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An Efficient Preparation of *N*-Methyl-α-amino Acids from *N*-Nosyl-α-amino Acid Phenacyl Esters

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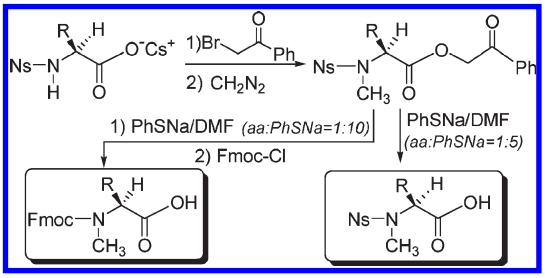

In this paper we describe a simple and efficient solution-phase synthesis of *N*-methyl-*N*-nosyl-α-amino acids and *N*-Fmoc-*N*-methyl-α-amino acids. This represents a very important application in peptide synthesis to obtain *N*-methylated peptides in both solution and solid phase. The developed methodology involves the use of *N*-nosyl-α-amino acids with the carboxyl function protected as a phenacyl ester and the methylating reagent diazomethane. An important aspect of this synthetic strategy is the possibility to selectively deprotect the carboxyl function or alternatively both amino and carboxyl moieties by using the same reagent with a different molar excess and under mild conditions. Furthermore, the adopted procedure keeps unchanged the acid-sensitive side chain protecting groups used in Fmoc-based synthetic strategies.

Introduction

The interesting biological activity of *N*-methylated peptides has encouraged the development of various synthetic procedures for both the preparation of *N*-methyl-α-amino acids[1] and their subsequent insertion into natural peptide chains to enhance and improve their activity. A successful synthesis of *N*-methyl-α-amino acid methyl esters has been recently achieved by a novel methodology based on the use of diazomethane as methylating reagent and α-amino acids protected on the amino function with the *p*-nitrobenzenesulfonyl (nosyl) group.[1a]

The developed procedure proved very effective also in the site-specific methylation of *N*-nosyl-peptides protected on the carboxyl function as methyl esters.[2]

The obtainment of *N*-methyl-*N*-nosyl-α-amino acids not protected on the carboxyl function represents an interesting goal to achieve. In fact, *N*-methyl-*N*-nosyl-α-amino acids and *N*-Fmoc-*N*-methyl-α-amino acids are useful building blocks in solution and solid phase synthesis of *N*-methylated peptides.[3]

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(2) Di Gioia, M. L.; Leggio, A.; Liguori, A. *J. Org. Chem.* **2005**, *70*, 3892. For previously reported *N*-methylation methods see also the references cited therein.

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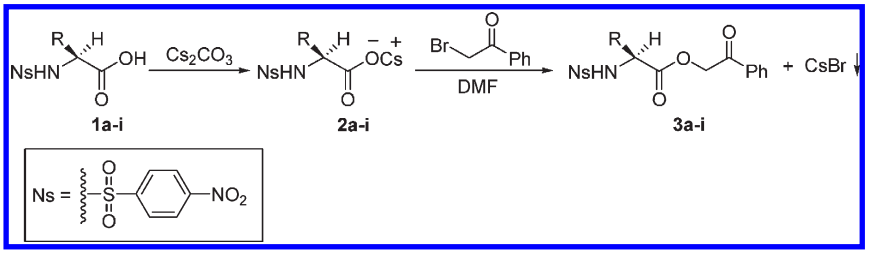
SCHEME 1



In solution phase synthesis of *N*-methyl-α-amino acids and *N*-methyl-peptides the protection of the carboxyl function as a methyl ester is advantageous since it is stable during the entire synthetic process.[1a,2] However, the regeneration of the free carboxyl function presents some difficulties. In peptide synthesis, the most used method for methyl ester cleavage is base hydrolysis.[4] This procedure, even when it is carried out under strictly controlled conditions, could cause racemization and other side reactions.[5]

Results and Discussion

In the light of the above-discussed limits, it is essential to use a carboxyl protecting group that remains stable during the *N*-methylation reaction and is easy to remove after the methylation of the sulfonamide nitrogen atom. Furthermore, the unblocking conditions of the carboxyl function should be compatible with the presence of protecting groups of side chain functionalized α-amino acids.

We developed a practical and general method for the preparation of *N*-methylated amino acids *N*-nosyl and *N*-Fmoc protected by using diazomethane as methylating reagent and protecting the carboxy function with an appropriate easily removable protecting group.

The synthetic strategy reported in this work is based on the use of *N*-nosyl-α-amino acids protected on the carboxyl function as phenacyl esters. The choice of the phenacyl group is due to the need to obtain not only the formation of *N*-methyl-*N*-nosyl-α-amino acids but also *N*-methyl-α-amino acids exploiting the same reagent to deprotect both the carboxyl and amino function. The phenacyl group is easily introduced on the carboxyl function and requires for its removal the treatment of the protected product with a sulfur nucleophile. This could also be used to remove the nosyl group from the amino functions.[6] The cleavage of the phenacyl ester is performed through the nucleophilic attack at the carbinol carbon atom under relatively mild conditions.

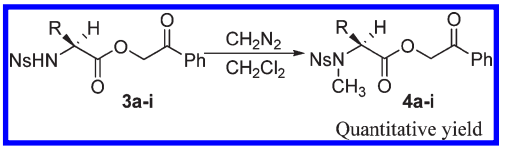
N-Nosyl-α-amino acid phenacyl esters **3a**−**i** were prepared in high yields by treatment of cesium salts **2a**−**i**, obtained from the reaction of the corresponding *N*-nosyl-α-amino acids[2,7] **1a**−**i** with cesium carbonate, with phenacyl bromide[8] in *N*,*N*-dimethylformamide (DMF) (Scheme 1, Table 1).

TABLE 1. Results of the Synthesis of *N*-Nosyl-α-amino Acid Phenacyl Esters 3a−i

entry	R	yield (%)[a]
3a	$CH(CH_3)CH_2CH_3$	95
3b	$CH(CH_3)_2$	98
3c	$CH_2CH(CH_3)_2$	95
3d	CH_3	99
3e	$CH_2(C_6H_5)$	92
3f	CH_2S-(Bzl)	89
3g	$(CH_2)_4NH$-(Boc)	86
3h	CH_2CONH-(Trt)	98
3i	$CH_2C_6H_4O$-(*t*-Bu)	79

[a]Isolated yield.

SCHEME 2



Subsequently, the *N*-methylation of **3a**−**i** performed with use of a dichloromethane solution of diazomethane[9] provided the corresponding *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4a**−**i** in quantitative yields (Scheme 2).

Sodium benzenethiolate (PhSNa) was used as sulfur nucleophile to deprotect the carboxyl function of phenacyl esters **4a**−**i**.[10]

The deprotection reaction of the carboxyl function was investigated by using lipophilic *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4a**−**e** as model systems.

In a preliminary experiment the deprotection reaction was performed with *N*-methyl-*N*-nosyl-L-isoleucine phenacyl ester (**4a**) as starting material. A DMF solution of **4a** was treated with PhSNa in the molar ratio 1:5 (Scheme 3, Table 2). After 30 min the TLC analysis (diethyl ether/petroleum ether 70:30 v/v) of the reaction mixture showed the disappearance of **4a**. The GC/MS analysis of the organic extracts obtained from the extraction of the basified reaction mixture revealed the formation of the deprotection adduct 1-phenyl-2-phenylsulfanylethanone (**6**). It was produced by the interaction of benzenethiolate with the carbinol carbon atom of the phenacyl ester function. The aqueous phase was then acidified and extracted to give the *N*-methyl-*N*-nosyl-L-isoleucine (**5a**) in 70% yield.

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(8) Clark, J. H.; Miller, J. M. *Tetrahedron Lett.* **1997**, *38*, 599.

(9) Arndt, F. *Org. Synth.* **1943**, *2*, 165. Diazomethane is dangerous because, in the presence of relatively acid protons, it forms the methyl diazonium ion that could methylate the alcoholic hydroxyl, amino, thiol, and carboxyl functions of biological structures. Diazomethane is explosive when it is prepared by distillation and used in hydrocarbon solvents which cannot solvate it. Commercially available trimethylsilyldiazomethane was also used as a methylating agent as a safe substitute for diazomethane (ref 3g).

(10) Seehan, J. C.; Daves, G. D. *J. Org. Chem.* **1964**, *29*, 2006.

SCHEME 3

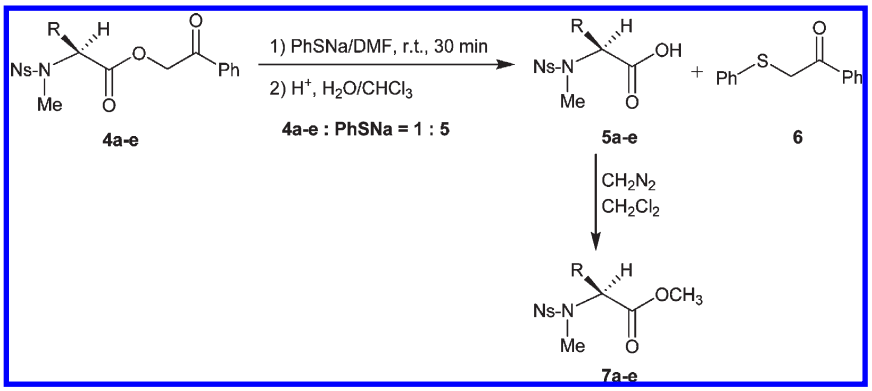


TABLE 2. Results of the Synthesis of *N*-Methyl-*N*-nosyl-α-amino Acids 5a−e

entry	R	yield (%)[a]
5a	$CH(CH_3)CH_2CH_3$	70
5b	$CH(CH_3)_2$	70
5c	$CH_2CH(CH_3)_2$	87
5d	CH_3	76
5e	$CH_2(C_6H_5)$	70

[a]Isolated yield.

The detailed examination of the spectroscopic data of the crude product *N*-methyl-*N*-nosyl-L-isoleucine (**5a**) allowed investigation of stereochemical aspects of the reaction. The ^{1}H NMR spectrum of **5a** showed resonances for a single reaction product as a single diastereoisomer.

N-Methyl-*N*-nosyl-L-isoleucine (**5a**) was converted into the corresponding methyl ester **7a** by treatment with diazomethane (Scheme 3). The GC/MS analysis of **7a**, showed also the presence of a single chromatographic peak and then of a single diastereoisomer, the mass spectrum of which corresponds to the *N*-methyl-*N*-nosyl-L-isoleucine methyl ester (**7a**).[1a]

Hence no measurable epimerization was observed in the deprotection reaction of the *N*-methyl-*N*-nosyl-L-isoleucine phenacyl ester **4a** with sodium benzenethiolate.

N-Methyl-*N*-nosyl-α-amino acids **5b−e** were also obtained in good yields and high purity (Scheme 3, Table 2) as described for **5a**.

Sodium benzenethiolate used in 5-fold molar excess allowed selective deprotection of the phenacyl esters **4a−e** providing the corresponding *N*-methyl-*N*-nosyl-α-amino acids **5a−e**.

Subsequently we also explored the possibility of obtaining from **4a−i** the corresponding *N*-methyl-α-amino acids deprotected on both the amino and the carboxyl function using the same deprotecting reagent. In this additional experiment the *N*-methyl-*N*-nosyl-L-valine phenacyl ester (**4b**) was treated in DMF with PhSNa in the molar ratio 1:5 respectively for longer times (Scheme 4).

After about 1 h the TLC analysis (diethyl ether/petroleum ether 70:30 v/v) of the reaction mixture showed the formation of a second product. It presented the typical coloration at the ninhydrin test, thus proving the deprotection of the amino function.

After 12 h the reaction mixture was acidified with a solution of 1 N HCl and extracted with chloroform. GC/MS analysis of the organic extract revealed the presence of the *N*-methyl-*N*-nosyl-L-valine (**5b**). The aqueous solution was then basified and treated with an excess of acetic anhydride to convert the completely deprotected *N*-methyl-L-valine (**8b**) into the corresponding *N*-acetyl derivative **9b** (Scheme 4). The latter was characterized by GC/MS analysis as methyl ester derivative (**10b**).

The conditions adopted to obtain *N*-methyl-L-valine starting from the *N*-methyl-*N*-nosyl-L-valine phenacyl ester (**4b**) led to a partial deprotection of the amino function. In fact two products corresponding to the *N*-methyl-*N*-nosyl-L-valine (**5b**) (40%) and the *N*-acetyl-*N*-methyl-L-valine (**9b**) (25%) were recovered.

On the basis of these results, the deprotection reaction was performed with use of a large excess of sodium benzenethiolate. *N*-Methyl-*N*-nosyl-L-valine phenacyl ester (**4b**) (1 mmol) was then treated in DMF with sodium benzenethiolate in the molar ratio 1:10, respectively (Scheme 5, Table 3).

The deprotection of the amino function went to completion in 3 h. GC/MS analysis of the organic extract of the acidified reaction mixture did not reveal the presence of the *N*-methyl-*N*-nosyl-L-valine (**5b**). This confirmed that the amino function was completely deprotected. The aqueous phase, containing the completely deprotected *N*-methyl-L-valine (**11b**), was then basified and treated with 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) in order to directly obtain the *N*-Fmoc-*N*-methyl-L-valine (**12b**). This was finally recovered in 72% yield (Scheme 5, Table 3).

N-Methyl-*N*-nosyl-α-amino acid phenacyl esters (**4a**, **4c−i**) treated under the same conditions of **4b** produced the corresponding *N*-Fmoc-*N*-methyl-α-amino acids (**12a**, **12c−i**) in 70−78% yield of isolated product (Scheme 5, Table 3).

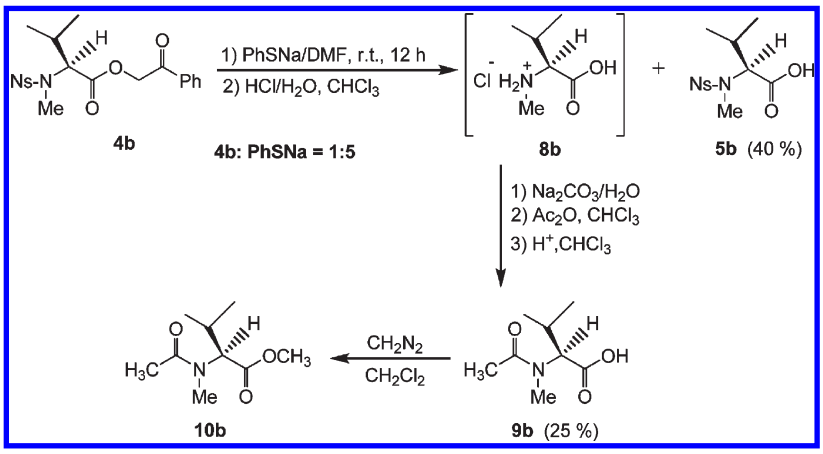
Such results show that using a large excess of sodium benzenethiolate both the amino and carboxyl functions of the *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters are effectively deprotected.

Furthermore, the treatment of the deprotected products **11a−i** with Fmoc-Cl afforded the corresponding *N*-Fmoc-*N*-methyl-α-amino acids (**12a−i**) with good yields and high purities.

The adopted procedure for obtaining *N*-Fmoc-*N*-methyl-α-amino acids was successfully applied also to *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4f−i** bearing functionalized side chains protected by acid labile protecting groups.

The recovery of *N*-Fmoc-*N*-methyl-α-amino acids **12f−i** required special attention due to the presence of the acid-sensitive side chain protecting groups. The workup was

SCHEME 4



SCHEME 5

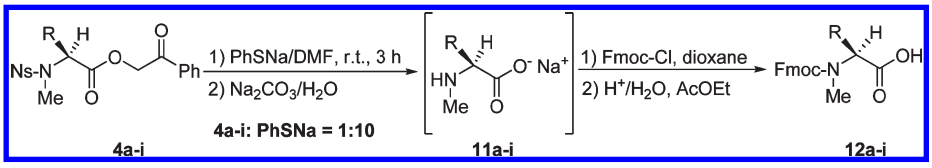


TABLE 3. Results of the Synthesis of *N*-Fmoc-*N*-methyl-α-amino Acids 12a−i

entry	R	yield (%)[a]
12a	$CH(CH_3)CH_2CH_3$	70
12b	$CH(CH_3)_2$	72
12c	$CH_2CH(CH_3)_2$	75
12d	CH_3	70
12e	$CH_2(C_6H_5)$	70
12f	CH_2S-(Bzl)	78
12g	$(CH_2)_4NH$-(Boc)	70
12h	CH_2CONH-(Trt)	70
12i	$CH_2C_6H_4O$-(*t*-Bu)	78

[a]Isolated yield.

performed with a 5% aqueous solution of $KHSO_4$ in order to prevent the undesired deprotection of side chain functionalities. *N*-Fmoc-*N*-methyl-α-amino acids **12f−i** were recovered in good yields keeping unchanged the protecting group on the side chains.

We measured the optical rotations of the *N*-Fmoc-*N*-methyl-α-amino acids **12b** ($[\alpha]^{25}_D$ −32.0), **12d** ($[\alpha]^{25}_D$ −21.2), and **12e** ($[\alpha]^{25}_D$ −54.5) and compared them with the corresponding values reported in literature.[11,1k] The comparison demonstrated that their optical purity is greater than 99%. Similarly, the optical rotations of the other products of the series **12** were measured (**12a**: $[\alpha]^{25}_D$ −51.4; **12c**: $[\alpha]^{25}_D$ −20.2; **12f**: $[\alpha]^{25}_D$ −67.5; **12g**: $[\alpha]^{25}_D$ −23.8; **12h**: $[\alpha]^{25}_D$ −6.5; **12i**: $[\alpha]^{25}_D$ −48.1) and compared with the values obtained, under the same conditions, from the corresponding authentic samples with defined stereochemistry and purity (**12a**: $[\alpha]^{25}_D$ −52.1; **12c**: $[\alpha]^{25}_D$ −20.5; **12i**: $[\alpha]^{25}_D$ −48.3). The results show that these products have an optical purity greater than 98%.

Furthermore in order to evaluate the enantiomeric purity of the compounds of series **12** the proton NMR spectra of these compounds were studied in deuterated methanol in the presence of chiral lanthanide NMR shift reagent europium-(III) tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorate] (Eu[hfc]$_3$).[12] In a typical experiment to 10 mg of *N*-Fmoc-*N*-methyl-L-valine (**12b**) dissolved in 0.5 mL of deuterated methanol was added the shift reagent with different molar ratio and the corresponding ^{1}H NMR spectra were obtained. The spectra obtained from each interaction complex that **12b** has formed with different amounts of the shift reagent are shown in Figure 1b−e. The effect of molar ratio of the shift reagent to the substrate on the change of the chemical shift of *N*-CH_3 protons (δ 2.76 − 2.82) and α-CH protons (δ 3.92 − 3.99) was investigated (Figure 1). Increasing the concentration of the shift reagent only a greater separation of the characteristic signals of the two rotational isomers is observed but no signals for *N*-CH_3 and α-CH protons corresponding to the enantiomer *N*-Fmoc-*N*-methyl-D-valine were detected. Decoupling experiments were used for the attribution of the signals of the two rotational isomers and thus to determine more accurately their ratio.

Additional experiments were performed to demonstrate that the two sets of signals, one for each rotational isomer, coalesce at higher temperature. The coalescence of the signals of the two rotational isomers of the *N*-Fmoc-*N*-methyl-L-valine (**12b**) was observed at 50 °C.[3f]

Equally in the spectra obtained, under the same conditions of **12b**, with the products **12a**, **12c−i** there was no evidence of the presence of enantiomeric species. For the products **12a**, **12c**, **12e**, and **12i** we observed a better separation of the signals corresponding to the rotational isomers. The adopted experiments exclude the presence in the products **12a−i** of the corresponding enantiomers in concentrations higher than that defined by the instrument sensitivity under the used conditions (3%).

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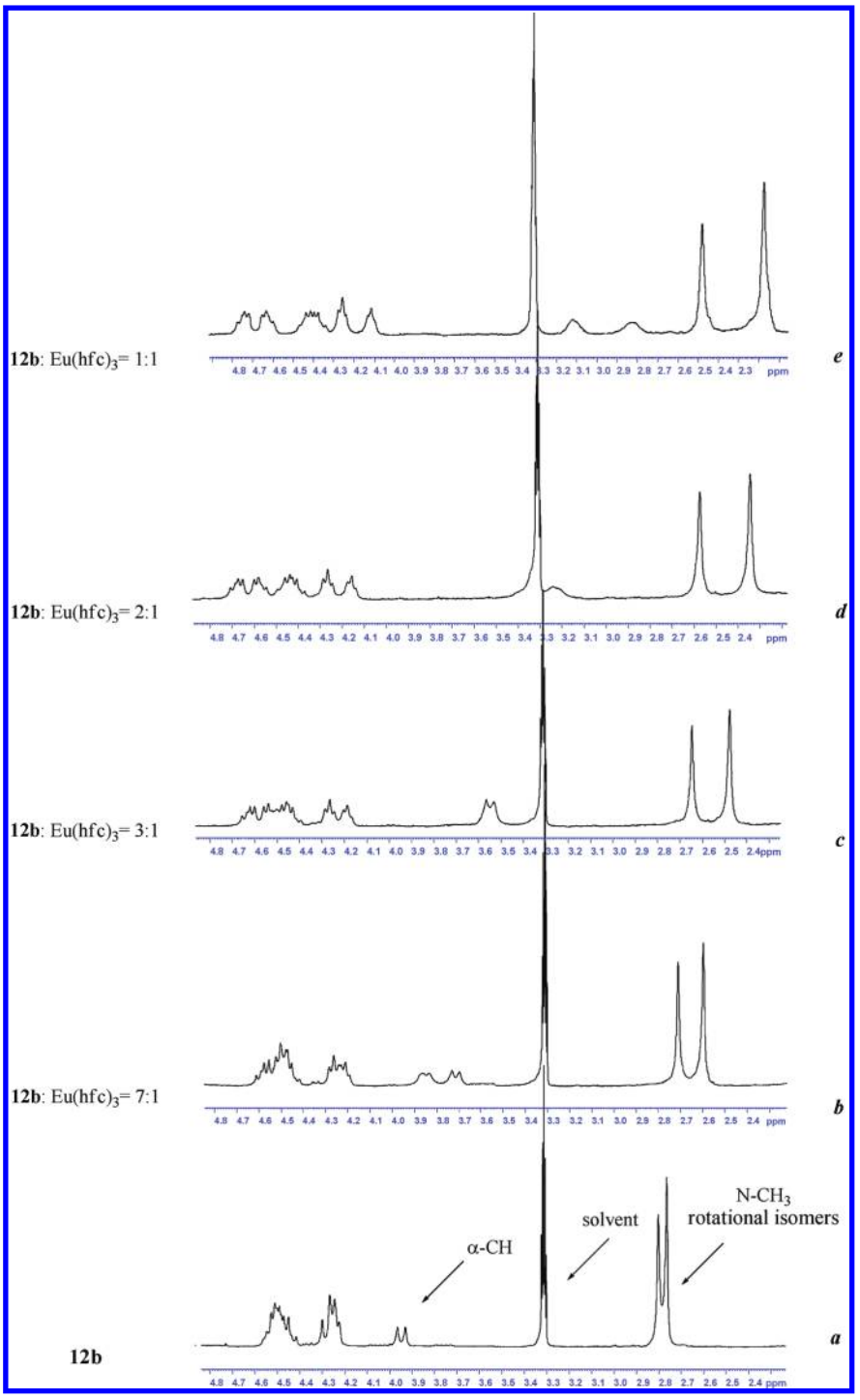

FIGURE 1. (a) The 300 MHz ^{1}H NMR spectrum of *N*-Fmoc-*N*-methyl-L-valine (**12b**) in CD_3OD. (b−e) Spectra of various mixtures of **12b** with the chiral shift reagent $Eu(hfc)_3$.

These results are consistent with what is observed in the NMR analysis of the *N*-Fmoc-*N*-methyl-L-Ile-OH (**12a**) that showed the absence of epimerization products.[3f] In the ^{1}H NMR and ^{13}C NMR spectra of **12a** only signals corresponding to one diastereoisomer were observed. The protection of the carboxylic function of *N*-nosyl-α-amino acids as phenacyl esters proved to be a successful choice to obtain the corresponding *N*-methyl-*N*-nosyl-α-amino acids. Sodium benzenethiolate used with a 5-fold molar excess deprotects rapidly and selectively the carboxylic function of the phenacyl esters **4a**−**e**. The observed selectivity can be explained by assuming that nucleophilic substitution by sodium benzenethiolate at the carbinol carbon atom of the phenacyl ester function is faster with respect to nucleophilic aromatic substitution involved in the removal of the nosyl group from the amino function. The deprotection of the amino function is subsequent to the removal of the phenacyl group and occurs completely only when sodium

benzenethiolate is used with a 10-fold molar excess and for longer times.

Conclusions

The present paper shows how we developed an efficient method to prepare *N*-methylated α-amino acids *N*-nosyl and *N*-Fmoc protected, which are, in turn, useful to obtain *N*-methylated peptides.

The synthetic strategy is based on the use of the phenacyl group to temporarily protect the carboxyl function of *N*-nosyl-α-amino acids. The phenacyl group has proved beneficial in that it is easily introduced, it is stable to the methylation reaction with diazomethane and it is easily removed.

An important and attractive aspect of this protecting system is represented by the possibility to employ for its removal a reagent also useful to deprotect the amino function of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters.

In fact, the simple use of sodium benzenethiolate allows the selective and rapid deprotection of the carboxyl function of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters, thus providing the corresponding *N*-methyl-*N*-nosyl-α-amino acids.

The same reagent, used with a larger molar excess, deprotects both the amino and the carboxy function of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters. The completely deprotected *N*-methyl-α-amino acids were converted easily into the corresponding *N*-Fmoc-*N*-methyl-α-amino acids.

Our approach was successfully applied also to a set of side chain functionalized α-amino acids bearing acid-sensitive protecting groups.

Experimental Section

General Experimental Methods. Solvents were purified and dried by standard procedures and distilled prior to use. Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, using CD_3OD, $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are reported in units of parts per million and all coupling constants are reported in hertz. GC/MS analyses were performed with an HP-5MS (30 m × 0.25 mm, PhMesiloxane 5%) capillary column. The mass detector was operated in the electron impact ionization mode (EI-MS) with an electron energy of 70 eV. All reactions were monitored by thin-layer chromatography, using silica gel 60-F_{254} precoated glass plates. When required, the reactions were carried out under an inert atmosphere (N_2). The dichloromethane solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea with a classical procedure.[9] The concentration of the diazomethane solution (0.66 M) was obtained by a back-titration performed with a standard benzoic acid solution.

Caution:[9] Diazomethane is highly toxic. Hence, this reagent must be handled carefully. Dichloromethane solutions of diazomethane are stable for long periods if stored on KOH pellets at −20 °C.

General Synthetic Procedure for *N*-Nosyl-α-amino Acid Phenacyl Esters 3a−i. The *N*-nosyl-α-amino acids[2,7] **1a−*l*** (1 mmol) were dissolved in ethanol and cooled to 0 °C. An aqueous solution of cesium carbonate (0.5 mmol) was added slowly and the reaction mixture was stirred for 1 h at room temperature. Then the solvent was evaporated under reduced pressure to afford the corresponding cesium salts of *N*-nosyl-α-amino acids **2a−i** as yellow solids in quantitative yields. To a solution of **2a−i** (1 mmol) in *N*,*N*-dimethylformamide (DMF) was added slowly a solution of phenacyl bromide (1 mmol) in DMF. During the reaction a white solid of cesium bromide was formed. The reaction mixture was stirred for about 1 h, monitoring the conversion of **2a−i** by TLC (diethyl ether/petroleum ether 70:30 v/v). The white solid was then separated by filtration and the solvent was evaporated under reduced pressure. The residue was treated with a 9% aqueous solution of sodium carbonate and extracted with chloroform (3 × 10 mL). The combined organic extracts were washed with water and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the corresponding *N*-nosyl-α-amino acid phenacyl esters **3a−i** as pale yellow solids in 79−99% overall yields.

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

General Synthetic Procedure for *N*-Methyl-*N*-nosyl-α-amino Acid Phenacyl Esters 4a−i. A 0.66 M solution of diazomethane[9] in dichloromethane (6 mmol) was added cautiously dropwise to a stirred solution of *N*-nosyl-α-amino acid phenacyl esters **3a−i** (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 1 h monitoring the conversion of the precursor by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4a−i** as oils in quantitative yields.

***N*-Methyl-*N*-nosyl-L-isoleucine phenacyl ester (4a):** yellow oil; $[\alpha]^{25}_D$ −33.0 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, 2H, J = 9.0 Hz), 8.07 (d, 2H, J = 9.0 Hz), 7.78−7.72 (m, 2H), 7.61 (m, 1H), 7.51−7.43 (m, 2H), 5.19 (d, 1H, J = 16.2 Hz), 5.11 (d, 1H, J = 16.2 Hz), 4.50 (d, 1H, J = 10.5 Hz), 2.98 (s, 3H), 2.02 (m, 1H), 1.68 (m, 1H), 1.23 (m, 1H), 1.04 (d, 3H, J = 6.3 Hz), 0.98 (t, 3H, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.5, 168.4, 149.9, 144.6, 134.2, 133.6, 129.1, 128.9, 127.5, 123.9, 65.9, 63.9, 33.6, 30.5, 24.9, 15.5, 10.7; MS (EI) *m*/*z* (rel intensity, %) 405 (0.1), 391 (3), 329 (0.9), 285 (100), 262 (13), 229 (26), 188 (13), 186 (12), 122 (20), 120 (23), 105 (44), 77 (20), 65 (3). Anal. Calcd for $C_{21}H_{24}N_2O_7S$: C, 56.24; H, 5.39; N, 6.25; S, 7.15. Found: C, 56.41; H, 5.41; N, 6.27; S, 7.18.

General Synthetic Procedure for *N*-Methyl-*N*-nosyl-α-amino Acid 5a−e. Sodium benzenethiolate (5 mmol) was added cautiously to a stirred solution of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4a−e** (1 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 30 min monitoring the conversion of the precursor by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with an aqueous solution of 1 N NaOH and extracted with chloroform (3 × 10 mL). The resulting aqueous basic solution was then acidified with a solution of 1 N HCl and then extracted with chloroform (3 × 10 mL). The combined organic extracts were washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the corresponding *N*-methyl-*N*-nosyl-α-amino acids **5a−e** as oils in 70−87% overall yields. *N*-Methyl-*N*-nosyl-α-amino acids **5a−e** (1 mmol) by treating with a

diazomethane solution (3 mmol) were converted into the corresponding methyl esters **7a**−**e** and analyzed by GC/MS.

***N*-Methyl-*N*-nosyl-L-isoleucine (5a):** yellow oil (70%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.40 (d, 2H, J = 9.0 Hz), 8.05 (d, 2H, J = 9.0 Hz), 4.16 (d, 1H, J = 10.5 Hz), 2.90 (s, 3H), 1.90 (m, 1H), 1.60 (m, 1H), 1.17 (m, 1H), 0.98−0.91 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.2, 149.9, 143.9, 128.6, 123.8, 63.7, 33.2, 29.6, 24.8, 14.3, 9.3. Anal. Calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; S, 9.71. Found: C, 47.32; H, 5.47; N, 8.46; S, 9.68.

***N*-Methyl-*N*-nosyl-L-isoleucine methyl ester (7a):** MS (EI) m/z (rel intensity, %) 287 (32), 285 (100), 229 (31), 186 (23), 122 (20), 57 (3).

Synthesis of *N*-Acetyl-*N*-methyl-L-valine (9b). Sodium benzenethiolate (2.37 mmol) was added cautiously to a stirred solution of *N*-methyl-*N*-nosyl-L-valine phenacyl esters (**4b**) (0.47 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 12 h monitoring the conversion of the precursor by TLC analysis (diethyl ether/petroleum ether 70:30 v/v) and verifying the deprotection of the amino function by ninhydrin test. After evaporation of the solvent under reduced pressure the obtained residue was treated with an aqueous solution of 1 N NaOH and extracted with chloroform (3 × 10 mL). The resulting aqueous basic solution was then acidified with a solution of 1 N HCl and then extracted with chloroform (3 × 10 mL). The evaporation of the organic solvent provided the *N*-methyl-*N*-nosyl-L-valine (**5b**) (0.061 g) in 40% yields. The aqueous phase was made basic with a saturated Na_2CO_3 solution (pH 9.0) and treated with acetic anhydride (0.95 mmol) in chloroform at room temperature for 1 h. The mixture, after extraction with chloroform, was acidified with 1 N HCl and again extracted with chloroform (3 × 8 mL). The combined organic extracts were dried and evaporated to dryness to afford the *N*-acetyl-*N*-methyl-L-valine (**9b**) (0.020 g) in 25% yield. The *N*-acetyl-*N*-methyl-L-valine (**9b**) (0.020 g, 0.12 mmol) by treating with a dichloromethane solution of diazomethane (0.36 mmol) was converted into the corresponding methyl esters **10b** and analyzed by GC/MS.

***N*-Acetyl-*N*-methyl-L-valine methyl ester (10b):** MS (EI) m/z (rel intensity, %) 187 (2), 144 (5), 128 (54), 102 (64), 86 (100), 43 (22).

General Synthetic Procedure for *N*-Fmoc-*N*-methyl-α-amino Acid 12a−e. Sodium benzenethiolate (10 mmol) was added to a stirred solution of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4a**−**e** (1 mmol) in DMF. The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 3 h monitoring the progress of the deprotection reaction by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with 1 N HCl (pH 2) and the aqueous solution was extracted with ethyl acetate (3 × 10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 (pH 9). To the basic liquors cooled at 0 °C, containing the corresponding completely deprotected *N*-methyl-α-amino acids **11a**−**e**, was added dropwise a solution of 9-fluorenylmethyloxycarbonyl chloride (1 mmol) in dioxane. The reaction mixture was stirred at 0 °C for 3 h with monitoring the complete conversion of the precursor **11a**−**e** by TLC analysis (chloroform/methanol 90:10 v/v). After evaporation of the solvent under reduced pressure the basic aqueous solution was extracted with diethyl ether. The aqueous solution was then acidified with 1 N HCl (pH 2) and extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness to afford the *N*-Fmoc-*N*-methyl-α-amino acids **12a**−**e** (70−75% overall yields).

***N*-Fmoc-*N*-methyl-L-isoleucine (12a):** white solid (70%); mp 182−183 °C; $[\alpha]^{25}_D$ −51.4 (c 1.00, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) [mixture of two rotational isomers A and B (80:20)] δ 7.94−7.24 (m, 8H), 4.48−3.92 (m, 4H), 2.71 (A) and 2.69 (B) (2s, 3H), 1.80 (m, 1H), 1.34−1.07 (m, 2H), 0.93−0.72 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) [two rotational isomers] δ 172.5, 156.4, 144.3, 144.2, 141.3, 128.1, 127.5, 125.4, 120.5, 67.2, 62.7, 59.9, 47.2, 33.1, 30.4, 25.1, 22.1, 16.2, 10.8. Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.69; H, 6.87; N, 3.82.

General Synthetic Procedure for *N*- Fmoc-*N*-methyl-α-amino Acid 12f−i. Sodium benzenethiolate (10 mmol) was added to a stirred solution of *N*-methyl-*N*-nosyl-α-amino acid phenacyl esters **4f**−**i** (1 mmol) in dimethylformamide (DMF). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature for 3 h monitoring the progress of the deprotection reaction by TLC analysis (diethyl ether/petroleum ether 70:30 v/v). After evaporation of the solvent under reduced pressure the obtained residue was treated with a 5% aqueous solution of $KHSO_4$ (pH 3−4) and extracted with ethyl acetate (3 × 10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3 (pH 9). To the basic liquor kept at 0 °C by an ice bath, containing the corresponding completely deprotected *N*-methyl-α-amino acids **11f**−**i**, was added dropwise a solution of 9-fluorenylmethyloxycarbonyl chloride (1 mmol) in dioxane. The reaction mixture was stirred at 0 °C for 3 h monitoring the complete conversion of the precursor **11f**−**i** by TLC analysis (chloroform/methanol 90:10 v/v). After evaporation of the solvent under reduced pressure the basic aqueous solution was extracted with diethyl ether. The aqueous solution was then acidified with a 5% aqueous solution of $KHSO_4$ (pH 3−4) and extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness to afford the *N*-Fmoc-*N*-methyl-α-amino acids **12f**−**i** (70−78% overall yields).

***N*-Fmoc-*N*-methyl-*O*-*tert*-butyl-L-tyrosine (12i):** (78%); $[\alpha]^{25}_D$ −48.1 (c 1.00, DMF); ^{1}H NMR (300 MHz, DMSO-d_6) [mixture of two rotational isomers A and B (60:40)] δ 12.85 (br s, 1H), 7.88−7.25 (m, 8H), 7.08 (d, 2H, J = 8.4 Hz), 6.80 (d, 2H, J = 8.4 Hz), 4.71 (A) and 4.63 (B) (2 dd, 1H, J = 4.8, 11.4 Hz), 4.31−4.10 (m, 3H), 3.19−2.89 (m, 2H), 2.68 (A) and 2.66 (B) (2s, 3H), 1.20 (B) and 1.15 (A) (2s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) [two rotational isomers] δ 172.5, 172.3, 156.1, 153.8, 144.2, 144.1, 141.2, 133.0, 132.8, 129.7, 128.1, 127.5, 125.4, 123.9, 120.6, 78.0, 67.2, 60.9, 47.1, 46.9, 33.7, 32.2, 31.7, 28.9. Anal. Calcd for $C_{29}H_{31}NO_5$: C, 73.55; H, 6.60; N, 2.96. Found: C, 73.69; H, 6.62; N, 2.97.

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Note Added after ASAP Publication. The Supporting Information file was incomplete in the version published ASAP on February 1, 2010; the complete file was posted on February 5, 2010.

Supporting Information Available: General experimental methods, experimental details for the synthesis of compounds **3b**−**i**, **4b**−**i**, **5b**−**e**, and **12b**−**h**, and copies of ^{1}H NMR and ^{13}C NMR spectra of compounds **3a**−**i** and **4a**−**i**. This material is available free of charge via the Internet at http://pubs.acs.org.