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N-Nosyl-α-amino acids in solution phase peptide synthesis

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Abstract—A highly efficient and practical synthesis of peptides in solution phase has been developed. The procedure is based on the use of *p*-nitrobenzenesulfonyl (nosyl) group for the protection of the amino function of α-amino acids. Every step of the procedure, protection of the amino function by the nosyl group, formation of the peptide bond, and removal of the sulfonamide group, is characterized by high yields and excellent purity of the final products. The described strategy allows the preparation of short peptide sequences keeping the chiral integrity of amino acid precursors. Compatibility of nosyl group with the side-chain protecting groups used in Fmoc-based strategy is demonstrated. The method here presented is an alternative strategy that could provide advantages for future peptide synthesis.

1. Introduction

In peptide chemistry the choice of amino protecting group of α-amino acids is critical. Many different protecting groups have been developed to protect the amino function of α-amino acids in peptide synthesis, but the use of carbamates has received considerable attention due to their ability to minimize base-catalyzed racemization during peptide synthesis.[1] Moreover, strategies for peptide synthesis based on the use of *tert*-butyl carbamate (Boc) and 9-fluorenylmethyl carbamate (Fmoc) are consolidated both in solution and solid phases.

Existing methodology based on the Boc protecting group requires trifluoroacetic acid for each step of Boc deprotection, and an acidic environment with harsher chemicals to remove side-chain protecting groups.[2]

The Fmoc[3,4] group is a base-labile protecting group that is readily cleaved by a variety of amines via base-promoted β-elimination; furthermore, acid-labile protecting groups can be removed in its presence using milder acidic reagents. However, Fmoc chemistry is better suited in solid phase than in solution due to the problems connected with the deprotection process. In fact, dibenzofulvene, released during Fmoc cleavage, can be inefficiently trapped by the base leading to alkene polymerization and resultant problems in purification.[5]

The use of *p*-nitrobenzenesulfonyl (nosyl) group to protect the amino function is of fundamental importance for obtaining *N*-methylated amino acids and peptides.[6] Therefore, of particular interest is the combination of the nosyl chemistry with Fmoc chemistry when the aim of the synthetic work is obtaining *N*-methylated peptides. In fact, the nosyl amino acids introduced in a specific position of the peptide chain are easily methylated on the α-amino function.[6b]

2. Results and discussion

The present work reports a new and alternative strategy for carrying out peptide synthesis using the nosyl group to protect the α-amino function and keeping on the side chain of amino acid protecting groups compatible with the Fmoc group.

The critical step when using the nosyl group concerns with the removal of the sulfonyl group probably by nucleophilic aromatic substitution (SNAr) with thiolate as nucleophile as reported for *N*-alkylated derivatives.[6c] With *N*-nosyl-*N*-methyl-α-amino acids, the deprotection of the amino function occurs easily at controlled temperature and in a short time using the reagent system mercaptoacetic acid/sodium methoxide.[6a,b] When the amino function is not methylated, the removal of the nosyl group is not so easy. In fact, the hydrogen atom on the sulfonamide function is relatively acidic and could reduce the efficiency of the sulfur nucleophile in the nucleophilic aromatic substitution that provides the unmasked amino function.

The feasibility of every step of planned methodology, including the protection of the α-amino acid, the elongation

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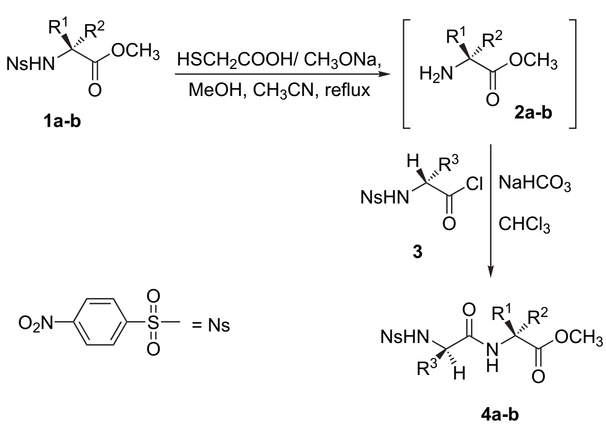
of the peptide chain, and deprotection of the terminal amino function, was previously assessed using lipophilic amino acid methyl esters of both D and L series as appropriate model compounds.

In a typical experiment, *N*-nosyl-L-valine (**1a**) and *N*-nosyl-D-valine (**1b**) methyl esters were prepared by treating the corresponding α-amino acid methyl ester hydrochlorides with *p*-nitrobenzenesulfonyl chloride (Ns-Cl).[6a] Then, to deprotect the amino function, **1a** and **1b** were treated with 3 equiv of mercaptoacetic acid in the presence of 8 equiv of sodium methoxide in acetonitrile and methanol solution at 50 °C.[6a] The unblocking reactions of **1a** and **1b** proceeded at a markedly reduced rate. Typically, the reaction time for all the deprotection processes of *N*-methylated *N*-nosyl-α-amino acid methyl esters by the reagent system mercaptoacetic acid/sodium methoxide was less than 10 min.[6a]

When the experiment was repeated using a larger amount of sodium methoxide and under reflux the nosyl group was removed more rapidly. In fact, treatment of *N*-nosyl-L-valine methyl ester **1a** with 3 equiv of mercaptoacetic acid in the presence of 12 equiv of sodium methoxide (Scheme 1) provided the corresponding L-valine methyl ester **2a** in 1 h. GC/MS analysis performed on an aliquot of the crude reaction product after acidic work up and treatment with diazomethane clearly showed the presence of the deprotection coproduct, methyl 2-(4-nitrophenylthio)acetate.[7] The formation of this compound supports the hypothesis that the removal of the nosyl group proceeds through a nucleophilic aromatic substitution as reported for *N*-alkylated analogues.[6c] Also *N*-nosyl-D-valine methyl ester **1b** was deprotected in the same way (Scheme 1).



Scheme 1.

The two deprotected products **2a** and **2b** were not isolated and directly coupled with *N*-nosyl-D-alanine chloride[6b] **3** in a chloroform solution containing aqueous $NaHCO_3$[6b] to obtain the corresponding diastereomeric dipeptides Ns-D-Ala-L-Val-OMe **4a** and Ns-D-Ala-D-Val-OMe **4b** (Scheme 1 and Table 1). The ^{1}H NMR analysis of both single crude products **4a** and **4b** revealed the presence of signals corresponding to only one diastereomer. Furthermore, in the ^{1}H NMR spectrum of an appropriately prepared mixture of the two diastereomers **4a** and **4b** (Fig. 1), distinct signals were observed for sulfonamidic and methyl ester protons of the two diastereomers.

Table 1. Results of the synthesis of *N*-nosyl-dipeptides **4a** and **4b**

Entry	R^1	R^2	R^3	Yield[a] (%)
4a	$CH(CH_3)_2$	H	CH_3	78
4b	H	$CH(CH_3)_2$	CH_3	71

[a] Isolated yield.

GC/MS analysis performed using the same mixture of **4a** and **4b** showed the presence of two peaks corresponding to the two diastereomers. The comparison of ^{1}H NMR spectra and GC/MS analyses of the mixture to those obtained from the single crude products **4a** and **4b** excluded the formation of epimerized products.

Diastereomeric tripeptides were also synthesized in order to explore the possibility of obtaining longer peptide chains, and to evaluate the stereochemical aspects of the entire process that consists of removing the nosyl group from the terminal amino function and coupling the deprotected peptide with another *N*-nosyl-α-amino acid. *N*-Nosyl-dipeptides **4a** and **4b** were then deprotected on the terminal amino function using 3 equiv of mercaptoacetic acid and 16 equiv of sodium methoxide (Scheme 2).

The deprotection reaction of the dipeptide systems required an increased amount of sodium methoxide and went to completion in 2 h. After this time the deprotected dipeptides **5a** and **5b** were treated with *N*-nosyl-L-isoleucine chloride[6b] **6** (Scheme 2). The tripeptides **7a** and **7b** were recovered in 89% and 84% yields, respectively (Table 2).

The diastereomeric tripeptides **7a** and **7b** were readily resolved by GC/MS (Fig. 2). Furthermore, the chromatograms recorded with the crude products **7a** and **7b** compared to that obtained from an appropriately prepared mixture of the two diastereoisomers excluded any detectable racemization process (Fig. 2).

^{1}H NMR spectra of both crude products **7a** and **7b** showed the presence of signals corresponding to only one diastereomer. In particular the peaks of the amide and the methyl ester protons of the *C*-terminal residue were selected to determine the presence of the other diastereomer in each spectrum. The corresponding signals were found with different chemical shifts in two diastereomers as also showed by ^{1}H NMR spectrum of a mixture of both **7a** and **7b**. Therefore, the obtained compounds showed retention of configuration of the chiral carbon atoms of the precursors.

Following these initial results, it seemed to be very attractive to investigate the full applicability of the proposed methodology to prepare short peptide chains using, as starting materials, *N*-nosyl-α-amino acids with functional groups in their side chains.

In particular, we examined the possibility of obtaining peptides containing α-amino acids protected on their side chain with suitable protecting groups, which should resist the conditions of protection and deprotection of the α-amino function, and formation of the peptide bond. To this end, acid-labile protecting groups were chosen to mask the functional groups of the α-amino acid side chains.

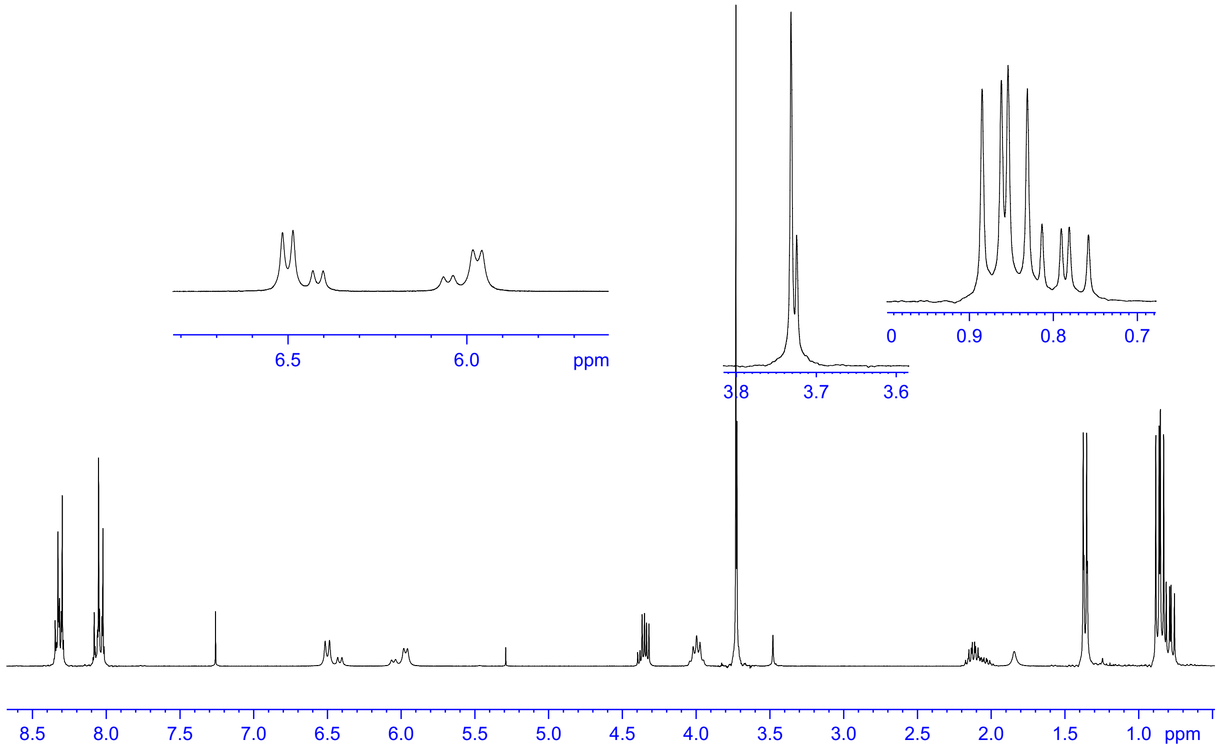
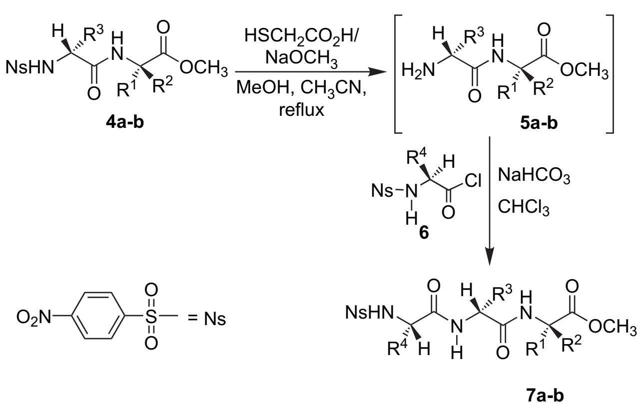


Figure 1. ^{1}H NMR spectrum of a mixture of N^{α}-Ns-D-Ala-L-Val-OMe (**4a**) and N^{α}-Ns-D-Ala-D-Val-OMe (**4b**).



Scheme 2.

The formation of the peptide bond was accomplished with the aid of coupling reagents to prevent the partial deprotection of side-chain functional groups during the formation of *N*-nosyl-α-amino acid chlorides by thionyl chloride.[6b] Therefore, *N*-nosyl-α-amino acids **9a–d** were prepared using the corresponding α-amino acids **8a–d** protected on their side chains with acid-labile protecting groups (Scheme 3).

In a typical experiment, N^{ε}-Boc-L-lysine **8a**, chosen as model compound, was dissolved in water/dioxane 1:1 and treated with *p*-nitrobenzenesulfonyl chloride (Ns-Cl) in the presence of triethylamine at 0 °C (Scheme 3). The reaction was completed within 30 min and afforded, after acidic treatment with a 5% aqueous solution of $KHSO_4$, N^{α}-nosyl-N^{ε}-Boc-L-lysine (**9a**) in 73% overall yield (Table 3).

Table 2. Results of the synthesis of *N*-nosyl tripeptides **7a** and **7b**

Entry	R^1	R^2	R^3	R^4	Yield[a] (%)
7a	$CH(CH_3)_2$	H	CH_3	$CH(CH_3)CH_2CH_3$	89
7b	H	$CH(CH_3)_2$	CH_3	$CH(CH_3)CH_2CH_3$	84

[a] Isolated yield.

The reaction was then extended to the amino acids **8b–d** and provided the corresponding *N*-nosyl-α-amino acids **9b–d** in 75–89% overall yields (Table 3). The synthesis of nosyl-dipeptides **11a–d** was then accomplished by coupling of *N*-nosyl-α-amino acids **9a–d** with α-amino acid methyl ester hydrochlorides **10a** and **10b** (Scheme 4) in the presence of dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, and *N*-methylmorpholine. The coupling reaction was initially carried out by treating N^{α}-nosyl-N^{ε}-Boc-L-lysine **9a** dissolved in dry tetrahydrofuran (THF) with L-alanine methyl ester hydrochloride **10a** at 0 °C (Scheme 4). After 2 h, the resulting dipeptide N^{α}-nosyl-N^{ε}-Boc-L-lysinyl-L-alanineOMe **11a** was isolated in quantitative yield (99%) as the sole reaction product.

^{1}H NMR spectroscopy and mass spectra of the crude product confirmed the structure of the dipeptide **11a**, in particular, the ^{1}H NMR spectrum showed two signals at δ 7.10 and 6.40 ppm corresponding to amidic and sulfonamidic protons, respectively.

The coupling reaction was also extended to the *N*-nosyl-α-amino acids **9b–d** (Scheme 4 and Table 4). The corresponding dipeptides **11b–d** were recovered in high yields (82–95%) and purity as confirmed by ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR spectra showed also the preservation of the enantiomeric integrity of the chiral centers.

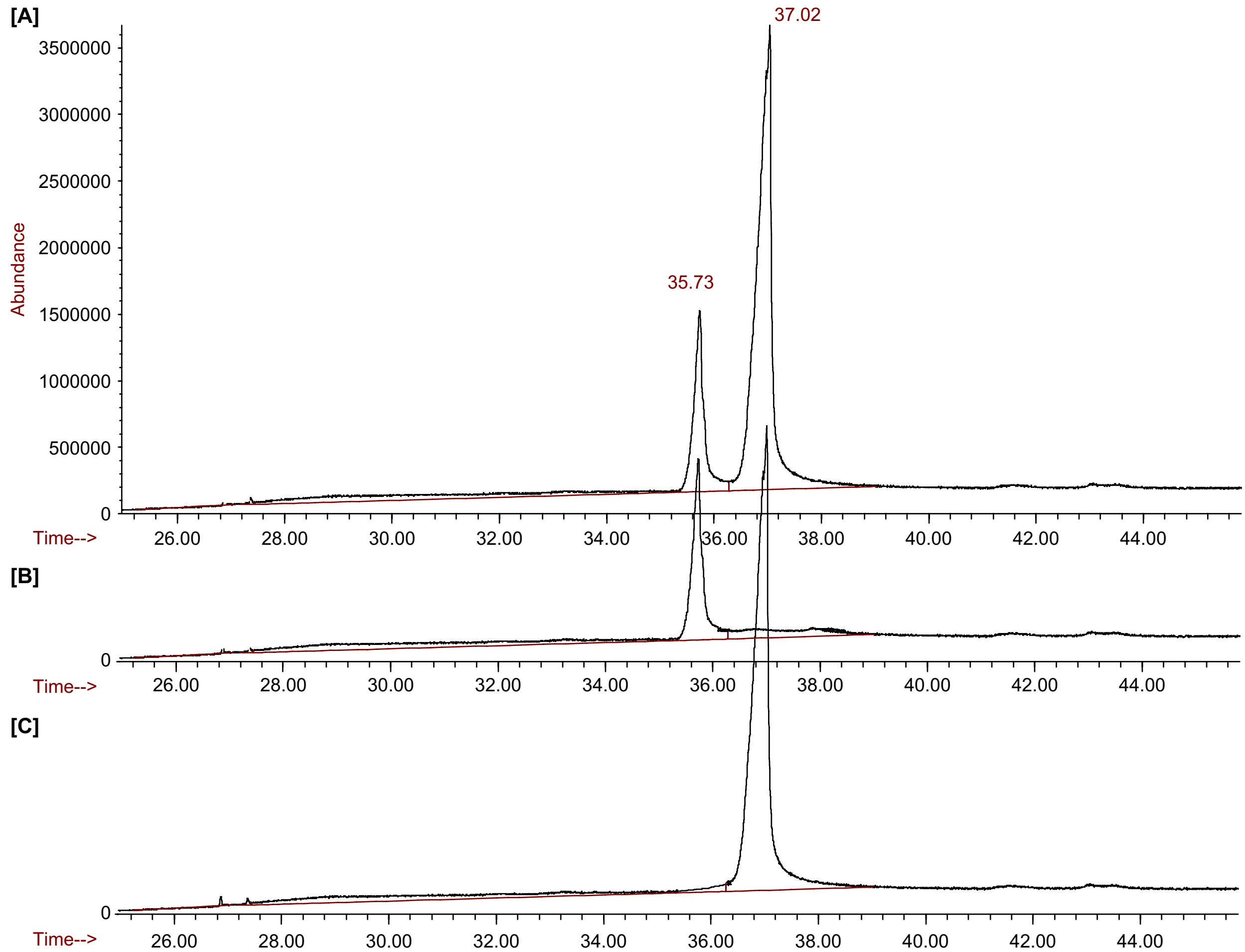
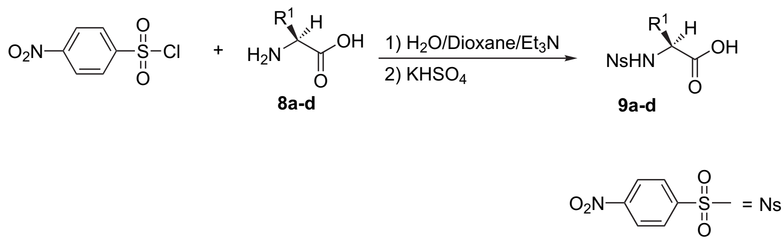


Figure 2. Comparison of gas chromatograms: [A] GC/MS analysis of a mixture (30:70) of *N*-nosyl-L-Ile-D-Ala-L-Val-OCH_3 (35.73 min) and *N*-nosyl-L-Ile-D-Ala-D-Val-OCH_3 (37.02 min); [B] GC/MS analysis of **7a**; [C] GC/MS analysis of **7b**.



Scheme 3.

Table 3. Results of the synthesis of side-chain protected *N*-nosyl amino acids **9a–d**

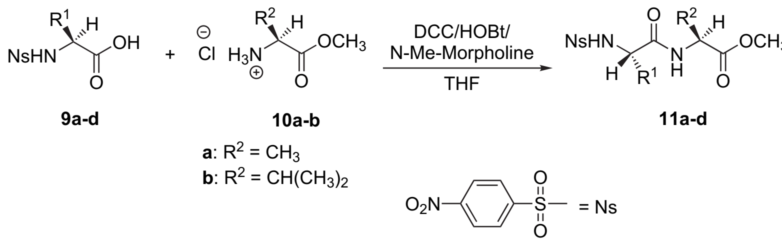
Entry	R^1	Yield[a] (%)
9a	$(CH_2)_4$NH-(Boc)	73
9b	$CH_2C_6H_4$O-(*t*-Bu)	75
9c	CH_2S-(Trt)	83
9d	CH_2CONH-(Trt)	89

[a] Isolated yield.

The selective deprotection of the terminal amino function of dipeptides **11a–c** to obtain subsequently the *N*-nosyl-tripeptides **14a–c** was also investigated.

Therefore, the dipeptide N^{α}-nosyl-N^{ε}-Boc-L-lysinyl-L-alanineOMe **11a** dissolved in acetonitrile/methanol was deprotected at the terminal amino function by the reagent system mercaptoacetic acid/sodium methoxide in the molar ratio 3:16 at reflux. The deprotection reaction was complete within 1 h, after this time the deprotected product **12a** was coupled, with no purification beyond the acid extraction, with N^{α}-nosyl-L-valine chloride (**13**) in a chloroform solution containing aqueous Na_2CO_3 (Scheme 5 and Table 5). After 30 min the corresponding tripeptide N^{α}-nosyl-L-valyl-N^{ε}-Boc-L-lysinyl-L-alanineOMe **14a** was recovered in 85% overall yield.

Analysis of the ^{1}H NMR spectrum of crude product showed the presence of signals referring to the protons of only one



Scheme 4.

Table 4. Results of the syntheses of side-chain protected N^{α}-nosyl-dipeptide methyl esters **11a–d**

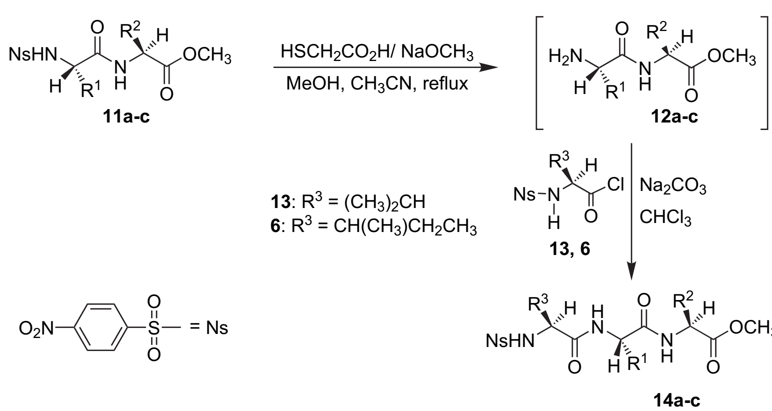
Entry	R^1	R^2	Yield[a] (%)
11a	$(CH_2)_4$NH-(Boc)	CH_3	99
11b	$CH_2C_6H_4O$-(*t*-Bu)	$CH(CH_3)_2$	82
11c	CH_2S-(Trt)	CH_3	87
11d	CH_2CONH-(Trt)	CH_3	95

[a] Isolated yield.

Table 5. Results of the syntheses of *N*-nosyl-tripeptide methyl esters **14a–c**

Entry	R^1	R^2	R^3	Yield[a] (%)
14a	$(CH_2)_4$NH-(Boc)	CH_3	$CH(CH_3)_2$	85
14b	$CH_2C_6H_4O$-(*t*-Bu)	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$	75
14c	CH_2S-(Trt)	CH_3	$CH(CH_3)_2$	71

[a] Isolated yield.



Scheme 5.

diastereomer. Particularly diagnostic for the structure of **14a** are the signals corresponding to the amidic protons of alanine and lysine at δ 7.10 and 7.30 ppm, respectively, and those ones corresponding to the sulfonamidic and urethanic protons at δ 6.36 and 4.92 ppm, respectively. Also the single signal at δ 3.75 ppm relative to the methyl ester protons confirmed the presence of a single diastereomer.

Afterward, tripeptides **14b** and **14c** were synthesized using the same protocol adopted for the preparation of **14a** (Scheme 5 and Table 5). ^{1}H NMR spectroscopic analysis of tripeptides **14a–c** excluded the deprotection of side-chain functional groups. In fact, no discernible extraneous signals were observed in the ^{1}H NMR spectra that revealed only signals referring to the structure of the tripeptides **14a–c**.

3. Conclusions

In conclusion we describe a solution phase synthetic strategy to prepare short peptide sequences using the nosyl group to protect the amino function of α-amino acids under mild conditions and in a rapid and efficient way.

The highly reactive and easily prepared[6b] nosyl-protected amino acid chlorides, used as reagents for peptide coupling with lipophilic amino acids, allow a practical and simple elongation of peptide chains.

The reagent system mercaptoacetic acid/sodium methoxide used for the removal of the nosyl group permits the efficient and specific deprotection of the α-amino function keeping the protecting group on the side chains.

The method described is particularly attractive because the adopted conditions, in every step of the entire process, do not cause any loss of the optical integrity at the chiral centers of the peptide.

The successful application of the developed methodology to the synthesis of peptides containing side-chain functionalized α-amino acids makes this method general for the solution phase peptide synthesis. In fact, amino acids bearing acid-labile protecting groups on their side chains were incorporated into the synthetic strategy to demonstrate the possibility of orthogonal-protection with the nosyl protecting group.

The results obtained demonstrate that the nosyl group combines very well with different side-chain protecting groups widely used in solution phase peptide synthesis based on the Fmoc strategy. Therefore, the nosyl group could be considered as an alternative to the Fmoc group in solution phase peptide synthesis because its removal from the terminal amino function of peptide chain provides the deprotected peptide with high purity without using complex purification procedures.

Furthermore, the reagent necessary to synthesize *N*-nosyl-α-amino acids, nosyl chloride, is commercially available and considerably cheaper than the reagent employed for the introduction of the Fmoc protecting group, 9-fluorenylmethoxycarbonyloxy succinimide.[8]

The procedure developed is particularly convenient to synthesize *N*-methylated peptide on specific amino acids because in this case it is possible to use the nosyl group as the sole α-amino function protecting group.

The results shown here demonstrate that the developed method based on the use of *N*-nosyl-α-amino acids could provide new potential synthetic strategies for peptide synthesis.

4. Experimental

4.1. General experimental procedures

All reagents were commercially obtained (Aldrich, Fluka) at highest commercial quality and used without further purification except where noted. 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 $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are reported in units of parts per million and all coupling constants are reported in Hertz. Optical rotations were measured on a Perkin–Elmer polarimeter at 20 °C. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

GC/MS analyses were performed using 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. Mass spectra were recorded on a Vacuum Generators ZAB-2F spectrometer, using 3-nitrobenzyl alcohol as matrix, by fast atom bombardment (FAB$^+$MS), with a neutral xenon beam operating at 8 keV and a total current of 10 μA. The MALDI mass spectrum was acquired on a 4700 proteomics analyzer mass spectrometer equipped with 200 Hz, Nd:YAG laser at 355 nm wavelength. The MS spectrum was acquired in reflectron mode (20 keV accelerating voltage), with 400 ns delayed extraction, averaging 2000 laser shots. α-Cyano-4-hydroxycinnamic acid (HCCA) was used as matrix. 0.45 μL of a premixed solution of HCCA and sample (800:1) dissolved in MeOH/H_20 (1:1) were spotted on the matrix target, dried at room temperature, and analyzed with the mass spectrometer.

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).

4.2. General synthetic procedure for *N*-nosyl-dipeptides 4a and 4b

Mercaptoacetic acid (3 mmol) was added to a solution of **1a** and **1b** (1 mmol) in dry acetonitrile (10 mL) under N_2 and stirred at reflux. Sodium methoxide (12 mmol) was then added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for ~1 h monitoring the conversion of **1a** and **1b** by TLC (diethyl ether/petroleum ether, 60:40 v/v). Then the solvent was evaporated under reduced pressure and the residue acidified with 1 M HCl and extracted with ethyl acetate (3×10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3. The basic liquors, containing the *N*-deprotected amino acid methyl esters **2a** and **2b**, were then treated with a solution of *N*-nosyl-D-alanine chloride **3** (1 mmol) in dry ethanol-free chloroform (10 mL). The reaction mixture was stirred at room temperature for ~1 h and the organic layer was separated. The aqueous phase was extracted with three additional portions of chloroform (3×10 mL). The combined organic extracts were washed with a 1 M aqueous solution of HCl and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the *N*-nosyl-tripeptides **4a** and **4b** as white solids in 71–78% yields.

4.2.1. *N*-Nosyl-D-alanyl-L-valine methyl ester 4a. The product was prepared by general procedure A using **1a** (0.20 g, 0.63 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (0.13 mL, 1.9 mmol), and sodium methoxide (0.41 g, 7.56 mmol) in methanol (5 mL). The reaction was stirred at reflux for 50 min. The afforded unmasked dipeptide in a 9% aqueous solution of $NaHCO_3$ was treated with **3** (0.18 g, 0.63 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 45 min. The subsequent work up afforded 0.14 g of the title compound **4a** as a white solid (0.49 mmol, 78%): mp 146–147 °C. $[\alpha]_D^{20}$ −19.7 (*c* 0.62, $CHCl_3$); IR (KBr): ν 3350, 3119, 2976, 1730, 1645, 1536, 1352, 1042, 856, 742 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (2H, d, *J*=8.6 Hz), 8.05 (2H, d, *J*=8.6 Hz), 6.51 (1H, d, *J*=8.8 Hz), 5.98 (1H, d, *J*=6.7 Hz), 4.33 (1H, dd, *J*=8.8, 4.7 Hz), 3.98 (1H, m), 3.73 (3H, s), 2.12 (1H, m), 1.37 (3H, d, *J*=7.0 Hz), 0.87 (3H, d, *J*=6.8 Hz), 0.83 (3H, d, *J*=6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.09, 170.76, 150.12, 145.41, 128.53, 124.43, 56.94, 52.65, 52.48, 31.27, 20.33, 18.91, 17.47. MS (EI) *m/z* (rel intensity %) 328 (M^+−COOCH$_3^{\cdot}$, 13), 229 (100), 186 (36), 158 (9), 130 (20), 122 (30), 72 (31). Anal. Calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; S, 8.28. Found: C, 46.31; H, 5.48; N, 10.88; S, 8.24.

4.2.2. *N*-Nosyl-D-alanyl-D-valine methyl ester 4b. The product was prepared following the general procedure described above using **1b** (0.20 g, 0.63 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (0.13 mL, 1.9 mmol), and sodium methoxide (0.41 g, 7.56 mmol) in methanol (5 mL). The reaction was stirred at reflux for 1 h. The afforded unmasked dipeptide in an aqueous 9% solution of

$NaHCO_3$ was treated with **3** (0.18 g, 0.63 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 45 min. The subsequent work up afforded 0.13 g of the title compound **4b** as a white solid (0.44 mmol, 71%): mp 160–162 °C. $[\alpha]_D^{20}$ +36.2 (*c* 0.64, $CHCl_3$); IR (KBr): ν 3350, 3119, 2976, 1730, 1645, 1536, 1352, 1042, 856, 742 cm−1. ^1H NMR (300 MHz, $CDCl_3$): δ 8.32 (2H, d, J=8.6 Hz), 8.08 (2H, d, J=8.6 Hz), 6.75 (1H, d, J=8.7 Hz), 6.50 (1H, d, J=8.5 Hz), 4.38 (1H, dd, J=8.7, 4.9 Hz), 4.06 (1H, m), 3.73 (3H, s), 2.02 (1H, m), 1.32 (3H, d, J=7.0 Hz), 0.89 (3H, d, J=6.9 Hz), 0.75 (3H, d, J=6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.06, 171.27, 149.98, 145.91, 128.34, 124.33, 57.25, 52.48, 52.38, 31.09, 19.76, 18.61, 17.54. MS (EI) *m/z* (rel intensity %) 328 ($M^+-COOCH_3^{\bullet}$, 13), 229 (100), 186 (36), 158 (9), 130 (20), 122 (30), 72 (31). Anal. Calcd for $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; S, 8.28. Found: C, 46.68; H, 5.45; N, 10.86; S, 8.24.

4.3. General synthetic procedure for *N*-nosyl-tripeptides 7a and 7b

Mercaptoacetic acid (3 mmol) was added to a solution of **4a** and **4b** (1 mmol) in dry acetonitrile (10 mL) under N_2 and stirred at reflux. Solid sodium methoxide (16 mmol) was then added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for ∼2 h monitoring the conversion of **4a** and **4b** by TLC (diethyl ether/petroleum ether, 60:40 v/v). Then the solvent was evaporated under reduced pressure and the residue acidified with 1 M HCl and extracted with ethyl acetate (3×10 mL). The aqueous phase was basified with saturated aqueous Na_2CO_3. The basic liquors, containing the *N*-deprotected amino acid methyl esters **5a** and **5b**, were then treated with a solution of *N*-nosyl-D-alanine chloride **6** (1 mmol) in dry ethanol-free chloroform (10 mL). The reaction mixture was stirred at room temperature for ∼1 h and the organic layer was separated. The aqueous phase was extracted with three additional portions of chloroform (3×10 mL). The combined organic extracts were washed with a 1 M aqueous solution of HCl and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the *N*-nosyl-tripeptides **7a** and **7b** as white solids in 79–92% yields.

4.3.1. *N*-Nosyl-L-isoleucyl-D-alanyl-L-valine methyl ester 7a. The product was prepared following the general procedure described above using **4a** (0.14 g, 0.36 mmol) in dry acetonitrile (15 mL), mercaptoacetic acid (0.08 mL, 1.08 mmol), and sodium methoxide (0.31 g, 5.8 mmol) in methanol (10 mL). The reaction was stirred at reflux for 2 h. The unmasked dipeptide in a 9% aqueous solution of $NaHCO_3$ was treated with **6** (0.12 g, 0.36 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 1 h. The subsequent work up afforded 0.16 g of the title compound **7a** as a white solid (0.32 mmol, 89%): mp 195–198 °C. $[\alpha]_D^{20}$ −24.5 (*c* 0.32, $CHCl_3$); IR (KBr): ν 3376, 1637, 1545, 1361, 1352, 856, 800, 742 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 8.22 (2H, d, J=8.6 Hz), 8.06 (2H, d, J=8.6 Hz), 7.51 (1H, d, J=8.4), 7.09 (1H, d, J=7.8 Hz), 6.75 (1H, d, J=9.0 Hz), 4.61 (1H, dd, J=8.0, 5.9 Hz), 4.19 (1H, m), 3.89 (3H, s), 3.71 (1H, t, J=8.1 Hz), 2.19 (1H, m), 1.58–1.78 (2H, m), 1.28 (3H, d, J=7.2 Hz), 1.12 (1H, m), 0.80–1.00 (12H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.52, 171.60, 169.81, 149.74, 146.26, 127.77, 124.11, 61.62. 56.85, 52.79, 48.36, 38.38, 31.74, 24.89, 19.15, 18.85, 17.59, 15.06, 11.09. MS (EI) *m/z* (rel intensity %) 500 ($M^{\bullet+}$, 1%), 441 (4), 370 (3), 342 (13), 271 (16), 241 (22), 215 (25), 187 (100), 186 (13), 156 (16), 122 (13), 72 (41). Anal. Calcd for $C_{21}H_{32}N_4O_8S$: C, 50.39; H, 6.44; N, 11.19; S, 6.41. Found: C, 50.54; H, 6.42; N, 11.29; S, 6.39.

4.3.2. *N*-Nosyl-L-isoleucyl-D-alanyl-D-valine methyl ester 7b. The product was prepared following the general procedure described above using **4b** (0.12 g, 0.31 mmol) in dry acetonitrile (15 mL), mercaptoacetic acid (0.06 mL, 0.93 mmol), and sodium methoxide (0.27 g, 4.96 mmol) in methanol (10 mL). The reaction was stirred at reflux for 2 h. The unmasked dipeptide in a 9% aqueous solution of $NaHCO_3$ was treated with **6** (0.05 g, 0.31 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 1 h. The subsequent work up afforded 0.13 g of the title compound **7b** as a white solid (0.26 mmol, 84%): mp 221–223 °C. $[\alpha]_D^{20}$ +31.7 (*c* 0.32, $CHCl_3$); IR (KBr): ν 3378, 1637, 1545, 1361, 1352, 858, 800, 741 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 8.32 (2H, d, J=8.6 Hz), 8.14 (1H, d, J=8.5 Hz), 8.04 (2H, d, J=8.6 Hz), 6.82 (1H, d, J=7.3 Hz), 6.51 (1H, d, J=8.2 Hz), 4.47 (1H, dd, J=8.0, 6.6 Hz), 4.38 (1H, m), 3.55–3.70 (4H, m), 1.97 (1H, m), 1.42–1.62 (2H, m), 1.08 (1H, m), 0.93 (3H, d, J=7.2 Hz), 0.73–0.86 (12H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.56, 172.25, 169.57, 149.77, 147.22, 128.77, 124.48, 60.70, 57.72, 52.09, 47.91, 37.32, 30.30, 24.70, 19.25, 18.55, 18.15, 15.40, 10.87. MS (EI) *m/z* (rel intensity %) 500 ($M^{\bullet+}$, 1%), 441 (2), 370 (3), 342 (14), 271 (15), 241 (30), 215 (23), 187 (100), 186 (12), 156 (26), 122 (12), 72 (40). Anal. Calcd for $C_{21}H_{32}N_4O_8S$: C, 50.39; H, 6.44; N, 11.19; S, 6.41. Found: C, 50.54; H, 6.42; N, 11.21; S, 6.39.

4.4. General synthetic procedure for *N*-nosyl-amino acids 9a–d

The side-chain protected α-amino acids **8a–d** (1 mmol) were dissolved in a dioxane/water solution and cooled to 0 °C. Dry triethylamine (20 mmol) and then a solution of *p*-nitrobenzenesulfonyl chloride (1.5–1.6 mmol) in dioxane was added slowly. The reaction mixture was stirred for 30–50 min, monitoring the conversion of **8a–d** by TLC (chloroform/methanol, 90:10 v/v). The solvent was removed under reduced pressure and the residue basified with a 5% aqueous solution of Na_2CO_3 and extracted with diethyl ether (3×10 mL). The aqueous phase was acidified with a 5% aqueous solution of $KHSO_4$ (pH=3–4) and extracted with ethyl acetate. The organic layer was washed with water and brine and then dried with Na_2SO_4. The solvent was evaporated to afford the corresponding side-chain protected *N*-nosyl amino acids **9a–d** as white solids in 73–89% overall yields.

4.4.1. N^{α}-Nosyl-N^{ε}-Boc-L-lysine 9a. The product was prepared following the general procedure described above using N^{ε}-Boc-L-lysine (**8a**) (0.4 g, 1.6 mmol) in dioxane/water solution (20 mL), triethylamine (4.52 mL, 32 mmol), and *p*-nitrobenzenesulfonyl chloride (0.57 g, 2.56 mmol). The reaction was stirred at room temperature for 30 min. The subsequent work up afforded 0.51 g of the title compound (1.18 mmol, 73%) as a white solid: mp 153–155 °C. $[\alpha]_D^{20}$ +20.2 (*c* 0.62, CH_3OH); IR (KBr): *ν* 3426, 3408, 3108, 2964, 1730, 1662, 1528, 1342, 1261, 1092, 800. ^{1}H NMR (300 MHz, DMSO-d_6): *δ* 12.72 (1H, br s), 8.58 (1H, d, *J*=8.7 Hz), 8.39 (2H, d, *J*=8.4 Hz), 8.00 (2H, d, *J*=8.9 Hz), 6.75 (1H, m), 3.70 (1H, dd, *J*=13.5, 8.4 Hz), 2.78 (2H, m), 1.40–1.60 (2H, m), 1.34 (9H, s), 1.10–1.27 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6): *δ* 172.99, 155.98, 149.85, 147.25, 144.76, 128.53, 124.79, 77.80, 56.10, 31.98, 29.20, 28.70, 22.76, 18.06. MS *m/z* (%) 453.8931 [(M+Na)$^+$, 100]. Anal. Calcd for $C_{17}H_{25}N_3O_8S$: C, 47.32; H, 5.84; N, 9.74; S, 7.43. Found: C, 47.41; H, 5.86; N, 9.72; S, 7.40.

4.4.2. N^{α}-Nosyl-*O-tert*-butyl-L-tyrosine 9b. The product was prepared following the general procedure described above using *O-tert*-butyl-L-tyrosine (**8b**) (0.4 g, 1.7 mmol) in dioxane/water solution (20 mL), triethylamine (4.7 mL, 34 mmol), and *p*-nitrobenzenesulfonyl chloride (0.56 g, 2.6 mmol). The reaction was stirred at room temperature for 50 min. The subsequent work up afforded 0.53 g of the title compound (1.25 mmol, 75%) as a pale yellow solid: mp 150–152 °C. $[\alpha]_D^{20}$ +1.9 (*c* 0.60, CH_3OH); IR (KBr): *ν* 3183, 2982, 1727, 1608, 1530, 1349, 1308, 1147, 850, 738. ^{1}H NMR (300 MHz, DMSO-d_6): *δ* 12.76 (1H, br s), 8.70 (1H, br s), 8.20 (2H, d, *J*=8.4 Hz), 7.75 (2H, d, *J*=8.9 Hz), 6.98 (2H, d, *J*=8.2 Hz), 6.65 (2H, d, *J*=8.2 Hz), 3.92 (1H, m), 2.92 (1H, m), 2.62 (2H, m), 1.37 (9H, s). ^{13}C NMR (75 MHz, DMSO-d_6): *δ* 171.62, 151.69, 145.83, 145.83, 131.11, 128.45, 128.23, 121.46, 114. 35, 78.05, 54.78, 51.97, 36.13, 29.53. MS *m/z* (%) 444.8162 [(M+Na)$^+$, 100]. Anal. Calcd for $C_{19}H_{22}N_2O_7S$: C, 54.02; H, 5.25; N, 6.63; O, 26.51; S, 7.59. Found: C, 53.80; H, 5.26; N, 6.62; S, 7.61.

4.4.3. N^{α}-Nosyl-*S*-trityl-L-cysteine 9c. The product was prepared following the general procedure described above using *S*-trityl-L-cysteine (**8c**) (0.5 g, 1.4 mmol) in dioxane/water solution (20 mL), triethylamine (3.8 mL, 27.5 mmol), and *p*-nitrobenzenesulfonyl chloride (0.46 g, 2 mmol). The reaction was stirred at room temperature for 40 min. The subsequent work up afforded 0.63 g of the title compound (1.15 mmol, 83%) as an orange solid: mp 99–101 °C. $[\alpha]_D^{20}$ +19.2 (*c* 0.60, CH_3OH); IR (KBr): *ν* 3271, 3100, 3056, 1725, 1606, 1530, 1348, 1164, 1090, 854, 740, 700. ^{1}H NMR (300 MHz, DMSO-d_6) *δ* 12.98 (1H, br s), 8.82 (2H, d, *J*=8.4 Hz), 8.35 (2H, d, *J*=8.4 Hz), 7.96 (1H, d, *J*=8.9 Hz), 7.16–7.34 (15H, m), 3.59 (1H, m), 2.32 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6): *δ* 174.90, 151.67, 145.83, 143.92, 129.38, 128.28, 128.21, 126.38, 121.44, 67.36, 55.74, 26.81. MS *m/z* (%) 571.9175 [(M+Na)$^+$, 100]. Anal. Calcd for $C_{28}H_{24}N_2O_6S_2$: C, 61.30; H, 4.41; N, 5.11; S, 11.69. Found: C, 61.42; H, 4.42; N, 5.09; S, 11.64.

4.4.4. N^{α}-Nosyl-N^{β}-trityl-L-asparagine 9d. The product was prepared following the general procedure described above using N^{β}-trityl-L-asparagine (**8d**) (0.3 g, 0.8 mmol) in dioxane/water solution (20 mL), triethylamine (2.23 mL, 16 mmol), and *p*-nitrobenzenesulfonyl chloride (0.27 g, 1.2 mmol). The reaction was stirred at room temperature for 40 min. The subsequent work up afforded 0.39 g of the title compound (0.7 mmol, 89%) as a white solid: mp 136–138 °C. $[\alpha]_D^{20}$ −11.2 (*c* 0.60, CH_3OH); IR (KBr): *ν* 3426, 3358, 3112, 2964, 1725, 1616, 1533, 1348, 1184, 1112, 850, 743, 700. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 12.81 (1H, br s), 8.15 (2H, d, *J*=8.6 Hz), 7.90 (2H, d, *J*=8.6 Hz), 7.28–7.02 (15H, m), 6.51 (1H, s), 6.00 (1H, br s), 4.02 (1H, m), 3.01 (1H, m), 2.83 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 34.15, 50.08, 72.77, 121.43, 126.33, 128.27, 128.32, 145.06, 145.81, 151.63, 173.78, 174.97. MS *m/z* (%) 567.8831 [(M+Na)$^+$, 100]. Anal. Calcd for $C_{29}H_{25}N_3O_7S$: C, 62.24; H, 4.50; N, 7.51; S, 5.73. Found: C, 62.11; H, 4.51; N, 7.48; S, 5.74.

4.5. General synthetic procedure for side-chain protected *N*-nosyl-dipeptides 11a–d

The α-amino acid methyl ester hydrochlorides **10a** and **10b** (1 mmol), 1-hydroxybenzotriazole (1.1 mmol), *N*-methylmorpholine (1 mmol), and the side-chain protected N^{α}-nosyl-α-amino acids **9a–d** (1 mmol) are dissolved in dry tetrahydrofuran (20 mL). The solution was stirred and cooled in an ice-water bath while dicyclohexylcarbodiimide (1.15 mmol) is added. Stirring was continued for 1 h at 0 °C and an additional hour at room temperature monitoring the reaction by TLC (chloroform/methanol, 80:20 v/v). *N,N′*-Dicyclohexylurea which separated was removed by filtration and the solvent evaporated in vacuo. A mixture of ethyl acetate (30 mL) and a saturated solution of $NaHCO_3$ in water (10 mL) was added to the residue and the organic phase extracted with a 10% solution of citric acid in water (10 mL), again with saturated $NaHCO_3$ (10 mL) and brine. The solution was dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo to afford the side-chain *N*-nosyl-dipeptides **11a–d** as pale yellow solids in 87–99% overall yields.

4.5.1. N^{α}-Nosyl-N^{ε}-Boc-L-lysinyl-L-alanineOMe 11a. The product was prepared following the general procedure described above using L-alanine methyl ester hydrochloride (**10a**) (0.1 g, 0.7 mmol), 1-hydroxybenzotriazole (0.1 g, 0.76 mmol), *N*-methylmorpholine (0.08 mL, 0.7 mmol), N^{α}-nosyl-N^{ε}-Boc-L-lysine (**9a**) (0.3 g, 0.7 mmol), and dicyclohexylcarbodiimide (0.16 g, 0.8 mmol) in dry tetrahydrofuran (20 mL). The solution was stirred and cooled in an ice-water bath for 1 h and then for an additional hour at room temperature. The subsequent work up afforded 0.35 g of the title compound (0.67 mmol, 90%) as a pale yellow solid: mp 102–104 °C. $[\alpha]_D^{20}$ +15.6 (*c* 0.64, $CHCl_3$); IR (KBr): *ν* 3336, 3260, 3108, 2931, 1741, 1686, 1647, 1529, 1450, 1352, 1261, 1166, 1090, 801. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 8.30 (2H, d, *J*=8.8 Hz), 8.03 (2H, d, *J*=8.7 Hz), 7.10 (1H, d, *J*=8.1 Hz), 6.40 (1H, d, *J*=5.5 Hz), 4.80 (1H, m), 4.31 (1H, m), 3.85 (1H, m), 3.68 (3H, s), 3.08–3.18 (1H, m), 2.82–3.03 (1H, m), 1.65 (2H, m), 1.25–1.46 (3H, m), 1.44 (9H, s), 1.06–1.17 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.84, 170.37, 156.74, 156.05, 150.05, 128.64, 124.22, 79.63, 56.33, 52.60, 49.31, 48.09, 38.98, 31.95, 29.01, 28.45, 24.90, 21.22, 17.90. MS *m/z* (%) 538.8431 [(M+Na)$^+$, 100]. Anal. Calcd for $C_{21}H_{32}N_4O_9S$: C, 48.83; H, 6.24; N, 10.85; S, 6.21. Found: C, 48.97; H, 6.23; N, 10.87; S, 6.18.

4.5.2. N^{α}-Nosyl-*O-tert*-butyl-L-tyrosinyl-L-valineOMe 11b. The product was prepared following the general procedure described above using L-valine methyl ester hydrochloride (**10b**) (0.12 g, 0.71 mmol), 1-hydroxybenzotriazole (0.1 g, 0.78 mmol), *N*-methylmorpholine (0.078 mL, 0.71 mmol), N^{α}-nosyl-*O*-*tert*-butyl-L-tyrosine (**9b**) (0.3 g, 0.71 mmol), and dicyclohexylcarbodiimide (0.168 g, 0.82 mmol) in dry tetrahydrofuran (20 mL). The solution was stirred and cooled in an ice-water bath for 1 h and then for an additional hour at room temperature. The subsequent work up afforded 0.31 g of the title compound (0.58 mmol, 82%) as a pale yellow solid: mp 64–66 °C. $[\alpha]_D^{20}$ −12.1 (*c* 0.58, $CHCl_3$); IR (KBr): *ν* 3394, 3213, 3119, 2963, 1749, 1637, 1535, 1351, 1261, 1093, 1024, 799. ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.20 (2H, d, *J*=8.9 Hz), 7.78 (2H, d, *J*=8.4 Hz), 6.91 (2H, d, *J*=8.5 Hz), 6.71 (2H, d, *J*=8.5 Hz), 6.62 (1H, d, *J*=8.2 Hz), 6.05 (1H, d, *J*=8.5 Hz), 4.38 (1H, dd, *J*=8.5, 4.8 Hz), 4.00 (1H, m), 3.72 (3H, s), 3.02 (1H, dd, *J*=14.1, 8.3 Hz), 2.88 (1H, dd, *J*=14.1, 5.9 Hz), 2.08 (1H, m), 1.30 (9H, s), 0.81 (6H, d, *J*=6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 171.67, 170.07, 154.87, 149.94, 145.27, 129.83, 129.70, 128.16, 124.33, 124.29, 58.70, 57.46, 52.35, 38.17, 33.83, 31.24, 28.74, 18.85, 17.70. MS *m*/*z* (%) 557.9979 [$(M+Na)^+$, 100]. Anal. Calcd for $C_{25}H_{33}N_3O_8S$: C, 56.06; H, 6.21; N, 7.85; S, 5.99. Found: C, 55.83; H, 6.19; N, 7.88; S, 6.00.

4.5.3. N^{α}-Nosyl-*S*-trityl-L-cysteinyl-L-alanineOMe 11c. The product was prepared following the general procedure described above using L-alanine methyl ester hydrochloride (**10a**) (0.076 g, 0.55 mmol), 1-hydroxybenzotriazole (0.08 g, 0.6 mmol), *N*-methylmorpholine (0.06 mL, 0.55 mmol), N^{α},N^{α}-nosyl-*S*-trityl-L-cysteine (**9c**) (0.3 g, 0.55 mmol), and dicyclohexylcarbodiimide (0.13 g, 0.63 mmol) in dry tetrahydrofuran (20 mL). The solution was stirred and cooled in an ice-water bath for 1 h and then for an additional hour at room temperature. The subsequent work up afforded 0.3 g of the title compound (0.47 mmol, 87%) as a pale yellow solid: mp 138–142 °C. $[\alpha]_D^{20}$ +21.9 (*c* 0.64, $CHCl_3$); IR (KBr): *ν* 3332, 3274, 3059, 2932, 1731, 1663, 1536, 1347, 1261, 1164, 1089, 800, 698. ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.18 (2H, d, *J*=8.8 Hz), 7.92 (2H, d, *J*=8.7 Hz), 7.28 (15H, m), 6.20 (1H, d, *J*=7.2 Hz), 5.70 (1H, d, *J*=7.5 Hz), 4.32 (1H, m), 3.68 (3H, s), 3.14 (1H, m), 2.60 (1H, dd, *J*=13.8, 5.4 Hz), 2.48 (1H, dd, *J*=13.8, 8.4 Hz), 1.22 (3H, d, *J*=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.25, 168.17, 149.90, 145.35, 143.90, 129.32, 128.57, 127.80, 126.98, 123.91, 67.30, 56.03, 52.43, 48.22, 24.77, 17.92. MS *m*/*z* (%) 656.9899 [$(M+Na)^+$, 100]. Anal. Calcd for $C_{32}H_{31}N_3O_7S_2$: C, 60.65; H, 4.93; N, 6.63; S, 10.12. Found: C, 60.83; H, 4.92; N, 6.64; S, 10.08.

4.5.4. N^{α}-Nosyl-N^{β}-trityl-L-asparaginyl-L-alanineOMe 11d. The product was prepared following the general procedure described above using L-alanine methyl ester hydrochloride (**10a**) (0.051 g, 0.37 mmol), 1-hydroxybenzotriazole (0.055 g, 0.4 mmol), *N*-methylmorpholine (0.04 mL, 0.37 mmol), N^{α}-nosyl-N^{β}-trityl-L-asparagine (**9d**) (0.2 g, 0.37 mmol), and dicyclohexylcarbodiimide (0.08 g, 0.42 mmol) in dry tetrahydrofuran (20 mL). The solution was stirred and cooled in an ice-water bath for 1 h and then for an additional hour at room temperature. The subsequent work up afforded 0.22 g of the title compound (0.35 mmol, 95%) as a pale yellow solid: mp 138–141 °C. $[\alpha]_D^{20}$ +36.6 (*c* 0.56, $CHCl_3$); IR (KBr): *ν* 3325, 3062, 2932, 2849, 1742, 1666, 1625, 1531, 1348, 1165, 1089, 853, 700. ^1H NMR (300 MHz, $CDCl_3$): *δ* 8.15 (2H, d, *J*=8.8 Hz), 7.90 (2H, d, *J*=8.9 Hz), 7.10–7.28 (15H, m), 6.53 (1H, s), 5.98 (1H, br s,), 5.56 (1H, d, *J*=9.8 Hz), 4.32 (1H, m), 4.05 (1H, m), 3.68 (3H, s), 3.09 (1H, m), 2.84 (1H, m), 1.25 (3H, d, *J*=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 172.28, 169.98, 169.09, 150.04, 145.46, 143.80, 128.45, 128.30, 127.91, 127.13, 124.34, 70.90, 52.90, 52.35, 48.40, 33.70, 17.36. MS *m*/*z* (%) 652.7740 [$(M+Na)^+$, 100]. Anal. Calcd for $C_{33}H_{32}N_4O_8S$: C, 61.48; H, 5.00; N, 8.69; S, 4.97. Found: C, 61.43; H, 4.98; N, 8.71; S, 4.96.

4.6. General synthetic procedure for side-chain protected *N*-nosyl-tripeptides 14a–c

Mercaptoacetic acid (3 mmol) was added to a solution of **11a–c** (1 mmol) in dry acetonitrile (10 mL) under N_2 and stirred at reflux. Sodium methoxide (16 mmol) was then added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for 1–2 h monitoring the conversion of **11a–c** by TLC (diethyl ether/petroleum ether, 90:10 v/v). Then the solvent was evaporated under reduced pressure and the residue acidified with a 5% solution of $KHSO_4$ in water and extracted with ethyl acetate (3×10 mL). The aqueous phase was basified with a 5% solution of Na_2CO_3 in water. The basic liquors, containing the *N*-deprotected amino acid methyl esters **12a–c**, were then treated with a solution of *N*-nosyl-α-amino acid chloride **13**, **6** (1 mmol) in dry ethanol-free chloroform (5 mL). The reaction mixture was stirred at room temperature for ∼2 h and the organic layer was separated. The aqueous phase was extracted with three additional portions of chloroform (3×10 mL). The combined organic extracts were washed with a 5% aqueous solution of citric acid and a saturated aqueous solution of NaCl, dried (Na_2SO_4), and evaporated to dryness to afford the *N*-nosyl-tripeptides **14a–c** as white solids in 71–85% yields.

4.6.1. N^{α}-Nosyl-L-valyl-N^{ε}-Boc-L-lysinyl-L-alanineOMe 14a. The product was prepared following the general procedure described above using **11a** (0.17 g, 0.33 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (0.07 mL, 0.99 mmol), and sodium methoxide (0.29 g, 5.3 mmol) in methanol (10 mL). The reaction was stirred at reflux for 1 h. The afforded unmasked dipeptide in a 5% aqueous solution of Na_2CO_3 was treated with N^{α}-nosyl-L-valine chloride (**13**) (0.105 g, 0.33 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 30 min. The subsequent work up afforded 0.17 g of the title compound **14a** as a white solid (0.27 mmol, 85%): mp 166–168 °C. $[\alpha]_D^{20}$ −11.7 (*c* 0.60, $CHCl_3$); IR (KBr): *ν* 3315, 3260, 3103, 2964, 2937, 1727, 1686, 1633, 1531, 1349, 1261, 1170, 1091, 798. ^1H NMR (300 MHz, DMSO-d_6): *δ* 8.31 (2H, d, *J*=7.5 Hz), 8.08 (2H, d, *J*=7.8 Hz), 7.15–7.28 (2H, m), 6.51 (1H, m), 4.90 (1H, m), 4.45–4.54 (2H, m), 3.65–3.80 (4H, m), 3.08 (2H, m), 2.18 (1H, m), 1.65 (2H, m), 1.45 (9H, s), 1.36 (3H, d, *J*=6.7 Hz), 1.10–1.27 (4H, m), 0.85 (6H, m). ^{13}C NMR (75 MHz, DMSO-d_6): *δ* 173.00, 171.00, 170.47, 156.50, 149.23, 146.10, 128.57, 124.18, 62.21, 52.61, 48.22, 39.72, 32.05, 31.84, 29.71, 29.37, 28.45, 22.15, 19.17, 17.79, 17.66. MS *m*/*z* (%) 637.9419 [$(M+Na)^+$]. Anal. Calcd for $C_{26}H_{41}N_5O_{10}S$: C, 50.72; H, 6.71; N, 11.37; S, 5.21. Found: C, 50.61; H, 6.72; N, 11.33; S, 5.22.

4.6.2. N^{α}-Nosyl-L-leucyl-*O*-*tert*-butyl-L-tyrosinyl-L-valine OMe 14b. The product was prepared following the general procedure described above using **11b** (0.20 g, 0.37 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (0.08 mL, 1.12 mmol), and sodium methoxide (0.32 g, 5.9 mmol) in methanol (10 mL). The reaction was stirred at reflux for 2 h. The afforded unmasked dipeptide in an aqueous 5% solution of Na_2CO_3 was treated with N^{α}-nosyl-L-isoleucine chloride (**6**) (0.12 g, 0.37 mmol) in ethanol-free chloroform. The reaction was stirred at room temperature for 1 h. The subsequent work up afforded 0.18 g of the title compound **14b** as a white solid (0.27 mmol, 75%): mp 203–205 °C. $[\alpha]_D^{20}$ −2.6 (*c* 0.58, $CHCl_3$); IR (KBr): ν 3324, 3270, 3177, 2963, 1743, 1644, 1530, 1261, 1165, 1094, 1024, 800. ^1H NMR (300 MHz, $CDCl_3$): δ 8.31 (2H, d, *J*=8.6 Hz), 8.04 (2H, d, *J*=8.6 Hz), 6.90–7.08 (5H, m), 6.50 (1H, d, *J*=8.4 Hz), 6.18 (1H, m), 4.61 (1H, m), 4.39 (1H, m), 3.88 (1H, m), 3.71 (3H, s), 2.85 (2H, m), 2.05 (2H, m), 1.39–1.50 (2H, m), 1.32 (9H, s), 0.70–0.87 (12H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.58, 171.03, 170.59, 154.60, 150.07, 145.62, 129.69, 128.57, 124.47, 124.32, 57.63, 55.67, 54.56, 52.34, 42.34, 37.69, 30.97, 28.81, 24.35, 22.85, 21.19, 18.81, 17.89. MS *m*/*z* (%) 670.9496 [$(M+Na)^+$, 100]. Anal. Calcd for $C_{31}H_{44}N_4O_9S$: C, 57.39; H, 6.84; N, 8.64; S, 4.94. Found: C, 57.50; H, 6.83; N, 8.66; S, 4.92.

4.6.3. N^{α}-Nosyl-L-valyl-*S*-trityl-L-cysteinyl-L-alanine OMe 14c. The product was prepared following the general procedure described above using **11c** (0.13 g, 0.25 mmol) in dry acetonitrile (10 mL), mercaptoacetic acid (0.052 mL, 0.75 mmol), and sodium methoxide (0.22 g, 4.0 mmol) in methanol (10 mL). The reaction was stirred at reflux for 90 min. The afforded unmasked dipeptide in a 5% aqueous solution of Na_2CO_3 was treated with 0.08 g (0.25 mmol) N^{α}-nosyl-L-valine chloride (**13**) in ethanol-free chloroform. The reaction was stirred at room temperature for 1 h. The subsequent work up afforded 0.13 g of the title compound **14c** as a yellow solid (0.18 mmol, 71%): mp 180–182 °C. IR (KBr): ν 3338, 3261, 2963, 1733, 1643, 1529, 1350, 1265, 1087, 1030, 806, 697. ^1H NMR (300 MHz, $CDCl_3$): δ 8.35 (2H, d, *J*=9.0 Hz), 8.05 (2H, d, *J*=9.0 Hz), 7.28 (15H, m), 6.20 (1H, d, *J*=7.2 Hz), 5.70 (1H, d, *J*=7.5 Hz), 5.56 (1H, d, *J*=9.8 Hz), 4.32 (1H, m), 3.95 (1H, m), 3.55 (3H, s), 3.14 (1H, m), 2.58–2.70 (2H, m), 2.11 (1H, m), 1.22 (3H, d, *J*=7.2 Hz), 0.98 (3H, d, *J*=6.8 Hz), 0.88 (3H, d, *J*=6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.84, 171.63, 171.17, 151.62, 145.83, 143.95, 128.41, 128.22, 127.23, 126.32, 124.07, 66.92, 61.96, 61.07, 51.92, 48.46, 31.47, 28.63, 18.85, 17.05, 13.88. MS *m*/*z* (%) 755.8488 [$(M+Na)^+$, 100]. Anal. Calcd for $C_{37}H_{40}N_4O_8S_2$: C, 60.64; H, 5.50; N, 7.64; S, 8.75. Found: C, 60.52; H, 5.49; N, 7.66; S, 8.72.

References and notes

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7. Methyl 2-(4-nitrophenylthio)acetate: MS (EI) *m*/*z* (rel intensity %) 227 (M^+, 82), 168 (100), 151 (8), 137 (6), 122 (56), 121 (58).
8. Based on the Aldrich 2007/08 catalog, where 4-nitrobenzenesulfonyl chloride is € 269/mol and 9-fluorenylmethyloxycarbonyloxy succinimide is € 3305/mol.