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Deprotection of *N*-Nosyl-α-amino Acids by Using Solid-Supported Mercaptoacetic Acid

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A simple and efficient synthesis of a solid-supported thiol has been developed. Mercaptoacetic acid was first protected by the dimethoxytrityl group and then anchored to Wang resin through an ester bond. Deprotection of the thiol function led to resin-supported mercaptoacetic acid, a useful supported thiol reagent that can be used in the polymer-assisted solution-phase removal of nosyl (Ns) groups from the amino function of α-amino acids in peptide synthesis.

Introduction

The deprotection of amino functions of *N*-nosyl-α-amino acids is generally performed by using sulfur nucleophiles by nucleophilic aromatic substitution.[1] The reaction is often complicated because of the difficult separation of the deprotected products from the adduct formed by the nucleophile and the aromatic derivative. For this reason the use of solid-supported thiols is important in the deprotection of the amino function of *N*-nosyl-protected amino acids. However, only one example of a resin-bound thiol used as a nucleophilic reagent for the deprotection of nitrobenzenesulfonamides has been reported.[2]

Solid-supported reagents and scavengers have been used in organic synthesis for many years, and the prominence of parallel synthesis has led to renewed interest in this group of reagents. In particular, polymer-supported thiols are used as scavengers for electrophilic reagents such as benzyl and allyl halides, aldehydes and ketones. The deprotection of Fmoc-protected amines has been realized successfully by using catalytic DBU in the presence of *N*-(2-mercaptoethyl)-aminomethylpolystyrene, which functions as a solid-supported scavenger to trap the released dibenzofulvene (DBF).[3]

Ion-exchange resins containing thiol groups directly bonded to aromatic ring resins have also been used to remove small quantities of mercury from waste water.[4]

Moreover, thiolated polymers have been employed in the development of controlled drug-release systems.[5] In aqueous solutions these modified polymers are capable of forming intramolecular disulfide bonds, thus producing matrix tablets based on thiolated polymers, which are useful as novel drug delivery systems.

Mercaptoacetic acid is generally used in solution under basic conditions for the removal of the nosyl protecting group from amine functions.[6] The carboxyl function of mercaptoacetic acid enables the separation, by appropriately varying the pH of the hydrolysis solutions, of acidic reaction products from those with a neutral or basic character.[7]

As a consequence, one approach that appears to offer considerable promise involves the attachment of mercaptoacetic acid to a suitable resin through its carboxy group; it was envisaged that an initial protection of the thiol group would take place and then a later deprotection reaction to release the SH groups, which should then act as nucleophiles. Deprotection of the thiol function just before its use prevents any oxidation processes.

Results and Discussion

To attach mercaptoacetic acid to a solid support, the former was loaded onto the Wang resin through an ester bond. The procedure required appropriate protection of the thiol function, which was realized by using the 4,4′-dimethoxytrityl protecting group. 4,4′-Dimethoxytrityl chloride (**1**) dissolved in dichloromethane was treated with mercaptoacetic acid (**2**; Scheme 1). After 6 h, the reaction mixture provided the corresponding protected product **3** in 75% yield.

Attachment of *S*-(4,4′-dimethoxytrityl)mercaptoacetic acid (**3**) through its carboxy group onto the Wang resin was performed in dimethylformamide (DMF) in the presence of a large molar excess of **3** (10:1 with respect to the free hydroxy groups). The formation of the ester bond between **3**

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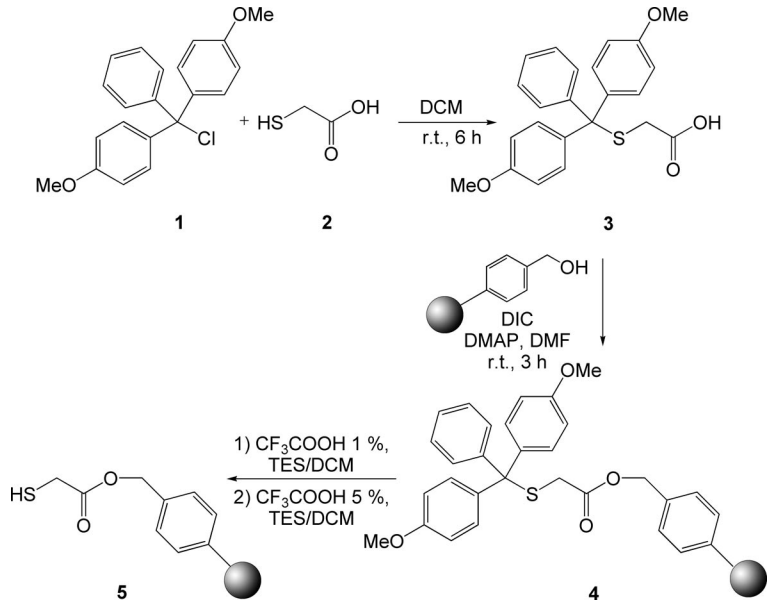


Scheme 1. Preparation of the thiol resin.

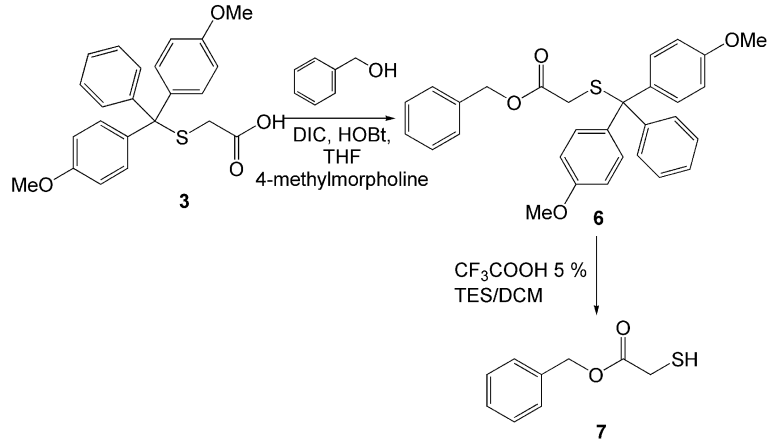
and the resin was greatly facilitated through the activation of the carboxy function by *N*,*N*′-diisopropylcarbodiimide (DIC) in the presence of 4-(dimethylamino)pyridine (DMAP) (Scheme 1).

The subsequent use of Wang-resin-bound *S*-(dimethoxytrityl)mercaptoacetic acid **4** required the unmasking of the thiol groups. To determine the appropriate conditions for the deprotection of the SH groups without affecting the ester bond, a model system was designed to mimic the removal of the trityl group in solution. For this purpose the *S*-protected mercaptoacetic acid **3** was treated with benzyl alcohol to afford benzyl *S*-(dimethoxytrityl)mercaptoacetate **6** (Scheme 2).

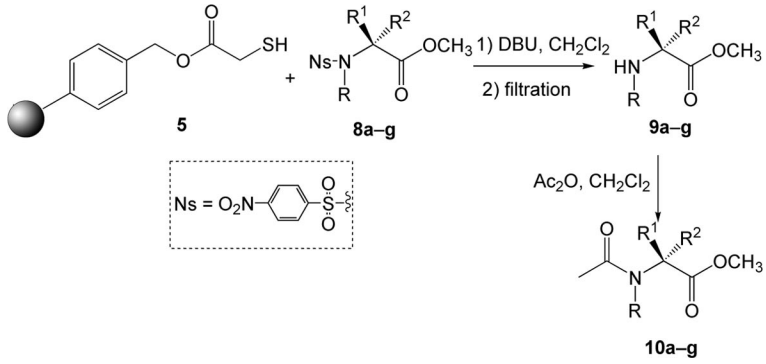
Removal of the trityl protecting group from the model system **6** was then tested by using trifluoroacetic acid in the presence of triethylsilane (TES) added as a scavenger for the stable trityl cation. Complete deprotection of the thiol-function was effected within 1 h by treatment with 5% trifluoroacetic acid (TFA) in the presence of TES. This reaction enabled the recovery of benzyl mercaptoacetate (**7**) without affecting the ester function.

In a similar fashion, the thiol resin **4** was treated with trifluoroacetic acid in dry dichloromethane in the presence of triethylsilane (Scheme 1). In this case a first cycle of 1 h with 1% TFA and a second cycle of 30 min with 5% trifluoroacetic acid resulted in the unmasking of the thiol function.

The success of this step was shown by the GC-MS analysis of the filtrate solution obtained by subjecting an aliquot of deprotected resin to 50% TFA to cleave the mercaptoacetic acid from the solid support: the chromatogram, in fact, revealed the total absence of *S*-(dimethoxytrityl)mercaptoacetic acid. This result confirms the complete deprotection of the thiol functions under mild acidic conditions (1 and 5% TFA).



Scheme 2. Removal of the trityl protecting group from the model system **6**.



Scheme 3. Use of the thiol resin for the removal of nosyl protecting groups.

To estimate the concentration of thiol groups supported on the Wang resin we used the thiol resin to deprotect the amino function of nosyl-protected amino acid methyl esters. Thus, the utility of resin-bound mercaptoacetic acid **5** was investigated in a typical experiment carried out to deprotect the α-amino function of *N*-nosyl-*N*-methylalanine methyl ester (**8a**). The reaction was performed in the presence of DBU in dichloromethane at room temperature (Scheme 3).

The first experiment was performed by using *N*-nosyl-*N*-methylalanine methyl ester (**8a**) in a 1:1 molar ratio with respect to the total active groups of the resin, assuming that the loading of the Wang resin with mercaptoacetic acid was quantitative. In this case the deprotection reaction of **8a** was not complete. A second reaction was then attempted by using a 0.8:1 ratio of **8a** with respect to the resin **5**. Again, in this experiment the reaction, monitored by TLC analysis, was not complete after 2 h. However, by using a 0.7:1 ratio of **8a**, complete deprotection of the starting substrate **8a** had occurred after 1 h with the formation of *N*-methylalanine methyl ester **9a**. Finally, the *N*-methylalanine methyl ester was recovered after acetylation of the free amino function. Treatment of the α-amino acid methyl ester with acetic anhydride afforded the *N*-acetylated derivative in quantitative yield (Scheme 3).

On the basis of the stoichiometry of the deprotection reaction, the equivalents of acetylated amino acid obtained correspond to the equivalents of thiol groups on the resin. Therefore it was possible to quantify the loading of resin-bound free thiol groups, which was at least 0.77 mmol/g.

This solid-supported solution-phase reaction adopted for the removal of the nosyl protecting group is advantageous in comparison with the reaction performed in solution. In fact, the excess reagents and byproducts trapped on the solid support are readily separated from the reaction mixture by filtration without need of conventional chromatographic purification of any intermediates. In addition, it is possible to monitor the reaction in real time by using conventional techniques, such as TLC and GC-MS analyses.

Subsequently, we sought to extend this methodology to other *N*-nosyl-protected amino acids. *N*-Nosyl-α-amino acid methyl esters **8b**–**g** were treated with the thiol resin **5** in 0.7:1 molar ratios under the same reaction conditions as adopted for **8a** (Table 1, Scheme 3). The *N*-acetylated products **10b**–**g** were obtained in quantitative yields.

Table 1. *N*-Nosyl-α-amino acid methyl esters used in the deprotection reaction.

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

Furthermore, the GC-MS and ^{1}H NMR analyses of the single crude products **10f** and **10g** revealed the total absence of optically inverted amino acid derivatives, the diastereoisomers **10g** and **10f**, respectively, thus confirming the retention of chiral integrity of the C-α stereocentres during the deprotection of the α-amino function of the *N*-nosyl amino acid methyl esters. The feasibility of the complete separation of **10g** and **10f** was confirmed by GC-MS analysis of an opportunely prepared mixture of **10g** (20 mg) and **10f** (20 mg).

Conclusions

In the light of these results, we can confirm the loading of resin-bound free thiol groups corresponding to 70% of the active groups of the starting Wang resin. Resin-bound mercaptoacetic acid **5** was obtained with a loading of 0.77 mmol/g. The usefulness of our resin has been successfully tested in the deprotection of the amino function of *N*-nosyl-protected α-amino acids.

The prepared solid-supported mercaptoacetic acid, combining the advantages inherent to both solid- and solution-phase synthesis, could make a substantial contribution to polymer-assisted solution-phase synthesis.

Experimental Section

General: Solvents were purified and dried by standard procedures and distilled prior to use. Commercially available reagents were

purchased from Aldrich Chemical Co. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, with a Bruker Avance 300 spectrometer by using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm, coupling constants (J) are reported in Hertz (Hz). Reaction mixtures were monitored by TLC using Merck silica gel $60F_{254}$ precoated glass plates. GC-MS analyses were performed with an HP-5MS instrument (30 m × 0.25 mm, PhMesiloxane capillary column). The mass detector was operated in the electron impact ionization mode (EI-MS) with an electron energy of 70 eV. Wang resin was purchased from Senn Chemicals (loading capacity 1.1 mmol/g).

Synthesis of *S*-(4,4′-Dimethoxytrityl)mercaptoacetic Acid (3): 4,4′-Dimethoxytrityl chloride (**1**; 1.50 g, 4.4 mmol) was added to a solution of mercaptoacetic acid (**2**; 0.3 mL, 4.4 mmol) in dry dichloromethane (20 mL). The resulting reaction mixture was stirred at room temperature and maintained under dry nitrogen for 6 h. The course of the reaction was monitored by TLC (chloroform/methanol, 90:10). Evaporation of the solvent afforded the corresponding *S*-(4,4′-dimethoxytrityl)mercaptoacetic acid (**3**) as an orange oil (1.30 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 9.50 (br. s, 1 H, COOH), 7.49–7.19 (m, 9 H, Ar-H), 6.90–6.80 (m, 4 H, Ar-H), 3.80 (s, 6 H, OCH_3), 3.06 (s, 2 H, 2-H_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.61, 158.29, 144.32, 136.23, 130.68, 129.26, 128.08, 126.87, 113.34, 66.01, 55.25, 34.56 ppm. $C_{23}H_{22}O_4S$ (394.48): calcd. C 70.03, H 5.82; found C 70.16, H 5.84.

Attachment of Mercaptoacetic Acid 2 to Wang Resin

Swelling of the Resin: A sample of the Wang resin (0.30 g, 1.1 mmol/g) was placed in a dried 20 mL glass reaction vessel, shaken in dry DMF (6 mL) for 1 h, and drained.

Attachment of *S*-Dimethoxytritylmercaptoacetic Acid 3 to the Wang Resin Through an Ester Linkage: *S*-(Dimethoxytrityl)mercaptoacetic acid (**3**, 1.30 g, 3.3 mmol) was dissolved in dry DCM (10 mL) and cooled in an ice bath. Diisopropylcarbodiimide (DIC; 3.3 mmol) dissolved in DCM (5 mL) was added, and the reaction mixture was stirred for 20 min. The DCM was removed under reduced pressure; the residue was dissolved in DMF (3 mL) and added to the resin. 4-(Dimethylamino)pyridine (DMAP; 0.03 mmol) dissolved in DMF (1 mL), was added and the reaction mixture was shaken at room temperature. for 3 h The operation was repeated for a further two cycles, after which the resin was washed with DCM (5 × 2 min), then with 2-propanol (IPA; 5 × 2 min) and then again with DCM (5 × 6 min).

Removal of the DMT Group: DCM/TES (95:5, 10 mL) and 1% TFA were added to the thiol resin, and the mixture was shaken for 1 h. It was then filtered, and the resin was treated with neat DCM/TES (95:5, 10 mL) and 5% TFA for 30 min. The resin was drained, washed with DCM (5 × 1 min), IPA (5 × 1 min), methanol (5 × 1 min), IPA (5 × 1 min) and DCM (5 × 1 min).

Synthesis of Benzyl *S*-(4,4′-Dimethoxytrityl)mercaptoacetate (6): *S*-(Dimethoxytrityl)mercaptoacetic acid (**3**, 0.39 g, 1 mmol) was added in one portion to a solution of benzyl alcohol (0.10 mL, 1 mmol), 1-hydroxybenzotriazole (0.15 g, 1.1 mmol) and 4-methylmorpholine (0.11 mL, 1 mmol) in dry THF (20 mL). This mixture was stirred under nitrogen and cooled in an ice bath. Then a slight excess of diisopropylcarbodiimide (0.18 mL, 1.15 mmol) was added, and the mixture was stirred for 1 h. The ice bath was removed, and the mixture was stirred at room temperature for a further 3 h, while monitoring the reaction by TLC (diethyl ether/petroleum ether, 60:40). The *N*,*N*-diisopropylurea was then filtered off and the solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL). After drying with anhydrous Na_2SO_4, the solvent was removed under reduced pressure to give **6** as a white solid (0.387 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.42–7.18 (m, 14 H, Ar-H), 6.85–6.75 (m, 4 H, Ar-H), 5.01 (s, 2 H, OCH_2Ph), 3.79 (s, 6 H, OCH_3), 3.03 (s, 2 H, SCH_2) ppm. $C_{30}H_{28}O_4S$ (484.61): calcd. C 74.35, H 5.82; found C 74.49, H 5.84.

Deprotection of Benzyl *S*-(4,4′-Dimethoxytrityl)mercaptoacetate (6): Benzyl *S*-(dimethoxytrityl)mercaptoacetate (**6**, 0.48 g, 1 mmol) was dissolved in dry DCM/TES (95:5) (10 mL) and 5% TFA (1 mL). The solution was stirred at room temperature for 1 h. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was washed with methanol. Purification by column chromatography on silica gel (diethyl ether/petroleum ether, 60:40) afforded benzyl mercaptoacetate (**7**) as an oil (0.16 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.23–7.15 (m, 5 H, Ar-H), 5.18 (s, 2 H, OCH_2), 3.30 (d, J = 8.4 Hz, 2 H, SCH_2), 2.02 (t, J = 8.1 Hz, 1 H, SH) ppm. GC-MS (EI): m/z (%) = 182 (1) $[M]^{+\cdot}$, 123 (30), 105 (2), 91 (100), 77 (6), 65 (10), 47 (4). $C_9H_{10}O_2S$ (182.24): calcd. C 59.32, H 5.53; found C 59.43, H 5.55.

Use of Wang-Resin-Bound Mercaptoacetic Acid for the Removal of Nosyl Protecting Groups

Deprotection of *N*-Nosyl-*N*-methylalanine Methyl Ester (8a): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol, assuming that the loading of Wang resin with mercaptoacetic acid was quantitative) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosyl-*N*-methylalanine methyl ester (**8a**) (0.10 g, 0.33 mmol) in dry DCM (20 mL). The reaction, monitored by TLC (diethyl ether/petroleum ether, 60:40), did not provide the complete conversion of **8a** into **9a** after 2 h. Another experiment was performed by adding resin-bound mercaptoacetic acid **5** and DBU (0.98 mL, 0.66 mmol) to a solution of 0.080 mg (0.26 mmol) of *N*-nosyl-*N*-methylalanine methyl ester (**8a**) in dry DCM. Also in this case, after 2 h, the reaction was not complete. An additional experiment was performed by adding resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) to a solution of *N*-nosyl-*N*-methylalanine methyl ester (**8a**, 0.07 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h to lead to the complete removal of the nosyl group. Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates, which contained the *N*-methylalanine methyl ester (**9a**), were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetyl-*N*-methylalanine Methyl Ester (10a): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9a** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-*N*-methylalanine methyl ester (**10a**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.25 (q, J = 7.5 Hz, 1 H, 2-H), 3.70 (s, 3 H, OCH_3), 2.96 (s, 3 H, N-CH_3), 2.12 (s, 3 H, CH_3CO), 1.38 (d, J = 7.5 Hz, 3 H, 3-H_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.50, 171.30, 55.98, 51.69, 32.19, 21.88, 14.52 ppm. GC-MS (EI): m/z (%) = 159 (4) $[M]^{+\cdot}$, 128 (1), 116 (5), 100 (53), 58 (100), 43 (17). $C_7H_{13}NO_3$ (159.18): calcd. C 52.82, H 8.23, N 8.80; found C 52.92, H 8.26, N 8.78.

Deprotection of *N*-Nosylleucine Methyl Ester (8b): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosylleucine methyl ester

(**8b**) (0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetylleucine Methyl Ester (10b): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **8b** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetylleucine methyl ester (**10b**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.20 (d, J = 8.1 Hz,1 H, NH), 4.60 (m, 1 H, 2-H), 3.69 (s, 3 H, OCH_3), 2.02 (s, 3 H, CH_3CO), 1.69–1.44 (m, 3 H, 2-H_2, 3-H), 0.92–0.89 [m, 6 H, $(CH_3)_2CH$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.84, 170.05, 52.27, 50.69, 41.54, 24.82, 23.05, 22.80, 21.91 ppm. GC-MS (EI): *m*/*z* (%) = 187 (2) $[M]^{+\cdot}$, 144 (4), 128 (76), 99 (16), 86 (100), 43 (46). $C_9H_{17}NO_3$ (187.24): calcd. C 57.73, H 9.15, N 7.48; found C 57.84, H 9.18, N 7.46.

Deprotection of *N*-Nosylvaline Methyl Ester (8c): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosylvaline methyl ester (**8c**; 0.073 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetylvaline Methyl Ester (10c): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9c** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetylvaline methyl ester (**10c**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.05 (br. s, 1 H, NH), 4.57 (dd, J = 4.8, J = 8.7 Hz, 1 H, 2-H), 3.74 (s, 3 H, OCH_3), 2.22–2.06 (m, 1 H, 3-H), 2.04 (s, 3 H, CH_3CO), 0.93 [d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$], 0.90 [d, J = 6.9 Hz, 3 H, $CH(CH_3)_2$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.21, 170.52, 57.02, 52.13, 31.22, 23.24, 18.87, 17.84 ppm. GC-MS (EI): *m*/*z* (%) = 173 (0.20) $[M]^{+\cdot}$, 142 (0.45), 131 (4.5), 114 (77), 99 (23), 88 (55), 72 (100). $C_8H_{15}NO_3$ (173.21): calcd. C 55.47, H 8.73, N 8.09; found C 55.57, H 8.76, N 8.07.

Deprotection of *N*-Nosylphenylalanine Methyl Ester (8d): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosylphenylalanine methyl ester (**8d**; 0.084 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetylphenylalanine Methyl Ester (10d): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9d** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetylphenylalanine methyl ester (**10d**) as a white solid in quantitative yield. M.p. 85–88 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.07–7.33 (m, 5 H, Ar-H), 6.01 (d, J = 7.12 Hz, 1 H, NH), 4.09 (m, 1 H, 2-H), 3.73 (s, 3 H, OCH_3), 3.18 (dd, J = 5.82, J = 13.82 Hz, 1 H, 3-H_2), 3.07 (dd, J = 5.97, J = 13.82 Hz, 1 H, 3-H_2), 1.99 (s, 3 H, CH_3CO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.14, 169.67, 135.81, 129.26, 128.61, 127.17, 53.11, 52.39, 37.82, 23.18 ppm. GC-MS (EI): *m*/*z* (%) = 221 (0.5) $[M]^{+\cdot}$, 162 (100), 131 (29), 120 (45), 91 (48), 88 (86), 43 (76). $C_{12}H_{15}NO_3$ (221.25): calcd. C 65.14, H 6.83, N 6.33; found C 65.22, H 6.86, N 6.31.

Deprotection of *N*-Nosylalanine Methyl Ester (8e): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosylalanine methyl ester (**8e**; 0.066 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetylalanine Methyl Ester (10e): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9e** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetylalanine methyl ester (**10e**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.15 (br. s, 1 H, NH), 4.60 (m, 1 H, 2-H), 3.75 (s, 3 H, OCH_3), 2.03 (s, 3 H, CH_3CO), 1.40 (d, J = 7.2 Hz, 3 H, 3-H_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.71, 171.62, 52.54, 48.02, 23.21, 18.60 ppm. GC-MS (EI): *m*/*z* (%) = 145 (5) $[M]^{+\cdot}$, 102 (3), 86 (72), 59 (3), 44 (100). $C_6H_{11}NO_3$ (145.16): calcd. C 49.65, H 7.64, N 9.65; found C 49.73, H 7.67, N 9.63.

Deprotection of *N*-Nosylisoleucine Methyl Ester (8f): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosylisoleucine methyl ester (**8f**; 0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetylisoleucine Methyl Ester (10f): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9f** (0.23 mmol) in dry DCM (10 mL) and the mixture stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetylisoleucine methyl ester (**10f**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.4 (d, J = 9.14 Hz, 1 H, NH), 4.66 (dd, J = 4.25, J = 9.14 Hz, 1 H, 2-H), 3.68 (s, 3 H, OCH_3), 1.99 (s,

3 H, CH_3CO), 1.82 (m, 1 H, 3-H), 1.30–1.42 (m, 2 H, 4-H_2), 0.90–1.05 (m, 6 H, 3-CH_3, 5-H_3) ppm. GC-MS (EI): *m*/*z* (%) = 187 (0.3) $[M]^{+\cdot}$, 131 (25), 128 (96), 99 (69), 88 (60), 86 (100), 43 (85). $C_9H_{17}NO_3$ (187.24): calcd. C 57.73, H 9.15, N 7.48; found C 57.82, H 9.17, N 7.46.

Deprotection of *N*-Nosyl-D-alloisoleucine Methyl Ester (8g): Resin-bound mercaptoacetic acid **5** (0.3 g, 0.33 mmol) and DBU (0.98 mL, 0.66 mmol) were added to a solution of *N*-nosyl-D-alloisoleucine methyl ester (**8g**; 0.076 g, 0.23 mmol) in dry DCM (20 mL). The reaction mixture was shaken at room temperature for 1 h, and the removal of the nosyl group was monitored by TLC (diethyl ether/petroleum ether, 60:40). Then the resin was drained and washed with DCM (5 × 1 min), 2-propanol (5 × 1 min) and DCM (5 × 1 min). The combined filtrates were concentrated to dryness under reduced pressure.

Synthesis of *N*-Acetyl-D-alloisoleucine Methyl Ester (10g): Acetic anhydride (0.1 mL, 1.15 mmol) was added to a solution of **9g** (0.23 mmol) in dry DCM (10 mL) and stirred at room temperature for 4 h. The reaction mixture was then acidified with 1 N hydrochloride acid and extracted with DCM (3 × 10 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford the corresponding *N*-acetyl-D-alloisoleucine methyl ester (**10g**) as a colourless oil in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.4 (d, *J* = 8.71 Hz, 1 H, NH), 4.55 (dd, *J* = 5.24, *J* = 8.71 Hz, 1 H, 2-H), 3.68 (s, 3 H, OCH_3), 1.97 (s, 3 H, CH_3CO) 1.82 (m,1 H, 3-H), 1.30–1.42 (m, 2 H, 4-H_2), 0.90–1.05 (m, 6 H, 5-H_3) ppm. GC-MS (EI): *m*/*z* (%) = 131 (25), 128 (86), 99 (70), 88 (55), 86 (100), 43 (85). $C_9H_{17}NO_3$ (187.24): calcd. C 57.73, H 9.15, N 7.48; found C 57.82, H 9.17, N 7.46.

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