 15 minutes. The formation of a black precipitate was indicative of the reduction of silver ions caused by exposure to light, whereas *N,N*-diethylbenzamide (**24**) was recovered after hydrolytic work-up of the reaction mixture in a totally unsatisfying yield (37%). We thus

repeated the experiment using glassware protected from exposure to light and maintaining the same reagent stoichiometry used in the previous test.

After 15 minutes, TLC showed the complete consumption of benzoyl chloride. The formation of a white precipitate indicated that silver cation reduction did not occur (no traces of black solid were observed) and the expected amide was isolated in an 87% yield.

Further optimization studies were carried out using the cheaper sodium acetate (two equivalents) together with silver acetate (one equivalent), instead of three equivalents of the more expensive silver salt.

This modification did not affect the efficiency of the method, and reaction times and yields remained practically unaltered.

We also experienced that the silver-assisted aminolysis of benzoyl chloride was strictly depending on the sequence adopted for reagent adding.

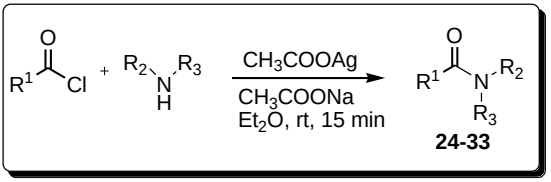
In fact, when a mixture of the acylating agent and silver acetate was stirred for five minutes at room temperature before the amine adding, a mixture of *N,N*-

diethylbenzamide (42%) and *N,N*-diethylacetamide (53%) was recovered.

The interaction between the acetate anion and benzoyl chloride can explain the reaction outcome.

A mixed anhydride was generated and this species further reacted with the amine affording the two observed acylated compounds.

Oppositely, the benzoyl derivative was exclusively isolated when the amine and silver acetate were added to an ethereal solution of the chloride.



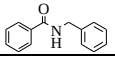
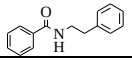
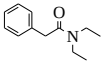
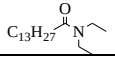
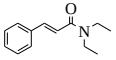
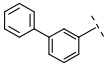
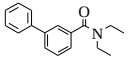
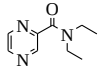
Scheme 14. The silver acetate-assisted aminolysis of acyl chlorides.

Benzoylation is a very important task in organic synthesis for the protection of amino groups.

Thus, we studied whether the aminolysis of benzoyl chloride can be performed using different primary and secondary amines. According to the optimized conditions, benzoyl chloride (one equivalent) dissolved in diethyl ether

was reacted with an equimolar amount of amine, in the presence of a mixture of sodium acetate (two equivalents) and silver acetate (one equivalent) at room temperature (Scheme 14).

Table 12 Amides produced via Scheme 14

Entry	R^1	R^2	R^3	Amide		Yield[a] (%)
a	C_6H_5	C_2H_5	C_2H_5	**24**		92
b	C_6H_5	C_2H_5	$CH(CH_3)_2$	**25**		82
c	C_6H_5	H	$C_6H_5CH_2$	**26**		87
d	C_6H_5	H	$C_6H_5(CH_2)_2$	**27**		89
e	C_6H_5	R^2– R^3 = $(CH_2)_4$		**28**		81
f	$C_6H_5CH_2$	C_2H_5	C_2H_5	**29**		93
g	$C_{15}H_{31}$	C_2H_5	C_2H_5	**30**		88
h	$C_6H_5CH=CH$	C_2H_5	C_2H_5	**31**		90
i		C_2H_5	C_2H_5	**32**		79
j		C_2H_5	C_2H_5	**33**		75

[a]Yields referred to the starting amount of the respective acyl chloride

The data summarized in Table 12 indicate that the protocol was found to be highly effective e in the direct preparation of structurally different amides under very mild conditions.

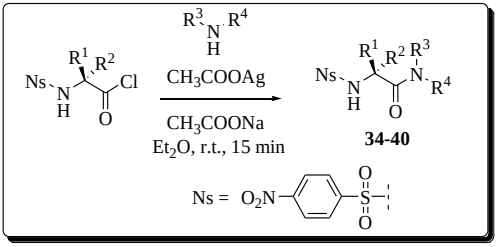
As expected, benzoyl chloride reacted with different amines to afford the respective amides **24-33** in good to excellent yields (Table 12, Entries a-e).
Lower yields were observed with *N*-ethyl-*N*-isopropyl amine and piperidine (Table 12, Entries b and e).
In these cases the corresponding amides were obtained in 82% and 81% yields, respectively, most likely due to the steric requirements imposed by the aliphatic cycle of piperidine and the ramified chain of the secondary amine.

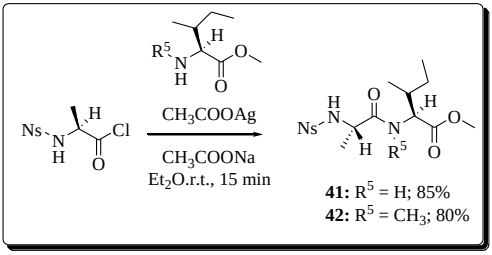
Other acylating agents different from benzoyl chloride were also investigated. The reaction of *N,N*-diethylamine with the commercial chlorides of phenylacetic, palmitic, 3-phenylbenzoic, cynnamic and pyrazine- 2-carboxylic acids (Table 12, Entries f-j) afforded the respective amides **29-33** in good yields confirming the synthetic potential of the protocol.

It is important to mention that all final products were easily isolated by a simple hydrolytic work-up of the respective reaction mixtures and obtained almost pure without need for chromatography.

^{1}H and ^{13}C NMR spectroscopy furnished data which were consistent with the structures assigned to the expected compounds.

The flexibility of the method and its possible application to other classes of acylating agents were verified by subjecting a series of *N*-(4-nitrobenzenesulfonyl)-α-amino acid chlorides (Table 13), prepared as previously reported[36] to the treatment with *N,N*-diethyl amine.

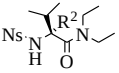
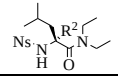


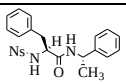
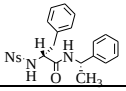


Scheme 15. The silver acetate-assisted aminolysis of *N-(*4-nitrobenzensulfonyl)-α-amino acid choloride and peptide bond formation

As desired, the treatment gave the corresponding amides 34-40 which were isolated in good to excellent yields by the simple hydrolytic work-up of the respective reaction mixtures and without need for chromatography.

Table 13. Amides produced via Scheme 15

	R^1	R^2	R^3	R^4	Amide		Yield(%)
a	CH_3	H	C_2H_5	C_2H_5	34		87
b	$CH(CH_3)_2$	H	C_2H_5	C_2H_5	35		85
c	$CH_2CH(CH_3)_2$	H	C_2H_5	C_2H_5	36		84
d	$CH(CH_3)CH_2CH_3$	H	C_2H_5	C_2H_5	37		81
e	$C_6H_5CH_2$	H	C_2H_5	C_2H_5	38		92

f	$C_6H_5CH_2$	H	H		39		82
g	H	$PhCH_2$	H		40		86

[a]Yields referred to the starting amount of the respective acyl chloride

All products were pure enough to the spectroscopic and GC-MS analysis, and the structures of products were assigned by ^{1}H and ^{13}C NMR.The reaction of the enantiomeric pair of N-protected amino acid chlorides prepared starting from L-Phe and D-Phe allowed us to investigate the effects of the experimental conditions of our protocol on the chiral configuration of the starting materials.The two α-amino acid derivatives reacted with the enantiomerically pure (S)-1-methylbenzylamine to afford the corresponding diastereomeric amides **39** and **40** which were recovered in good yields (82% and 86%, respectively) without need for chromatography.

^{1}H and ^{13}C NMR spectroscopy demonstrated that each amide was obtained as a unique diastereomer; no traces of the other diastereomer were individuated in each sample within the sensitivity limits of the spectroscopic technique.

LC-MS runs definitively confirmed that **39 and 40** were formed without racemization.

N-(4-Nitrobenzenesulfonyl)-α-amino acid chlorides are largely employed in the synthesis of peptides.[37] Therefore, we thought that the silver acetate-assisted protocol should also be helpful in preparing short peptides. Exemplificative of the preparation of *N*-(4-nitrobenzenesulfonyl)-dipeptides, the coupling of *N*-(4-nitrobenzenesulfonyl)-alanine chloride with the methyl ester of isoleucine was investigated. The corresponding *N*-protected dipeptide methyl ester **41** was isolated in high yields (85%) without need for chromatography. ^{1}H and ^{13}C NMR spectroscopy confirmed the structure attributed to **41.** For completeness, we investigated the reaction between *N*-(4-nitrobenzenesulfonyl)-alanine and *N*-methyl-isoleucine methyl ester. The coupling of *N*-methyl amino acid derivatives to obtain *N*-methylated dipeptides is often bothersome.[7]

The method was successful in giving the desired *N*-methylated dipeptide derivative (**42**) which was isolated in very good yields (80%) avoiding further chromatographic purification.

In summary, the use of silver acetate was proposed for the high-yielding aminolysis of a variety of acyl chlorides under heterogeneous phase conditions. In all cases, amides are easily recovered and isolated pure in high to excellent yields without need for chromatography. The silver cation assists the amide bond formation, and the acetate anion as well as silver chloride is easily removed at the end of the reaction. The operative conditions circumvent unwanted side-reactions and racemization that are commonly observed when acylating agents are used in the presence of tertiary amines. The aminolysis of *N*-(4-nitrobenzenesulfonyl)-α-amino acid chlorides by α-amino acid methyl esters or their *N*-methylated derivatives is advantageously used to install peptide bonds avoiding the use of expensive coupling reagents. This simple method could be widespread in a variety of applications, opening up previously unavailable synthetic routes to important target molecules.

(The work has been published: Leggio, A.; Belsito, E. L.; Di Gioia, M. L.; Leotta, V.; Romio, E.; Siciliano C.; Liguori, A. "Silver acetate-assisted formation of amides from acyl chlorides" Tetrahedron Letters 2015, 56, 199–202).

2.1.2 *One-pot synthesis of amide bond from carboxylic acids activated using thionyl chloride*

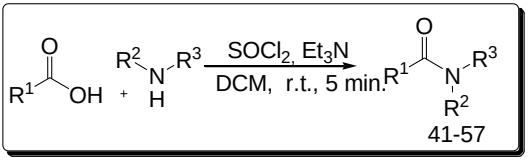
The amidation of carboxylic acids via acyl chlorides is usually a two-step process, involving first the conversion of the acid into the acyl chloride followed by the coupling itself with the amine.

The conversion of acid chlorides into the corresponding amides requires an excess of amine, or the presence of a tertiary amine able to neutralize the acid environment generated during the reaction.[38]

Thionyl chloride ($SOCl_2$) is the most popular reagent to activate carboxylic function because it is volatile and the excess can be removed easily by distillation, finally it is non-expensive. Usually, for the preparation of acid chlorides, thionyl chloride is used, neat or dissolved in a solvent, in the presence of the corresponding acid and the reaction requires heating.

This work exploited the possibility to carry out the synthesis of amide bond by using as coupling regent $SOCl_2$ in a single step. In order to confirm this hypothesis and verify the formation of the amide, we designed an experiment in which benzoic acid, the carboxylic acid chosen as model system, is treated with thionyl chloride in the presence of the amine (Scheme 16).

Scheme 16. Formation of carboxylic amides.



1 mmol of benzoic acid (Table 14, entry a) was added to 1 mmol of diethylamine (Et_2NH) and 3 mmol of triethylamine (Et_3N) in dichloromethane, then 1 mmol of $SOCl_2$ was added.

After 5 minutes stirring at room temperature, TLC analysis of the reaction mixture showed the complete conversion of benzoic acid. The recovery of the reaction product was performed by evaporating the solvent under reduced pressure to remove traces of unreacted thionyl chloride. The resulting residue was taken up in DCM and washed with 1N HCl, and then with 1N NaOH. The organic phase was dried (Na_2SO_4), and evaporated to dryness to provide the corresponding *N,N* diethylbenzamide (**41**) in 86% overall yield.

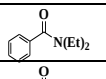
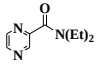
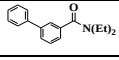
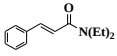
The molecular structure of **41** was assigned by ^{1}H and ^{13}C NMR spectroscopy and GC/MS analysis. An additional

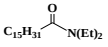
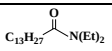
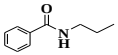
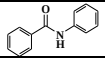
experiment was performed to investigate the reaction progress in absence of the tertiary amine Et_3N.

To this aim 1 mmol of $SOCl_2$ was added to 1 mmol of benzoic acid and 1 mmol of diethylamine (Et_2NH) in dichloromethane at room temperature. After 20 minutes the reaction was not yet complete, as showed by TLC analysis (Et_2O/petroleum ether, 7:3, v/v), and a mixture of benzoic acid (65% yield) and *N*,*N*-diethylbenzamide (31% yield) was recovered.

The use of stoichiometric amounts of diethylamine, in absence of Et_3N, leads to a lower conversion to amides since the diethylamine works also as a base to neutralize the hydrochloric acid that is generated during the reaction. This result demonstrated that the presence of the tertiary amine is essential to obtain the amide in high yields.

Table 24a. Amides produced via Scheme 16

	R^1	R^2	R^3	RT	Amide		Yields[a]
a	C_6H_5	C_2H_5	C_2H_5	5		**41**	86%
b		C_2H_5	C_2H_5	5		**42**	88%
c		C_2H_5	C_2H_5	5		**43**	91%
d	$C_6H_5CH=CH$	C_2H_5	C_2H_5	5		**44**	86%

e	$C_{15}H_{31}$	C_2H_5	C_2H_5	5		**45**	90%
f	$C_{13}H_{27}$	C_2H_5	C_2H_5	5		**46**	88%
g	C_6H_5	C_2H_5	$CH(CH_3)_2$	5		**47**	86%
h	C_6H_5	$R^2R^3 = (CH_2)_5$		5		**48**	88%
i	C_6H_5	C_2H_5	$C_6H_5CH_2$	5		**49**	85%
j	C_6H_5	C_3H_7	H	5		**50**	92%
k	C_6H_5	C_6H_5	H	5		**51**	89%

[a]Yields based on starting amount of the respective carboxylic acid

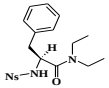
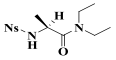
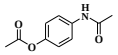
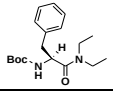
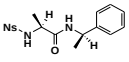
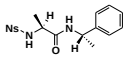
	R^1	R^2	R^3	RT	Amides		Yield
l	$NsNHCH(CH_2Ph)$	C_2H_5	C_2H_5	20		**52**	81%
m	$NsNHCH(CH_3)$	C_2H_5	C_2H_5	20		**53**	83%
n	CH_3	C_6H_4OH	H	5		**54**	48%
o	$BocNHCH(CH_2Ph)$	C_2H_5	C_2H_5	15		**55**	78%
p	(S)-$NsNHCH(CH_3)$	(R)-$C_6H_5CH($	H	20		**56**	75%
q	(S)-$NsNHCH(CH_3)$	(S)-$C_6H_5CH($	H	20		**57**	68%

Table 34b. Amides produced via Scheme 16

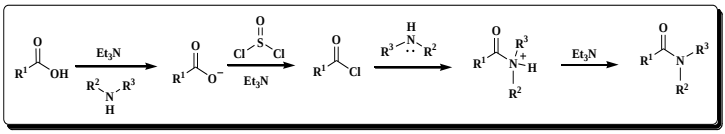
[a]Yields based on starting amount of the respective carboxylic acid

We also experienced that the outcome of the reaction is strongly dependent on the order of reagent addition. In fact, if benzoic acid is preliminarily added to thionyl chloride and Et_2NH and Et_3N are added subsequently, the reaction yield is lowered and after 5 minutes the reaction is not yet complete. The crude reaction product, obtained after work up of the reaction mixture, also contains the benzoyl chloride with a percentage of 35% as detected by GC/MS analysis.

Furthermore it was verified that $SOCl_2$ does not react with the amine. In fact, the *N*-benzyl-*N*-ethylamine was recovered unchanged after it was treated with $SOCl_2$ at room temperature for 1 hour.

On the basis of the obtained results it was hypothesized that the reaction probably proceeds through the formation of the acylchloride which then reacts with the nucleophilic amine in a single step.

In order to give an addition confirm to this hypotesis, the amide synthesis reaction has been carried out in a NMR tube. The reaction's monitoring by using ^{13}C-NMR has confirmed the clear presence of acyl chlorid as intermediate. (Scheme 17).



Scheme 17. A proposed mechanism of the one pot amide synthesis

The reaction was subsequently extended to other aromatic substrates (Table 14) characterized by a lower electrophilicity at the carbonyl carbon atom and therefore less reactive toward the amine (Table 14; entries b-d). In all experimented cases the reaction is complete within 5 minutes and has provided excellent yields (86-91%). The methodology was then applied to two long chain aliphatic carboxylic acids, palmitic and myristic acid (Table 14; entries e-f). Also with these systems no changes were observed during the reaction, in fact the amide formation occurs even in short times and with yields (88-90%) comparable to those previously observed (Table 14; **45-46)**. In order to evaluate the possible effects due to the steric hindrance of the amine, *N*-ethyl-*N*-isopropylamine, piperidine and *N*-benzyl-*N*-ethylamine (Table 14; entries g-i) were treated with benzoic acid under the described reaction conditions obtaining in all cases excellent yields (85-88%).

Significant effects due to steric hindrance of the amines were not detected.

Then, with the purpose to investigate the steric hindrance present on the carboxylic acid, the developed procedure was applied to two *N*-protected α-amino acids, *N*-nosyl-L-phenylalanine and *N*-nosyl-L-alanine (Table 14; entries l-m). *N*-nosyl-L-phenylalanine and *N*-nosyl-L-alanine were converted into the corresponding *N*,*N*-diethylamides (Table 12; **52-53**) in 20 minutes and with yields slightly lower than those of other examined systems (Table 14).

Based on these results it can be argued that steric hindrance offered by the groups on the nitrogen atom of the amine does not affect the reaction progress while the reaction times are longer and the reaction yields are a little lower when sterically hindered carboxylic acids are used.

The adopted procedure for obtaining *N*,*N*-diethylamides was also applied successfully to *N*-Boc-L-phenylalanine, a substrate bearing the acid labile group *tert*-butoxyxorbonyl (Boc). The corresponding *N*,*N*-diethylamide (**55**) was obtained in 78 % yield keeping unchanged the acid-sensitive group (entry o, Table 14). The application of the two step amide synthesis, through the formation of acyl chloride, to

N-Boc-L-phenylalanine doesn't work well due to the instability of the corresponding acyl chloride.

The described protocol was also applied to obtain secondary amides by using primary amines as nucleophilic reagents (Table 14; entries j-k). To this aim *N*-propylamine and aniline were selected as nucleophiles. The reactions proceeded as described previously and afforded the corresponding amides in short times and high yields (Table 14; **50-51**) by demonstrating that the adopted one-pot procedure is also applicable successfully for obtaining secondary amides.

We also investigated the stereochemical aspects of the reaction by extending the developed procedure to the formation of a couple of diastereoisomeric amides. To this aim *N*-((R)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (**56**) and *N*-((S)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (**57**) were synthesized by treating *N*-(4-nitrobenzenesulfonyl)-L-alanine with (R)-1-phenylethylamine and (S)-1-phenylethylamine respectively according the adopted conditions. The corresponding amides **56** and **57** were obtained in 68-75 % yields after 20 minutes (entry p and

entry q Table 14). The ^{1}H NMR and ^{13}C NMR spectroscopic data of crude amides **56** and **57** did not show any signals from possible epimers resulting from an inversion of the configuration at the α-carbon atom of the alanine.

The absence of racemization was also monitored by HPLC analysis of the amides **56** and **57** and of a suitably prepared mixture of the two diastereisomers **56** and **57** (approx. 30 % : 70 %). The two diastereoisomers were readily resolved by HPLC: the presence of two chromatographic peaks with retention times of 15.558 and 16.867 minutes was observed (Figure 1 B). Chromatograms of the single amide **56** (Figure 4 A) and **57**(Figure 4 C) showed the presence of a unique peak with retention times of 16.908 and 15.433 minutes respectively.

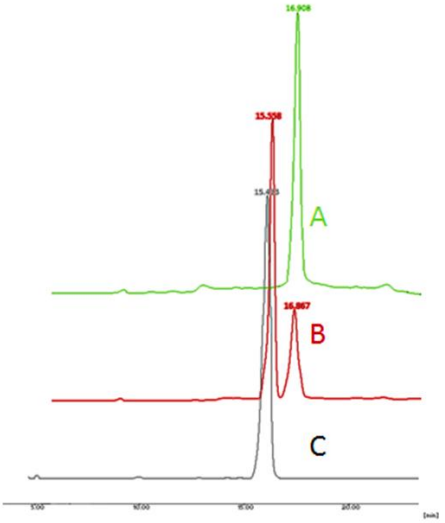


Figure 4. HPLC analyses of crude amides **56** and **57** : **(A)** *N*-((R)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (**56**); **(B)** a mixture of **56** and **57** (approx. 30 % : 70 %); **(C)** *N*-((S)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (**57**)

HPLC analysis in combination with NMR data of the two diastereomeric amides **56** and **57**, demonstrated the absence of epimerization products excluding any detectable racemization throughout the synthetic process.

Finally, we evaluated the chemoselectivity of the reaction by using a substrate containing both an amino and a phenolic function (Table 14; entry **n**).

According to the developed procedure, *p*-amino phenol was treated with an equimolar amount of acetic acid and thionyl chloride. After 15 minutes, TLC analysis (Et_2O/ petroleum ether, 7:3, v/v) showed the complete conversion of the starting material. The reaction workup afforded *p*-acetamidophenyl acetate in 48% yield. Therefore the acylation reaction takes place both on the amino and phenolic function without any chemoselectivity.

The thionyl chloride works as coupling reagent and allows to obtain, at room temperature and in short times, secondary and tertiary amides in high yields without need of further purification. Furthermore the adopted conditions did not cause any detectable loss of the optical integrity at the chiral centres of the precursors as confirmed by HPLC and NMR analysis.

The reaction progress is affected by steric hindrance only when bulky groups are present on the carboxylic acid molecule. The synthesis of amides is performed in an efficient one-pot procedure under mild reaction conditions, the order of reagent addition is predetermined for obtaining the amides in high yields.

Our approach was successfully extented to α-amino acids bearing acid-sensitive groups by demonstrating an important applicative validity also in the peptide synthesis.

2.2 Reduction of amide bond and formation of modified dipeptides

The reduction of carboxylic amides is an important process in organic chemistry.[39]

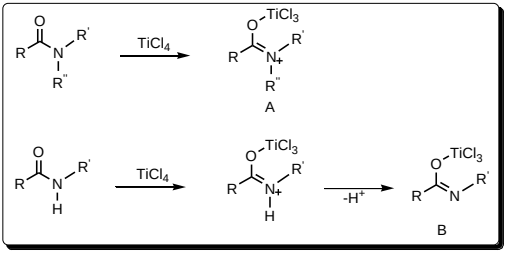
In particular, the reduction of amide bond has captured much attention as a practical method for modified peptide.[40]

Moreover, the bioorganic chemistry of reduced-peptide-containing compounds represents an area of growing interest for the design and development of peptide agonists and antagonists for numerous peptide-receptor systems.[41]

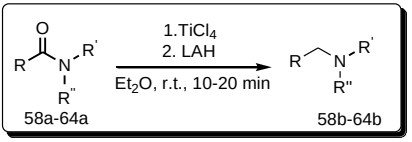
In this context, the reduction of the amide carbonyl to a methylene group is one of the most common types of motifs for the modification of the natural peptide backbone. Pseudopeptides having $\Psi[CH_2NH]$ moieties show significant biological and pharmaceutical properties.

In fact, the introduction of the CH_2NH peptide bond isostere often induces changes on the backbone conformation and increases the resistance to peptidases. In our preliminary evaluation, we decided to activate the carbonyl amide group by addition of titanium tetrachloride, in order to conduct the reductive reaction under mild conditions.

Scheme 18. Activation of the carbonyl group by $TiCl_4$.



Scheme 19. General procedure for the reduction of amides



Titanium tetrachloride is a powerful activator of carbonyl groups, in fact, it has high affinity for the oxygen atom of the carbonyl group thus promoting the attack of lithium aluminum hydride (Scheme 18).

Therefore, the possible attack of hydride ions as nucleophiles on the electrophilic carbon atoms of species A and B occurs smoothly even if B, being a neutral compound is less reactive than A.

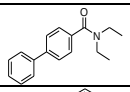
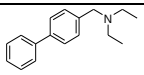
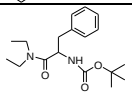
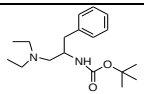
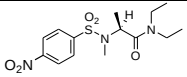
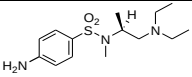
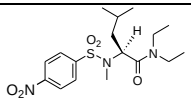
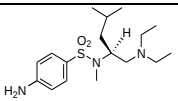
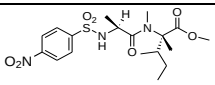
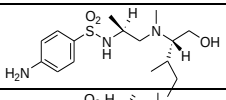
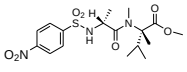
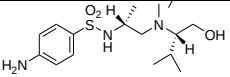
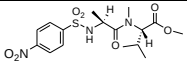
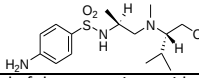
The proposed mechanism is in agreement with experimental observations reported in the literature.[42]

We carried out an experiment choosing the 4-phenyl-*N,N*-diethylbenzamide (**58a**) as a model substrate to investigate the reduction. Hence, the carbonyl group of **58a** was activated with an equimolar amount of titanium tetrachloride ($TiCl_4$) in diethyl ether; then, lithium aluminum hydride (LAH) (3 mol equiv) was added and the mixture was left under magnetic stirring at room temperature (25 ^oC) (Scheme 19).

The combination of these two reagents proved to be an effect way to reduce the carbonyl amide in short reaction times. In fact, complete conversion of the substrate was observed in only ten minutes; moreover, the corresponding

amine, 4-phenyl-*N,N*-diethylamine (**58b**) was obtained in high yield (85%) and without need for chromatography.

Table 15. Amines produced via scheme 19

Substrate		Product		R.t	Yield [a] %
	**58a**		**58b**	**10**	**85**
	**59a**		**59b**	**20**	**83**
	**60a**		**60b**	**20**	**92**
	**61a**		**61b**	**20**	**87**
	**62a**		**62b**	**15**	**86**
	**63a**		**63b**	**15**	**72**
	**64a**		**64b**	**15**	**64**

[a] Yields reffered to the starting material of the respective amide

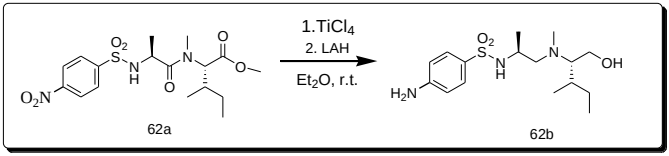
On the basis of these results, the use of LAH in the presence of $TiCl_4$ at room temperature was investigated for the reduction of *N*-α-protected amino acid derived amides. To this aim, the *N*-α-Boc-*N,N*-diethyl-phenylalanyl amide

(Table 15**, 59a**) was treated under the above adopted conditions affording, after 20 min, the corresponding Boc-diamine **59b** in 83% isolated yield after hydrolysis of the reaction mixture.

It is noteworthy that excellent chemoselectivity was observed in the presence of the urethane amide of the Boc protecting group. We next examined the ability of the reductive system to reduce the carbonyl amide of α-amino acids in the presence of a different *N*-α-protecting group. We selected the *N*-(4-Nitrobenzenesulfonyl). Therefore, the *N*-α-methyl-*N*-α-(4-nitrobenzenesulfonyl)-*N,N*-di-ethylaminoacid amides **60a-61a** were subjected to the treatment with $TiCl_4$/LAH at room temperature. The reactions went to completion in 20 min furnishing the reduced compounds **60b-61b** in 92 and 87% yields, respectively (Table 15). The reduction of the nitro group to amine represents an unusual aspect for these substrates, despite the general observation that treatment of an aryl nitro compound with metal hydrides normally gives azo compounds. The structures of amines **60b-61b** were proposed based on the NMR spectroscopic analysis.

After demonstrating the applicability of our procedure to the reduction of different kinds of amides and of amino acid derivatives, we extended the methodology to the reduction of the amide bond in peptide systems. The dipeptide *N*-(4-nitrobenzenesulfonyl)-alanyl-*N*-methyl-isoleucine methyl ester (**62a**) was chosen as the model substrate and treated with our reducing system (Scheme 20).

Scheme 20. Reduction of the *N*-Nosyl protected dipeptide **62a** with $TiCl_4$/LAH.



The transformation of the peptide bond occurred in short reaction times (15 min). However it was observed that the reaction proceeded without any chemoselectivity as other sensitive functional groups present in the starting compound were affected by the reduction. In fact, the aryl nitro as well as the ester group were both reduced to amine and alcohol, respectively, resulting in the formation of (**62b**) 4-amino-*N*-(1-(1-hydroxy-3-methylpentan-2-ylamino)propan-2-yl)benzenesulfonamide. Compound **62b** was

recovered after work-up of the reaction mixture in 86% yield (Table 15) and without the need for chromatography. Despite the absence of chemoselectivity, the reduction reaction enabled the preparation of chiral molecules containing a 1,2-diamino moiety that are attractive as peptide surrogates and more generally as synthetic intermediates in organic synthesis. [43]

The final aim of our work was to investigate the retention of configuration in the compounds subjected to the proposed reduction protocol. It is well known, in fact, that the use of LAH for reductions can cause racemization of the stereocenters in the systems.[44]Although the rate of our reduction protocol was found to be very fast we decided to check the stability of the chiral centers of the substrates especially in the case of the peptide frames. We thus synthesized the couple of diastereomeric *N*-nosyl protected dipeptides **63a** and **64a** in which the two L- and D optical forms of alanine were inserted (Table 15). Afterward **63a and 64a** were treated with the reducing system and samples of the crude amines obtained were analyzed by ^{1}H and ^{13}C NMR. The spectroscopic analysis of **63b** (Fig. 5, [A]) did not reveal signals attributable to the other diastereomer

64b (Fig. 1, [B]). In fact, for example, it is evident that the two CH_2N methylene protons give a unique NMR signal at 2.51 ppm in **64b** (Fig. 5, spectrum [B]) while they are well separated in the spectrum of **63b** (Fig. 5, 244 and 267 ppm, spectrum [A]). In addition, the signal relative to the CH_2OH proton at 3.33 ppm in **63b** (spectrum [A], Fig. 5) was not revealed in the spectrum of the crude product **63b**; on the other hand, the signal relative to the CH_2OH proton at 3.40 ppm in the spectrum of **64b** is absent in the spectrum of the crude **63b**. Within the limits of the ^1H NMR methodology used, these data demonstrated that the configuration of the chiral centers was preserved during the reductive process.

To further confirm this result, an appropriately prepared mixture of **63b** and **64b** was analyzed by LC/MS. The chromatogram shows that the two compounds **63b** and **64b** are readily resolved and neither is found in the MS spectrum of the other compound.

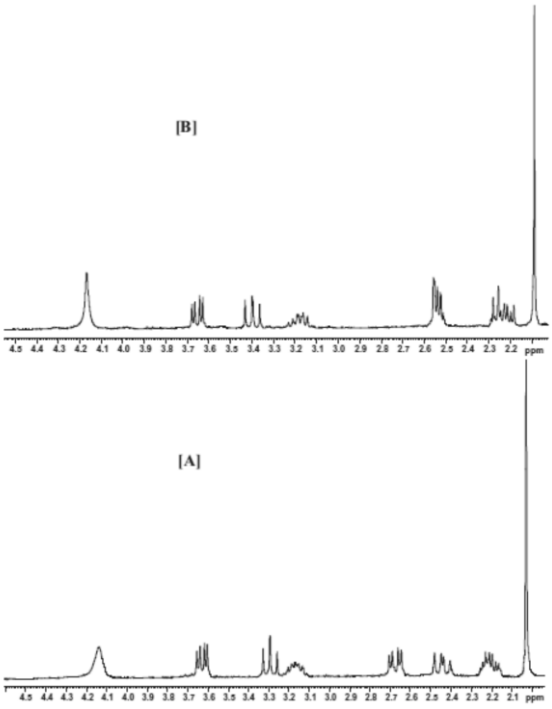


Figure 5. ^{1}H NMR analysis of crude samples of reduced compounds 63b [A] and 64b [B].

In conclusion, the proposed methodology is a modified version of the classical reduction of amides with lithium aluminum hydride. The activation of the carbonyl by the preliminary use of titanium tetrachloride makes the reduction reaction mild, very fast, and thus without

modification of the stereochemistry of the chiral centers of the starting compounds.

(The work has been published: Di Gioia, M. L.; Belsito, E. L.; Leggio, A.; Leotta, V.; Romio, E.; Siciliano C.; Liguori, A. "Reduction of amide carbonyl group and formation of modified amino acids and dipeptides" Tetrahedron Letters 2015, 56, 2062-2066)

Chapter 3
Other research lines

3.1 New methodology for the measurement of analytes in complex matrices

3.1.1 Analysis of Fatty Acids in meat product

Dry fermented sausages are traditional products mainly manufactured in Countries of the Southern Europe like Italy.[45]

In Calabria region (Southern Italy) several types of fermented sausages are still produced using traditional manufacturing processes but there is an increase in the demand for industrial production of these products. In the last decade numerous studies concerning technological, chemical, and sensory characterization of traditional dry fermented meat products have been performed.[46]

The analysis of dry fermented sausages generally points out on some important parameters: breakdown products of lipolysis and proteolysis[47] i.e. peptides, amino acids[48], free

fatty acids (FFAs) that contribute to the nutritional characteristics of fermented meat.

Much attention is also paid to the determination of products of further degradation such as biogenic amines[49] or compounds derived from oxidation of polyunsaturated fatty acids (PUFAs).[50]

The analysis of fatty acids has become increasingly important in a modern society with dietary recommendations favoring a low intake of fats because more people are aware of their nutritional implication.

Furthermore, the growing interest in the control of the composition in acylglycerols and FFAs of sausages mainly is attributable to the necessity of producing "healthy" foods characterized by saturated/unsaturated fatty acid ratios all in favor of the latter ones. Epidemiological and biochemical studies have provided a great deal of evidence about the protective effect of ω-3 PUFAs against some common tumors, rheumatoid arthritis and cardiovascular diseases (CVD)[51].

The investigation of fatty acid composition in the lipid fraction of sausages could provide information about changes in the content in saturated fatty acids (SFAs),

monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) occurring during the ripening.

This point should be of interest for the evaluation of the changes of nutritional characteristics of these products.

In fact, modifications of the PUFA and MUFA content can have possible effects related to a low or high consumption of the fermented sausages.

In this context, detailed information about the fatty acid profile provides researchers with the necessary knowledge in this field, as well as consumers about the shelf-life and acceptance of this kind of meat products.

In this part of work, the total lipid fraction extracted from three different Calabrian fermented sausages (known as “salsiccia”, “spianata” and “soppressata”) was analyzed by GC/MS.

The investigation covered the determination of fatty acid profiles in the respective acylglycerol portions as well as the composition of free fatty acids during a long time period of ripening (80 days).

In dry sausages fatty acids are present both as esters linked to glycerol and phospholipids and as free (unesterified) fatty acids.

The determination of fat composition in dry fermented sausages either in the acylglycerol or in the unesterified form was carried out by transesterification of the total lipid extract using a large excess of sodium methoxide.

The methods which utilize methanol in acidic medium for the transesterification of acylglycerols cannot be adopted because the results could be affected by the esterification of the FFAs present in the fat.[52]

On the other hand, base-catalyzed transesterification of acylglycerols can suffer from the presence of FFAs that could neutralize the reaction environment thus leading to an incomplete transesterification.[53]

The use of a large excess of sodium methoxide in dry environment enables the neutralization of the FFAs converting them rapidly into the corresponding sodium salts that are retained on the methanolic phase.

At the same time, the use of an excess sodium methoxide ensures, through the complete transesterification of acylglycerols, the formation of fatty acid methyl esters (FAMEs) that are soluble in hexane.

Although, the determination of fat composition in dry fermented sausages either in the acylglycerol or in the

unesterified form was carried out by transesterification of the total lipid extract using a large excess of sodium methoxide in *n*-esane and methanol.

The hexane, separated from the methanolic phase, contains fatty acid methyl esters (FAMEs).

FAMEs were analyzed by GC/MS and identified by comparing their retention times of the chromatographic peaks with those obtained with the methyl esters from a mixture prepared with standard fatty acids.

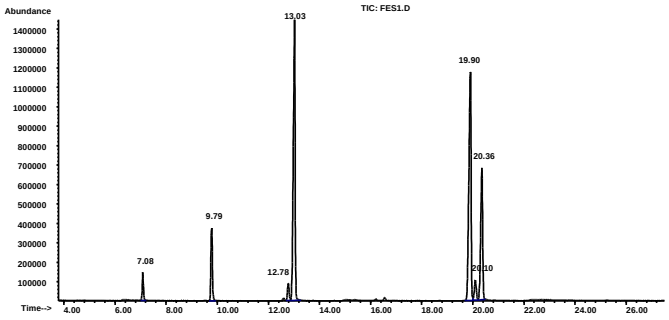


Figure 6. GC/MS analysis of FAMEs obtained from the transesterification of sample sal20 (r.t. 7.08 myristic acid methyl ester; r.t. 9.79 pentadecanoic acid methyl ester (reference standard); r.t. 12.78 palmitoleic acid methyl ester; r.t. 13.03 palmitic acid methyl ester; r.t. 19.90 oleic acid methyl ester; r.t. 20.10 linoleic acid methyl ester; r.t. 20.36 stearic acid methyl ester).

FFAs, instead, were recovered after acidification of the methanolic solution and were then subjected to the

derivatization procedure and measurement. In fact, fatty acids need to be converted into FAMEs in order to improve their volatility and thus ensuring better gas chromatographic peak shape. Derivatization was performed with diazomethane according to the method of Schlenlg and Gellerman (1960)[54] that enables the conversion of the FFAs into the corresponding methyl esters. Table 16 reports the data obtained for FFAs in all products considered. The three “salsiccia” batches showed a lower content of FFAs (about 6% of total fat) than those recovered in the samples from “soppressata” and “spianata” (7.5-10% ca of total fat). The percentage of FFAs in the total fat was calculated as the sum of individual fatty acids. The main identified fatty acids in the three different types of sausages were: oleic, palmitic, linoleic, stearic, palmitoleic and myristic acid. This profile coincides basically with that found by other authors in pork fat.[55] All the examined samples (Table 16) showed a higher content of MUFAs and PUFAs than SFAs. In particular, the saturated/unsaturated free fatty acid ratio (S/U) achieves values around 0.22 and 0.36. Oleic acid (C18:1 cis-9) was predominant among MUFAs, being also the most prevalent individual FFA in all kinds of sausages,

with values around 58-68%. Linoleic acid (C18:2 cis-9, cis-12) was the dominating PUFA with values around 9-18%. SFAs were also determined, with palmitic acid (C16:0) contributing for the highest amounts (12-18%); stearic acid (C18:0) being below 8% and the myristic acid (C14:0) concentration being less than 2%.

Table 16. FFAs profile in acylglycerols of the analyzed sausages form Southern Italy, determined as FAMEs by GC/MS.

	"Salsiccia"			"Soppressata"		"Spianata"		
	Sal20	**Sal40**	**Sal80**	**Sop40**	**Sop80**	**Spi20**	**Spi40**	**Spi80**
FAMEs	89.29 ± 1.94	88.72 ± 1.68	88.37± 1.25	87.20± 1.51	90.08± 1.61	90.34 ± 1.15	87.18 ± 1.37	87.14 ± 1.16
Myristic acid ($C_{14:0}$)	1.72 ± 0.06	1.74 ± 0.06	1.74 ± 0.05	1.53 ± 0.05	1.54 ± 0.04	1.63 ± 0.04	1.49 ± 0.03	1.48 ± 0.04
Palmitic acid ($C_{16:0}$)	28.49 ± 0.31	26.94 ± 0.13	25.04 ±0.08	23.71± 0.15	25.55± 0.28	25.71± 0.24	23.85± 0.25	23.42 ± 0.43
Stearic acid ($C_{18:0}$)	13.17 ± 0.48	12.83 ± 0.17	12.28 ±0.25	12.39± 0.29	13.49± 0.18	10.46± 0.22	11.61± 0.42	11.99 ± 0.18
Palmitoleic acid ($C_{16:1}$)	4.10 ± 0.06	4.37 ± 0.07	3.77 ± 0.03	4.70 ± 0.12	3.25 ± 0.07	3.58 ± 0.11	3.39 ± 0.10	3.66 ± 0.08
Oleic acid ($C_{18:1}$)	50.33 ± 0.64	51.71 ± 0.35	54.64 ±0.44	55.19± 0.21	54.07± 0.36	55.93± 0.04	57.07± 0.55	56.75 ± 0.24
Linoleic acid ($C_{18:2}$)	2.19 ± 0.07	2.41 ± 0.06	2.53 ± 0.04	2.49 ± 0.04	2.42 ± 0.03	2.35 ± 0.03	2.58 ± 0.10	2.71 ± 0.10
S/U	0.77	0.71	0.64	0.60	0.68	0.61	0.59	0.58

Data are expressed as mean value ± SD in percentage, (n=3). Measures were performed in triplicate. S/U: saturated/unsaturated free fatty acid ratio.

To this aim the FAMEs recovered in the hexane phase obtained from the transesterification were subjected to quantitative analysis.

The individual FAMEs measurement allowed to understand the composition of the fatty acids in the acylglycerols fraction of the examined sausages (Table 16).

These results show a different profile of fatty acids contained in acylglycerols respect to the FFAs. The Kruskal-Wallis test (for "salsiccia" and "spianata") and Mann-Whitney U test (for "soppressata") were applied to both FAME and FFA concentration data grouped in three categories for each type of sausage corresponding to the three ageing periods in order to find possible statistical differences between groups and, therefore, to study the influence of ageing period on FAME and FFA contents.

Table 17. Significant differences between the three different ageing periods (20 days (1), 40 days (2), 80 days (3)) obtained by applying the Kruskal-Wallis test (for Salsiccia and Spianata) and Mann-Whitney U test (for Soppressata) to both FAME and FFA data.

FAME		
Variable	**Sausage**	**Periods**[a]
Linoleic acid	Salsiccia	1-3
Palmitoleic acid	Soppressata	1-2
Stearic acid	Soppressata	1-2
FFA		
Variable	**Sausage**	**Periods**[a]
Total amount	Salsiccia	1-3
Miristic acid	Salsiccia	1-2
Palmitoleic acid	Salsiccia	1-2
Palmitic acid	Salsiccia	1-2
Oleic acid	Salsiccia	1-3
Linoleic acid	Salsiccia	1-2
Stearic acid	Soppressata	1-2

[a] Periods between which the significant difference was observed

As shown in Table 17, many more differences were found in the FFA amounts than in the FAME ones.

In particular, the concentration values of all the FFAs analyzed in the three types of sausages, except for stearic acid in salsiccia, were significantly affected by ageing process.The decrease in the content of ω-6 fatty acids mostly represented by linoleic acid is consistent with the fact that there is a significant lipase activity. Linoleic acid represents together with oleic acid one of the preferred substrates for the hydrolytic activity of the lipases[56]. Therefore, lipases act selectively releasing MUFAs and PUFAs with 18 carbon atoms. This enzymatic selectivity can explain the FFAs profile found in the examined sausages.

The dry fermented sausages cases of study are known as MUFAs and PUFAs rich foods. PUFAs are responsible for the oxidative phenomena. Oxidative stress and thermally induced conditions provoke lipid peroxidation of PUFAs in appreciable extents, with particular regard for linoleic acid, since its molecular structure displays a series of double carbon-carbon bonds which can undergo oxidative disruption.

In order to assess the extent of oxidation processes the Kreis assay (Table 18) was performed on the three different type of sausages.

This classical assay has a qualitative validity in establishing the presence of aldehydes in the lipid portions extracted from meat products. In particular, the oxidative processes that occur in sausages are responsible for the formation of alkanals and alkenals by oxidation of the double bonds of linoleic acids present in the sausages. [57]

Table 18. Kreis test of sausages

Sample	Kreis test[a]
Sal20	Negative
Sal40	Negative
Sal80	Positive ++
Sop40	Negative
Sop80	Positive ++
Spi20	Negative
Spi40	Negative
Spi80	Positive +

[a] + = pink; ++ = deep pink.

One sample for each kind of sausages gave positive test (samples **sal80**, **sop80**, **spi80**) indicating the presence of

aldehydes in the sausages. However, the purity of reagents used for the test are sometimes responsible for false determination of the presence of aldehydes, especially when the concentration of these compounds in the sample is very moderate.

We decided, at this point, to subject one of the lipid extract positive to the Kreis test to further investigation using other more sensitive instrumental techniques[58]. As an example of analysis, we subjected to the nuclear magnetic resonance investigation the sample **sal80**. It has been shown that high resolution ^{1}H NMR is an appropriate tool for the evaluation in fats and edible oils of primary oxidation compounds, as well as of secondary oxidation products.[58]

The lipid extracts were analyzed directly after their obtainment, with no work-up, to make the procedure as short in time as possible and to zeroing any further undesired lipid oxidation.

The proton NMR spectrum of the total lipid extract obtained from the sausage case of study displayed the expected set of signals attributable to the content of acyl chains, which were present in the sausage fat either as components of acylglycerols and as FFAs (Fig. 7A).

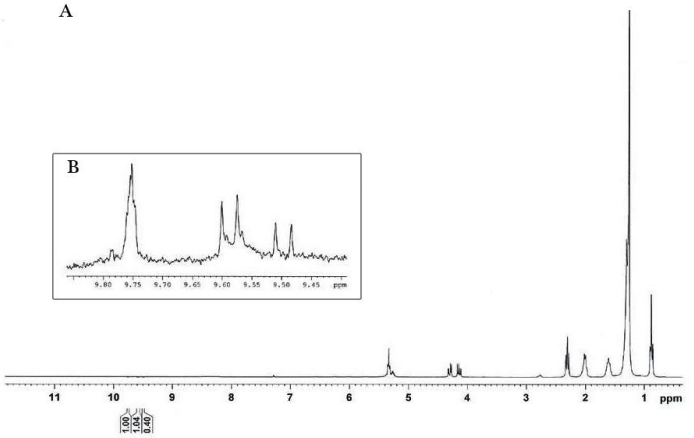


Figure 7. High resolution ^{1}H NMR spectrum of a sample of total lipid extract obtained from sample **sal80** (**A**), and region of the aldehydic protons (**B**).

All these signals were unambiguously assigned to the corresponding spin systems of the different types of protons belonging to the various acyl chains, according to the literature values.[59]

No other signals were visible in the recorded spectrum, at least upon normally scaled graphic conditions. Afterward, we undertook the analysis of the sub-spectrum: the downfield region between 9.00 and 10.00 ppm showed some interesting peculiarities, principally due to the presence of a series of well-distinct signals (Fig. 7B). All

these resonances were attributed to the formyl protons of aldehydes generated from the lipid secondary oxidation pathway. In the absence of specimens of these aldehydes, the data already published[60] supported us in establishing by analogy the nature of compounds generating the above set of signals. Therefore, the signal appearing at 9.75 ppm was attributed to the series of overlapped triplets generated by the aldehydic proton in n-alkanals. The two lines at 9.56 and 9.59 ppm were indicative of the presence of 4-hydroxy-trans-2-alkenals in the products of oxidation of the mature sausage fat. For this family of compounds the values of 9.560 and 9.586 ppm are reported, demonstrating the perfect matching between our analysis and the literature data. The spectrum displayed two further doublets: the first, with lines at 9.57 and 9.60 ppm, was attributable to the aldehydic protons of 4-hydroperoxy-trans-2-alkenals; the second, generated by the aldehydic group of trans-2-alkenals, showed its lines at 9.48 and 9.51 ppm.

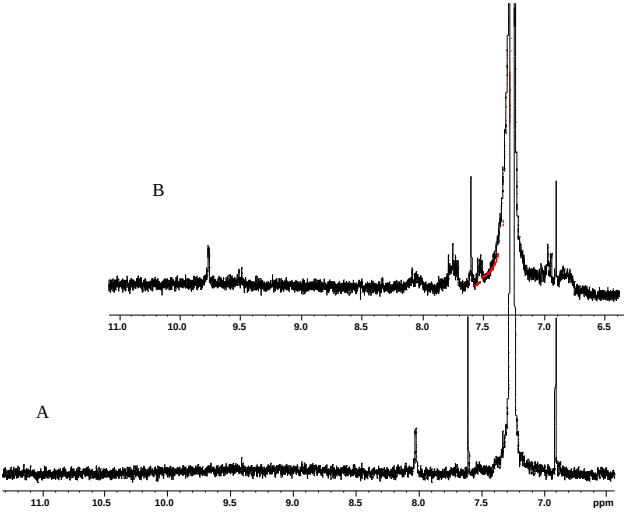


Figure 8. Aldehydic proton windows of the high resolution ^{1}H NMR spectra recorded on samples **spi40** (spectrum **A**) and **spi80** (spectrum **B**).

The appearance of the discussed signals in the spectrum, together with the absence of the triplet typically recognized for the methyl group of linolenic acid at 0.97 ppm indicated that PUFAs underwent oxidation generating the observed series of aldehydes.

^{1}H NMR analysis was also performed on samples **spi40** and **spi80**. The respective aldehydic proton windows are displayed in Figure 8. As it can be observed from spectrum A the analysis of **spi40**, sample negative to Kreis test, did not show signals attributable to secondary lipid oxidation

products. Aldehydic proton resonances of weak intensity were instead observed in the case of the slightly oxidized sample **spi80** (spectrum B).

In conclusion, GC/MS analysis was used for determining the FFA content and the fatty acid profiles in acylglycerols of three kinds of dry fermented sausages from Calabria during their ripening. The data obtained indicates that lipolysis plays an essential role in developing FFAs during ripening. A preferential hydrolysis of linoleic and oleic acid was observed.

The generation of aldehydic products by the oxidative degradation of unsaturated fatty acid chains was also studied by ^{1}H NMR. The spectroscopic investigation confirmed the data obtained by Kreis test.

(The work has been published: Angelo Liguori, Emilia Lucia Belsito, Maria Luisa Di Gioia, Antonella Leggio, Francesca Malagrinò, Emanuela Romio, Carlo Siciliano, Antonio Tagarelli. "GC/MS Analysis of Fatty Acids in Italian Dry Fermented Sausages". The Open Food Science Journal, 2015, 9, 5-13)

3.1.2 GC/MS methodology for the direct analysis of volatile compounds of bergamot essential oil

Phytotherapy employs fully characterized active ingredients extracted from plants for the treatment and prevention of many diseases.

Essential oils and their components exhibit various biological activities and are also used for human disease prevention and treatment. They exert antiviral, antidiabetic, antimicrobial and cancer suppressive activities[61], furthermore they play a key role in cardiovascular diseases prevention including atherosclerosis and thrombosis.[62]

Today aromatherapy, a branch of phytotherapy, is gaining momentum as complementary therapy to the traditional medicine[63]. Aromatherapy uses essential oils via inhalation or massage as the main therapeutic agents to treat several diseases. The inhalation of volatile aromatic substances extracted from plants can affect the mood and state of health of the person by inducing psychological and physical effects.[64]

Recently, many papers[65] have tried to give scientific value to the aromatherapy, traditionally based on empirical observations and evaluations also poorly stringent, by

establishing criteria similar to those that support the rigorous scientific research.[66]

It has been verified in fact, that among hundreds of papers related to aromatherapy inhalation only a few are scientifically significant.

It is important to know the chemical compositions and characteristics of the essential oil in order to use it appropriately.

It seems clear, however, that if the essential oils are delivered by inhalation knowing the composition of the gas phase (or headspace) above the liquid essential oil sample becomes important.[67]

The migration of volatile molecules into the headspace phase does not just depend on their volatility but also on their affinity for the liquid sample phase, their relative concentrations between the two phases will reach an equilibrium value. At equilibrium, the partial pressure of each volatile component in the headspace vapor will be equivalent to its vapor pressure that is directly proportional to its mole fraction in the liquid phase. In essence, the concentration of a compound in the headspace is proportional to its concentration in the liquid phase and can

be affected by temperature, respective volumes of the sample and the headspace and other factors.

Thus, headspace phase composition can be very different from that of the liquid phase.

Over the years many studies designed to identify a procedure of analysis of headspace gas at equilibrium with liquid essential oil have been reported.[68]

These works are mostly based on use of solid-phase microextraction (SPME) by which the headspace gas is extracted by a fused silica fiber coated with a suitable stationary phase (HS-SPME).[69] The volatile compounds adsorbed on the fiber are then thermally desorbed in the GC injector port of a GC/MS instrument to perform the qualitative analysis and GC/FID for the quantitative determination.

However, the composition of volatile compounds adsorbed on the fiber is different from that of headspace gas in equilibrium with the essential oil since the adsorption on the fiber depends on the fiber characteristics and extraction conditions used for the analysis. Therefore, this procedure is not sufficient to define the actual composition of the vapor phase in equilibrium with the essential oil, and hence

poorly applicable to the study of aromatherapy. Headspace gas composition determination in bergamot essential oil is extremely useful in aromatherapy. Nevertheless, greater efforts are still needed to develop a simple, and objective methodology.

In the present work we studied the composition of the gaseous phase at equilibrium with the liquid phase of bergamot essential oil by developing a gas chromatography-mass spectrometry (GC/MS) method useful for the determination of the volatile aroma components.

Bergamot (Citrus Bergamia) is an endemic plant of the Calabria region in the South of Italy and its fruit is used for the extraction of bergamot essential oil (BEO). Bergamot essential oil is the basic component of perfumes and is used also in the formulation of cosmetic products, food and confections as a flavouring.Furthermore, it is employed in aromatherapy as an antidepressant to reduce anxiety and stress by improving mood and facilitating sleep induction.[70]

The used bergamot essential oil was preliminarily analyzed to define its composition. The individual analytes present in the oil were identified by GC/MS methodology by

comparing the corresponding retention times and mass spectra with those of authentic samples (table 19, figure 9). Anisole was chosen as internal standard for the quantitative measurement of the individual analytes.

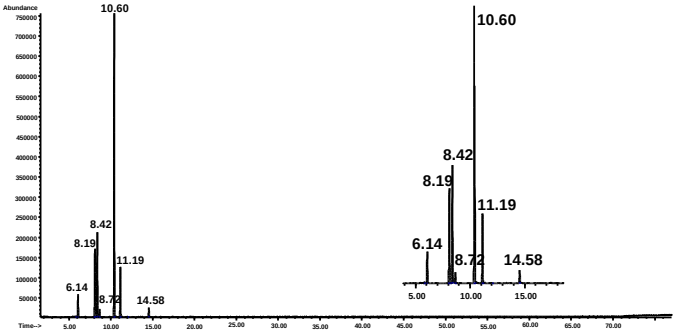


Figure 9. GC/MS analysis of BEO

For the quantitative analysis six standard stock solution containing different concentration levels of each identified analyte and the same amount of internal standard were prepared. Each solution was injected in triplicate in the GC/MS system under optimized conditions. For each measurement, the concentration and the peak area of the analytes are compared with those of the internal standard. High contents of limonene, linalool, linalyl acetate, and ɣ-

terpinene, are observed in analogy with the data reported in literature.[71]

Table 19. Composition of BEO and Gaseous Phase.

	Compound	**TR**	**Composition essential oil[a]**	**Gaseous Phase composition**	
				GC-MS[b]	**GC-FID[c]**
	Cyclic hydrocarbon monoterpenes				
1	α-Pinene	6.14	1,03 ±0.10[e]	6.90±0.10[e]	7.06
2	β-Pinene	8.19	6.56 ± 0.14	25.90± 0.40	26.68
3	α-Fellandrene	9.48	0.04 ± 0.01	-	-
4	α-Terpinene	9.94	0.16 ± 0.02	-	-
5	Limonene	10.60	30.20 ± 0.77	58.07± 0.38	57.12
6	p-cimene	11.19	0.18 ± 0.01	6.36±0.04	6.02
7	ɣ-Terpinene	12.15	11.95 ± 0.32	-	-
8	Terpinolene	13.36	0.27 ± 0.03	-	-
	Acyclic hydrocarbon monoterpenes				
9	Mircene	8.72	0.82 ± 0.02	2.19± 0.22	2.16
10	Ocimene	11.03	0.08 ± 0.01	-	-
	Acyclic oxygenated hydrocarbon monoterpenes				
11	Linalool	14.58	21.82 ± 0.87	3.04± 0.54	2.96
12	Linalyl Acetate	21.42	16.21 ± 0.84	-	-
13	Neral	22.94	0.21 ± 0.01	-	-
14	Geranial	24.46	0.11 ± 0.01	-	-
15	Neryl Acetate	28.14	0.28± 0.02	-	-
	Cyclic oxygenated hydrocarbon monoterpenes				
16	α-Terpineol	20.01	0.87 ± 0.08	-	-
	Esters				
17	Octyl Acetate	19.63	0,10±0.01	-	-
	Sesquiterpenes				
18	β-Caryophyllene	27.85	0.14 ± 0.02	-	-

a= The w/w percents were determined by the internal standard method and referred to the amount of each component contained in 100 g of oil, b= reported only for the analytes present in the gaseous phase of the essential oil bergamot analized by GC-MS, c= reported only for the analytes present in the gaseous phase of the essential oil bergamot analized by GC-FID, e=standard deviation

The determination of gas phase composition above the liquid oil has preliminarily required controlled temperature and pre-established equilibrium conditions.

To this aim, a weighed amount of essential oil was placed in a headspace vial, after adding a given amount of anisole the vial was sealed and then allowed to stand for 30 min at 0 ^{0}C to establish the equilibrium at that temperature. Once the volatile compounds have equilibrated, an aliquot of the headspace gas was withdrawn using a gas tight syringe, injected into the gas chromatograph injection port and analysed by GC/MS. The individual analytes present in the headspace gas were identified through comparison of retention times and mass spectral data with those of authentic standards. For the quantitative analysis a mixture of reference analytes at a known concentration to which is added the same amount of anisole as internal standard was used. An aliquot (1 µl) of this stock solution was injected into the GC/MS injection port where fast and completely turned to gas and analysed. All the analyses were performed in splitless conditions in triplicate.

The determination of each analyte concentration level in the headspace gas of essential oil sample was performed by

comparing the peak area of each individual analyte with the corresponding peak area in the reference mixture, the peak area of the analytes are always compared with those of the internal standard.

The adopted methodology assumes that the total reference mixture amount introduced into the injection port is vaporized and that all the produced gas reaches the ion source (splitless conditions).

In this study the headspace gas in equilibrium with the bergamot oil sample has been also investigated by means of GC–FID in order to validate the proposed methodology.

The results of GC/FID analysis are comparable to those obtained by GC/MS (Table 19). It can be observed that the gaseous phase composition is quite different from that of the liquid phase at equilibrium with it.

The comparison between the bergamot essential oil composition (Table 19) and headspace gas at equilibrium shows how the linalool and the linalyl acetate amounts decrease dramatically in the gas phase on the contratry the concentration of limonene is almost double (approximately 60%).

Furthermore, β-pinene content that is very low in the liquid oil is particularly high in gaseous phase.

These results suggest that the determination of the gaseous phase composition in equilibrium with the liquid essential oil is critical for establishing the correlation between the volatile components and their activity.

This study showed that for employing bergamot essential oil in aromatherapy it is not enough to know the essential oil composition but is essential to know the volatile fraction composition in equilibrium with it.

This paper reports a GC/MS methodology for the direct analysis of volatile compounds of bergamot essential oil.

The method can also be applied to environments of greater volume provided that the parameters relating to temperature are maintained and operates in conditions such that the gaseous phase is in equilibrium with the essential oil.

EXPERIMENTAL SECTION

General

Commercially available reagents were purchased from Sigma-Aldrich Chemical Co. (Milano, Italy) and used as supplied unless stated otherwise. Solvents were purified and dried by the standard procedures and distilled prior to use. All the reactions were carried out under an inert atmosphere (N_2). Melting points were determined on a melting point apparatus Stuart SMP20 (Staffordshire, UK) and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz, while ^{13}C NMR spectra were measured at 75 MHz on a Bruker Avance 300 spectrometer (Faellanden, Switzerland). Spectral analysis was performed at 293 K on diluted solutions of each compound by using $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm and referenced to $CDCl_3$ (7.25 ppm for ^{1}H and 77.0 ppm for ^{13}C spectra). Coupling costants (J) are reported in Hertz (Hz). The dichloromethane solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea with a classical procedure.[72] The concentration of the diazomethane solution (0.66 M)

was obtained by a back titration performed with a standard benzoic acid solution. Reaction mixtures were monitored by thin layer chromatography (TLC) using Merck Silica gel 60-F254 precoated glass plates, and UV light (254 nm) or 0.2% ninhydrin in ethanol as visualizing agent. Kieselgel 60H without gypsum was used for flash column chromatography.

Evaporation of solvents was performed at reduced pressure using a rotary vacuum evaporator.

LC-MS analyses were carried out using an Agilent 6540 UHD Accurate - Mass Q-TOF LC/MS (Santa Clara, CA) fitted with a electrospray ionization source (Dual AJS ESI) operating in positive ion mode. GC-MS analyses were performed using a 30 m × 0.25 mm, PhMesiloxane capillary column. GC-FID analyses were performed using a HP6890 A series 2 GC System (Agilent Technologies Inc., Palo Alto, CA) equipped with a HP-35MS (35% diphenyl siloxane;l=20 m, d= 0.25 mm 0.25 μm). The mass detector was operated in the electron impact ionization mode (EIMS) with an electron energy of 70 eV. The injection port was heated to 250 °C. The oven temperature program was initially set at 100 °C

with a hold of 2 min and ramped to 280 °C at 14 °C/min with a hold of 10 min.

Deprotection of 1a-d and N-Acetylation of 2a-d. General Procedure. Mercaptoacetic acid (3 mmol) and Et_3N (5 mmol) were added to a solution of **1a-d** (1 mmol) in [bmim][BF_4] (1 mL). After the addition, the reaction mixture was stirred magnetically at room temperature. The consumption of the starting materials was monitored by TLC (diethyl ether/petroleum ether 60:40) and was complete in 4-6 min. The reaction mixture was then extracted with Et_2O (5 × 4 mL) and the combined Et_2O extracts were concentrated *in vacuo*. The crude residue, containing the respective free α-amino acid methyl esters **2a-d**, was solubilized in dry CH_2Cl_2 (5 mL). Acetic anhydride (1 mL) and a 9% aqueous solution of $NaHCO_3$ (5 mL) were then added and the mixture was maintained under stirring at room temperature for 4 h. The organic layer was separated and the aqueous phase was extracted with three additional portions of methylene chloride (3 × 10 mL). The combined organic layers were washed twice a 9% aqueous solution of $NaHCO_3$, twice with aqueous HCl 1N, once with brine and finally dried (Na_2SO_4). Filtration and

concentration under reduced pressure gave **4a-d** as colorless oils in 89-96 % overall yields referred to the starting materials **1a-d**.

***N*-Acetyl-L-Valine Methyl Ester (4a)** colorless oil (104.6 mg, 96 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 [d, J = 6.9 Hz, 3H, CH(CH_3)$_2$], 0.91 [d, J = 6.9 Hz, 3H, CH(CH_3)$_2$], 2.01 (s, 3H, CH_3CO), 2.12 [m, 1H, CH(CH$_3$)$_2$], 3.71 (s, 3H, OCH_3), 4.54 (dd, J = 8.7 and 5.1 Hz, 1H, α-CH), 6.15 (d, J = 6.9 Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.8, 18.8, 23.1, 31.2, 52.0, 57.0, 169.9, 172.7. GC/MS (CI): *m/z* (%) 214 (17), 202 (27) [(M + C_2H_5)$^+$], 174 (65) [(M + H)$^+$], 156 (8), 142 (64), 132 (49), 114 (100), 101 (6). Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.32; H, 8.75; N, 8.06.

***N*-Acetyl-L-Phenylalanine Methyl Ester (4b)** colorless oil (111.3 mg, 92 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98 (s, 3H, CH_3CO), 3.03-3.18 (m, 2H, CH_2Ph), 3.71 (s, 3H, OCH_3), 4.88 (m, 1H, α-CH), 6.08 (d, J = 6.9 Hz, 1H, NH), 7.06-7.11 (m, 2H, ArH), 7.22-7.32 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1, 37.8, 52.6, 53.1, 127.2, 128.4, 129.2, 135.8, 169.8, 172.1. GC/MS (CI): *m/z* (%) 262 (13), 250 (31) [(M + C_2H_5)$^+$], 222 (100) [(M + H)$^+$], 190 (38), 180 (69), 162 (76),

120 (8). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.38; H, 6.80; N, 6.35.

***N*-Acetyl-L-Leucine Methyl Ester (4c)** colorless oil (101.1 mg, 89 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 [d, J = 6.3 Hz, 6H, $CH_2CH(CH_3)_2$], 1.41-1.69 [m, 3H, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 2.01 (s, 3H, CH_3CO), 3.71 (s, 3H, OCH_3), 4.61 (m, 1H, α-C*H*), 6.07 (d, 1H, J = 7.8 Hz, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.9, 22.7, 24.8, 41.6, 50.7, 52.3, 169.9, 173.7; GC/MS (CI*): m/z* (%) 228 (26), 216 (41) [$(M + C_2H_5)^+$], 188 (100) [$(M + H)^+$], 170 (7), 156 (58), 146 (54), 128 (100), 86 (7). Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.58; H, 9.18; N, 7.46.

***N*-Acetyl-L-Isoleucine Methyl Ester (4d)** colorless oil (102.3 mg, 90 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85-0.95 [m, 6H, $CH(CH_3)CH_2CH_3$, $CH(CH_3)CH_2CH_3$], 1.20 [m, 1H, $CH(CH_3)CH_2CH_3$], 1.44 [m, 1H, $CH(CH_3)CH_2CH_3$], 1.83 [m, 1H, $CH(CH_3)CH_2CH_3$], 2.01 (s, 3H, CH_3CO), 3.72 (s, 3H, OCH_3), 4.57 (dd, J = 8.7 and 5.1 Hz, 1H, α-C*H*), 6.12 (d, J = 8.7 Hz, 1H, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 15.3, 23.2, 24.9, 37.9, 52.0, 56.4, 169.8, 172.6. GC/MS (CI): *m/z* (%) 228 (21), 216 (35) [$(M + C_2H_5)^+$], 188 (79) [$(M + H)^+$], 170 (5), 156 (51),

146 (31), 128 (100), 86 (5). 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.

One Pot Synthesis of Boc-amino acid methyl esters 5a-h in [bmim][BF_4]. General Procedure. Mercaptoacetic acid (3 mmol) and Et_3N (5 mmol) were added to a solution of **1a-h** (1 mmol) in [bmim][BF_4] (1 mL). The solution was magnetically stirred for 3-7 min at room temperature, until the complete denosylation of starting compounds, as monitored by TLC (diethyl ether/petroleum ether 60:40). Boc_2O (1 mmol) was then added and the stirring was further maintained for 5-10 min at room temperature and the progress of the reaction was monitored by TLC and GC/MS. After a total time of 8-15 min the reaction mixture was diluted with Et_2O and the IL settled at the bottom. The supernatant was decanted off and the IL was washed with Et_2O (5 × 2 mL). The combined Et_2O extracts were washed twice with a 5% aqueous solution of $NaHSO_4$ and once with brine (10 mL), dried over Na_2SO_4 and filtered. Evaporation of the solvent to dryness under reduced pressure conditions afforded **5a-h** in 92-96% yields. Compounds **5a-h** displayed NMR spectral characteristics similar to those elsewhere

published for the same compounds obtained by other methods.[20]

***N*-Boc-L-Valine Methyl Ester (5a)** viscous colorless oil (137.2 mg, 94 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 [d, J = 6.9 Hz, 3H, CH(CH_3)$_2$], 0.90 [d, J = 6.9 Hz, 3H, CH(CH_3)$_2$,], 1.39 (s, 9H, *t*-Bu), 2.07 [m, 1H, CH(CH_3)$_2$,], 3.68 (s, 3H, OCH_3), 4.16 (dd, J = 8.7 and 4.9 Hz, 1H, α-CH), 5.05 (d, J = 8.7 Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.6, 18.9, 27.3, 28.2, 54.4, 58.4, 79.6, 155.6, 173.1. GC/MS (EI*): m/z* (%) 231 (2), 172 (24), 158 (4), 130 (21), 116 (61), 88 (25), 72 (100), 57 (99). Anal. Calcd for $C_{11}H_{21}NO_4$: C, 57.12; H, 9.15; N, 6.06. Found: C, 57.34; H, 9.12; N, 6.08.

***N*-Boc-L-Phenylalanine Methyl Ester (5b)** viscous colorless oil (147.8 mg, 96 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9H, *t*-Bu), 2.98-3.14 (m, 2H, CH_2Ph), 3.69 (s, 3H, OCH_3), 4.57 (m, 1H, α-CH), 5.01 (d, J =7.9 Hz, 1H, NH), 7.10 (d, J = 6.5 Hz, 2H, ArH), 7.18-7.31 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.1, 38.0, 51.9, 54.4, 79.5, 126.7, 128.3, 129.1, 136.1, 155.0, 172.2. GC/MS (EI*): m/z* (%) 279 (2), 223 (7), 206 (10), 178 (6), 162 (100), 131 (7), 120 (26), 91 (37), 57 (56). Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.24; H, 7.57; N, 5.02

***N*-Boc-L-Leucine Methyl Ester (5c)** viscous colorless oil (139.4 mg, 95 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 [d, J = 6.3 Hz, 3H, $CH_2CH(CH_3)_2$], 0.85 [d, J = 6.3 Hz, 3H, $CH_2CH(CH_3)_2$], 1.32 (s, 9H, *t*-Bu), 1.39-1.68 [m, 3H, $CH_2CH(CH_3)_2$, $CH_2CH(CH_3)_2$], 3.64 (s, 3H, OCH_3), 4.22 (dt, J = 8.7 and 6.9 Hz, 1H, α-CH), 4.98 (d, J =8.4 Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 22.6, 24.5, 27.2, 28.1, 41.5, 51.9, 79.5, 155.3, 173.9. GC/MS (EI*)*: *m/z* (%) 186 (15), 172 (2), 144 (12), 130 (57), 86 (100), 57 (73). Anal. Calcd for $C_{12}H_{23}NO_4$: C, 58.75; H, 9.45; N, 5.71. Found: C, 58.97; H, 9.43; N, 5.69.

***N*-Boc-L-Isoleucine Methyl Ester (5d)** viscous colorless oil (138.7 mg, 94 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 [t, J = 7.2 Hz, 3H, $CH(CH_3)CH_2CH_3$], 0.82 [d, J = 6.9 Hz, 3H, $CH(CH_3)CH_2CH_3$], 1.08 (m, 1H, $CH(CH_3)CH_2CH_3$], 1.32 (m, 1H, $CH(CH_3)CH_2CH_3$], 1.34 (s, 9H, *t*-Bu), 1.72 (m, 1H, $CH(CH_3)CH_2CH_3$], 3.63 (s, 3H, OCH_3), 4.13 (m, 1H, α-CH), 5.01 (s broad, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.3, 15.3, 24.9, 28.1, 37.9, 51.7, 57.7, 79.5, 155.4, 172.6. GC/MS (EI*)*: *m/z* (%) 186 (24), 172 (3), 144 (17), 130 (92), 86 (78), 57 (100). Anal. Calcd for $C_{12}H_{23}NO_4$: C, 58.75; H, 9.45; N, 5.71. Found: C, 58.53; H, 9.49; N, 5.72.

***N*-Boc-L-Alanine Methyl Ester (5e)** viscous colorless oil (132.1 mg, 94 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, *J* = 7.2 Hz, 3H, CHC*H*$_3$), 1.41 (s, 9H, *t*-Bu), 3.71 (s, 3H, OC*H*$_3$), 4.27 (quintet, *J* = 7.2 Hz, 1H, α-C*H*), 5.05 (s broad, 1H, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.4, 28.1, 48.1, 52.1, 79.6, 155.2, 172.8. GC/MS (EI*)*: *m/z* (%) 144 (52), 130 (9), 116 (6), 102 (16), 88 (40), 59 (36), 57 (100). Anal. Calcd for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.39; H, 8.44; N, 6.87.

***N*-Boc-D-Phenylalanine Methyl Ester (5f)** viscous colorless oil (148 mg, 96 % yield). Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.70; H, 7.56; N, 5.00.

***N*-Boc-L-Tyrosine(*O*Bn) Methyl Ester (5g)** viscous colorless oil (150.4 mg, 93% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9H, *t*-Bu), 3.02 (m, 2H,CHC*H*$_2$), 3.69 (s, 3H, OC*H*$_3$), 4.55 (m, 1H, α-C*H*), 5.02 (s, 2H, C*H*$_2$Ph), 6.89 (d, *J* = 8.7 Hz, 2H, *o*-OBnAr*H*), 7.03 (d, *J*= 8.7 Hz, 2H, *m*-OBnAr*H*), 7.27-7.43 (m, 5H, Bn-Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 37.3, 52.0, 54.4, 69.9, 79.7, 114.8, 127.3, 127.8, 128.2, 128.4, 130.2, 136.9, 146.6, 157.8, 172.28. Anal. Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.82; H, 7.04; N, 3.62.

***N*-Boc-L-Cysteine(*S*Bn) Methyl Ester (5h)** viscous colorless oil (147.1 mg, 92% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 9H, *t*-Bu), 2.56 (dd, *J* = 13.9 and 5.1 Hz, 1H, CH_2SBn), 2.61 (dd, *J*= 13.9 and 5.1 Hz, 1H, CH_2SBn), 3.62 (s, 2H,SCH_2Ph), 3.64 (s, 3H, OCH_3), 4.43 (dt, *J* = 7.8 and 5.1 Hz, 1H, α-C*H*), 5.21 (d, *J*= 7.8 Hz, 1H, N*H*), 7.08-7.28 (m, 5H, Ar*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 33.5, 36.5, 52.6, 53.0, 80.0, 127.1, 128.5, 128.9, 137.6, 155.1, 171.5. Anal. Calcd for $C_{16}H_{23}NO_4S$: C, 59.05; H, 7.12; N, 4.30. Found: C, 59.27; H, 7.10; N, 4.29.

***Synthesis of N-Boc-dipeptide methyl esters 7 and 8 via EDCI/HOBt assisted coupling. General Procedure*.[73]** To a solution of *N*-Boc-L-phenylalanine methyl ester **1b** or *N*-Boc-D-phenylalanine methyl ester **1f** (1 mmol) in ethyl acetate (10 mL), LiI (5 mmol) was added and the resulting solution was heated at reflux for 24 h. After complete conversion of the starting amino acid methyl ester, a saturated aqueous Na_2CO_3 solution and the mixture was extracted twice with EtOAc. The aqueous phase was made acidic by adding 5% aq. $KHSO_4$, then extracted three times with EtOAc. The organic layers were collected, washed once with brine, dried over Na_2SO_4 and evaporated to dryness.

The crude residue, containing the free carboxylic acid derivatives, was directly used for the subsequent coupling reaction. To a solution of *N*-Boc-L-Phe-OH or *N*-Boc-D-Phe-OH (1 mmol) in dry methylene chloride (3 mL) HOBt monohydrate (1 mmol), EDCI (1 mmol) and DIEA (2.5 mmol) was added. The resulting mixture was maintained under magnetic stirring for 45 min at °0 C. L-Alanine methyl ester hydrochloride (**6**) (1 mmol) was dissolved in dry methylene chloride (2 mL) and added dropwise to the reaction mixture over a period of 15 min. The final solution was stirred overnight at room temperature. The mixture was thus evaporated to dryness under vacuum and the residue dissolved in AcOEt (5 mL). The organic solution was washed with 5% aqueous $NaHSO_4$ (3 × 5 mL), 5% aqueous $NaHCO_3$ (3 × 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 **7** (228.2 mg, 91 % yield) or **8** (232.4 mg, 93 % yield). Each product displayed ^1H and ^{13}C NMR characteristics that matched those observed for the same compound from the [bmim][BF_4] procedure.

***N*-Boc-L-Phenylalanyl-L-Alanine Methyl Ester (7)** Colorless oil, 92% yield, ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (d, 3H, J =

7.2 Hz,CHC*H*$_3$), 1.33 (s, 9H, *t*Bu), 2.88-3.09 (m, 2H, C*H*$_2$Ph), 3.63 (s, 3H, OC*H*$_3$), 4.38 (m, 1H, α-C*H*Phe), 4.47 (quintet, *J* = 7.2 Hz, α-C*H*Ala), 5.29 (d, 1H, *J* = 8.2 Hz, N*H*CHCH$_3$), 6.84 (s broad, 1H, OCON*H*), 7.10-7.23 (m, 5H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 17.8, 28.1, 38.3, 47.9, 52.1, 55.7, 79.8, 126.6, 128.3, 129.2, 136.6, 155.4, 171.0, 172.7. GC/MS (EI*)*: *m/z* (%) 294 (5), 277 (3), 233 (10), 174 (11), 164 (37), 159 (21), 120 (90), 91 (19), 77 (4), 57 (100). Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.48; H, 7.50; N, 7.97.

***N*-Boc-D-Phenylalanyl-L-Alanine Methyl Ester (8)** viscous colorless oil, 91% yield, ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 1.21 (d, 3H, *J* = 7.3 Hz, CHC*H*$_3$), 1.34 (s, 9H, *t*Bu), 2.93-3.07 (m, 2H, C*H*$_2$Ph), 3.64 (s, 3H, OC*H*$_3$), 4.35 (m, 1H, α-C*H*Phe), 4.46 (quintet, *J* = 7.3 Hz, 1H, α-C*H*Ala), 5.31 (d, *J* = 8.1 Hz, N*H*CHCH$_3$, 1H), 6.58 (d, *J* = 7.3 Hz, 1H, OCON*H*), 7.11-7.24 (m, 5H, Ar*H*); GC/MS (EI*)*: *m/z* (%) 294 (5), 277 (3), 233 (11), 174 (12), 164 (41), 159 (20), 120 (92), 91 (21), 77 (4), 57 (100). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 17.8, 28.1, 38.3, 47.9, 52.0, 55.6, 79.9, 126.6, 128.4, 129.2, 136.7, 155.2, 170.8, 172.8. Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.60; H, 7.46; N, 8.01.

Mixture of 8 and 9 Mixture of diastereomers (1:1 ratio): 1H NMR (300 MHz, CDCl3) δ 1.20 (d, J = 7.2 Hz, 3H,CHCH3), 1.28 (d, J = 7.2 Hz, 3H, CHCH3,), 1.33 (s, 9H, tBu), 1.34 (s, 9H, tBu), 2.96-3.11 (m, 4H, CH2Ph), 3.63 (s, 3H OCH3), 3.64 (s, 3H, OCH3), 4.28-4.41 (m, 2H, α-CHPhe), 4.41-4.55 (m, 2H, α-CHAla), 5.12 (m, 2H, NHCHCH3), 6.40 (d, J = 7.3 Hz, 1H, OCONH), 6.55 (d, J = 7.3 Hz, 1H, OCONH), 7.10-7.23 (m, 10H, ArH); 13C NMR (75 MHz, CDCl3) δ 17.8, 18.0, 28.1, 38.2, 38.6, 47.8, 47.9, 52.1, 55.7, 55.8, 79.9, 126.7, 128.4, 129.2, 136.5, 136.6, 155.2, 170.6, 170.8, 172.6, 172.8.

One pot synthesis of N-Boc-N-Methyl-L-Valine Methyl Ester in [bmim][BF_4]. Mercaptoacetic acid (3 mmol) and Et_3N (5 mmol) were added to a solution of *N*-nosyl-*N*-methyl-L-valine methyl ester (1 mmol) in [bmim][BF_4] (1 mL). The solution was magnetically stirred for 30 min at room temperature, until the complete denosylation of starting compounds, as monitored by TLC (diethyl ether/petroleum ether 60:40). Boc_2O (1 mmol) was then added and the stirring was further maintained for 5 min at room temperature and the progress of the reaction was monitored by TLC and GC/MS. The reaction mixture was

diluted with Et_2O and the IL settled at the bottom. The supernatant was decanted off and the IL was washed with Et_2O (5 × 2 mL). The combined Et_2O extracts were washed twice with a 5% aqueous solution of $NaHSO_4$ and once with brine (10 mL), dried over Na_2SO_4 and filtered. Evaporation of the solvent to dryness under reduced pressure conditions afforded *N*-Boc-*N*-methyl-L-valine methyl ester as a viscous colorless oil in 90% yield: ^{1}H NMR (300 MHz, $CDCl_3$, a mixture of rotamers) δ 0.87 [d, J = 6.9 Hz, 3H, $CH(CH_3)_2$], 0.98 [d, J = 6.9 Hz, 3H, $CH(CH_3)_2$], 1.43 (s, 9H, *t*Bu), 1.99-2.21 [m, 1H, $CH(CH_3)_2$], 2.78 and 2.82 (s, 3H, NCH_3), 3.69 (s, 3H, OCH_3), 4.08 and 4.42 (d, J = 10.6 Hz, 1H, α-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.1, 27.8, 28.3, 30.3, 51.5 and 51.6, 70.9, 79.7, 155.3, 171.5. GC/MS (EI*): m/z* (%) 245 (2), 186 (15), 144 (17), 130 (79), 102 (49), 86 (92), 57 (100), 41 (34). Anal. Calcd for $C_{12}H_{23}NO_4$: C, 58.75; H, 9.45; N, 5.71. Found: C, 58.62; H, 9.47; N, 5.69.

General procedure for the N-Fmoc and N-Cbz protection of amines 10a-e in [Bmim][BF$_4$] To a magnetically stirred mixture of amine **10a-e** (1 mmol) and [bmim[BF_4] (1 mL) (for amines **10b-e** 0.5 mL of $NaHCO_3$ aqueous solution were

added), Fmoc-OSu or Cbz-Osu (1 mmol) was added and the mixture was stirred at ambient temperature for 5-9 min. The reaction was monitored by TLC. After the completion of reaction, diethyl ether was added, and the IL settled at the bottom. The supernatant was decanted off and the IL was washed with Et_2O (3 × 2 mL). The combined Et_2O extracts were washed once with a 1N aqueous solution of HCl (3 mL), dried over Na_2SO_4 and filtered. The products were isolated after evaporation of the diethyl ether to yield the highly pure *N*-Fmoc derivatives **11a-e** in 85-90 % yields or the *N*-Cbz-derivatives **12a-e** in 86-90 % yields. ^{1}H NMR and ^{13}C NMR were consistent with the assigned structures.

***N*-Fmoc-aniline (11a)** solid pale brown, obtained in 86% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.30 (t, *J* = 6.4 Hz, 1H CHFmoc), 4.59 (d, *J* = 6.4 Hz, 2H, CH_2Fmoc), 6.70 (br s, 1H, NH), 7.09 (t, *J* = 7.1 Hz, 1H, ArH), 7.24 - 7.48 (m, 8H, ArH), 7.63 (d, *J* = 7.6 Hz, 2H, ArH), 7.80 (d, *J* =7.4 Hz, 2H, ArHFmoc). ^{13}C NMR: (75 MHz, $CDCl_3$) δ 47.1, 66.8, 118.8, 120.1, 123.6, 124.9, 127.1, 127.8, 129.1, 138.3, 141.4, 143.8, 153.9. ESI(+)-MS: calcd for $([C_{21}H_{17}NO_2] + Na)^+$ $[M+Na]^+$ 338.1157; found: 338.1151

***N-Cbz*-aniline (12a)** pale brown solid obtained in 87% yield. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): 5.3 (s, 2H, OCH_2), 6.84 (br s, 1H, N*H*), 7.10 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.30 - 7.48 (m, 9H, Ar*H*). ^{13}C-NMR: (75 MHz, $CDCl_3$) δ 67.1, 118.8, 123.6, 128.3, 128.4, 128.6, 129.1, 136.1, 137.8, 153.4. EIMS m/z (%) 227 (18, M+•), 183 (16), 119 (42), 108 (17), 91(100), 79 (18), 77(16), 65(9).

***N*-Fmoc-glycine methyl ester (11b)** white solid obtained in 90% yield. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): 3.76 (s, 3H, OCH_3) 4.01 (d, J = 5.4 Hz, 2H, $NHCH_2$), 4.23 (t, J = 7.2 Hz, 1H, CHFmoc) 4.42 (d, J = 7.2 Hz, 2H, CH_2Fmoc), 5.40 (br s, 1H, NH), 7.27 - 7.42 (m, 4H, ArH), 7.61 (d, J = 7.2 Hz, 2H, ArH), 7.77 (d, J = 7.5 Hz, 2H, ArH). ^{13} C-NMR: (75 MHz, $CDCl_3$) δ 42.6, 47.0, 52.4, 67.1, 120.0, 125.0, 127.0, 127.7, 141.2, 143.7, 156.3, 170.5. ESI(+)-MS: calcd for $([C_{18}H_{17}NO_4] + Na)^+$ $[M+Na]^+$ 334.1055; found: 334.1049

***N*-Cbz-glycine methyl ester (12b)** White solid obtained in 87% yield.*^{1}H-NMR*: (300 MHz, $CDCl_3$, 25°C): 3.72 (s, 3H, OCH_3) 4.01 (d, *J* = 5.7 Hz, 2H, NHCH_2), 5.12 (s, 2H, OCH_2), 5.40 (br s, 1H, N*H*), 7.30 - 7.40 (m, 5H, Ar*H*). *^{13}C-NMR:* (75 MHz, $CDCl_3$) δ 42.6, 52.4, 67.1, 127.9, 128.3, 128.6, 136.1,

156.3, 170.5. EIMS m/z (%) 223 (8, M+•), 164 (2), 120 (2), 108 (86), 91 (100), 79 (18), 77 (15), 65 (8).

***N*-Fmoc-alanine methyl ester (11c)** pale yellow solid obtained in 85% yield. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): 1.45 (d, J = 7.2 Hz, 3H, $CHCH_3$), 3.77 (s, 3H, OCH_3) 4.22 (t, J = 6.7 Hz, 1H, CHFmoc) 4.32 – 4.48 (m, 3H, CH_2Fmoc and $CHCH_3$), 5.38 (d, J = 7.5 Hz, 1H, NH), 7.30 - 7.48 (m, 4H, ArHFmoc), 7.58 - 7.62 (m, 2H, ArHFmoc), 7.78 (d, J = 7.5 Hz, 2H, ArHFmoc). ^{13}C-NMR: (75 MHz, $CDCl_3$) δ 18.7, 30.2, 47.1, 52.5, 66.9, 119.9, 125.0, 127.0, 127.7, 136.6, 141.8, 158.0, 172.1. ESI(+)-MS: calcd for $([C_{19}H_{19}NO_4] + Na)^+$ $[M+Na]^+$ 348.1212; found: 348.1200

***N*-Cbz-alanine methyl ester (12c)** colorless solid obtained in 86% yield. *^{1}H-NMR*: (300 MHz, $CDCl_3$, 25°C): 1.36 (d, *J*= 7.2 Hz, 3H, CHC*H_3*), 3.75 (s, 3H, OC*H_3*), 4.30-4.50 (m, 1H, C*H*CH_3), 5.11 (s, 2H, OC*H_2*), 5.34 (br s, 1H, N*H*), 7.30 - 7.40 (m, 5H, Ar*H*). *^{13}C-NMR:* (75 MHz, $CDCl_3$) *δ* 18.6, 49.5, 52.4, 66.9, 127.7, 128.3, 128.6, 136.2, 155.5, 173.4. EIMS m/z (%) 237 (3, M+•), 178 (8), 134 (12), 108 (45), 91 (100), 79 (21), 77 (16), 70 (35), 65(7).

***N*-Fmoc-valine methyl ester (11d)** Pale yellow solid obtained in 86% yield.^{1}H-NMR (300 MHz, $CDCl_3$, 25°C): 0.93

[d, J = 6.9 Hz, 3H, $CH(CH_3)_2$], 0.99 [d, J = 6.6 Hz, 3H, $CH(CH_3)_2$], 2.09 -2.49 [m, 1H, $CH(CH_3)_2$], 3.77 (s, 3H, OCH_3), 4.25 (t, J = 7.2 Hz, 1H, CHFmoc), 4.38 (m, 1H, α-CH), 4.42 (d, J = 7.2 Hz, 2H, CH_2Fmoc), 5.38 (d, J = 9.9 Hz, 1H, NH), 7.31-7.44 (m, 4 H, ArHFmoc), 7.62 (d, J = 7.2 Hz, ArHFmoc), 7.78 (d, J = 7.2 Hz, ArHFmoc). ^{13}C-NMR: (75 MHz, $CDCl_3$) δ 17.7, 18.9, 31.3, 47.2, 52.2, 59.0, 67.0, 120.0, 125.1, 127.1, 127.7, 141.3, 143.8, 156.3, 172.6. ESI(+)-MS: calcd for $([C_{21}H_{23}NO_4] + Na)^+$ $[M+Na]^+$ 376.1525; found: 376.1516

***N*-Cbz-valine methyl ester (12d)** colorless solid obtained in 90% yield.*^{1}H-NMR*: (300 MHz, $CDCl_3$, 25°C): 0.89 [d, *J*= 6.9 Hz, 3H, $CH(CH_3)_2$], 0.95 [d, *J*= 6.9 Hz, 3H, $CH(CH_3)_2$], 2.10-2.20 [m, 1H, $CH(CH_3)_2$], 3.72 (s, 3H, OCH_3), 4.25-4.35 (m, 1H, NHC*H*), 5.12 (s, 2H, OCH_2), 5.32 (br s, 1H, N*H*), 7.32-7.42 (m, 5H, Ar*H*). *^{13}C-NMR:* (75 MHz, $CDCl_3$) *δ* 17.5, 18.9, 25.4, 52.2, 58.9, 67.0, 128.1, 128.6, 129.3, 136.1, 156.2, 172.5. EIMS m/z (%) 265 (5, M+•), 206 (14), 162 (21), 116 (21), 108 (24), 91 (100), 79 (13), 77(10), 65 (5).

***N*-Fmoc-isoleucine methyl ester (11e)** yellow solid obtained in 85% yield. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): 0.90 – 0.99 [m, 6H, $CH(CH_3)CH_2CH_3$ and $CH(CH_3)CH_2CH_3$], 1.20-1.40 (m, 1H, $CH(CH_3)CH_2CH_3$), 1.44-1.51 (m, 1H, $CH(CH_3)CH_2CH_3$),

1.90-2.03 (m, 1H, $CH(CH_3)CH_2CH_3$), 3.75 (s, 3H, OCH_3), 4.24 (t, J = 7.2 Hz, 1H, CHFmoc), 4.36 – 4.43 (m, 3H, CH_2Fmoc and α-CH), 5.37 (d, J = 9.3 Hz, 1H, NH), 7.30 – 7.48 (m, 4H, ArHFmoc), 7.68 (d, J = 6.3 Hz, 2H, ArHFmoc), 7.76 (d, J = 6.3 Hz, 2H, ArHFmoc). ^{13} C-NMR: (75 MHz, $CDCl_3$) δ 11.6, 15.4, 25.3, 37.9, 47.1, 52.1, 58.3, 66.9, 119.9, 125.2, 127.0, 128.1, 141.2, 143.8, 156.0, 172.6. ESI(+)-MS: calcd for $([C_{22}H25NO_4] + Na)^+$ $[M+Na]^+$ 390.1681; found: 390.1695

***N*-Cbz-isoleucine methyl ester (12e)** colorless solid obtained in 88% yield. *^{1}H-NMR*: (300 MHz, $CDCl_3$, 25°C): 0.83-0.97 [m, 6H, $CH(CH_3)CH_2CH_3$ and $CH(CH_3)CH_2CH_3$], 1.16-1.22 (m, 1H, $CH(CH_3)CH_2CH_3$), 1.40-1.49 (m, 1H, $CH(CH_3)CH_2CH_3$), 1.90-1.95 (m, 1H, $CH(CH_3)CH_2CH_3$), 3.75 (s, 3H, OCH_3), 4.30 – 4.50 (m, 1H, α-CH), 5.10 (s, 2H, OCH_2), 5.32 (d, *J* = 8.4 Hz, 1H, NH), 5.33 -5.47 (m, 5H, ArH). ^{13}C-NMR*:* (75 MHz, $CDCl_3$) *δ* 11.5, 15.4, 24.9, 37.9, 52.1, 58.3, 66.9, 127.9, 128.1, 128.5, 136.2, 156.0, 172.5. EIMS m/z (%) 279 (2, M+•), 220 (25), 176 (36), 162 (5), 108 (20), 91 (100), 79 (8), 77 (7), 65 (6).

Synthesis of N-9-Fluorenylmethanesulfonyl-α-amino acid methyl esters (16a-g). General Procedure

Diisopropylethylamine (2.2 mmol) was added to a suspension of the α-amino acid methyl ester hydrochloride **16a-g** (1 mmol) in dry DCM (20 mL). The reaction mixture was cooled in an ice-bath and a dichloromethane solution of 9-fluorenylmethanesulfonyl chloride (**14**) (1 mmol) was then added dropwise. The mixture was left at room temperature under nitrogen atmosphere for 1-2 h and the progress of the reaction was monitored by TLC (eluent: petroleum ether/Et_2O 70:30). The final mixture was acidified to pH 1 with aqueous 1 N HCl* and extracted with DCM (3 x 10 mL). The combined organic extracts were washed twice with brine, dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure conditions. The corresponding *N*-9-Fluorenylmethanesulphonyl α-amino acid methyl esters **16a-g** were obtained in very good yields (66–84%).

*For the synthesis of *N*-9-Fluorenylmethanesulfonyl-*O*-benzyl-L-tyrosine methyl ester **(16g)** the acidic work up was performed by using 5% aqueous $NaHSO_4$.

***N*-9-Fluorenylmethanesulfonyl-L-alanine methyl ester (16a)** yellow solid (0.27 g, 78% yield); mp 132-133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (d, J = 7.2 Hz, 3H, CHCH_3),

3.54 (dd, *J* = 14.4 and 6.0 Hz, 1H, C*H*$_2$Fms), 3.60 (dd, *J* = 14.4 and 5.7 Hz, 1H, C*H*$_2$Fms), 3.75 (s, 3H, OC*H*$_3$), 4.29 (m, 1H, α-C*H*), 4,48 (t$_{app}$, *J* = 6.0 Hz, 1H, C*H*Fms), 5.15 (d, *J* = 8.1 Hz, 1H, NH), 7.32-7.46 (m, 4H, Ar*H*), 7.73-7.83 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.06, 42.53, 51.87, 52.92, 58.34, 119.94, 125.14, 127.51, 127.96, 140.73, 144.79, 173.09; anal. calcd for $C_{18}H_{19}NO_4S$: C, 62.59; H, 5.54; N, 4.06, found: C, 62.84; H, 5.52; N, 4.04.

***N*-9-Fluorenylmethanesulfonyl-D-alanine methyl ester (16b)** yellow solid (0.16 g, 66% yield); mp 130-132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (d, *J* = 7.2 Hz, 3H, CHC*H*$_3$) 3.54 (dd, J=14.6 and 6.3 Hz, 1H, C*H*$_2$Fms), 3.61 (dd, *J* = 14.6 and 5.4 Hz, 1H, C*H*$_2$Fms), 3.76 (s, 3H, OC*H*$_3$), 4.30 (m, 1H, α-C*H*), 4,48 (t$_{app}$, *J* = 5.8 Hz, 1H, C*H*Fms), 5.18 (d, *J* = 7.8 Hz, 1H, N*H*), 7.32-7.43 (m, 4H, Ar*H*), 7.72-7.85 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.92, 42.52, 51.87, 52.85, 58.30, 119.90, 125.13, 127.47, 127.91, 140.81, 144.60, 172.90; anal. calcd for $C_{18}H_{19}NO_4S$: C, 62.59; H, 5.54; N, 4.06, found: C, 62.80; H, 5.53; N, 4.04.

***N*-9-Fluorenylmethanesulfonyl-L-valine methyl ester (16c)** yellow solid (0.34 g, 77% yield); mp 158-160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (d, *J* = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$), 1.06

(d, *J* = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$), 2.22 (m, 1H, C*H*(CH$_3$)$_2$), 3.42 (dd, *J* = 14.4 and 6.6 Hz, 1H, C*H*$_2$Fms), 3.55 (dd, *J* = 14.4 and 5.4 Hz, 1H, C*H*$_2$Fms), 3.74 (s, 3H, OC*H*$_3$), 4.07 (dd, *J*= 9.6 and 4.5 Hz, 1H, α-C*H*), 4.49 (t_{app}, *J* = 6.0 Hz, 1H, C*H*Fms), 5.19 (d, *J* = 9.6 Hz, 1H, N*H*), 7.30-7.44 (m, 4H, Ar*H*), 7.71-7.86 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 17.27, 19.16, 31.56, 42.43, 52.70, 58.02, 61.41, 119.94, 125.14, 127.51, 127.96, 140.73, 144.81, 172.53; anal. calcd for $C_{20}H_{23}NO_4S$: C, 64.32; H, 6.21; N, 3.75, found: C, 64.12; H, 6.23; N, 3.70.

***N*-9-Fluorenylmethanesulfonyl-L-isoleucine methyl ester (16d)** yellow solid (0.35 g, 82% yield); mp 135-137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 0.93 (t, *J* = 7.2 Hz, 3H, C*H*$_3$CH$_2$), 1.02 (d, *J* = 6.6 Hz, 3H, C*H*$_3$CH), 1.24 (m, 1H, CH$_3$C*H*$_2$), 1.43 (m, 1H, CH$_3$C*H*$_2$), 1.95 (m, 1H, CH$_3$C*H*), 3.43 (dd, *J* = 14.4 and 6.6 Hz, 1H, C*H*$_2$Fms), 3.56 (dd, *J* = 14.4 and 5.4 Hz, 1H, C*H*$_2$Fms), 3.75 (s, 3H, OC*H*$_3$), 4.12 (dd, *J* = 9.6 and 4.8 Hz, 1H, α-C*H*), 4.50 (t_{app}, *J* = 6.0 Hz, 1H, C*H*Fms), 5.17 (d, *J* = 9.6 Hz, 1H, N*H*), 7.29-7.45 (m, 4H, Ar*H*), 7.69-7.75 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 11.54, 15.67, 24.67, 38.47, 42.47, 52.61, 58.07, 60.80, 119.93, 125.50, 127.55, 127.95, 140.78, 144.76, 172.49; anal. calcd for $C_{21}H_{25}NO_4S$: C, 65.09; H, 6.50; N, 3.61, found: C, 64.82; H, 6.51; N, 3.62.

***N*-9-Fluorenylmethanesulfonyl-L-phenylalanine methyl ester (16e)** yellow oil (0.30 g, 75% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06 (dd, *J* = 13.8 and 7.2 Hz, 1H, CH_2Ph), 3.19 (dd, *J* = 13.8 and 5.1 Hz, 1H, CH_2Ph), 3.26 (d, *J* = 5.7 Hz, 2H, CH_2Fms), 3.76 (s, 3H, OCH_3), 4.35 (t, *J* = 5.7 Hz, 1H, C*H*Fms), 4.49 (ddd, *J* = 9.0, 7.2 and 5.1 Hz, 1H, α-C*H*), 5.03 (d, *J* = 9.0 Hz, 1H, N*H*), 7.12-7.44 (m, 9H, Ar*H*), 7.59-7.77 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.56, 42.39, 52.80, 57.29, 58.17, 119.86, 125.18, 125.46, 127.42, 127.90, 128.85, 129.43, 135.20, 140.73, 144.64, 171.89; anal. calcd for $C_{24}H_{23}NO_4S$: C, 68.39; H, 5.50; N, 3.32, found: C, 68.13; H, 5.49; N, 3.33.

***N*-9-Fluorenylmethanesulfonylglycine methyl ester (16f)** yellow solid (0.44 g, 84% yield); mp 114-115°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (d, *J* = 5.7 Hz, 2H, CH_2Fms), 3.75 (s, 3H, OCH_3), 3,97 (d, *J* = 5.4 Hz, 2H, CH_2COOMe), 4.47 (t, *J* = 5.7 Hz, 1H, C*H*Fms), 5.18 (t, *J* = 5.4 Hz, 1H, NH), 7.29-7.44 (m, 4H, ArH), 7.71-7.81 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.54, 44.20, 52.82, 57.91, 119.98, 125.27, 127.54, 127.98, 140.76, 144.65, 170.25; anal. calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23, found: C, 61.85; H, 5.15; N, 4.21.

***N*-9-Fluorenylmethanesulfonyl-O-benzyl-L-tyrosine methyl**

ester (16g) yellow solid (0.26 g, 72% yield); mp 96-97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.00 (dd, J = 13.8 and 7.2 Hz, 1H, CH_2C$_6$H$_4$OBzl), 3.14 (dd, J = 13.8 and 5.1 Hz, 1H, CH_2C$_6$H$_4$OBzl), 3.28 (d, J = 5.7 Hz, 2H, CH_2Fms), 3.77 (s, 3H, OCH_3), 4.34 (t, J = 5.7 Hz, 1H, C*H*Fms), 4.46 (ddd, J = 9, 7.2 and 5.1 Hz, 1H,α-C*H*), 4.89 (s, 2H, PhCH_2O), 5.06 (d, J = 9.0 Hz, 1H, NH), 6.84 (d, J = 8.7 Hz, 2H, ArH), 7.09 (d, J = 8.7 Hz, 2H, ArH), 7.29-7.43 (m, 9H, ArH), 7.62-7.78 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.74, 42.41, 52.81, 57.47, 58.17, 69.91, 115.17, 119.86, 125.14, 125.45, 127.45, 127.89, 127.99, 128.58, 130.52, 136.76, 140.67, 146.70, 172.10; anal. calcd for $C_{31}H_{29}NO_5S$: C, 70.57; H, 5.54; N, 2.65, found: C, 70.30; H, 5.56; N, 2.66.

Synthesis of *N*-9-Fluorenylmethanesulfonyl-*N*-methyl-α-amino acid methyl esters (17a-g). General Procedure.

Aluminium chloride (1.30 mmol) was added to a solution of *N*-9-fluorenylmethanesulfonyl-α-amino acid **16a-g** (1 mmol) in dry DCM. A 0.66 M solution of diazomethane in DCM (6 mmol) was added cautiously dropwise to the reaction mixture. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred for 1-3 h monitoring the

complete conversion of the precursor by TLC analysis (petroleum ether/Et_2O, 70:30 v/v). After evaporation of the solvent under reduced pressure, aqueous 1 N HCl was added and the aqueous phase was extracted with dichloromethane (3 x 10 mL). The combined organic extracts were washed once with aqueous 1N NaOH and twice with brine. The resulting organic extracts were finally dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure conditions. The crude product was purified by flash column chromatography (Et_2O:petroleum ether) to afford the correspondent *N*-9-Fluorenylmethanesulfonyl-*N*-methyl-α-amino acid methyl esters **17a-g** in 68-73% overall yields.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-L-alanine methyl ester (17a)** white solid (0.11 mg, 70% yield); mp 126-127°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, J = 7.2 Hz, 3H, CHCH_3), 2.97 (s, 3H, NCH_3), 3.50 (dd, J = 14.7 and 6.6 Hz, 1H, CH_2Fms), 3.55 (dd, J = 14.7 and 6.0 Hz, 1H, CH_2Fms), 3.73 (s, 3H, OCH_3), 4.49 (t_{app}, J = 6.0 Hz, 1H, C*H*Fms), 4.84 (q, J = 7.2 Hz, 1H, α-C*H*), 7.32-7.45 (m, 4H, Ar*H*), 7.71-7.85 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.03, 29.98, 42.36, 52.51, 55.14, 55.39, 119.88, 125.25, 127.47, 127.86, 140.76,

145.14, 172.02; anal. calcd for $C_{19}H_{21}NO_4S$: C, 63.49; H, 5.89; N, 3.90, found: C, 63.72; H, 5.86; N, 3.88.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-D-alanine methyl ester (17b)** white solid (0.12 g, 73% yield); mp 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, J = 7.2 Hz, 3H, CHCH_3), 2.97 (s, 3H, NCH_3), 3.47-3.61 (m, 2H, CH_2Fms), 3.71 (s, 3H, OCH_3), 4.50 (t_{app}, J = 6.0 Hz, 1H, CHFms), 4.84 (m, 1H, α-CH), 7.31-7.45 (m, 4H, ArH), 7.71-7.85 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.98, 29.96, 42.35, 53.41, 55.13, 55.41, 119.85, 125.22, 127.48, 127.83, 140.74, 145.13, 171.84; anal. calcd for $C_{19}H_{21}NO_4S$: C, 63.49; H, 5.89; N, 3.90, found: C, 63.68; H, 5.88; N, 3.89.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-L-valine methyl ester (17c)** white solid (0.10 g, 72% yield); mp 127-129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (d, J = 6.6 Hz, 3H, CH(CH_3)$_2$), 1.08 (d, J = 6.6 Hz, 3H,CH (CH_3)$_2$), 2.25 (m, 1H, CH(CH$_3$)$_2$), 3.01 (s, 1H, NCH_3), 3.42 (d, J = 5.7 Hz, 2H, CH_2Fms), 3.66 (s, 3H, OCH_3), 4.24 (d, J = 10.8 Hz, 1H, α-CH), 4.49 (t, J = 5.7 Hz, 1H, CHFms), 7.34-7.45 (m, 4H, ArH), 7.75-7.84 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.17, 19.40, 27.52, 30.30, 42.25, 52.00, 54.99, 65.09, 119.93, 125.15, 127.52, 127.91, 140.72, 145.04, 171.06; anal. calcd for

$C_{21}H_{25}NO_4S$: C, 65.09; H, 6.50; N, 3.61, found: C, 64.84; H, 6.52; N, 3.62.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-L-isoleucine methyl ester (17d)** white solid (0.10 g, 69% yield); mp 134-135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (d, J = 6.6 Hz, 3H, CHCH_3), 0.97 (t, J = 7.2 Hz, 3H, CH_2CH_3), 1.24 (m, 1H, CH_2CH_3), 1.65 (m, 1H, CH_2CH_3), 2.01 (m, 1H, CHCH$_3$), 2.99 (s, 3H, NCH_3), 3.39 (d, J = 6.0 Hz, 2H, CH_2Fms), 3.66 (s, 3H, OCH_3), 4.32 (d, J = 10.5 Hz, 1H, α-CH), 4.48 (t, J = 6.0 Hz, 1H, CHFms), 7.34-7.45 (m, 4H, ArH), 7.75-7.83 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.56, 24.99, 30.40, 33.47, 42.19, 52.04, 54.81, 63.76, 65.95, 119.93, 125.34, 127.54, 127.93, 140.70, 145.04, 171.22; anal. calcd for $C_{22}H_{27}NO_4S$: C, 65.81; H, 6.78; N, 3.49, found: C, 66.02; H, 6.75; N, 3.48.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-L-phenylalanine methyl ester (17e)** white solid (0.11 g, 70% yield); mp 213-214 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (dd, J = 14.4 and 5.7 Hz, 2H, CH_2Fms), 2.98 (dd, J = 14.4 and 10.2 Hz, 1H, CH_2Ph), 2.99 (s, 3H, NCH_3), 3.17 (dd, J = 14.4 and 6.3 Hz, 1H, CH_2Fms), 3.39 (dd, J = 14.4 and 5.7 Hz, 1H, CH_2Ph), 3.75 (s, 3H, OCH_3), 4.31 (t_{app}, J = 6.0 Hz, 1H, CHFms), 5.01 (dd, J = 10.2 and 5.7 Hz, 1H, α-CH), 7.21-7.27 (m, 5H, ArH), 7.30-

7.42 (m, 4H, Ar*H*), 7.60-7.83 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.28, 35.54, 42.17, 52.57, 55.23, 61.03, 119.77, 125.39, 127.30, 127.36, 127.78, 128.75, 128.98, 136.53, 140.63, 144.91, 170.96; anal. calcd for $C_{25}H_{25}NO_4S$: C, 68.94; H, 5.79; N, 3.22, found: C, 69.18; H, 5.77; N, 3.20.

***N*-9-Fluorenylmethanesulfonyl-*N*-methylglycine methyl ester (17f)** white solid (0.050 g, 68% yield); mp 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (s, 3H, NC*H*$_3$), 3.63 (d, *J* = 6.0 Hz, 2H, C*H*$_2$Fms), 3.73 (s, 3H, OC*H*$_3$), 4.12 (s, 2H, C*H*$_2$COOMe), 4.50 (t, *J* = 6.0 Hz, 1H, C*H*Fms), 7.33-7.44 (m, 4H, ArH), 7.47-7.83 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.43, 42.42, 52.38, 55.72, 65.89, 119.89, 125.38, 127.47, 127.87, 140.78, 144.92, 169.84; anal. calcd for $C_{18}H_{19}NO_4S$: C, 62.59; H, 5.54; N, 4.06, found: C, 62.82; H, 5.51; N, 4.04.

***N*-9-Fluorenylmethanesulfonyl-*N*-methyl-O-benzyl-L-tyrosine methyl ester (17g).** ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (m, 1H, C*H*$_2$Fms), 2.93 (m, 1H, C*H*$_2$C$_6$H$_4$OBzl), 2.99 (s, 3H, NC*H*$_3$), 3.17 (dd, *J* = 14.6 and 6.1 Hz, 1H, C*H*$_2$Fms), 3.33 (dd, *J* = 14.4 and 5.5 Hz, 1H, C*H*$_2$C$_6$H$_4$OBzl), 3.76 (s, 3H, OC*H*$_3$), 4.26 (t_{app}, *J* = 6.1 Hz, 1H, C*H*Fms), 4.78 (s, 2H, OC*H*$_2$Ph), 5.01 (dd, *J* = 10.4 and 5.5 Hz, 1H, α-C*H*), 6.81 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.17 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.20-7.43

(m, 9H, Ar*H*), 7.61-7.78 (m, 4H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.68, 42.16, 52.56, 55.20, 61.15, 65.87, 69.82, 115.10, 119.79, 125.27, 125.37, 127.40, 127.77, 127.91, 128.52, 128.73, 129.99; 136.93, 140.62, 145.05, 157.92, 171.03; anal. calcd for $C_{32}H_{31}NO_5S$: C, 70.96; H, 5.77; N, 2.59, found: C, 70.69; H, 5.79; N, 2.62.

Removal of 9-Fluorenylmethanesulfonyl protecting group and synthesis of *N*-Nosyl dipeptides 20a and 20b. General procedure. Diethylamine (10 mmol) was added to a solution of **17a-b** (1 mmol) in dry DCM. The resulting mixture was stirred for about 2 h at room temperature under nitrogen atmosphere monitoring the removal of the 9-fluorenylmethanesulfonyl protecting group by TLC (diethyl ether/petroleum ether, 60:40 v/v). After evaporation of the solvent under reduced pressure, aqueous 1N HCl was added and the aqueous solution was extracted with AcOEt (3 x 10 mL). The aqueous phase was made basic (pH 8) with a saturated aqueous solution of $NaHCO_3$. The basic liquors, containing the *N*-deprotected products **18a-b**, were then treated with a solution of *N*-Nosyl-L-phenylalanine chloride (**19**) (1 mmol) in dry DCM (20 mL). The reaction mixture was stirred at room

temperature for about 2 h monitoring the formation of the dipeptides by TLC (eluent: petroleum ether/Et_2O 50:50). The organic layer was separated and the aqueous phase was extracted with three additional portions of DCM (3 x 10 mL). The combined organic extracts were washed once with 1N HCl and once with brine. The resulting organic extracts were finally dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure conditions to give a crude reaction product. The subsequent chromatographic purification (diethyl ether/petroleum ether) afforded the corresponding *N*-Nosyl-dipeptides **20a-b** in 72-75% yields.

***N*-Nosyl-L-phenylalanyl-*N*-methyl-L-alanine methyl ester (20a)** pale yellow oil; 72% yield ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (d, J = 7.2 Hz, 3H, CHCH_3), 2.82 (s, 3H, NCH_3), 2.90 (dd, J = 14.1 and 6.9 Hz, 1H, CH_2Ph), 3.05 (dd, J = 14.1 and 5.7 Hz, 1H, CH_2Ph), 3.70 (s, 3H, OCH_3), 4.52 (m, 1H, CHCH$_2$Ph), 5.01 (q, J = 7.2 Hz, 1H, CHCH$_3$), 6.25 (d, J = 9.6 Hz, 1H, NH), 7.09-7.27 (m, 5H, C_6H_5CH$_2$), 7.80 (d, J = 9.0 Hz, 2H, ArH-Ns), 8.19 (d, J = 9.0 Hz, 2H, ArH-Ns); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.03, 31.19, 39.30, 52.38, 52.67, 54.90, 124.03, 127.21, 128.06, 128.58, 129.51, 135.27, 146.08, 149.76, 170.89, 171.32; ESI-QTOF-MS: 450.1355 $(M+H)^+$, 472.1187 $(M+Na)^+$.

***N*-Nosyl-L-phenylalanyl-*N*-methyl-D-alanine methyl ester (20b)** pale yellow oil, 75% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 7.5 Hz, 3H, CHCH_3), 2.51 (s, 3H, NCH_3), 2.90-3.08 (m, 2H, CH_2Ph), 3.61 (s, 3H, OCH_3), 4.55 (m, 1H, CHCH$_2$Ph), 4.90 (q, J = 7.5 Hz, 1H, CHCH$_3$), 5.90 (d, J = 9.0 Hz, 1H, NH), 7.11-7.29 (m, 5H, C$_6H_5$CH$_2$), 7.91 (d, J = 8.7 Hz, 2H, ArH-Ns), 8,27 (d, J = 8.7 Hz, 2H, ArH-Ns); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.35, 31.05, 40.87, 52.23, 52.31, 54.48, 124.40, 127.53, 128.07, 128.66, 129.56, 134.93, 145.52, 149.79, 170.59, 171.25; ESI-QTOF-MS: 450.1356 $(M+H)^+$, 472.1177 $(M+Na)^+$.

Synthesis of amines 22a-i, 23d,*g*-i. General Procedure.

$LiAlH_4$ (5 mmol) is added to a stirred suspension of $TiCl_4$ (1 mmol) in diethyl ether. The resulting sospension was stirred at room temperature for 15 minutes. Then a solution in diethyl ether of the corresponding nitro compound (**21a-i**, 1 mmol) was added slowly to the obtained black reducing suspension under inert nitrogen atmosphere. The reaction was kept stirring at room temperature and monitored by TLC (diethyl ether/ petroleum ether 60:40 v/v). After complete conversion of the starting material, the reaction mixture was paper filtered, washed with 1 N aqueous NaOH

(3 × 5 mL) and once with brine (5 mL). The ethereal layers were dried (Na_2SO_4) and evaporated to dryness under reduced pressure conditions to give the corresponding product (**22a-i, 23d,g-i**).

4-amino-*N,N*-diethylbenzenesulfonamide (22a) 92% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.55 (d, J=9.0 Hz, 2H, Ha, Ha'), 6.66 (d, J=9,0 Hz, 2H, Hb, Hb'), 4.15 (s_{broad}, 2H, NH_2), 3.18 (q, J=6.6 Hz, 4H, NCH_2), 1.11 (t, J=6.6 Hz, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 150.2, 129.0, 128.5, 114.0, 42.0, 14.6; GC/MS m/z (% rel.): 228[M+.] (33), 213(40), 156(100), 108(33), 92(35), 65(18); Anal. Calcd for $C_{10}H_{16}N2O_2S$: C, 52.61; H, 7.06; N, 12.27; S, 14.04; Found: C, 52.70; H, 7.04; N, 11.35; S, 14.05

3-amino- *N,N*-diethylbenzenesulfonamide (22b) 89% yield, Rf=0.26; TLC (diethyl ether/ petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.24 (m, 1H, Hd); 7.13-7.08 (m, 2H, Ha, Hc); 6.81 (ddd, J'=7.9 Hz, J''=2.5 Hz, J'''=1.05 Hz, 1H, Hb); 3.98 (s_{broad}, 2H, NH_2); 3.21 (q, J= 7.2 Hz, 4H, NCH_2), 1.11 (t, J= 7.2 Hz, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 147.2, 140.8, 129.9, 118.5, 116.4, 112.8, 42.1, 14.1; GC/MS *m/z* (% rel.): 228[$M^{+\cdot}$] (25), 213(45), 156(60), 92(100), 72(50), 65(25); Anal. Calcd for $C_{10}H_{16}N_2O_2S$: C, 52.61; H, 7.06; N,

12.27; S, 14.04; Found: C, 52.72; H, 7.06; N, 11.39; S, 14.11

2-amino-*N,N*-diethylbenzenesulfonamide (22c) 90% yield, Rf=0.26, TLC (diethyl ether/ petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.56 (dd, J'=7.9 Hz, J"=1.5 Hz, 1H, H_b), 7.20 (td, J'=7.9 Hz, J"=1.5 Hz, 1H, H_c), 7.09 (td, J'=7.9 Hz, J"=1.5 Hz, 1H, H_d), 6.66 (m, 1H, H_a) 4.92 (s_{broad}, NH_2, 2H), 3.23 (q, J=7.0 Hz, 4H, NCH_2), 1.06 (t, J=7.0 Hz, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.5, 136.8, 133.7, 131.6, 129.8, 117.2, 41.3, 13.7; GC/MS *m/z* (% rel.): 228[$M^{+\cdot}$](12), 156(50), 108(25), 93(65), 92(70), 72(100), 65(30). Anal. Calcd for $C_{10}H_{16}N_2O_2S$: C, 52.61; H, 7.06; N, 12.27; S, 14.04; Found: C, 52.76; H, 7.08; N, 11.38; S, 14.06

4-fluoroaniline (22d) 98% yield, Rf=0.62, TLC (diethyl ether/ petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.87 (m, 2H, Hb, Hb'), 6.63 (dd, $J_{Ha\text{-}F}$=4.5 Hz, $J_{Ha\text{-}Hb}$=9.0 Hz, 2H, Ha, Ha'), 3.45 (s_{broad}, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.4 (d, *J* = 234 Hz), 142.3, 116.0 (d, J = 7.5 Hz), 115.6 (d, J = 23.2 Hz); GC/MS *m/z* (% rel.): 111[$M^{+\cdot}$] (100), 84(60), 83(40), 57(10). Anal. Calcd for C_6H_6FN: C, 64.85; H, 5.44; N, 12.61; Found: C, 64.96; H, 5.42; N, 11.66

4-chloroanilina (22e) 91% yield, Rf=0.65, TLC (diethyl ether/ petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ:

7.11 (d, J=8.1 Hz, 2H, Hb, Hb'), 6.61 (d, J=8.1 Hz, 2H, Ha, Ha'), 3.65 (s_{broad}, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.9, 129.1, 123.1, 116.2; GC/MS *m/z* (% rel.): 129(34), 127[$M^{+\cdot}$] (100), 100(15), 92(20), 65(25). Anal. Calcd for C_6H_6ClN: C, 56.49; H, 4.74; N, 10.98; Found: C, 56.58; H, 4.72; N, 11.35

4-bromoaniline (22f) 94% yield, Rf=0.77; TLC (diethyl ether/petroleum ether: 60:40 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.23 (d, J=8.4 Hz, 2H, Hb, Hb'), 6.56 (d, J=8.4 Hz, 2H, Ha, Ha'), 3.68 (s_{broad}, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.4, 131.9, 116.6, 110.1; GC/MS *m/z* (% rel.): 173(97), 171[$M^{+\cdot}$](100), 92(50), 65(60). Anal. Calcd for C_6H_6BrN: C, 41.89; H, 3.52; N, 8.14; Found: C, 41.96; H, 3.50; N, 8.15.

aniline (22g) 48% yield, Rf=0.64; TLC (diethyl ether/petroleum ether 60:40 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.35 (m, 2H, Hb, Hb'); 6.99 (m, 1H, Hc,); 6.81 (m, 2H, Ha, Ha'); 3.75 (s_{broad}, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 146.5, 129.3, 118.5, 115.2; GC/MS *m/z* (% rel.): 93[$M^{+\cdot}$] (100), 66(31); Anal. Calcd for C_6H_7N: C, 77.38; H, 7.58; N, 15.04; Found: C, 77.51; H, 7.56; N, 13.91

***p*-toluidine (22h)** 45% yield, Rf=0.67, TLC (diethyl ether/petroleum ether 60:40 v/v); ^1H NMR (300 MHz,

$CDCl_3$) δ: 7.02 (d, J=8.4 Hz, 2H, Hb, Hb'); 6.65 (d, , J=8.4 Hz, 2H, Ha, Ha'); 3.53 (s_{broad}, 2H, NH_2); 2.30 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 143.7, 129.5, 127.5, 115.1, 20.3; GC/MS *m/z* (% rel.): 107[$M^{+\cdot}$] (67), 106(100), 77(16). Anal. Calcd for C_7H_9N: C, 78.46; H, 8.47; N, 13.07; Found: C, 78.59; H, 8.45; N, 12.09

4-methoxyaniline (22i) 43% yield, Rf=0.62; TLC (diethyl ether/petroleum ether 60:40 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ: 6.75 (d, J=9 Hz, 2H, Hb, Hb'); 6.64 (d, J=9 Hz, 2H, Ha, Ha'); 3.73 (s, 3H, OCH_3); 3.29 (s_{broad}, 2H, NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.6, 139.8, 116.3, 114.6, 55.6; GC/MS *m/z* (% rel.): 123[$M^{+\cdot}$](73), 108(100), 80(36). Anal. Calcd for C_7H_9NO: C, 68.27;

1,2-bis(4-fluorophenyl)diazene (23d) 88% yield, Rf=0.94; TLC (diethyl ether/petroleum ether 60:40 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.92 (dd, J=5.1 Hz, J=9.0 Hz, 4H, Ha, Ha'); 7.18 (m, 4H, Hb, Hb'); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.3 (d, J = 249.7 Hz), 148.9, 124.8 (d, J=9.0 Hz), 116.0 (d, J=23.2 Hz); GC/MS: *m/z* 218[$M^{+\cdot}$](42), 123(24), 95(100). Anal. Calcd for $C_{12}H_8F_2N_2$: C, 66.05; H, 3.70; N, 12.84; Found: C, 66.16; H, 3.69; N, 11.88.

1,2-diphenyldiazene (23g) 89% yield, Rf=0.91; TLC (diethyl

ether/petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.95 (dd, J'=8.4, J''=1.5 Hz, 4H, Ha, Ha'); 7.58-7.45 (m, 6H, Hb, Hb', Hc); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 152.5, 130.9, 129.0, 122.8 ; GC/MS *m/z* (% rel.): 182[$M^{+\cdot}$](47), 152(20), 105(21), 77(100), 51(25); Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.10; H, 5.53; N, 15.37; Found: C, 79.23; H, 5.51; N, 14.22

1,2-di-*p*-tolyldiazene (23h) 92% yield, Rf=0.93; TLC (diethyl ether/petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.83 (d, J=8.4 Hz, 4H, Ha, Ha'); 7.32 (d, J=8.4 Hz, 4H, Hb, Hb'); 2.45 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 150.8, 141.2, 129.7, 122.7, 21.5; GC/MS *m/z (% rel.):* 210[$M^{+\cdot}$] (55), 119(16), 91(100), 65(20). Anal. Calcd for $C_{14}H_{14}N_2$: C, 79.97; H, 6.71; N, 13.32; Found: C, 80.10; H, 6.69 N, 12.32

1,2-bis(4-methoxyphenyl)diazene (23i) 20% yield, Rf=0.85; TLC (diethyl ether/petroleum ether 60:40 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90 (d, J=9.0 Hz, 4H, Ha, Ha'); 7.00 (d, J=9.0 Hz, 4H, Hb, Hb'); 3.85 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.5, 147.0, 124.3, 114.1, 55.5; GC/MS *m/z (% rel.):* 242[$M^{+\cdot}$] (90), 135(36), 107(100), 92(27), 77(40), 64(13). Anal. Calcd for $C_{14}H_{14}N_2O_2$: C, 69.41; H, 5.82; N, 11.56;

Found: C, 69.52; H, 5.82; N, 11.57

Synthesis of amides (24-40) and dipeptide methyl ester (41-42). General Procedure.

Amine (1 mmol), silver acetate (1 mmol) and sodium acetate (2 mmol) were added to a solution of acyl chloride (1 mmol) in diethyl ether (10 mL). The heterogeneous mixture was magnetically stirred at room temperature for 15 minutes, preserving the reaction flask from exposure to light. After this time, a white precipitate was formed and TLC (diethyl ether/ P. E. 70:30 v/v) showed the complete conversion of the starting chloride. The mixture was paper filtered, washed with 1 N aqueous HCl (3 × 5 mL), 1 N aqueous NaOH (3 × 5 mL) and once with brine (5 mL). The ethereal layers were dried (Na_2SO_4) and evaporated to dryness under reduced pressure conditions.

***N,N*-diethylbenzamide** (**24**) viscous colorless oil without need for chromatography. Yield: 92%; TLC: R_f = 0.77; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31-7.40 (m, 5H, ArH), 3.52 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 1.25 (m, 3H, CH_3) 1.10 (m, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.3, 137.2, 129.1, 128.4, 126.2, 43.3, 39.2, 14.2, 12.9. MS (EI, 70 eV) m/z (% rel.): 177

[$M^{+\cdot}$] (), 176 (49), 162 (8), 148 (16), 134 (12), 105 (100), 77 (48), 51 (21). Anal. Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.65; H, 8.51; N, 7.88.

***N*-ethyl-*N*-isopropylbenzamide** (**25**) viscous colorless oil, 82%, TLC (eluent: diethyl ether/P.E. 70/30 v/v): R_f = 0.71; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.26-7.42 (m, 5H, ArH), 3.92 (m, 1H, NCH), 3.40 (m, 2H, CH_2), 1.05-1.34 (m, 9H, $CHC\underline{H}_3$ and $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.0, 132.4, 129.7, 128.8, 125.8, 50.1, 39.4, 21.0, 14.6; Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.90. Found: C, 75.48; H, 8.94; N, 7.31.

***N*-benzylbenzamide** (**26**): viscous colorless oil, 87%, TLC (eluent: diethyl ether/P.E. 70/30 v/v): R_f = 0.59; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.81 (m, 2H, ArH), 7.20-7.52 (m, 8H, ArH), 6.05 (bs, 1H, NH), 4.42 (d, J = 6.9 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.4, 138.2, 134.4, 131.6, 128.9, 128.7, 127.6, 127.3, 126.4, 44.1. Anal. Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.50; H, 6.20; N, 6.61.

***N*-phenethylbenzamide** (**27**): viscous colorless oil, 89%, TLC (eluent: diethyl ether/P.E. 70/30 v/v): R_f = 0.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.71 (m, 2H, ArH), 7.20-7.50 (m, 10H, ArH), 6.23 (bs, 1H, NH), 3.74 (q, J = 5.8 Hz, 2H, $NHC\underline{H}_2$), 2.94

(t, *J* = 5.8 Hz, 2H, CH_2Ph) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 167.5, 138.9, 134.6, 131.4, 128.8, 128.7, 128.5, 126.6, 41.7, 35.7; MS (EI, 70 eV) *m/z* (% rel.): 225 [$M^{+\cdot}$] (35), 105 (100), 77 (40). Anal. Calcd for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22. Found: C, 79.78; H, 6.73; N, 6.20.

***N*-benzoyl piperidine** (**28**): viscous colorless oil, 81%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.50; ^1H NMR (300 MHz, $CDCl_3$) *δ*: 7.24 (m, 5H, ArH), 3.58 (m, 2H, NCH_2), 3.20 (m, 2H, NCH_2), 1.61 (m, 4H, NCH2C$\underline{H_2}$), 1.35 (m, 2H, $NCH_2CH_2C\underline{H_2}$); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 169.5, 135.8, 127.5, 126.4, 48.0, 42.3, 25.8, 25.0, 23.9; MS (EI, 70 eV) *m/z* (% rel.): 189 [$M^{+\cdot}$] (35), 188 (100), 105 (75), 77 (41). Anal. Calcd for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.29; H, 8.01; N, 7.41.

***N,N*-diethyl-2-phenylacetamide** (**29**) viscous colorless oil, 93%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.68; ^1H NMR (300 MHz, $CDCl_3$) *δ*: 6.89-7.05 (m, 5H, ArH), 3.10 (q, *J* = 5.9 Hz, 2H, C$\underline{H_2}CH_3$), 2.99 (q, *J* = 5.9 Hz, 2H, C$\underline{H_2}CH_3$), 0.83 (t, *J* = 5.9 Hz, 3H, CH_3), 0.78 (t, *J* = 5.9 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 169.1, 134.8, 128.6, 128.4, 125.7, 41.5, 40.0, 39.2, 14.4, 13.3; Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.44; H, 8.94; N, 7.34.

***N,N*-diethylpalmitamide** (**30**) viscous colorless oil, 88%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.68; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.30 (t, *J* = 7.0 Hz, 2H, NCH_2), 3.24 (t, *J* = 7.0 Hz, 2H, NCH_2), 2.20 (t, *J* = 6.9 Hz, 2H, $COCH_2$), 1.58 (m, 2H, CH_2), 1.20 (m, 24H, CH_2), 1.10 (t, *J* = 7.0 Hz, 3H, $NCH_2C\underline{H}_3$), 1.03 (t, *J* = 7.0 Hz, 3H, $NCH_2C\underline{H}_3$), 0.81 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2, 41.8, 39.9, 33.0, 31.8, 29.5, 29.4, 29.3, 29.2, 25.4, 22.5, 14.2, 14.0, 13.0; MS (EI, 70 eV) *m/z* (% rel.): 311 [$M^{+\cdot}$] (12), 128 (25), 115 (100), 100 (28), 72 (16). Anal. Calcd for $C_{20}H_{41}NO$: C, 77.10; H, 13.26; N, 4.50. Found: C, 77.18; H, 13.29; N, 4.48.

***N,N*-diethylcinnamamide** (**31**) viscous colorless oil, 90%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 15.3 Hz, 1H, CH vinyl), 7.55 (m, 2H, ArH), 7.39 (m. 3H, ArH), 6.85 (d, J = 15.3 Hz, 1H, CH vinyl), 3.40-3.50 (m, 4H, CH_2), 1.25 (m, 6H, CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.7, 142.3, 135.5, 129.4, 128.7, 127.7, 117.7, 42.3, 41.1, 15.1, 13.2 ppm; MS (EI, 70 eV) *m/z* (% rel.): 203 [$M^{+\cdot}$] (31), 131 (100), 103 (40), 77 (23). Anal. Calcd for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.76; H, 8.41; N, 6.90.

***N,N*-diethyl-3-phenylbenzamide** (**32**) viscous colorless oil, 79%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.75; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.23-7.45 (m, 9H, ArH), 3.73 (m, 1H, CH_2), 2.91 (m, 2H, CH_2), 2.60 (m, 1H, CH_2), 0.84 (t, J = 6.8 Hz, 3H, CH_3), 0.67 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.7, 139.2, 138.2, 135.7, 128.9, 128.6, 128.5, 128.3, 127.4, 126.8, 42.2, 38.3, 13.1, 11.7; MS (EI, 70 eV) *m/z* (% rel.): 253 [$M^{+\cdot}$] (37), 252 (75), 181 (100), 152 (51), 77 (38). Anal. Calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 80.72; H, 7.58; N, 5.54.

***N,N*-diethylpyrazine-2-carboxamide** (**33**) viscous colorless oil, 75%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.39; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.82 (m, 1H, ArH), 8.68 (m, 1H, ArH), 8.51 (m, 1H, ArH), 3.52 (q, J = 6.5 Hz, 2H, CH_2), 3.32 (q, J = 6.5 Hz, 2H, CH_2), 1.21 (t, J = 6.5 Hz, 3H, CH_3), 1.08 (t, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.1, 150.1, 144.6, 144.4, 142.7, 43.3, 40.5, 14.1, 12.5. Anal. Calcd for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45. Found: C, 60.40; H, 7.29; N, 23.51.

Amide 34 Pale yellow oil, 87%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.72; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32 (d, J = 8.1 Hz, 2H, ArH), 8.05 (d, J = 8.1

Hz, 2H, ArH), 6.55 (d, *J* = 9.0 Hz, 1H, NH), 4.28 (m, 1H, NCH), 3.20 (m, 4H, $C\underline{H}_2CH_3$), 1.30 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H}_3$), 0.98 (m, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8, 149.9, 146.4, 128.1, 124.3, 49.1, 42.3, 40.7, 20.4, 14.3, 13.6; MS (EI, 70 eV) *m/z* (% rel.): 329 [$M^{+\cdot}$] (65), 229 (69), 100 (95), 72 (100). Anal. Calcd for $C_{13}H_{19}N_3O_5S$: C, 47.41; H, 5.81; N, 12.76; S, 9.74. Found: C, 47.55; H, 5.82; N, 12.80; S, 9.76.

Amide 35 Pale yellow oil, 85%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.61; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.28 (d, *J* = 8.1 Hz, 2H, ArH), 8.02 (d, *J* = 8.1 Hz, 2H, ArH), 6.27 (d, *J* = 9.0 Hz, 1H, NH), 3.92 (dd, *J* = 9.0, 5.4 Hz, 1H, NCH), 2.95-3.26 (m, 4H, $C\underline{H}_2CH_3$), 1.87 (m, 1H, $C\underline{H}CH_3$), 0.99 (m, 6H, CH_3), 0.86 (m, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.1, 149.9, 146.0, 127.7, 123.2, 58.2, 41.7, 40.3, 31.5, 19.7, 16.5, 14.1, 12.5. Anal. Calcd for $C_{15}H_{23}N_3O_5S$: C, 50.41; H, 6.49; N, 11.76; S, 8.97. Found: C, 50.49; H, 6.47; N, 11.72; S, 8.99.

Amide 36 Pale yellow oil, 84%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.60; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 8.1 Hz, 2H, ArH), 8.01 (d, *J* = 8.1 Hz, 2H, ArH), 6.07 (bs, 1H, NH), 4.12 (m, 1H, NCH), 3.10 (m, 4H, $NC\underline{H}_2CH_3$), 1.98 (m, 1H, $C\underline{H}_2CH$), 1.72 (m, 1H, $C\underline{H}_2CH$),

1.55 (m, 1H, $CH_2C\underline{H}$), 1.10 (t, *J* = 6.9 Hz, 3H, $NCH_2C\underline{H_3}$), 0.98 (m, 6H, CH_3), 0.85 (t, *J* = 6.9 Hz, 3H, $NCH_2C\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 169.9, 149.9, 145.9, 128.5, 124.0, 52.0, 42.9, 40.6, 23.4, 20.9, 14.2, 12.6; MS (EI, 70 eV) *m/z* (% rel.): 371 [$M^{+\cdot}$] (100), 215 (45), 186 (28), 100 (30), 72 (25).

Anal. Calcd for $C_{16}H_{25}N_3O_5S$: C, 51.74; H, 6.78; N, 11.31; S, 8.63. Found: C, 51.69; H, 6.80; N, 11.33; S, 8.61.

Amide 37 Pale yellow oil, 81%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.77; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 8.32 (d, *J* = 8.1 Hz, 2H, ArH), 8.02 (d, *J* = 8.1 Hz, 2H, ArH), 5.99 (d, 1H, *J* = 9.0 Hz, NH), 3.96 (m, 1H, NCH), 2.97-3.28 (m, 4H, $NC\underline{H_2}CH_3$), 1.61 (m, 1H, $C\underline{H}CH_3$), 1.46 (m, 1H, $CHC\underline{H_2}CH_3$), 1.22 (m, 1H, $CHC\underline{H_2}CH_3$), 1.05 (m, 6H, CH_3), 0.95 (m, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 169.0, 149.9, 146.6, 129.5, 124.0, 59.0, 41.6, 40.3, 38.3, 23.1, 16.1, 14.1, 12.5, 11.5; MS (EI, 70 eV) *m/z* (% rel.): 371 [$M^{+\cdot}$] (100), 215 (39), 156 (36), 100 (34), 72 (35). Anal. Calcd for $C_{16}H_{25}N_3O_5S$: C, 51.74; H, 6.78; N, 11.31; S, 8.63. Found: C, 51.72; H, 6.76; N, 11.28; S, 8.65.

Amide 38 Pale yellow oil, 92%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 8.21 (d, *J* = 8.1 Hz, 2H, ArH), 7.90 (d, *J* = 9.1

Hz, 1H, NH), 7.75 (d, *J* = 8.1 Hz, 2H, ArH), 7.20 (m, 2H, ArH), 7.10 (m, 3H, ArH), 4.45 (m, 1H, NCH), 3.27-3.40 (m, 1H, CH_2Ph), 2.88-3.09 (m, 5H, CH_2Ph and $C\underline{H}_2CH_3$), 0.90 (m, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.5, 149.7, 146.6, 135.5, 129.5, 128.6, 128.1, 127.2, 123.9, 54.5, 41.7, 40.6, 29.6, 13.1, 12.5; MS (EI, 70 eV) *m/z* (% rel.): 405 [$M^{+\cdot}$] (2), 314 (51), 305 (100), 203 (44), 100 (55), 72 (61). Anal. Calcd for $C_{19}H_{23}N_3O_5S$: C, 56.28; H, 5.72; N, 10.36; S, 7.91. Found: C, 56.43; H, 5.74; N, 10.33; S, 7.88.

Amide 39 Pale yellow oil, 82%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.69; ^1H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.0 Hz, 2H, ArH), 7.75 (d, *J* = 8.0 Hz, 2H, ArH), 6.95-7.40 (m, 10H, ArH and $NHSO_2$), 6.39 (bs, 1H, NHCO), 4.89 (quintet, *J* = 5.3 Hz, 1H, $C\underline{H}CH_3$), 4.04 (q, *J* = 5.8 Hz, 1H, $C\underline{H}CH_2$), 2.77-2.98 (m, 2H, CH_2Ph), 1.39 (d, *J* = 5.3 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.4, 148.9, 145.3, 135.5, 129.3, 128.7, 128.4, 128.3, 127.5, 127.4, 125.8, 125.6, 123.6, 52.0, 48.7, 24.7, 21.7; ESI(+)-MS: expected for $C_{23}H_{24}N_3O_5S$ [M+H]+ 454.1437; Found: 454.1430. Anal. Calcd for $C_{23}H_{23}N_3O_5S$: C, 60.91; H, 5.11; N, 9.27; S, 7.07. Found: C, 60.88; H, 5.13; N, 9.25; S, 7.08.

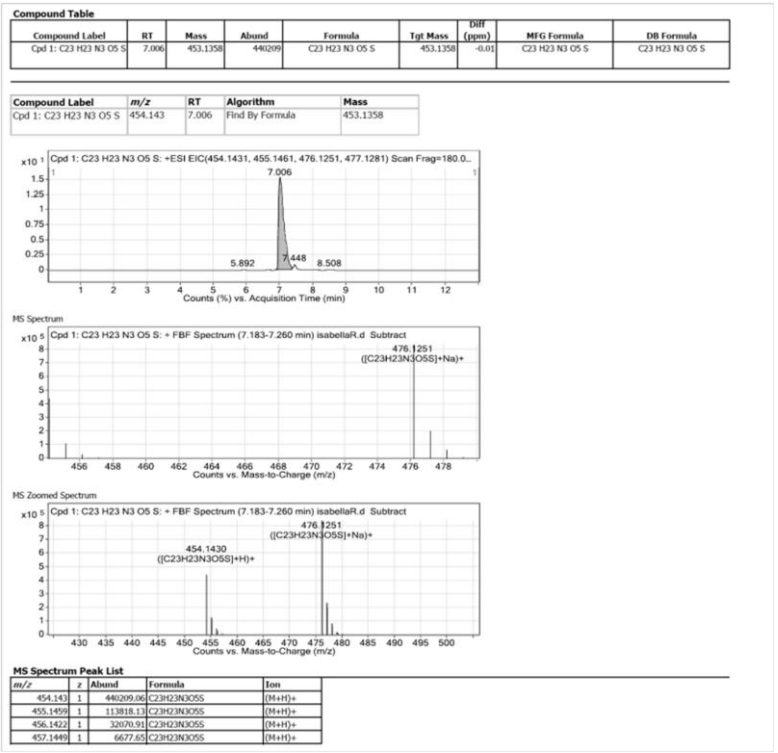
Amide 40 Pale yellow oil, 86%, TLC (eluent: chloroform/methanol 90/10 v/v): R_f = 0.68; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.0 Hz, 2H, ArH), 7.75 (d, *J* = 8.0 Hz, 2H, ArH), 6.10 (d, *J* = 8.7 Hz, 1H, $NHSO_2$), 5.99 (d, *J* = 7.0 Hz, 1H, NHCO), 4.81 (quintet, *J* = 7.0 Hz, 1H, $CHCH_3$), 3.92 (m, 1H, $CHCH_2$), 2.98 (m, 2H, CH_2Ph), 1.24 (d, *J* = 5.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.6, 149.7, 144.9, 135.5, 129.3, 129.2, 128.9, 128.6, 128.0, 127.7, 127.4, 125.8, 124.1, 58.6, 49.3, 39.5, 21.7; ESI(+)-MS: expected for $C_{23}H_{24}N_3O_5S$ $[M+H]^+$ 454.1437; Found: 454.1431. Anal. Calcd for $C_{23}H_{23}N_3O_5S$: C, 60.91; H, 5.11; N, 9.27; S, 7.07. Found: C, 61.07; H, 5.10; N, 9.29; S, 7.08.

Dipeptide methyl ester 41 Pale yellow foam, 85%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.72; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 8.1 Hz, 2H, ArH), 8.05 (d, *J* = 8.1 Hz, 2H, ArH), 6.30 (d, *J* = 9.1 Hz, 1H, $NHSO_2$), 5.53 (d, *J* = 8.8 Hz, 1H, NHCO), 4.40 (m, 1H, $NC\underline{H}CH_3$), 3.95 (m, 1H, $NC\underline{H}CH$), 3.71 (s, 3H, OCH_3), 1.66 (m, 1H, $NCHC\underline{H}$), 1.38 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H_3}$), 1.10-1.40 (m, 1H, $C\underline{H_2}CH_3$), 1.05 (m, 1H, $C\underline{H_2}CH_3$), 0.85 (t, *J* = 7.3 Hz, 3H, $CH_2C\underline{H_3}$), 0.70 (d, *J* = 6.9 Hz, 3H, $CHC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.8, 172.1, 149.9, 146.6, 128.4, 124.3, 56.7, 52.5, 50.4, 37.8, 25.1, 20.1,

15.2, 11.5. Anal. Calcd for $C_{16}H_{23}N_3O_7S$: C, 47.87; H, 5.77; N, 10.47; S, 7.99. Found: C, 48.16; H, 5.78; N, 10.44; S, 8.00.

Dipeptide methyl ester 42 Pale yellow foam, 80%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.53; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.35 (d, J = 8.1 Hz, 2H, ArH), 8.05 (d, J = 8.1 Hz, 2H, ArH), 6.05 (d, J = 7.9 Hz, 1H, $NHSO_2$), 4.74 (d, J = 8.1 Hz, 1H, C<u>H</u>NCH_3), 4.32 (m, 1H, C<u>H</u>NH), 3.69 (s, 3H, OCH_3), 2.90 (s, 3H, NCH_3), 1.85 (m, 1H, CHC<u>H</u>CH_3), 1.35 (d, J = 6.5 Hz, 3H, NHCHC<u>H</u>$_3$), 0.95 (m, 2H,C<u>H</u>$_2$$CH_3$), 0.85 (d, J = 6.5 Hz, 3H, CH_2C<u>H</u>$_3$), 0.78 (t, J = 6.4 Hz, 3H, CHC<u>H</u>$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.3, 172.0, 146.8, 128.3, 124.3, 124.1, 51.9, 49.5, 33.2, 31.0, 25.0, 20.8, 19.8, 15.5, 10.7. Anal. Calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; S, 7.72. Found: C, 49.08; H, 6.05; N, 10.09; S, 7.69.

Ion current chromatogram (*m/z* 454, $[M+H]^+$) and ESI(+)-MS spectrum of compound 41.



Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C23 H23 N3 O5 S	7.006	453.1358	440209	C23 H23 N3 O5 S	453.1358	-0.01	C23 H23 N3 O5 S	C23 H23 N3 O5 S

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C23 H23 N3 O5 S	454.143	7.006	Find By Formula	453.1358

MS Spectrum Peak List

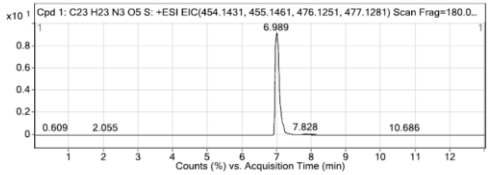
m/z	z	Abund	Formula	Ion
454.143	1	440209.06	C23H23N3O5S	(M+H)+
455.1459	1	113818.13	C23H23N3O5S	(M+H)+
456.1422	1	32670.91	C23H23N3O5S	(M+H)+
457.1449	1	6677.65	C23H23N3O5S	(M+H)+

Ion current chromatogram (*m/z* 454, $[M+H]^+$) and ESI(+)-MS spectrum of compound 42

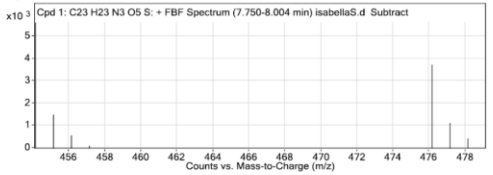
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C23 H23 N3 O5 S	7.828	453.1347	5613	C23 H23 N3 O5 S	453.1358	-2.46	C23 H23 N3 O5 S	C23 H23 N3 O5 S

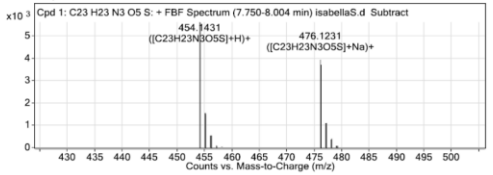
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C23 H23 N3 O5 S	454.1431	7.828	Find By Formula	453.1347



MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
454.1431	1	5613.25	C23H23N3O5S	(M+H)+
455.1461	1	1492.72	C23H23N3O5S	(M+H)+
456.1431	1	566.44	C23H23N3O5S	(M+H)+
457.1372	1	124.24	C23H23N3O5S	(M+H)+

General procedure for one pot synthesis of amides 41-57

1 mmol of carboxylic acid is added to 1 mmol of amine and 3 mmol of triethylamine (Et_3N) in dichloromethane, then 1 mmol of $SOCl_2$ is added at room temperature. The mixture is stirred for 5-20 minutes at room temperature. The recovery of the reaction product is performed by evaporating the solvent under reduced pressure. The resulting residue is taken up in dichloromethane and washed first with 1N HCl* and then with 1N NaOH. The organic phase was dried (Na_2SO_4), and evaporated to dryness to afford the corresponding carboxylic amide.

*For the synthesis of *N,N*-diethyl-(S)-2-(*tert*-butoxycarbonyl)-amino-3-phenylpropanamide (**55**) the acidic work up was performed by using 5% aqueous $NaHSO_4$.

***N,N* diethylbenzamide (41)** Viscous red oil, 86%, R_f = 0.77, TLC (eluent: diethyl ether/P.E. 70:30 v/v): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43-7.34 (m, 5H, ArH), 3.63-3.43 (m, 2H, NCH_2), 3.38-3.18 (m, 2H, NCH_2), 1.32-1.18 (m, 3H, $CH_2C\underline{H}_3$) 1.17-1.02 (m, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.3, 137.2, 129.1, 128.4, 126.2, 43.3, 39.2, 14.2, 12.9. MS (EI, 70 eV) m/z (% rel.): 177 [$M^{+\cdot}$] (20), 176 (49), 162 (8), 148

(16), 105 (100), 77 (48), 51 (21). Anal. Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.65; H, 8.51; N, 7.88.

***N,N*-diethylpyrazine-2-carboxamide (42)** Viscous colorless oil, 88%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.39; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83-8.78 (m, 1H, ArH), 8.56-8.50 (m, 1H, ArH), 8.49-8.43 (m, 1H, ArH), 3.49 (q, J = 6.5 Hz, 2H, NCH_2), 3.31 (q, J = 6.5 Hz, 2H, NCH_2), 1.19 (t, J = 6.5 Hz, 3H, $CH_2C\underline{H}_3$), 1.08 (t, J = 6.5 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.1, 150.2, 144.9, 144.7, 142.5, 43.2, 40.4, 14.2, 12.6. GC/MS: m/z 179[$M^{+\cdot}$](5), 107(30), 79(35), 72(100). Anal. Calcd for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45. Found: C, 60.40; H, 7.29; N, 23.51

***N,N*-diethyl-3-phenylbenzamide (43)** Viscous colorless oil, 91%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.75; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50-7.23 (m, 9H, ArH), 3.85-3.70 (m, 1H, NCH_2), 3.10-2.89 (m, 2H, NCH_2), 2.71-2.52 (m, 1H, NCH_2), 0.89 (t, J = 6.8 Hz, 3H, $CH_2C\underline{H}_3$), 0.74 (t, J = 6.8 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.7, 139.7, 138.4, 136.1, 128.9, 128.6, 128.5, 128.3, 127.5, 126.8, 42.3, 38.4, 13.3, 11.9; MS (EI, 70 eV) m/z (% rel.): 253 [$M^{+\cdot}$] (37), 252 (75), 181 (100), 152 (51), 77 (38). Anal. Calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 80.72; H, 7.58; N, 5.54.

***N,N*-diethylcinnamamide (44)** Viscous colorless oil, 86%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.73 (d, J = 15.3 Hz, 1H, CH vinyl), 7.61-7.50 (m, 2H, ArH), 7.40-7.34 (m. 3H, ArH), 6.84 (d, J = 15.3 Hz, 1H, CH vinyl), 3.60-3.40 (m, 4H, NCH_2), 1.33-1.15 (m, 6H, $CH_2C\underline{H}_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.7, 142.3, 135.5, 129.4, 128.7, 127.7, 117.7, 42.3, 41.1, 15.1, 13.2 ppm; MS (EI, 70 eV) m/z (% rel.): 203 [$M^{+\cdot}$] (31), 188 (20), 131 (100), 103 (40), 77 (23). Anal. Calcd for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.76; H, 8.41; N, 6.90.

***N,N*-diethylpalmitamide (45)** Viscous colorless oil, 90%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.68; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.42-3.20 (m, 4H, NCH_2), 2.40-2.30 (t, J = 7.2 Hz, 2H, CH_2CO), 1.67-1.50 (m, 2H, $C\underline{H}_2CH_2CO$), 1.34-1.10 (m, 30H, $CH_3(C\underline{H}_2)_{12}CH_2CH_2CO$ and $(C\underline{H}_3CH_2)_2N$), 0.81 (t, J = 6.6 Hz, 3H, $(CH_2)_{14}C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 34.2, 32.5, 31.8, 29.6, 29.4, 29.3, 29.2, 25.7, 24.8, 22.6, 14.0; MS (EI, 70 eV) m/z (% rel.): 311 [$M^{+\cdot}$] (12), 128 (25), 115 (100), 100 (28), 72 (16). Anal. Calcd for $C_{20}H_{41}NO$: C, 77.10; H, 13.26; N, 4.50. Found: C, 77.18; H, 13.29; N, 4.48.

***N,N*-diethylmyristamide (46)** Viscous colorless oil, 88%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.67, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.38 (q, J = 7.2 Hz, 2H, NCH_2), 3.31 (q, J = 7.2 Hz, 2H, NCH_2), 2,29 (t, J=7.2 Hz, 2H, CH_2CO), 1.70-1.59 (m, 4H, $(C\underline{H}_2)_2CH_2CO$), 1.38-1.23 (m, 18H, $CH_3(C\underline{H}_2)_9(CH_2)_2CH_2CO$), 1.18 (t, J = 7.2 Hz, 3H, $CH_2C\underline{H}_3$), 1.12 (t, J = 7.1 Hz, 3H, $NCH_2C\underline{H}_3$), 0.89 (t, J = 6.6 Hz, 3H, $C\underline{H}_3(CH_2)_{12}CO$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2, 41.9, 39.9, 33.1, 31.9, 29.6, 29.5, 29.4, 29.3, 25.5, 22.6, 14.4, 14.1, 13.1; MS (EI, 70 eV) m/z (% rel.): 283 [$M^{+\cdot}$] (12), 128 (25), 115 (100), 100 (28), 72 (16). Anal. Calcd for $C_{18}H_{37}NO$: C, 76.26; H, 13.16; N, 4.94. Found: C, 76.37; H, 13.27; N, 4.95

***N*-ethyl-*N*-isopropylbenzamide (47)** Viscous colorless oil, 86%, TLC (eluent: diethyl ether/P.E. 70/30 v/v): R_f = 0.71; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50-7.20 (m, 5H, ArH), 4.10-3.80 (m, 1H, NCH), 3.52-3.25 (m, 2H, NCH_2), 1.39-0.95 (m, 9H, $CH(C\underline{H}_3)_2$ and $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.1, 129.9, 128.9, 128.4, 125.9, 50.2, 35.2, 29.6, 21.1, 14.8; m/z (% rel.): [$M^{+\cdot}$] 191 (22), 190 (25), 176 (5), 162 (10), 148 (5), 105 (100), 77 (35). Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.48; H, 8.94; N, 7.31.

***N*-benzoylpiperidine (48)** Viscous colorless oil, 88%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.50; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.29-7.15 (m, 5H, ArH), 3.65-3.45 (m, 2H, NCH_2), 3.28-3.10 (m, 2H, NCH_2), 1.58-1.27 (m, 6H, $NCH_2(C\underline{H}_2)_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1, 136,3, 129.2, 128.2, 126.6, 48.5, 42.9, 26.3, 25.5, 24.4; MS (EI, 70 eV) m/z (% rel.): 189 [$M^{+\cdot}$] (35), 188 (100), 105 (75), 77 (41). Anal. Calcd for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.29; H, 8.01; N, 7.41.

***N*-benzyl-*N*-ethylbenzamide (49)** Viscous colorless oil, 85%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f =0.59; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.60-6.90 (m, 10H, ArH), 4.90-4.42 (m, 2H, NCH_2Ph), 3.78-3.05 (m, 2H, NCH_2), 1.38-1.02 (m, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ:170.8, 133.4, 130.1, 129.8, 128.9, 128.5, 128.3, 127.6, 126.6, 53.9, 42.9, 13.9; MS (EI, 70 eV) m/z (% rel.): 239 [$M^{+\cdot}$] (55), 238 (40), 210 (10), 148 (5), 134 (6), 105 (100), 91 (20), 77 (35). Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.41; H, 7.17 ; N, 5.86.

***N*-propylbenzamide (50)** Viscous colorless oil, 92%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f = 0.38 ,^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.95-7.70 (m, 2H, ArH), 7.51-7.28 (m,

3H, ArH), 3.36 (t, J=7.2 Hz, 2H, NHC$\underline{H}_2$), 1.72-1.54- (m, 2H, NHCH$_2$C$\underline{H}_2$), 0.90 (t, J=7.2, Hz, 3H, C$\underline{H}_3$CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.9, 131.9, 129.9, 128.5, 127.4, 42.3, 22.5, 11.3; MS (EI, 70 eV) m/z (% rel.): 163 [$M^{+\cdot}$] (40), 148 (5), 134 (10), 105 (100), 77 (40). Anal. Calcd for $C_{10}H_{13}NO$: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.69; H, 8.04; N, 8.59

***N*-phenylbenzamide (51)** Viscous colorless oil, 89%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): R_f =0.58 , ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.35-7.05 (m, 11H, ArH and NH), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.9, 137.9, 134.9, 130.1, 129.0, 128.7, 128.1, 124.5, 120.3 MS (EI, 70 eV) m/z (% rel.): 197 [$M^{+\cdot}$] (60), 105 (100), 77 (50). Anal. Calcd for $C_{13}H_{11}NO$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.27; H, 5.63; N, 7.11

***N,N*-diethyl-(S)-2-(4-nitrophenylsulfonamido)-3-phenylpropanamide (52)** Pale yellow oil, 81%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.22 (d, J = 9.0 Hz, 2H,), 7.88 (d, J = 9.0 Hz, 2H, ArH), 7.25-7.15 (m, 3H, ArH), 7.14-7.04 (m, 2H, ArH), 6.24 (d, J =8.1 Hz, 1H, NH), 4.48-4.32 (m, 1H, CHCO), 3.39-3.23 (m, 1H, CH_2Ph), 3.09-2.83 (m, 5H, CH_2Ph and NCH_2), 0.99-0.80 (m, 6H, CH$_2$C$\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.3, 149.8, 146.3, 135.4, 129.5, 128.6, 128.2, 127.3,

123.9, 54.5, 41.5, 40.7, 13.9, 12.5; MS (EI, 70 eV) m/z (% rel.): 405 [$M^{+\cdot}$] (2), 314 (51), 305 (100), 203 (44), 100 (55), 72 (61). Anal. Calcd for $C_{19}H_{23}N_3O_5S$: C, 56.28; H, 5.72; N, 10.36; S, 7.91. Found: C, 56.43; H, 5.74; N, 10.33; S, 7.88.

***N,N*-diethyl-(S)-2-(4-nitrophenylsulfonamido)-propanamide (53)** Pale yellow oil, 83%, TLC (eluent: chloroform/methanol 90:10 v/v): R_f = 0.72; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.32 (d, J = 8.1 Hz, 2H, ArH), 8.04 (d, J = 8.1 Hz, 2H, ArH), 6.24 (d, J = 8.7 Hz, 1H, NH), 4.32-4.18 (m, 1H, CHCO), 3.30-3.05 (m, 4H, NCH_2), 1.33 (d, J = 6.9 Hz, 3H, $CHC\underline{H}_3$), 1.09 (t, J = Hz, 3H, $CH_2C\underline{H}_3$), 0.92 (t, J = Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1, 149.9, 146.3, 128.4, 124.1, 49.2, 41.6, 40.6, 20.5, 14.3, 12.6; MS (EI, 70 eV) m/z (% rel.): 229 (69), 186 (15), 122 (10), 100 (95), 72 (100). Anal. Calcd for $C_{13}H_{19}N_3O_5S$: C, 47.41; H, 5.81; N, 12.76; S, 9.74. Found: C, 47.55; H, 5.82; N, 12.80; S, 9.76.

4-acetamidophenylacetate (54) Viscous colorless oil, 48%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): Rf= 0.88; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.91 (s, 1H, NH), 7.55 (d, J = 9.0 Hz, 2H, ArH), 7.01 (d, J = 9.0 Hz, 2H, ArH), 2.29 (s, 3H, CH_3CO), 2.17 (s, 3H, CH_3CO), ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 168.6, 147.0, 135.9, 121.8, 120.8, 24.4, 21.1; MS (EI, 70 eV) m/z (%

rel.): 193 [$M^{+\cdot}$] (10), 151(55), 109 (100), 80 (10). Anal. Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.26; H, 5.75; N, 7.26

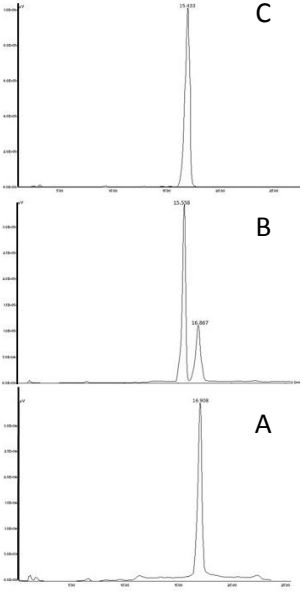
***N,N*-diethyl-(S)-2-(*tert*-butoxycarbonyl)-amino-3-phenylpropanamide (55)** Pale yellow oil, 78%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): Rf= 0.81; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.26-7.10 (m, 5H, ArH), 5.39 (d, J=9 Hz, 1H, NH), 4.74-4.64 (m, 1H, CHCO), 3.53-3.41 (m, 1H $\underline{CH_2}CH_3$); 3.11-2.80 (m, 5H CH_2Ph, $\underline{CH_2}CH_3$), 1.37 (s, 9H, tBu), 0.99 (t, J=7.2 Hz, 3H, $\underline{CH_3}CH_2N$), 0.92 (t, J=7.2 Hz, 3H, $\underline{CH_3}CH_2N$), ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 154.9, 136.5, 129.5, 128.3, 126.7, 79.5, 51.3, 41.5, 40.4, 28.2, 14.0; MS (EI, 70 eV) *m/z* (% rel.): 320 [$M^{+\cdot}$] (2), 229(6), 220(20), 164(45), 129(40), 120(100), 100(35), 91(20), 72(40), 57(70). Anal. Calcd for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.51; H, 8.87; N, 8.75

***N*-((R)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (56)** Pale yellow oil, 75%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): Rf= 0.88; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.17 (d, J=8.7 Hz, 2H, ArH), 7.93 (d, J=8.7 Hz, 2H, ArH), 7.34-7.23 (m, 5H, ArH), 5.94 (d, J=8.2, 1H, NH), 5.55 (d, J=8.1, 1H, NH), 4.97-4.86 (m, 1H, CHPh), 3.90-3.79 (m, 1H, CHCO), 1.44 (d, J=6.9 Hz, 3H, $\underline{CH_3}$CHPh), 1.39 (d, J=6.9 Hz, 3H, $\underline{CH_3}$CHCO);

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 158.1, 142.1, 137.9, 128.8, 128.2, 127.8, 125.9, 124.3, 52.6, 49.4, 21.8, 20.3; Anal. Calcd for $C_{17}H_{19}N_3O_5S$: C, 54.10; H, 5.07; N, 11.13; S, 8.50. Found: C, 54.18; H, 5.15; N, 11.28; S, 8.61. ESI-QTOF-MS: 378.1063 $(M+H)^+$, 400.0944 $(M+Na)^+$.

***N*-((S)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)-propanamide (57)** Pale yellow oil, 68%, TLC (eluent: diethyl ether/P.E. 70:30 v/v): Rf= 0.88; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.35 (d, J=9 Hz, 2H, ArH), 8.05 (d, J=9 Hz, 2H, ArH), 7.38-7.21 (m, 5H, ArH), 5.97 (d, J=7.2 Hz 1H, NH), 5.60 (s_{broad}, 1H, NH), 4.98-4.88 (m, 1H, CHPh), 3.91-3.82 (m, 1H, CHCO), 1.40 (d, J=6.9 Hz, 3H, $\underline{CH_3}$CHPh), 1.35 (d, J=6.9 Hz, 3H, $\underline{CH_3}$CHCO); ^{3}C NMR (75 MHz, $CDCl_3$) δ 168.6, 157.8, 136.9, 142.2, 128.8, 128.4, 127.8, 125.9, 124.3, 51.9, 48.8, 21.1, 19.4; Anal. Calcd for $C_{17}H_{19}N_3O_5S$: C, 54.10; H, 5.07; N, 11.13; S, 8.50. Found: C, 54.15; H, 5.09; N, 11.23; S, 8.59. ESI-QTOF-MS: 378.1092 $(M+H)^+$, 400.0939 $(M+Na)^+$.

HPLC analyses of crude amides 56 and 57 : (A) N-((R)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido)propanamide (56); (B) a mixture of 56 and 57 (approx. 30% : 70%); (C) N-((S)-1-phenylethyl)-2-(S)-(4-nitrophenylsulfonamido) propanamide (**57**)



General procedure for the reduction of amides 58b-61b and dipeptides 62b-64b.

Typical procedure for the reduction of amides to amines is presented as follows. To a solution of amide **58a-61a** or **62a-64a** (1.0 mmol) in dry diethyl ether (10 mL) was added 1.0 molar equivalent of titanium tetrachloride at room temperature. Then, lithium aluminum hydride (3.0 mmol) was added to the mixture and the reaction was magnetically stirred at rt for 10-20 min. TLC (diethyl ether/petroleum ether 70:30 v/v) showed the complete conversion of the starting amide. After completion of the reaction, the mixture was washed with 1 N aqueous NaOH (3 × 5 mL) and once with brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the corresponding amine **58b-61b** as oils in 75-94 overall yields and polyamines **62b-64b** in 64-86% overall yields. No chromatographic purification is required.

***N*-(Biphenyl-3-ylmethyl)-*N*-ethylethanamine (58b)** colorless oil, yield = 94%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (t, J = 7.2 Hz , 6H, NCH_2CH_3), 2.45 (q, J = 7.2 Hz, 4H, NCH_2CH_3), 3.54 (s, 2H, $PhCH_2$), 7.22 – 7.48 (m, 8H, ArH), 7.70 (d, J = 7.4 Hz, 1H, ArH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ

11.9, 46.7, 54.5, 126.3, 126.7, 127.2, 127.9, 129.5, 129.6, 129.8, 137.6, 141.6, 142.2 ppm. Anal. Calcd for $C_{17}H_{21}N$: C, 85.30; H, 8.84; N, 5.85. Found: C, 85.62; H, 8.80; N, 5.82.

1-*tert*-butoxycarbonylamino-1-benzyl-2-*N,N*,-diethylamino ethane (**59b**) colorless oil, yield 83%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, J = 7.0 Hz, 6H, NCH_2CH_3), 1.41 (s, 9H, *t*Bu), 2.18-2.59 (m, 6H, NCH_2CH_3 + $CHCH_2N$ + $CHCH_2Ph$), 2.80-2.90 (m, 2H, $CHCH_2N$ + $CHCH_2Ph$), 3.82 (m, 1H, α-CH), 4.74 (m, 1H, NH), 7.17-7.32 (m, 5H, Ar*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9, 28.4, 39.2, 47.1, 55.9, 59.7, 79.0, 126.1, 128.4, 129.2, 138.4, 155.8. Anal. Calcd for $C_{18}H_{30}N_2O_2$: C, 70.55; H, 9.87; N, 9.14. Found: C, 70.80; H, 9.83; N, 9.11.

(*S*)-4-amino-*N*-(1-(diethylamino)propan-2-yl)-*N*-methylbenzenesulfonamide (60b) colorless oil, yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90-1.02 (m, 9H, NCH_2CH_3 + $CHCH_3$), 2.32 (m, 1H, $CH_2NCH_2CH_3$), 2.42 (m, 1H, $CH_2NCH_2CH_3$), 2.54 (m, 4H, NCH_2CH_3), 2.77 (s, 3H, NCH_3), 4.10 (m, 3H, -CH + NH_2), 6.58 (d, J = 8.7 Hz, 2H, ArH), 7.52 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.6, 15.4, 27.9, 47.2, 50.6, 56.6, 114.0, 129.2, 130.0, 150.1. Anal. Calcd for $C_{14}H_{25}N_3O_2S$: C, 56.16; H, 8.42; N, 14.03. Found: C, 56.38; H, 8.38; N, 13.99.

(S)-4-Amino-*N*-(1-(diethylamino)-4-methylpentan-2-yl)-*N*-methylbenzenesulfonamide (61b) colorless oil, yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83-0.98 [m, 12H, NCH_2CH_3 + $CH(CH_3)_2$], 1.20-130 [m, 2H, $CHCH_2CH(CH_3)_2$], 1.57 [m, 1H, $CH(CH_3)_2$], 2.23-2.55 (m, 6H, NCH_2CH_3 + $CH_2NCH_2CH_3$), 2.60 (s, 3H, NCH_3), 4.02-4.18 (m, 3 α-CH + NH_2), 6.66 (d, J = 8.7 Hz, 2H, ArH), 7.68 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.6, 23.5, 24.5, 29.7, 39.3, 47.1, 53.2, 55.2, 113.8, 129.2, 129.5, 150.0. Anal. Calcd for $C_{17}H_{31}N_3O_2S$: C, 59.79; H, 9.15; N, 12.30; Found: C, 60.01; H, 9.11; N, 12.32.

4-amino-*N*-(1-((1-hydroxy-3-methylpentan-2-yl)(methyl)amino)propan-2-yl)benzenesulfonamide (62b) colorless oil, yield 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (d, J = 6.6 Hz, 3H, $CHCH_3$), 0. 87 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.08 (d, J = 6.6 Hz, 3H, $NHCHCH_3$), 1.21-1.28 (m, 2H, CH_2CH_3), 1.42 (m, 1H, $CH(CH_3)CH_2CH_3$), 2.08 (s, 3H, NCH_3), 2.30-2.53 (m, 3H, $CHCH_2OH$ + CH_2NCH_3), 3.18 (m, 1H, $NHCHCH_3$), 3.45 (m, 1H, CH_2OH), 3.62 (m, 1H, CH_2OH), 4.10 (brs, 1H, NH), 6.68 (d, J = 8.7 Hz, 2H, ArH), 7.65 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.9, 15.9, 19.5, 27.3, 33.3, 36.7, 47.5, 60.0, 60.5, 69.5, 114.0, 128.6, 129.3, 150.3. Anal.

Calcd for $C_{16}H_{29}N_3O_3S$: C, 55.95; H, 8.51; N, 12.23;Found: C, 55.95; H, 8.48; N, 12.18.

4-amino-*N*-((R)-1-(((S)-1-hydroxy-3-methylbutan-2-yl)(methyl)amino)propan-2-yl)benzenesulfonamide (63b) colorless oil, yield 86; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 [d, J= 6.6 Hz, 3H, CH(C*H3*)2], 0.88 [d, J = 6.6 Hz, 3H, CH(C*H3*)2], 1.02 (d, J = 6.6 Hz, 3H, CH*CH3*), 1.70 [m, 1H, *CH*(CH3)2], 2.04 (s, 3H, NCH_3), 2.21 (m, 1H, C*H*CH_2OH), 2.44 (m, 1H, CH_2NCH_3), 2.67 (dd, J = 13.2, 4.8 Hz, 1H, CH_2NCH_3), 3.16 (m, 1H, C*H*CH3), 3.33 (m, 1H, CH_2OH), 3.62 (dd, J = 10.8, 4.5 Hz, 1H, CH_2OH), 4.08-4.21 (m, 2H, NH_2), 6.65 (d, J = 8.7 Hz, 2H, ArH), 7.64 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.2, 20.0, 21.7, 28.0, 34.1, 47.8, 60.5, 63.1, 72.5, 113.9, 129.3, 129.7, 150.3.Anal. Calcd for $C_{15}H_{27}N_3O_3S$: C, 54.68; H, 8.26; N, 12.75; Found: C, 54.82; H, 8.22; N, 12.70.

4-amino-*N*-((S)-1-(((S)-1-hydroxy-3-methylbutan-2-yl)(methyl)amino)propan-2-yl)benzenesulfonamide (64b) colorless oil, yield 64%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 [d, J= 6.9 Hz, 3H, CH(C*H3*)2], 0.91 [d, J = 6.9 Hz, 3H,CH(C*H3*)2], 1.06 (d, J = 7.2 Hz, 3H, CH*CH3*), 1.70 [m, 1H, *CH*(CH3)2], 2.09 (s, 3H, NCH_3), 2.24 (m, 1H, C*H*CH_2OH), 2.49-2.53 (m, 2H, CH_2NCH_3), 3.18 (m, 1H, C*H*CH3), 3.40 (m, 1H, CH_2OH), 3.66

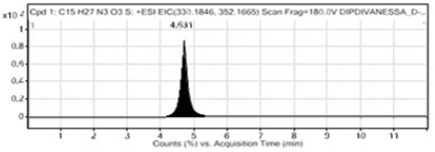
(m, 1H, CH_2OH), 4.12-4.20 (m, 2H, NH_2), 6.65 (d, J = 8.7 Hz, 2H, ArH), 7.63 (d, J = 8.7 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.4, 20.2, 21.8, 27.9, 36.6, 47.7, 60.4, 63.8, 71.7, 113.9, 128.3, 129.2, 150.4. Anal. Calcd for $C_{15}H_{27}N_3O_3S$: C, 54.68; H, 8.26; N, 12.75; Found: C, 54.82; H, 8.22; N, 12.70.

Ion current chromatogram (*m/z* 330, $[M+H]^+$) and ESI(+)-MS spectrum of compound 63b

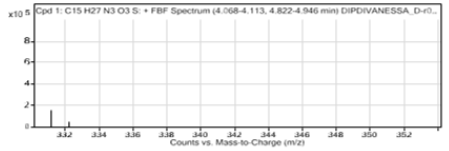
Compound Table

CompoundLabel	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C15 H27 N3 O3 S	4.631	329.1782	998415	C15 H27 N3 O3 S	329.1775	2.66	C15 H27 N3 O3 S	C15 H27 N3 O3 S

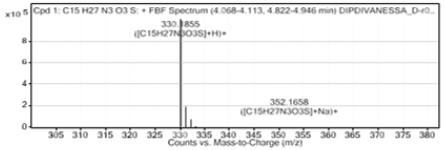
CompoundLabel	m/z	RT	Algorithm	Mass
Cpd 1: C15 H27 N3 O3 S	330.1855	4.631	Find By Formula	329.1782



MS Spectrum



MS Zoomed Spectrum



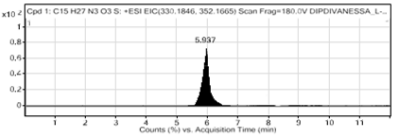
m/z	z	Abund	Formula	Ion
330.1855	1	998415.81	C15H27N3O3S	(M+H)+
331.1883	1	164573.31	C15H27N3O3S	(M+H)+
332.1848	1	51710.89	C15H27N3O3S	(M+H)+
333.1851	1	7927.94	C15H27N3O3S	(M+H)+
334.1867	1	912.91	C15H27N3O3S	(M+H)+
352.1658	1	2396.74	C15H27N3O3S	(M+Na)+
353.1693	1	504.81	C15H27N3O3S	(M+Na)+
354.1659	1	193.61	C15H27N3O3S	(M+Na)+

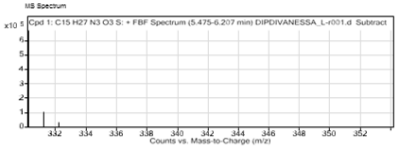
Ion current chromatogram (*m/z* 330, $[M+H]^+$) and ESI(+)-MS spectrum of compound 64b

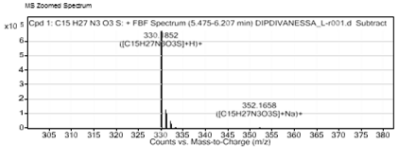
Compound Table

CompoundLabel	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C15 H27 N3 O3 S	5.937	329.1779	668183	C15 H27 N3 O3 S	329.1779	1.76	C15 H27 N3 O3 S	C15 H27 N3 O3 S

CompoundLabel	m/z	RT	Algorithm	Mass
Cpd 1: C15 H27 N3 O3 S	330.1852	5.937	Find By Formula	329.1779







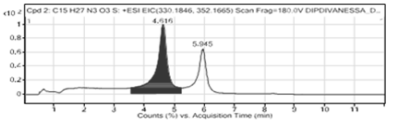
m/z	z	Abund	Formula	Ion
330.1852	1	668183	C15H27N3O3S	(M+H)+
331.1879	1	107616.8	C15H27N3O3S	(M+H)+
332.1846	1	33574.78	C15H27N3O3S	(M+H)+
333.1851	1	5256.87	C15H27N3O3S	(M+H)+
334.1849	1	753.53	C15H27N3O3S	(M+H)+
352.1658	1	1714.99	C15H27N3O3S	(M+Na)+
353.1709	1	371.79	C15H27N3O3S	(M+Na)+
354.1649	1	179.32	C15H27N3O3S	(M+Na)+

Ion current chromatogram (*m/z* 330, $[M+H]^+$) and ESI(+)-MS spectrum of a mixture of compounds 63b and 64b.

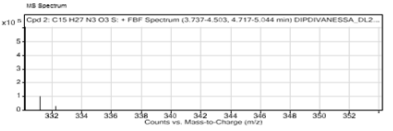
Compound Table

CompoundLabel	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFGFormula	DB Formula
Cpd 2: C15 H27 N3 O3 S	4.616	329.1777	609493	C15 H27 N3 O3 S	329.1773	1.03	C15 H27 N3 O3 S	C15 H27 N3 O3 S
Cpd 1: C15 H27 N3 O3 S	5.945	329.1774	821339	C15 H27 N3 O3 S	329.1773	0.17	C15 H27 N3 O3 S	C15 H27 N3 O3 S

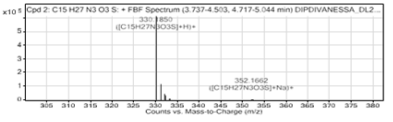
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 2: C15 H27 N3 O3 S	330.185	4.616	Find By Formula	329.1777



MS Spectrum

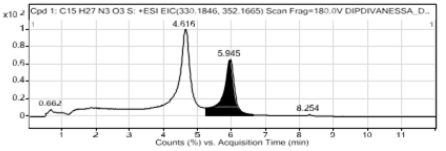


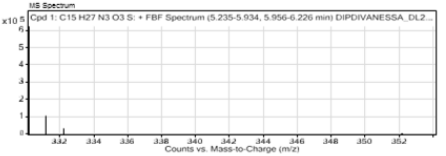
MS Zoomed Spectrum

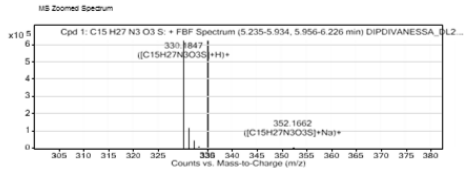


m/z	z	Abund	Formula	Ion
330.185	1	609494.88	C15H27N3O3S	(M+H)+
331.1875	1	102946.38	C15H27N3O3S	(M+H)+
332.1843	1	32458.63	C15H27N3O3S	(M+H)+
333.185	1	5097.14	C15H27N3O3S	(M+H)+
334.1812	1	818.79	C15H27N3O3S	(M+H)+
352.1662	1	2067.01	C15H27N3O3S	(M+Na)+
353.171	1	508.26	C15H27N3O3S	(M+Na)+
354.1671	1	228.37	C15H27N3O3S	(M+Na)+

CompoundLabel	m/z	RT	Algorithm	Mass
Cpd 1: C15 H27 N3 O3 S	330.1847	5.945	Find By Formula	329.1774







m/z	z	Abund	Formula	Ion
330.1847	1	621338.88	C15H27N3O3S	(M+H)+
331.1873	1	104342.95	C15H27N3O3S	(M+H)+
332.184	1	32968.87	C15H27N3O3S	(M+H)+
333.1846	1	5356.76	C15H27N3O3S	(M+H)+
334.1847	1	882.87	C15H27N3O3S	(M+H)+
352.1662	1	1991.77	C15H27N3O3S	(M+Na)+
353.1735	1	514.8	C15H27N3O3S	(M+Na)+
354.1666	1	253.54	C15H27N3O3S	(M+Na)+

Analysis of Fatty Acids in meat product

Sausages formulation and processing

The dry fermented sausages used in this study were prepared at an industrial establishment sited in the province of Cosenza (Calabria region, Southern Italy) following the current Italian industrial processing technology. Pork meat was ground and then mixed. Once out of the grinder and before mixing, sodium chloride (2.4% w/w) was added. Moreover, sodium nitrite (0.01% w/w), sodium nitrate (0.01% w/w), antioxidants (ascorbic acid, 0.02% w/w), sugars (dextrose and sucrose, 0.45% w/w), and spices (red hot pepper for “salsiccia” and “spianata”, 0.6% w/w; black pepper and other spices for “soppressata” 0.6% w/w), and aromas (1% w/w) were added during mixing. Microbial starter formulations were also added as follows: i) SAGA T (0.01% w/w, 2 × 1010 ufc/g Pediococcus pentosaceus and Staphylococcus carnosus, Kerry Ingredients & Flavours Italia S.p.A., Mozzo, Bergamo, Italy) for “salsiccia” and “soppressata”; ii) LH30 (Lactobacillus helveticus fratelli Pagani, Milano, Italy) for “spianata”. Mixtures were stuffed in casings and then spiked to allow

entrapped air to escape. The sausages were stored in a warm room to dry under the following conditions: 24-26 °C at 75-80% relative humidity (RH) for 3 days, then 12-14 °C at 60-70% RH in the last days of the curing process. After 20 days, sausages were then stored under vacuum-packaging for the entire ripening period (80 days).

Sampling

The sausages were sampled and analyzed in triplicate (three different sources) during three different periods of ripening (20 days: samples sal20 and spi20; 40 days: samples sal40, sop40 and spi40; 80 days: samples sal80, sop80 and spi80). Samples were homogenized in a household kitchen-type blender before analysis.

Determination of fatty acid composition

An aliquot of lipid extract (200 mg) was dissolved in 3 mL of n-hexane. A methanolic solution (5 mL) of sodium methoxide (10.1 mg/mL) was then added. A 20% excess sodium methoxide was used: this excess was calculated assuming that fat is constituted exclusively by triolein. The mixture was stirred at room temperature for 5 min. Afterwards, the hexane was separated from the methanolic phase. Then the methanolic phase was washed twice with

hexane. The hexane was finally removed under reduced pressure conditions. The residue was dissolved in dichloromethane and analyzed by GC/MS. Fatty acid methyl esters (FAMEs) were identified by comparing the retention times of the chromatographic peaks with those obtained with the methyl esters from a mixture prepared with fatty acids from Sigma-Aldrich (Milano, Italy). The structural assignment was further confirmed by comparing their mass spectra with those of reference standards.

In a second time, the methanolic phase, containing the FFAs as carboxylate anions, was evaporated under reduced pressure. The residue was acidified with aqueous HCl 1N and then extracted with diethyl ether (5 x 3 mL). The combined ethereal extracts were washed once with a saturated sodium chloride aqueous solution, dried over anhydrous sodium sulfate (Na_2SO_4) and paper filtered and evaporated to dryness under vacuum.

FFAs quantitative analysis

Fatty acids were identified by comparison of their retention times to standards. Methyl pentadecanoate (C15:0) purchased from Sigma-Aldrich, normally not present in the lipid extracts, was used as the internal standard (20

mg/mL). This was added to aliquots of lipid extracts in order to determine the quantity of each fatty acid in the sample analyzed. FFAs were quantified as their corresponding FAMEs. To this aim, the residue obtained from transesterification was treated with a large excess of a 0.66N dichloromethane solution of diazomethane. Until the solution exhibited a constant pale yellow color. After 30 min the organic solvent was removed under reduced pressure conditions, the residue was dissolved in 5 mL of dichloromethane and analyzed by GC/MS.

FAMEs standard solutions: A mixture of standard free fatty acids (21.8 mg of myristic acid; 22.0 mg of palmitic acid; 26.9 mg of palmitoleic acid; 25.3 mg of linolenic acid; 23.8 mg of linoleic acid; 30.4 mg of oleic acid; 22.4 mg of stearic acid) was treated with a large excess of a 0.66 N dichloromethane solution of diazomethane using the procedure described above. The residue was dissolved in 10 mL of methylene chloride (solution A). Four stock solutions were then prepared starting from solution A: an aliquot of 0.1 mL of methyl pentadecanoate standard solution was added to 1, 2, 3 and 4 mL of solution A and each stock solution was diluted to 5 mL with methylene chloride to

create standard solutions. 1µL of each FAMEs standard solution was analyzed by GC/MS.

Kreis Test Methodology

In each experiment, a known quantity of lipid extract (2 g) was mixed with 2 mL of concentrated HCl in a test tube for 30 s. Then 2 mL of a 0.1% solution of phloroglucinol in diethyl ether were added and mixed thoroughly with the lipid-acid mixture.[74] A pink color formation indicated that the fat was slightly oxidized while a red color indicated that the fat was definitely oxidized.

GC/MS methodology for the direct analysis of volatile compounds of bergamot essential oil

Solution bergamot essential oil analysis

Sample preparation: Three aliquots of the essential oil bergamot (55, 95 and 147 mg), containing anisole (0.1 mL) as internal standard, were diluted to in 5mL with diethyl ether and then subjected to the quantitative analysis. Quantitative data were obtained by comparing the

analyte/anisole area ratios in the standard solutions with the corresponding ratios in the oil samples solutions.

Internal standard solution: 40 mg of anisole were diluted to 100 mL with diethyl ether

Preparation of stock solutions A-D: For the quantitative analysis of β-pinene (2) limonene (5), γ-terpinene (7), linalool (11), linalyl acetate (12), five stock solutions A were prepared using 150 mg of each analytes and dissolving them in 5 mL of diethyl ether. Solutions A were further used to prepare diluted working solutions B. In particular, 0.1, 0.2, 0.5, 1, 1.3 and 1.5 mL of each stock solution A was made up to volume with diethyl ether to 5 mL. The final concentration of each analyte in working solutions B were 0.6, 1.2, 3, 6, 7.8, 9.6 mg/mL respectively.

Then, 0.1 mL of the internal standard solution was added to each solution B.

For the quantitative analysis of α-pinene (1), α-phellandrene (3), α-terpinene (4), p-cimene (6), terpinolene (8), myrcene (9), Ocimene (10), neral (13), geranial (14), neryl acetate (15), α-terpineol (16), octyl acetate (17), caryophyllene (18) stock solutions C were prepared as follows: 50 mg of each analyte was diluted to 100 mL with

diethyl ether. Aliquots of these solutions C were then used to prepare diluted working solutions D. In particular, 0.2, 0.5, 1, 1.3, 1.7 and 2.5 mL of each analyte was diluted to 5 mL with diethyl ether. 0.1 mL of the internal standard solution was then added to solutions D. The final concentration of each analyte in working solutions D were 0.02, 0.05, 0.10, 0.13, 0.17, 0.21 mg/mL.

Quantitative analysis of the gaseous phase of bergamot essential oil

Sample preparation: the gaseous phase of the bergamot essential oil was prepared as follows: 100 mg of bergamot essential oil and 7 mg of anisole used as the internal standard, were transferred to a 10 mL vial that was sealed and then maintained at 0 °C.

After 30 minutes, a gastight syringe was used to weigh out the gaseous phase (0.4 mL) and then subjected to the quantitative analysis both by GC-MS and GC-FID. Quantitative data were obtained by comparing the analyte/anisole area ratios in the standard solutions with the corresponding ratios in the essential oil samples solutions.

Internal standard solution: 20 mg of anisole was diluted to 500 mL with diethyl ether.

Preparation of stock solutions E-H: For the quantitative analysis of α-pinene (1), p-cimene (6), mircene (9), linalool (11), linalyl acetate (12), five stock solutions E were prepared using 50 mg of each analytes and dissolving them in 100 mL of diethyl ether. Solutions E were further used to prepare diluted working solutions F. In particular, 0.3, 0.5, 1, 1.3, 1.5, 1.7 mL of each stock solution E was made up to volume with diethyl ether to 10 mL. The final concentration of each analyte in working solutions F were 0.015, 0.025, 0.05, 0.06, 0.07 and 0.08 mg/mL respectively. Then, 0.1 mL of the internal standard solution was added to each solution F.

For the quantitative analysis of : limonene and β-pinene, two stock solutions G were prepared using 150 mg of each analytes and dissolving them in 100 mL of diethyl ether. Solutions G were further used to prepare diluted working solutions H. In particular, 1.5, 2, 2.5, 3, 3.5 and 4 mL of each stock solution G was made up to volume with diethyl ether to 10 mL. The final concentration of each analyte in working solutions G were 0.15, 0.225, 0.30, 0.375, 0.45, 0.525 and

0.60 mg/mL respectively. Then, 0.1 mL of the internal standard solution was added to each solution G.

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