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C → *N* and *N* → *C* solution phase peptide synthesis using the *N*-acyl 4-nitrobenzenesulfonamide as protection of the carboxylic function†

Rosaria De Marco, Mariagiovanna Spinella, Anna De Lorenzo, Antonella Leggio* and Angelo Liguori*

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In this paper we describe a solution phase peptide synthesis strategy using the 4-nitrobenzenesulfonamido/*N*-methyl-4-nitrobenzenesulfonamido group as a protecting/activating system of the carboxyl function. The 4-nitrobenzenesulfonamido group is stable during peptide chain elongation (Fmoc chemistry). The *N*-aminoacyl or *N*-dipeptidyl-4-nitrobenzensulfonamides, when activated by methylation, can be easily coupled with another amino acid or reconverted into the free-carboxyl function amino acids or peptides. This activatable protecting group allows both the *C* → *N* and the *N* → *C* direction solution phase peptide synthesis. We also verified that the absolute configuration at the chiral centers does not change during the coupling reactions.

Introduction

A solution phase peptide synthesis is typically carried out in the *C* → *N* direction by sequential incorporation of N^{α}-protected amino acids followed by removal of the protecting group on the amino function. In this strategy, the protecting group of the carboxyl function should be stable during the peptide elongation and easily removed at the end of the synthesis. In contrast, the *N* → *C* direction peptide synthesis is rarely used because of the intrinsic danger of epimerization at the C-terminal amino acid *via* oxazolone formation.[1] Nonetheless the reverse strategy has the advantage to directly generate C-terminal modified peptides. Many of these are biologically relevant and show potential therapeutic properties.[2] Only a few synthetic strategies for solid phase peptide synthesis have been provided to couple in the reverse *N* → *C* direction and all these examples give racemization at the chiral centers.[3]

The choice of a suitable activating group of the carboxylic function is a critical step in the reverse peptide synthesis.

A carboxyl function protection which is stable during elongation in the *C* → *N* direction and that can be opportunely converted into an activating group of the same function, allowing elongation in the *N* → *C* direction or easy deprotection of the carboxyl function at the end of the synthesis, could represent an important improvement in the solution phase peptide synthesis, especially for the assembly of a large peptide using convergent peptide synthesis.

An *N*-acylsulfonamide bond was successfully used in solid phase peptide synthesis. An alkanesulfonamide "safety-catch" linker has been developed by Kenner for tethering carboxylic groups of α-amino acids to the support.[4] Acylation of the sulfonamide support provides a support-bound *N*-acylsulfonamide that is stable to both basic and strongly nucleophilic reaction conditions. At the end of a solid-phase synthesis of a peptide sequence, the acylsulfonamide function can be *N*-alkylated to become cleavable under mild reaction conditions.[5] An alkyl sulfonamide resin was used by Triola *et al.* for the synthesis of lipidated Ras peptides.[6] This strategy was based on the release of the peptide from the resin, previously activated by alkylation, using the amine functionality of the C-terminal amino acid as a nucleophile.

On the other hand, in solution phase peptide synthesis, the *p*-nitrobenzenesulfonyl group is commonly employed as a protecting group of the amino function when this last needs to be activated for the alkylation. This strong electron-withdrawing masking group, in fact, enhances the reactivity of the N–H function toward diazomethane or other alkylating agents allowing the easy formation of the species responsible for alkylation.[7–10] The *N*-alkylation of the *p*-nitrobenzenesulfonamide function enables easy deprotection of the amino function due to the absence of an acidic proton on the nitrogen atom.[7]

Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Ed. Polifunzionale, 87036, Arcavacata di Rende, Italy.
E-mail: a.liguori@unical.it; Fax: +39 0984 493265; Tel: +39 0984 493205

†Electronic supplementary information (ESI) available: ESI features detailed analytical data of all compounds. Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds **2**, **4a–j**, **5a–i**, **7a–i**, **8j**, **11j** and of ^{1}H NMR spectra of **9j** and **10j**. See DOI: 10.1039/c3ob40169c

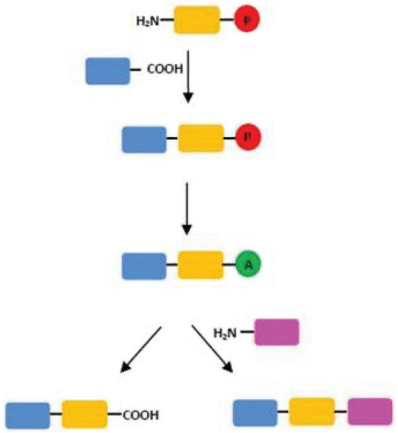


Fig. 1 Graphical description of an "activatable protecting group" of carboxyl function in convergent solution phase peptide synthesis.

Based on a previous consideration we decided to use the *N*-acylsulfonamide function in solution-phase peptide synthesis exploiting its structural and reactivity properties to design an "activatable protecting group" of the carboxyl function. This group should be stable during peptide chain elongation and should be easily converted into an activated group to allow the regeneration of the free carboxyl function or the introduction of another amino acid or peptide on the C-terminal (Fig. 1).

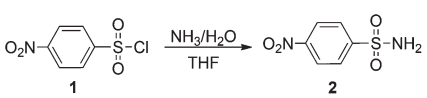
In particular, we chose as a protecting group of the carboxyl function the *p*-nitrobenzensulfonamido group, obtaining the corresponding *N*-acyl 4-nitrobenzenesulfonamide. The easy *N*-alkylation of the latter could convert the sulfonamide function into a group activated towards the nucleophilic acyl substitution.

Results and discussion

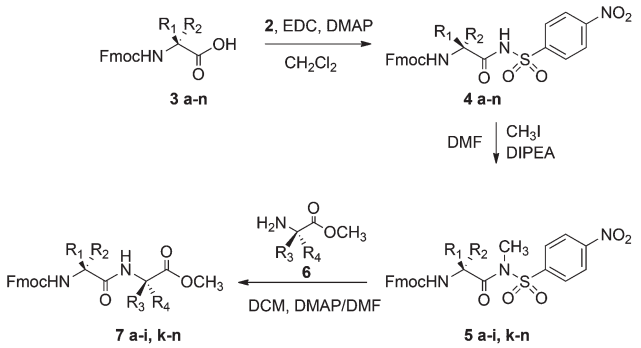
In order to verify the suitability of the *p*-nitrobenzensulfonamido group as a protecting group of the carboxylic function and of the *N*-methyl-*p*-nitrobenzensulfonamido group as an activating group of the carboxyl function in solution phase peptide synthesis, we chose to synthesize a series of dipeptides to test and validate this protecting/activating reagent system.

In this field we attempted to generate *N*-acyl 4-nitrobenzenesulfonamides of α-amino acids Fmoc-protected on amino function.

First, following a classical literature procedure,[11] we prepared 4-nitrobenzenesulfonamide (nosylamide) by treatment of 4-nitrobenzenesulfonyl chloride (nosyl chloride) with an aqueous solution of ammonia (Scheme 1).



Scheme 1 Synthesis of 4-nitrobenzenesulfonamide (**2**).



Scheme 2 Synthesis of *N*-Fmoc-α-aminoacyl-4-nitrobenzenesulfonamides (**4a–n**), *N*-Fmoc-α-aminoacyl-*N*-methyl-4-nitrobenzenesulfonamides (**5a–i, k–n**), and *N*-Fmoc-dipeptide methyl esters (**7a–i, k–n**).

The 4-nitrobenzenesulfonamide **2** was obtained in excellent yield (97%) and the spectroscopic data confirmed its purity. The obtained 4-nitrobenzenesulfonamide was used to synthesize *N*-acylsulfonamide derivatives. *N*-Fmoc-L-valine (**3a**) was chosen as a model system and treated with 4-nitrobenzenesulfonamide **2** in the presence of the condensing agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and the dimethylaminopyridine (DMAP) as the base (Scheme 2). The corresponding *N*-Fmoc-L-valine 4-nitrobenzenesulfonamide (**4a**) was recovered in high yield after 1 h stirring at room temperature (Table 1). In order to verify its activatability by *N*-alkylation in solution phase, we decided to methylate the sulfonamide nitrogen atom of **4a**. To achieve this aim, we treated *N*-Fmoc-L-valinyl-4-nitrobenzenesulfonamide (**4a**) with methyl iodide and diisopropylethyl amine (DIPEA) for 1 h at room temperature. After a simple work-up, the corresponding *N*-methyl derivative **5a** was recovered in very good yield (85%, Table 1). The subsequent coupling of the C-activated Fmoc-L-valine **5a** with the L-alanine methyl ester (**6a**) required the use of a base (DMAP) and warming (Scheme 2). The completion of the reaction was monitored by TLC. After column chromatography, the corresponding dipeptide **7a** was recovered in good yield and high purity (Table 1). In light of the successful result and to validate this carboxylic function activation procedure, the same reactions were applied to a series of *N*-Fmoc-α-amino acids (**3b–n**, Table 1). In each experiment the corresponding dipeptide was obtained in good yield. These results make the 4-nitrobenzenesulfonamido group methylated on a nitrogen atom a good activating agent of the carboxyl function. The coupling, in fact, proceeds successfully also in the case of amino acids sterically hindered or functionalized in the side chain (see Table 1).

We also wanted to investigate whether the configuration of the amino acid chiral centers was retained during the coupling reaction by synthesizing various couples of diastereoisomeric dipeptides: the Fmoc-L-valinyl-L-alanine methyl ester (**7a**)/Fmoc-L-valinyl-D-alanine methyl ester (**7e**), the Fmoc-L-phenylalanyl-L-alanine methyl ester (**7b**)/Fmoc-D-phenylalanyl-L-alanine methyl ester (**7f**), the Fmoc-L-valinyl-L-alanine methyl ester (**7a**)/Fmoc-D-valinyl-L-alanine methyl ester (**7k**), the Fmoc-

Table 1 Results of the reactions of synthesis of *N*-Fmoc-α-aminoacyl-4-nitrobenzenesulfonamides (**4a–i, k–n**), *N*-Fmoc-α-aminoacyl-*N*-methyl-4-nitrobenzenesulfonamides (**5a–i, k–n**), and *N*-Fmoc-dipeptide methyl esters (**7a–i, k–n**)

Entry	R^1	R^2	R^3	R^4	Yield **4** (%)	Yield **5** (%)	Yield **7** (%)
a	$CH(CH_3)_2$	H	H	CH_3	95	85	70
b	CH_2Ph	H	H	CH_3	94	86	68
c	CH_3	H	H	$CH_2CH(CH_3)_2$	80	92	75
d	$CH_2CH(CH_3)_2$	H	H	CH_3	85	90	76
e	$CH(CH_3)_2$	H	CH_3	H	95	85	72
f	H	CH_2Ph	H	CH_3	90	80	70
g	CH_2OtBu	H	H	CH_3	92	84	80
h	$CH_2C_6H_4OtBu$	H	H	CH_3	86	85	74
i	$CH_2CH_2CO_2tBu$	H	H	CH_3	80	87	75
k	H	$CH(CH_3)_2$	H	CH_3	90	82	75
l	CH_3	H	H	$CH(CH_3)_2$	80	92	78
m	H	CH_3	H	$CH(CH_3)_2$	82	89	76
n	H	CH_2OtBu	H	CH_3	89	82	82

L-alanyl-L-valine methyl ester (**7l**)/Fmoc-D-alanyl-L-valine methyl ester (**7m**), the Fmoc-L-serinyl(*Ot*Bu)-L-alanine methyl ester (**7g**)/Fmoc-D-serinyl(*Ot*Bu)-L-alanine methyl ester (**7n**). Coupling of compounds **5** with compounds **6** was performed under the conditions usually adopted for this methodology. Each crude dipeptide was analyzed by ^{1}H NMR.

However, if any racemization occurs during the peptide coupling, that would have been on the C-activated amino acid.

Therefore we graphically compared the ^{1}H NMR signals relative to the two diastereoisomers Fmoc-L-phenylalanyl-L-alanine methyl ester (**7b**) and Fmoc-D-phenylalanyl-L-alanine methyl ester (**7f**) (Fig. 2). We observed a good separation of some signals, in particular of the signal at 6.43 and 6.16 ppm relative to the amidic proton and of the doublets relative to the methyl side chain of alanine at 1.23 and 1.35 ppm.

This last result was confirmed by overlapping ^{1}H NMR spectra of another diasteroisomeric couple: **7a**/**7k** (Fig. 3). In this case a good separation of signals relative to the amidic proton at 6.27 and 6.76 ppm, to the urethane protons at 5.40 and 5.58 ppm and to the α-proton of valine at 4.02 and 4.11 ppm was observed.

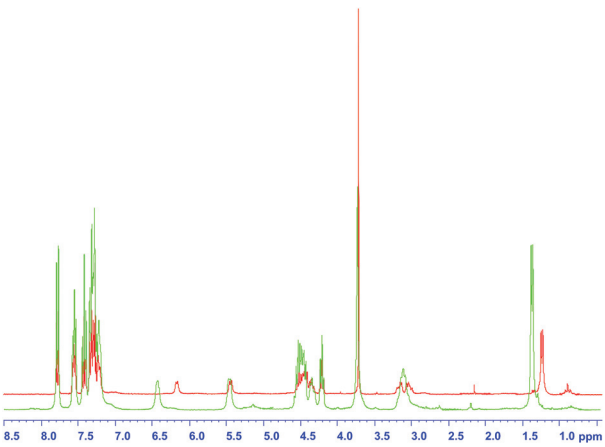


Fig. 2 Overlapped ^{1}H NMR spectra of crude Fmoc-L-phenylalanyl-L-alanine methyl ester (**7b**, green) and crude Fmoc-D-phenylalanyl-L-alanine methyl ester (**7f**, red).

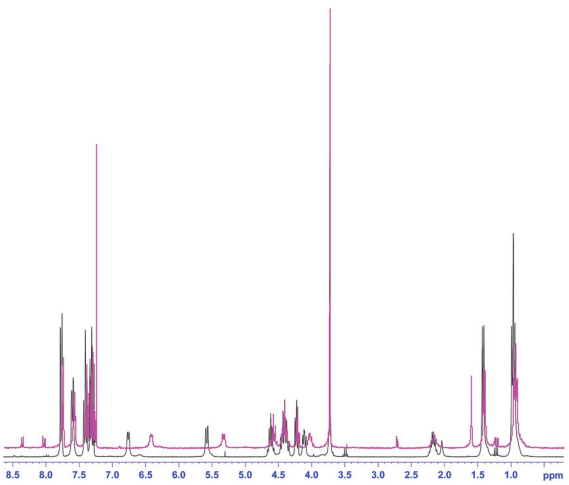


Fig. 3 Overlapped ^{1}H NMR spectra of crude Fmoc-L-valinyl-L-alanine methyl ester (**7a**, pink) and crude Fmoc-D-valinyl-L-alanine methyl ester (**7k**, black).

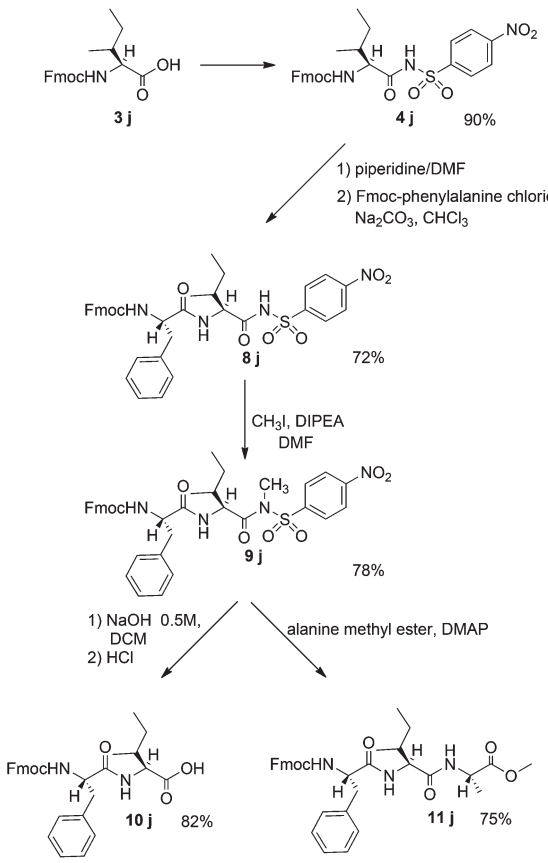
^{1}H NMR analysis performed on the couples of diastereoisomeric systems **7b**/**7f**, **7a**/**7k**, **7l**/**7m** and **7g**/**7n** showed that the absolute configuration of the C-activated amino acids used remains unchanged.

Furthermore, a sample containing a mixture of crude Fmoc-L-valinyl-L-alanine methyl ester (**7a**, 11 mg) and Fmoc-L-valinyl-D-alanine methyl ester (**7e**, 19 mg) was analyzed by ^{1}H-NMR and the corresponding spectrum showed the complete separation of the signal at 6.27 and 6.46 ppm relative to the amidic proton of the two dipeptides.

Within the limits of the ^{1}H NMR methodology used, all these data confirmed the absence of any signal relative to possible epimers in the spectra of the single crude dipeptide systems analyzed and, in this way, showed the total retention of configuration of the chiral centers during the coupling reaction.

The absence of racemization observed is probably due to the rapid coupling reaction under mild conditions, which avoids possible side reactions. In fact, a similar coupling reaction reported in the literature, performed using a sulfonamide resin *N*-alkylated, furnished dipeptides with a 25% racemization.[6]

After verifying the possibility of activation of the *N*-acyl sulfonamide group towards acyl nucleophilic substitution through *N*-alkylation, we wanted to confirm the stability of the same group unmethylated under the conditions usually adopted in solution-phase peptide synthesis (Fmoc-chemistry). As a consequence, the *N*-Fmoc-L-isoleucinyl-4-nitrobenzenesulfonamide (**4j**) was synthesized as previously described. **4j** was then deprotected on the amino function using a solution of pyperidine in DMF. The corresponding isoleucinyl-4-nitrobenzenesulfonamide free on the amino function was immediately coupled with the *N*-Fmoc-L-phenylalanine activated as a chloride. The dipeptide *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide **8j** was recovered in high yield (Scheme 3, 72%). This confirmed the stability of *N*-acyl sulfonamide towards Fmoc deprotection and coupling condition and, in this way, the suitability of the 4-nitrobenzenesulfonamido group as a protecting group of the carboxyl function in peptide synthesis. The subsequent *N*-methylation of the sulfonamido nitrogen of **8j** gave the corresponding methylated *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide (**9j**), a dipeptide activated on the carboxyl function (Scheme 3). In this form the peptide could be enabled for the coupling with another amino acid in the $N \rightarrow C$ direction or for the basic hydrolysis to obtain the free carboxyl function. To confirm this trend, we performed two experiments in parallel. In a first experiment **9j** was treated with the L-alanine methyl ester (Scheme 3) adopting the coupling condition previously developed. The tripeptide methyl ester **11j** was recovered in high yield (75%, Scheme 3). Another reaction was performed to verify the activation of carboxyl function also towards basic hydrolysis conditions. In this case, the same activated dipeptide **9j** was treated with a 0.5 M aqueous solution of NaOH (Scheme 3). After a 2 h reaction at room temperature, the dipeptide *N*-Fmoc-L-phenylalanyl-L-lisoleucine (**10j**) was recovered in very good yield (82%).



Scheme 3 Synthesis of *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide (**8j**), its conversion into *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-*N*-methyl-4-nitrobenzenesulfonamide (**9j**) and the basic hydrolysis to obtain the dipeptide **10j** or the coupling in $N \rightarrow C$ direction to obtain the tripeptide methyl ester **11j**.

The ^{1}H NMR spectrum of the recovered product **10j** was identical to that of an authentic sample of *N*-Fmoc-L-phenylalanyl-L-lisoleucine.

Moreover, the ^{1}H NMR spectrum of **10j** showed the presence of signals corresponding to only one diastereomer.

Our attention then turned to the study of the coupling reaction in the $N \rightarrow C$ direction and, in particular, to the preservation of configuration of chiral centers. It is well known, in fact, that peptide synthesis should be performed in the $C \rightarrow N$ direction because of the troubles relative to the formation of an oxazolone derivative and the consequent partial enantiomerization of the activated residue during peptide elongation when the latter is performed in the $N \rightarrow C$ direction, which leads to the production of an amount of epimerized peptide in addition to the desired product.

The spectroscopic analysis of the crude tripeptide **11j** showed a total absence of signal relative to the corresponding epimer. Both ^{1}H NMR and ^{13}C NMR spectra, in fact, had signals relative to proton and carbon atoms of only one diasteroisomer. These data confirmed the total retention of configuration of the chiral centers during the coupling of the growing peptide chain with an amino acid in the $N \rightarrow C$ direction. This last experiment confirmed the usefulness of the *N*-acyl-*N*-methyl-4-nitrobenzenesulfonamide function as an activating agent of the carboxyl function also for the carboxyl function of peptide and, in this way, its crucial role in $N \rightarrow C$ direction peptide synthesis.

Conclusions

In this paper we adopted a protection strategy of the carboxyl function of α-amino acids in solution phase peptide synthesis, which is of great utility for the obtainment of peptides in the $C \rightarrow N$ direction and in the $N \rightarrow C$ direction such as in convergent peptide synthesis.

In the procedure, the 4-nitrobenzenesulfonamido group was used as an "activatable protecting group" in the synthesis of short peptides in solution phase. The methodology is effective and achieves peptides in high yield and does not show any inversion of configuration at the chiral centers under the adopted analytical conditions. The success of the activating agent is attributed to the use of nosyl group as the

aromatic moiety of the sulfonamide, a good leaving group which allows a rapid coupling reaction.

Experimental section

General

Solvents were purified and dried by standard procedures and distilled prior to use. Commercially available reagents were purchased by Aldrich Chemical Co. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker Avance 300 spectrometer by using $CDCl_3$ and DMSO-d_6 as the solvents. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (J) are reported in hertz (Hz). Reaction mixtures were monitored by TLC using Merck Silica gel 60-F_{254} precoated glass plates, and UV light (254 nm) or 0.2% ninhydrin in ethanol and charring as a visualizing agent. Kieselgel 60H without gypsum was used for flash column chromatography.

Synthesis of 4-nitrobenzenesulfonamide (2)

4-Nitrobenzenesulfonyl chloride (**1**, 1.35 mmol) dissolved in 15 mL of dry THF was added dropwise to a 37% aqueous solution of ammonia (5.4 mmol) in a two-necked round-bottomed flask (100 mL) immersed in an ice bath. The reaction, monitored by TLC (chloroform–methanol, 95 : 5, v/v), was completed after 1 h. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in a 1 N aqueous solution of HCl (10 mL). The aqueous solution was extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, washed with brine solution (1 × 10 mL), dried with Na_2SO_4, and filtered. Evaporation of the solvent afforded the 4-nitrobenzenesulfonamide (**2**) in 97% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (s, NH_2, 2H), 8.06 (d, J = 8.7 Hz, Ar-H, 2H), 8.41 (d, J = 8.7 Hz, Ar-H, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 124.89, 128.21, 149.67, 149.90. Anal. Calcd for $C_6H_6N_2O_4S$ (202.19): C, 35.64; H, 2.99; N, 13.86%. Found: C, 35.72; H, 3.00; N, 13.90%.

Synthesis of *N*-Fmoc-α-aminoacyl-4-nitrobenzene sulfonamides (4a–n)

N-Fmoc amino acid (**3a–n**, 1 mmol) was dissolved in dry DCM (10 mL) in a 100 mL round-bottomed flask and 4-nitrobenzenesulfonamide (**2**, 1 mmol) together with EDC (1.1 mmol) and DMAP (1.1 mmol) was then added. The mixture was stirred under an inert atmosphere at room temperature. The reaction, monitored by TLC (chloroform–methanol, 85 : 15, v/v), was completed after 1 h. The reaction mixture was washed with a 5% $NaHSO_4$ aqueous solution (3 × 10 mL), then with a 5% $NaHCO_3$ aqueous solution (3 × 10 mL) and with a brine solution (1 × 10 mL). The organic solution was dried with Na_2SO_4, and filtered and evaporated to dryness under reduced pressure to afford the corresponding *N*-Fmoc-α-aminoacyl-4-nitrobenzenesulfonamides (**4a–n**) in very good yield (80–95%).

***N*-Fmoc-L-valinyl-4-nitrobenzenesulfonamide (4a, e).** Yield: 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.66 (d, J = 6.9 Hz, CH-(CH_3)$_2$, 3H), 0.75 (d, J = 6.9 Hz, CH(CH_3)$_2$, 3H), 2.02–2.09 (m, β-CH, 1H), 3.68 (dd, J = 5.1 Hz, J = 7.2 Hz, α-CH, 1H), 4.14–4.26 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 6.61 (d, J = 9.0 Hz, NH, 1H), 7.26–7.33 (m, Ar-H_{Fmoc}, 2H), 7.35–7.44 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.73 (dd, J = 3.0 Hz, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 7.87 (d, J = 7.8 Hz, Ar-H_{Fmoc}, 2H), 8.16 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.23 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.89, 20.07, 31.54, 47.17, 62.08, 66.04, 120.50, 123.41, 125.87, 127.53, 128.03, 128.73, 141.12, 144.30, 148.43, 152.72, 156.45, 176.15. Anal. Calcd for $C_{26}H_{25}N_3O_7S$ (523.56): C, 59.65; H, 4.81; N, 8.03%. Found: C, 59.91; H, 4.80; N, 8.05.

***N*-Fmoc-L-phenylalanyl-4-nitrobenzenesulfonamide (4b).** Yield: 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (app t, J = 6.9 Hz, CH_2Ph, 2H), 4.12–4.26 (m, CH_{2Fmoc}, 2H), 4.38–4.45 (m, CH_{Fmoc}, 1H), 4.48–4.59 (m, α-CH, 1H), 5.44 (d, J = 8.4 Hz, NH, 1H), 6.89–6.91 (m, Ar-H_{Ph}, 2H), 6.95–7.06 (m, Ar-H_{Ph}, 3H), 7.27–7.35 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.39–7.57 (m, Ar-H_{Fmoc}, 4H), 7.80 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 8.01 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H), 8.23 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.91, 46.74, 56.20, 67.96, 120.15, 124.08, 125.06, 127.25, 127.44, 128.02, 128.75, 129.08, 129.73, 134.65, 141.30, 141.36, 143.56, 143.90, 162.44, 169.92. Anal. Calcd for $C_{30}H_{25}N_3O_7S$ (571.60): C, 63.04; H, 4.41; N, 7.35%. Found: C, 63.28; H, 4.43; N, 7.33.

***N*-Fmoc-L-alanyl-4-nitrobenzenesulfonamide (4c, l).** Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (d, J = 7.0 Hz, CHCH_3, 3H), 3.92–4.09 (m, α-CH, 1H), 4.12–4.26 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 7.24–7.34 (m, Ar-H_{Fmoc}, 2H), 7.35–7.43 (m, Ar-H_{Fmoc}, 2H), 7.63–7.69 (m, Ar-H_{Fmoc}, 2H), 7.74 (d, J = 7.0 Hz, NH, 1H), 7.88 (d, J = 7.7 Hz, Ar-H_{Fmoc}, 2H), 8.15 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H), 8.31 (s, $NHSO_2Ar$, 1H), 8.42 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.86, 46.51, 47.20, 65.95, 120.52, 123.39, 125.85, 127.51, 128.02, 128.71, 141.10, 144.25, 148.13, 152.70, 156.11, 176.05. Anal. Calcd for $C_{24}H_{21}N_3O_7S$ (495.50): C, 58.17; H, 4.27; N, 8.48%. Found: C, 58.28; H, 4.28; N, 8.51%.

***N*-Fmoc-L-leucinyl-4-nitrobenzenesulfonamide (4d).** Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81 (d, J = 6.6 Hz, CH(CH_3)$_2$, 3H), 0.84 (d, J = 6.6 Hz, CH(CH_3)$_2$, 3H), 1.26–1.47 (m, CH_2, 2H), 1.49–1.65 (m, γ-CH, 1H), 3.98–4.10 (m, CH_{Fmoc}, 1H), 4.13–4.28 (m, CH_{2Fmoc}, α-C*H*, 3H), 7.22–7.32 (m, Ar-H_{Fmoc}, 2H), 7.33–7.44 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.64 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.70 (d, J = 7.5 Hz, NH, 1H), 7.86 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 8.15 (d, J = 8.1 Hz, Ar-H_{Ns}, 2H), 8.40 (d, J = 8.1 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.32, 23.42, 24.67, 47.01, 53.74, 66.12, 79.63, 120.58, 124.92, 125.66, 127.49, 128.10, 129.63, 141.13, 144.08, 144.81, 150.67, 156.45, 173.01. Anal. Calcd for $C_{27}H_{27}N_3O_7S$ (537.58): C, 60.32; H, 5.06; N, 7.82%. Found: C, 60.21; H, 5.08; N, 7.84%.

Synthesis of *N*-Fmoc-D-phenylalanyl-4-nitrobenzenesulfonamide (4f). Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.72 (dd, J = 9.3 Hz, J = 13.8 Hz, CH_2Ph, 1H), 3.03 (dd, J = 5.1 Hz, J = 13.8 Hz, CH_2Ph, 1H), 3.89–3.98 (m, α-CH, 1H), 4.04–4.17 (m, CH_{Fmoc}, CH_{2Fmoc}, 3H), 6.86 (d, J = 8.9 Hz, NH, 1H), 7.04–7.17 (m, Ar-H_{Ph}, 5H), 7.23–7.42 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 5H), 7.59–7.66 (m, Ar-H_{Fmoc}, 2H), 7.86 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H),

7.97 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.22 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.20, 47.09, 58.96, 65.91, 120.49, 123.62, 125.82, 126.33, 127.74, 128.01, 128.26, 128.84, 129.39, 139.06, 141.09, 144.33, 148.72, 155.20, 155.87, 175.81. Anal. Calcd for $C_{30}H_{25}N_3O_7S$ (571.60): C, 63.04; H, 4.41; N, 7.35%. Found: C, 63.25; H, 4.40; N, 7.32%.

***N*-Fmoc-*O*-*t*Bu-L-serinyl-4-nitrobenzenesulfonamide (4g).** Yield: 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.02 (s, C(CH_3)$_3$, 9H), 3.46 (dd, J = 6.3 Hz, J = 8.7 Hz, CH_2O*t*Bu, 1H), 3.55 (dd, J = 3.3 Hz, J = 8.7 Hz, CH_2O*t*Bu, 1H), 3.80–3.84 (m, α-CH, 1H), 4.16–4.23 (m, CH_{Fmoc}, CH_{2Fmoc}, 3H), 6.50 (d, J = 8.4 Hz, NH, 1H), 7.25–7.44 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 5H), 7.69 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.86 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.96 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.19 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.76, 47.14, 50.98, 63.65, 66.01, 72.61, 120.50, 123.41, 125.79, 127.53, 128.02, 128.57, 141.13, 144.41, 148.12, 174.77. Anal. Calcd for $C_{28}H_{29}N_3O_8S$ (567.61): C, 59.25; H, 5.15; N, 7.40%. Found: C, 59.48; H, 5.17; N, 7.43%.

***N*-Fmoc-*O*-*t*Bu-L-tyrosinyl-4-nitrobenzenesulfonamide (4h).** Yield: 86%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, C(CH_3)$_3$, 9H), 2.65–2.98 (m, CH_{2Tyr}, 2H), 3.79–4.20 (m, CH_{Fmoc}, CH_{2Fmoc}, 3H), 4.23–4.40 (m, α-CH, 1H), 5.50 (br s, NH, 1H), 6.62 (d, J = 8.7 Hz, Ar-H_{Tyr}, 2H), 6.77 (d, J = 8.7 Hz, Ar-H_{Tyr}, 2H), 6.92–7.09 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 5H), 7.18–7.29 (m, Ar-H_{Fmoc}, 2H), 7.56–7.67 (m, Ar-H_{Fmoc}, 2H), 7.70–7.89 (m, Ar-H_{Ns}, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.73, 36.71, 46.77, 60.42, 67.26, 78.34, 119.98, 123.53, 123.90, 124.85, 126.99, 127.65, 129.56, 141.08, 143.53, 148.31, 149.15, 154.10, 156.95, 176.43. Anal. Calcd for $C_{34}H_{33}N_3O_8S$ (643.71): C, 63.44; H, 5.17; N, 6.53%. Found: C, 63.29; H, 5.19; N, 6.56%.

***N*-Fmoc-*O*-*t*Bu-L-glutamyl-4-nitrobenzenesulfonamide (4i).** Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (s, C(CH_3)$_3$, 9H), 1.63–2.20 (m, CH_2CH_2, 4H), 3.60–3.69 (m, α-CH, 1H), 3.71–3.82 (m, CH_{Fmoc}, 1H), 4.13–4.32 (m, CH_{2Fmoc}, 2H), 6.74 (d, J = 9.0 Hz, NH, 1H), 7.25–7.34 (m, Ar-H_{Fmoc}, 2H), 7.35–7.44 (m, Ar-H_{Fmoc}, 2H), 7.63–7.72 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.81–7.90 (m, Ar-H_{Fmoc}, 2H), 7.96 (d, J = 8.4 Hz, Ar-H_{Ns}, 2H), 8.21 (d, J = 8.4 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.05, 28.21, 28.79, 47.17, 56.05, 62.12, 79.81, 120.49, 123.44, 125.85, 127.51, 128.02, 128.65, 141.12, 144.10, 144.72, 152.02, 157.60, 172.56, 173.01. Anal. Calcd for $C_{30}H_{31}N_3O_9S$ (609.65): C, 59.10; H, 5.13; N, 6.89%. Found: C, 59.30; H, 5.14; N, 6.91%.

***N*-Fmoc-L-isoleucinyl-4-nitrobenzenesulfonamide (4j).** Yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71–0.83 (m, CH_2CH_3, CHCH_3, 6H), 0.95–1.09 (m, CH_2CH$_3$, 1H), 1.26–1.44 (m, CH_2CH$_3$, 1H), 1.64–1.76 (m, C*H*CH$_3$, 1H), 4.12–4.28 (m, CH_{Fmoc}, α-C*H*, 2H), 4.40 (d, J = 7.2 Hz, CH_{2Fmoc}, 2H), 5.65 (d, J = 9.2 Hz, NH, 1H), 7.18–7.29 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.33–7.41 (m, Ar-H_{Fmoc}, 2H), 7.52 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.75 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 8.11 (d, J = 8.8 Hz, Ar-H_{Ns}, 2H), 8.23 (d, J = 8.8 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.90, 15.05, 24.46, 37.49, 46.76, 59.40, 67.71, 120.28, 123.88, 124.89, 127.09, 127.83, 129.59, 141.15, 143.08, 143.93, 150.89, 156.85, 171.00. Anal. Calcd for $C_{27}H_{27}N_3O_7S$ (537.58): C, 60.32; H, 5.06; N, 7.82%. Found: C, 60.23; H, 5.07; N, 7.81%.

***N*-Fmoc-D-valinyl-4-nitrobenzenesulfonamide (4k).** Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.65 (d, J = 6.9 Hz, CH(CH_3)$_2$, 3H), 0.75 (d, J = 6.9 Hz, CH(CH_3)$_2$, 3H), 1.93–2.15 (m, β-CH, 1H), 3.65–3.74 (m, α-CH, 1H), 4.06–4.44 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 6.69 (s broad, NH, 1H), 7.23–7.31 (m, Ar-H_{Fmoc}, 2H), 7.32–7.42 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 3H), 7.73–7.78 (m, Ar-H_{Fmoc}, 2H), 7.86 (d, J = 7.8 Hz, Ar-H_{Fmoc}, 2H), 7.98 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.22 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.89, 19.86, 31.45, 46.90, 62.12, 66.06, 120.48, 123.54, 125.86, 127.53, 128.00, 128.61, 141.09, 144.26, 148.35, 152.11, 157.01, 176.20. Anal. Calcd for $C_{26}H_{25}N_3O_7S$ (523.56): C, 59.65; H, 4.81; N, 8.03%. Found: C, 59.88; H, 4.82; N, 8.04.

***N*-Fmoc-D-alanyl-4-nitrobenzenesulfonamide (4m).** Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.14 (d, J = 7.2 Hz, CHCH_3, 3H), 3.68–3.86 (m, α-CH, 1H), 4.13–4.23 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 6.89 (d, J = 7.0 Hz, NH, 1H), 7.25–7.46 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 5H), 7.63–7.76 (m, Ar-H_{Fmoc}, 2H), 7.90 (d, J = 7.7 Hz, Ar-H_{Fmoc}, 2H), 7.96 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H), 8.22 (d, J = 8.9 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.93, 46.41, 47.03, 65.98, 120.48, 123.54, 125.65, 127.52, 128.00, 128.61, 141.09, 144.12, 148.15, 152.00, 155.20, 176.20. Anal. Calcd for $C_{24}H_{21}N_3O_7S$ (495.50): C, 58.17; H, 4.27; N, 8.48%. Found: C, 58.25; H, 4.29; N, 8.50%.

***N*-Fmoc-*O*-*t*Bu-D-serinyl-4-nitrobenzenesulfonamide (4n).** Yield: 89%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.04 (s, C(CH_3)$_3$, 9H), 3.42–3.51 (m, CH_2O*t*Bu, 1H), 3.55 (dd, J = 3.3 Hz, J = 8.7 Hz, CH_2O*t*Bu, 1H), 3.81–3.88 (m, α-CH, 1H), 4.17–4.25 (m, CH_{Fmoc}, CH_{2Fmoc}, 3H), 6.63 (d, J = 8.4 Hz, NH, 1H), 7.27–7.43 (m, Ar-H_{Fmoc}, $NHSO_2Ar$, 5H), 7.71 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.87 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.98 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.21 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.78, 47.11, 50.23, 63.09, 65.99, 72.60, 120.48, 123.40, 125.76, 127.51, 128.00, 128.55, 141.11, 144.33, 148.75, 175.03. Anal. Calcd for $C_{28}H_{29}N_3O_8S$ (567.61): C, 59.25; H, 5.15; N, 7.40%. Found: C, 59.45; H, 5.18; N, 7.41%.

Synthesis of *N*-Fmoc-α-aminoacyl-*N*-methyl-4-nitrobenzenesulfonamides (5a–i, k–n)

N-Fmoc-α-aminoacyl-4-nitrobenzenesulfonamide (**4a–i**, **k–n**, 1 mmol) was dissolved in 10 mL of dry DMF in a 50 mL round-bottomed flask. Methyl iodide (5 mmol) and DIPEA (5 mmol) were added and the mixture was stirred at room temperature under an inert atmosphere and monitored by TLC (chloroform–methanol, 90 : 10, v/v). The reaction was completed after 1 h. To the reaction mixture, 10 mL of a 5% $NaHSO_4$ aqueous solution were added and the resulting solution was extracted with ethyl acetate (3 × 10 mL). The organic layers were collected and washed with a 5% $NaHCO_3$ aqueous solution (3 × 10 mL), with a brine solution (1 × 10 mL) and then dried (Na_2SO_4), filtered and evaporated to dryness. The corresponding *N*-Fmoc-aminoacyl-4-nitrobenzenesulfonamides *N*-methylated (**5a–i**, **k–n**) were recovered in very good yield (80–92%).

***N*-Fmoc-L-valinyl-*N*-methyl-4-nitrobenzenesulfonamide (5a, e).** Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (d, J = 6.6 Hz,

CH(CH_3)$_2$, 6H), 1.93–2.06 (m, CH(CH$_3$)$_2$, 1H), 3.46 (s, NCH$_3$, 3H), 4.10–4.32 (m, CH$_{Fmoc}$, CH$_{2Fmoc}$, 3H), 4.45 (app t, J = 7.8 Hz, α-CH, 1H), 7.23–7.44 (m, Ar-H$_{Fmoc}$, 4H), 7.51–7.53 (m, Ar-H$_{Fmoc}$, 2H), 7.81–7.90 (m, Ar-H$_{Fmoc}$, NH, 3H), 8.20 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H), 8.35 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 18.35, 19.13, 30.61, 34.02, 47.02, 58.94, 66.36, 120.56, 124.76, 127.48, 128.08, 129.89, 141.18, 144.12, 144.20, 150.73, 174.01. Anal. Calcd for $C_{27}H_{27}N_3O_7S$ (537.58): C, 60.32; H, 5.06; N, 7.82%. Found: C, 60.56; H, 5.06; N, 7.80%.

***N*-Fmoc-L-phenylalanyl-*N*-methyl-4-nitrobenzenesulfonamide (5b).** Yield: 86%. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.60–2.98 (m, CH$_2$Ph, 2H), 3.20 (s, NCH$_3$, 3H), 4.05–4.16 (m, CH$_{Fmoc}$, 1H), 4.20–4.40 (m, α-CH, CH$_{2Fmoc}$, 3H), 5.48 (br s, NH, 1H), 7.10–7.45 (m, Ar-H$_{Fmoc}$, Ar-H$_{Phe}$, 9H), 7.48 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 7.86 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 8.17 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H), 8.36 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 33.23, 39.15, 46.93, 55.35, 67.30, 120.07, 124.41, 124.98, 127.08, 127.75, 127.80, 128.82, 129.32, 129.36, 135.09, 141.28, 143.53, 143.60, 150.81, 156.98, 173.41. Anal. Calcd for $C_{31}H_{27}N_3O_7S$ (585.63): C, 63.88; H, 5.19; N, 6.98%. Found: C, 64.12; H, 5.21; N, 6.95%.

***N*-Fmoc-L-alanyl-*N*-methyl-4-nitrobenzenesulfonamide (5c, l).** Yield: 92%. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.48 (d, J = 6.9 Hz, CH(CH_3), 3H), 3.32 (s, NCH$_3$, 3H), 4.09–4.27 (m, CH$_{Fmoc}$, 1H), 4.34 (d, J = 7.2 Hz, CH$_{2Fmoc}$, 2H), 5.10–5.23 (m, α-CH, 1H), 5.36 (d, J = 9.0 Hz, NH, 1H), 7.25–7.46 (m, Ar-H$_{Fmoc}$, 4H), 7.65 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 7.81 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 8.26 (d, J = 9.0 Hz, Ar-H$_{Ns}$, 2H), 8.38 (d, J = 9.0 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 18.88, 33.27, 47.01, 50.29, 67.24, 120.06, 124.47, 125.00, 127.07, 127.81, 129.42, 141.30, 143.02, 143.86, 155.87, 174.60. Anal. Calcd for $C_{25}H_{23}N_3O_7S$ (509.53): C, 58.93; H, 4.55; N, 8.25%. Found: C, 58.72; H, 4.57; N, 8.22%.

***N*-Fmoc-L-leucinyl-*N*-methyl-4-nitrobenzenesulfonamide (5d).** Yield: 90%. ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.94–1.07 (m, CH(CH_3)$_2$, 6H), 1.47–1.85 (m, CH_2CH(CH$_3$)$_2$, 3H), 3.32 (s, NCH$_3$, 3H), 4.13–4.21 (m, CH$_{Fmoc}$, 1H), 4.28–4.43 (m, CH$_{2Fmoc}$, 2H), 5.10–5.30 (m, NH, α-CH, 2H), 7.27–7.34 (m, Ar-H$_{Fmoc}$, 2H), 7.37–7.42 (m, Ar-H$_{Fmoc}$, 2H), 7.56 (d, J = 7.2 Hz, Ar-H$_{Fmoc}$, 2H), 7.77 (d, J = 7.2 Hz, Ar-H$_{Fmoc}$, 2H), 8.25 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H), 8.36 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.06, 23.37, 24.92, 33.26, 41.72, 47.04, 53.04, 67.18, 120.05, 124.45, 124.98, 127.06, 127.82, 129.43, 141.30, 143.55, 143.60, 150.73, 156.27, 174.73. Anal. Calcd for $C_{28}H_{29}N_3O_7S$ (551.61): C, 60.97; H, 5.30; N, 7.62%. Found: C, 61.17; H, 5.32; N, 7.64%.

***N*-Fmoc-D-phenylalanyl-*N*-methyl-4-nitrobenzenesulfonamide (5f).** Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 2.77 (dd, J = 9.3 Hz, J = 13.8 Hz, CH$_2$Ph, 1H), 2.98 (dd, J = 5.1 Hz, J = 13.8 Hz, CH$_2$Ph, 1H), 3.30 (s, NCH$_3$, 3H), 3.98–4.32 (m, CH$_{Fmoc}$, CH$_{2Fmoc}$, 3H), 4.77–4.86 (m, α-CH, 1H), 7.06–7.43 (m, Ar-H$_{Fmoc}$, Ar-H$_{Phe}$, 9H), 7.54–7.66 (m, Ar-H$_{Fmoc}$, 2H), 7.82 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 8.01 (m, NH, 1H), 8.17 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H), 8.37 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 14.54, 21.21, 55.56, 60.20, 79.63, 120.54, 124.84, 127.48, 128.08, 128.64, 129.71, 137.76, 141.15, 143.84, 143.98, 156.88, 173.71. Anal. Calcd for $C_{31}H_{27}N_3O_7S$ (585.63): C, 63.88; H, 5.19; N, 6.98%. Found: C, 64.14; H, 5.20; N, 6.96%.

***N*-Fmoc-*O*-*t*Bu-L-serinyl-*N*-methyl-4-nitrobenzenesulfonamide (5g).** Yield: 84%. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.05 (s, C(CH_3)$_3$, 9H), 3.34 (s, NCH$_3$, 3H), 4.12–4.32 (m, CH$_{Fmoc}$, CH$_{2Fmoc}$, 3H), 4.39–4.49 (m, CH$_2$O*t*Bu, 2H), 4.67–4.76 (m, α-CH, 1H), 7.27–7.34 (m, Ar-H$_{Fmoc}$, 2H), 7.36–7.45 (m, Ar-H$_{Fmoc}$, 2H), 7.58–7.71 (m, Ar-H$_{Fmoc}$, NH, 3H), 7.90 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 8.18 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H), 8.37 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 27.93, 34.01, 46.96, 53.90, 60.21, 66.88, 73.59, 120.57, 124.71, 127.51, 128.10, 130.01, 141.18, 144.12, 150.96, 156.67, 182.11. Anal. Calcd for $C_{29}H_{31}N_3O_8S$ (581.64): C, 59.88; H, 5.37; N, 7.22%. Found: C, 60.11; H, 5.35; N, 7.24%.

***N*-Fmoc-*O*-*t*Bu-L-tyrosinyl-*N*-methyl-4-nitrobenzenesulfonamide (5h).** Yield: 85%. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.35 (s, CH(CH_3)$_3$, 9H), 2.84–2.95 (m, CH$_{2Tyr}$, 1H), 3.04–3.11 (m, CH$_{2Tyr}$, 1H), 3.18 (s, NCH$_3$, 3H), 4.08–4.50 (m, CH$_{Fmoc}$, CH$_{2Fmoc}$, 3H), 4.61–4.68 (m, α-CH, 1H), 5.40 (d, J = 8.7 Hz, NH, 1H), 6.91 (d, J = 8.7 Hz, Ar-H$_{Tyr}$, 2H), 7.09 (d, J = 8.7 Hz, Ar-H$_{Tyr}$, 2H), 7.22–7.43 (m, Ar-H$_{Fmoc}$, 4H), 7.53 (d, J = 7.2 Hz, Ar-H$_{Fmoc}$, 2H), 7.80 (d, J = 7.2 Hz, Ar-H$_{Fmoc}$, 2H), 8.19 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H), 8.35 (d, J = 8.9 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 29.01, 33.18, 38.65, 46.94, 55.36, 60.42, 67.31, 120.06, 124.21, 125.00, 127.08, 127.80, 129.53, 129.81, 141.28, 143.54, 143.76, 150.66, 154.81, 155.86, 173.52. Anal. Calcd for $C_{35}H_{35}N_3O_8S$ (657.73): C, 64.17; H, 5.83; N, 6.24%. Found: C, 64.37; H, 5.85; N, 6.26%.

***N*-Fmoc-*O*-*t*Bu-L-glutamyl-*N*-methyl-4-nitrobenzenesulfonamide (5i).** Yield: 87%. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.38 (s, CH(CH_3)$_3$, 9H), 1.74–2.02 (m, CH$_{2Glu}$, 2H), 2.19–2.31 (m, CH$_{2Glu}$, 2H), 3.33 (s, NCH$_3$, 3H), 4.02–4.33 (m, α-CH, CH$_{Fmoc}$, CH$_{2Fmoc}$, 4H), 7.25–7.44 (m, Ar-H$_{Fmoc}$, 4H), 7.73 (d, J = 7.5 Hz, Ar-H$_{Fmoc}$, 2H), 7.80–7.95 (m, Ar-H$_{Fmoc}$, 2H), 7.96 (d, J = 8.9 Hz, NH 1H), 8.21 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H), 8.38 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 26.76, 28.20, 30.61, 47.11, 53.01, 66.20, 80.05, 120.58, 126.05, 127.51, 128.09, 141.21, 144.56, 154.89, 157.81, 176.21, 178.16. Anal. Calcd for $C_{31}H_{33}N_3O_9S$ (623.67): C, 59.70; H, 5.33; N, 6.74%. Found: C, 59.76; H, 5.34; N, 6.72%.

***N*-Fmoc-D-valinyl-*N*-methyl-4-nitrobenzenesulfonamide (5k).** Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 0.85 (d, J = 7.0 Hz, CH(CH_3)$_2$, 6H), 1.95–2.10 (m, CH(CH$_3$)$_2$, 1H), 3.40 (s, NCH$_3$, 3H), 4.11–4.32 (m, CH$_{Fmoc}$, CH$_{2Fmoc}$, 3H), 4.49 (app t, J = 7.8 Hz, α-CH, 1H), 7.24–7.49 (m, Ar-H$_{Fmoc}$, 4H), 7.55–7.70 (m, Ar-H$_{Fmoc}$, 2H), 7.79–7.90 (m, Ar-H$_{Fmoc}$, NH, 3H), 8.16 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H), 8.36 (d, J = 8.7 Hz, Ar-H$_{Ns}$, 2H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 18.26, 19.12, 30.46, 34.12, 47.05, 59.01, 66.50, 120.65, 124.80, 127.49, 128.05, 129.92, 141.20, 144.15, 144.23, 150.65, 174.11. Anal. Calcd for $C_{27}H_{27}N_3O_7S$ (537.58): C, 60.32; H, 5.06; N, 7.82%. Found: C, 60.50; H, 5.08; N, 7.79%.

***N*-Fmoc-D-alanyl-*N*-methyl-4-nitrobenzenesulfonamide (5m).** Yield: 89%. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.49 (d, J = 7.0

Hz, CH(CH_3), 3H), 3.34 (s, NCH_3, 3H), 4.12–4.25 (m, CH_{Fmoc}, 1H), 4.33 (d, J = 7.2 Hz, CH_{2Fmoc}, 2H), 5.14–5.24 (m, α-CH, 1H), 5.37 (d, J = 9.0 Hz, NH, 1H), 7.15–7.38 (m, Ar-H_{Fmoc}, 4H), 7.53 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.79 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 8.25 (d, J = 9.0 Hz, Ar-H_{Ns}, 2H), 8.37 (d, J = 9.0 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.60, 32.95, 47.05, 51.26, 67.25, 120.08, 125.01, 125.90, 127.15, 127.78, 129.10, 141.45, 143.05, 143.44, 155.85, 174.75. Anal. Calcd for $C_{25}H_{23}N_3O_7S$ (509.53): C, 58.93; H, 4.55; N, 8.25%. Found: C, 58.76; H, 4.56; N, 8.22%.

***N*-Fmoc-*O*-*t*Bu-D-serinyl-*N*-methyl-4-nitrobenzenesulfonamide (5n).** Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (s, C(CH_3)$_3$, 9H), 3.40 (s, NCH_3, 3H), 4.11–4.35 (m, CH_{Fmoc}, CH_{2Fmoc}, CH_2O*t*Bu, 5H), 4.71–4.80 (m, α-CH, 1H), 7.25–7.37 (m, Ar-H_{Fmoc}, 2H), 7.38–7.48 (m, Ar-H_{Fmoc}, 2H), 7.59–7.71 (m, Ar-H_{Fmoc}, NH, 3H), 7.89 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 8.19 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.37 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.73, 33.82, 47.12, 54.00, 63.02, 66.06, 73.56, 120.48, 124.66, 127.50, 128.01, 129.99, 141.11, 144.36, 150.65, 155.97, 177.86. Anal. Calcd for $C_{29}H_{31}N_3O_8S$ (581.64): C, 59.88; H, 5.37; N, 7.22%. Found: C, 60.02; H, 5.39; N, 7.25%.

Synthesis of *N*-Fmoc-dipeptide methyl esters (7a–i, k–n)

N-Fmoc-α-aminoacyl-*N*-methyl-4-nitrobenzenesulfonamide (1 mmol, **5a–i, k–n**) was dissolved in 10 mL of dry dichloromethane in a 100 mL two-necked round-bottomed flask under a nitrogen inert atmosphere. α-Amino acid methyl ester (1 mmol, **6**), DMAP (2 mmol) and 1 mL of DMF were added. The reaction mixture was stirred at reflux and the reaction monitored by TLC (Et_2O–petroleum ether 70 : 30 v/v). After about 1 h the reaction was completed and the reaction mixture was washed with a 9% aqueous solution of Na_2CO_3 (2 × 10 mL), then with a 5% $NaHSO_4$ aqueous solution (2 × 10 mL) and finally with a brine solution (1 × 10 mL). The organic layer was dried (Na_2SO_4), paper-filtered and evaporated under reduced pressure. The crude reaction product was purified by column chromatography (Et_2O–petroleum ether 70 : 30 v/v) and the *N*-Fmoc-dipeptide methyl ester (**7a–i, k–n**) was recovered in good yield (68–80%).

***N*-Fmoc-L-valinyl-L-alanine methyl ester (7a).** Yield: 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (m, CH(CH_3)$_2$, 6H), 1.43 (d, J = 7.2 Hz, CHCH_3, 3H), 2.07–2.19 (m, CH(CH$_3$)$_2$, 1H), 3.75 (s, OCH_3, 3H), 4.02 (dd, J = 6.6 Hz, J = 9.0 Hz, CH_{val}, 1H), 4.19–4.27 (m, CH_{Fmoc}, 1H), 4.33–4.48 (m, CH_{2Fmoc}, 2H), 4.60 (app quin, J = 7.2 Hz, CH_{ala}, 1H), 5.40 (d, J = 9.0 Hz, $NH_{urethane}$, 1H), 6.27 (d, J = 7.5 Hz, NH, 1H), 7.22–7.32 (m, Ar-H_{Fmoc}, 2H), 7.36–7.48 (m, Ar-H_{Fmoc}, 2H), 7.61 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.25, 19.00, 31.05, 47.20, 48.15, 52.53, 60.27, 67.07, 119.98, 125.08, 127.09, 127.73, 141.32, 143.98, 173.07, 173.46. Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60%. Found: C, 67.67; H, 6.68; N, 6.62%.

***N*-Fmoc-L-phenylalanyl-L-alanine methyl ester (7b).** Yield: 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (d, J = 7.2 Hz, CHCH_3, 3H), 3.02–3.19 (m, CH_{2Phe}, 2H), 3.72 (s, OCH_3, 3H), 4.16–4.25 (m, CH_{Fmoc}, 1H), 4.28–4.39 (m, CH_{Phe}, 1H), 4.41–4.58 (m, CH_{ala}, CH_{2Fmoc}, 3H), 5.44 (br s, $NH_{urethane}$, 1H), 6.43 (br s, NH, 1H), 7.16–7.37 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, 7H), 7.38–7.46 (m, Ar-H_{Fmoc}, 2H), 7.51–7.59 (m, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.31, 38.62, 47.12, 48.19, 52.48, 56.00, 67.11, 120.00, 125.02, 127.75, 128.01, 128.56, 128.98, 136.24, 141.30, 143.71, 156.01, 170.32, 172.76. Anal. Calcd for $C_{28}H_{28}N_2O_5$ (472.53): C, 71.17; H, 5.97; N, 5.93%. Found: C, 71.35; H, 5.95; N, 5.95%.

***N*-Fmoc-L-alanyl-L-leucine methyl ester (7c).** Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–0.94 (m, CH(CH_3)$_2$, 6H), 1.32–1.45 (m, CHCH$_3$, 1H), 1.47 (d, J = 6.9 Hz, CHCH_3, 3H), 1.55–1.68 (m, CH_2, 2H), 3.31 (s, OCH_3, 3H), 4.12–4.26 (m, CH_{leu}, 1H), 4.31–4.45 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 5.16 (m, CH_{ala}, 1H), 5.32 (d, J = 9.5 Hz, $NH_{urethane}$, 1H), 7.25–7.34 (m, Ar-H_{Fmoc}, NH, 3H), 7.37–7.45 (m, Ar-H_{Fmoc}, 2H), 7.55 (d, J = 7.4 Hz, Ar-H_{Fmoc}, 2H), 7.77 (d, J = 7.4Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.19, 18.21, 22.99, 24.70, 41.60, 47.20, 48.10, 52.40, 53.44, 67.15, 120.00, 127.10, 127.70, 129.56, 134.66, 141.48, 153.55, 171.99, 173.10. Anal. Calcd for $C_{25}H_{30}N_2O_5$ (438.52): C, 68.47; H, 6.90; N, 6.39%. Found: C, 68.62; H, 6.92; N, 6.37%.

***N*-Fmoc-L-leucinyl-L-alanine methyl ester (7d).** Yield: 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70–1.05 (m, CH(CH_3)$_2$, 6H), 1.43 (d, J = 7.2 Hz, CHCH_3, 3H), 1.51–1.76 (m, CH_2CH(CH$_3$)$_2$, 3H), 3.71 (s, OCH_3, 3H), 4.18–4.29 (m, CH_{Fmoc}, CH_{leu}, 2H), 4.35–4.48 (m, CH_{2Fmoc}, 2H), 4.60 (app quin, J = 7.2 Hz, CH_{ala}, 1H), 5.29 (d, J = 7.8 Hz, $NH_{urethane}$, 1H), 6.53 (d, J = 6.9 Hz, NH, 1H), 7.24–7.37 (m, Ar-H_{Fmoc}, 2H), 7.38–7.46 (m, Ar-H_{Fmoc}, 2H), 7.61 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.29, 18.31, 22.91, 24.63, 41.63, 47.17, 48.07, 52.50, 53.41, 67.05, 119.98, 127.08, 127.73, 129.09, 134.70, 141.31, 153.97, 171.90, 173.46. Anal. Calcd for $C_{25}H_{30}N_2O_5$ (438.52): C, 68.47; H, 6.90; N, 6.39%. Found: C, 68.14; H, 6.91; N, 6.42%.

***N*-Fmoc-L-valinyl-D-alanine methyl ester (7e).** Yield: 72%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99–1.02 (m, CH(CH_3)$_2$, 6H), 1.43 (d, J = 7.2 Hz, CHCH_3, 3H), 2.12–2.24 (m, CH(CH$_3$)$_2$, 1H), 3.76 (s, OCH_3, 3H), 4.01–4.10 (m, CH_{val}, 1H), 4.22–4.27 (m, CH_{Fmoc}, 1H), 4.36–4.50 (m, CH_{2Fmoc}, 2H), 4.61 (app quin, J = 7.2 Hz, CH_{ala}, 1H), 5.36 (d, J = 8.9 Hz, $NH_{urethane}$, 1H), 6.46 (d, J = 7.0 Hz, NH, 1H), 7.30–7.47 (m, Ar-H_{Fmoc}, 4H), 7.61 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.39, 19.14, 29.98, 47.20, 48.15, 52.57, 60.27, 67.07, 120.01, 125.05, 127.09, 127.74, 141.33, 143.88, 170.37, 170.91. Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60%. Found: C, 68.17; H, 6.62; N, 6.63%.

***N*-Fmoc-D-phenylalanyl-L-alanine methyl ester (7f).** Yield: 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, J = 7.2 Hz, CHCH_3, 3H), 2.98–3.07 (m, CH_2Phe, 1H), 3.11–3.19 (m, CH_2Phe, 1H), 3.71 (s, OCH_3, 3H), 4.20 (t, J = 6.9 Hz, CH_{Fmoc}, 1H), 4.29–4.57 (m, CH_{ala}, CH_{2Fmoc}, CH_{Phe}, 4H), 5.44 (d, J = 8.1 Hz, $NH_{urethane}$, 1H), 6.16 (d, J = 7.5 Hz, NH, 1H), 7.17–7.36 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, 7H), 7.37–7.45 (m, Ar-H_{Fmoc}, 2H), 7.52–7.59 (m, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.13, 38.60, 47.11, 47.96, 52.56, 56.10,

67.10, 120.02, 125.06, 127.10, 127.18, 127.76, 128.79, 129.36, 136.32, 141.31, 143.70, 170.03, 172.91. Anal. Calcd for $C_{28}H_{28}N_2O_5$ (472.53): C, 71.17; H, 5.97; N, 5.93%. Found: C, 71.43; H, 5.95; N, 5.96%.

***N*-Fmoc-*Ot*Bu-L-serinyl-L-alanine methyl ester (7g).** Yield: 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, $C(CH_3)_3$, 9H), 1.44 (d, J = 7.2 Hz, $CHCH_3$, 3H), 3.34–3.43 (m, CH_{2ser}, 1H), 3.76 (s, OCH_3, 3H), 3.80–3.86 (m, CH_{2ser}, 1H), 4.20–4.30 (m, CH_{Fmoc}, CH_{ser}, 2H), 4.40 (d, J = 7.2 Hz, CH_{2Fmoc}, 2H), 4.52–4.65 (m, CH_{ala}, 1H), 5.82 (d, J = 6.0 Hz, $NH_{urethane}$, 1H), 7.26–7.45 (m, Ar-H_{Fmoc}, 4H), 7.51 (d, J = 6.9 Hz, NH, 1H), 7.61 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 7.77 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.46, 27.38, 47.10, 48.32, 52.53, 54.01, 61.73, 67.12, 74.48, 120.03, 125.18, 127.10, 127.75, 141.29, 143.73, 156.04, 169.98, 173.07. Anal. Calcd for $C_{26}H_{32}N_2O_6$ (468.54): C, 66.65; H, 6.88; N, 5.98%. Found: C, 66.87; H, 6.89; N, 6.00%.

***N*-Fmoc-*Ot*Bu-L-tyrosinyl-L-alanine methyl ester (7h).** Yield: 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21–1.39 (m, $CHCH_3$, $C(CH_3)_3$, 12H), 2.94–3.05 (m, CH_{2tyr}, 1H), 3.06–3.17 (m, CH_{2tyr}, 1H), 3.72 (s, OCH_3, 3H), 4.15–4.24 (m, CH_{Fmoc}, 1H), 4.31–4.53 (m, CH_{ala}, CH_{2Fmoc}, CH_{tyr}, 4H), 5.43 (d, J = 7.9 Hz, $NH_{urethane}$, 1H), 6.33 (d, J = 6.7 Hz, NH, 1H), 6.92 (d, J = 8.1 Hz, Ar-H_{Tyr}, 2H), 7.10 (d, J = 8.1 Hz, Ar-H_{Tyr}, 2H), 7.25–7.44 (m, Ar-H_{Fmoc}, 4H), 7.54–7.60 (m, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.33, 29.73, 38.02, 47.11, 48.20, 52.53, 56.10, 67.10, 78.47, 120.01, 125.05, 127.11, 127.76, 129.85, 141.29, 143.70, 154.47, 156.04, 170.33, 172.75. Anal. Calcd for $C_{32}H_{36}N_2O_6$ (544.64): C, 70.57; H, 6.66; N, 5.14%. Found: C, 70.71; H, 6.64; N, 5.12%.

***N*-Fmoc-*Ot*Bu-L-glutamyl-L-alanine methyl ester (7i).** Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31–1.59 (m, $CHCH_3$, $C(CH_3)_3$, 12H), 1.90–2.19 (m, CH_{2glu}, 2H), 2.41–2.54 (m, CH_{2glu}, 2H), 3.78 (s, OCH_3, 3H), 4.20–4.31 (m, CH_{glu}, CH_{Fmoc}, 2H), 4.36–4.48 (m, CH_{2Fmoc}, 2H), 4.52–4.68 (m, CH_{ala},1H), 5.75 (d, J = 7.5 Hz, $NH_{urethane}$, 1H), 6.89 (d, J = 7.2 Hz, NH, 1H), 7.26–7.48 (m, Ar-H_{Fmoc}, 4H), 7.61 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.14, 28.52, 29.70, 31.60, 47.13, 48.19, 52.50, 53.99, 67.01, 81.18, 120.00, 125.14, 127.09, 127.73, 141.30, 143.96, 156.05, 172.71, 173.06, 173.12. Anal. Calcd for $C_{28}H_{34}N_2O_7$ (510.58): C, 65.87; H, 6.71; N, 5.49%. Found: C, 66.11; H, 6.73; N, 5.50%.

***N*-Fmoc-D-valinyl-L-alanine methyl ester (7k).** Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (app t, J = 7.2 Hz, $CH(CH_3)_2$, 6H), 1.42 (d, J = 7.2 Hz, $CHCH_3$, 3H), 2.11–2.24 (m, $CH(CH_3)_2$, 1H), 3.72 (s, OCH_3, 3H), 4.11 (dd, J = 6.3 Hz, J = 8.7 Hz, CH_{val}, 1H), 4.19–4.26 (m, CH_{Fmoc}, 1H), 4.33–4.47 (m, CH_{2Fmoc}, 2H), 4.61 (app quin, J = 7.2 Hz, CH_{ala}, 1H), 5.58 (d, J = 8.7 Hz, $NH_{urethane}$, 1H), 6.76 (d, J = 7.2 Hz, NH, 1H), 7.27–7.35 (m, Ar-H_{Fmoc}, 2H), 7.36–7.44 (m, Ar-H_{Fmoc}, 2H), 7.60 (d, J = 7.2 Hz, Ar-H_{Fmoc}, 2H), 7.77 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.30, 19.13, 31.17, 47.14, 48.05, 52.51, 60.13, 67.05, 119.97, 125.06, 127.07, 127.71, 141.28, 143.74, 156.41, 170.75, 173.13. Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60%. Found: C, 67.68; H, 6.67; N, 6.59%.

***N*-Fmoc-L-alanyl-L-valine methyl ester (7l).** Yield: 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84–0.96 (m, $CH(CH_3)_2$, 6H), 1.42 (d, J = 6.9 Hz, $CHCH_3$, 3H), 2.11–2.24 (m, $CH(CH_3)_2$, 1H), 3.75 (s, OCH_3, 3H), 4.18–4.26 (m, CH_{Fmoc}, 1H), 4.30–4.44 (m, CH_{2Fmoc}, CH_{ala}, 3H), 4.56 (dd, J = 5.1 Hz, J = 9.0 Hz, CH_{val}, 1H), 5.50 (d, J = 7.5 Hz, $NH_{urethane}$, 1H), 6.60 (d, J = 9.0 Hz, NH, 1H), 7.27–7.36 (m, Ar-H_{Fmoc}, 2H), 7.37–7.45 (m, Ar-H_{Fmoc}, 2H), 7.60 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.70, 18.94, 31.23, 47.07, 50.45, 52.25, 57.16, 67.16, 120.01, 125.08, 127.10, 127.75, 141.29, 143.74, 156.01, 172.20, 173.01. Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60%. Found: C, 67.72; H, 6.67; N, 6.61%.

***N*-Fmoc-D-alanyl-L-valine methyl ester (7m).** Yield: 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (d, J = 6.6 Hz, $CH(CH_3)_2$, 3H), 0.94 (d, J = 6.6 Hz, J = $CH(CH_3)_2$, 3H), 1.45 (d, J = 6.9 Hz, $CHCH_3$, 3H), 2.11–2.23 (m, $CH(CH_3)_2$, 1H), 3.74 (s, OCH_3, 3H), 4.19–4.26 (m, CH_{Fmoc}, 1H), 4.32–4.48 (m, CH_{2Fmoc}, CH_{ala}, 3H), 4.57 (dd, J = 4.8 Hz, J = 8.7 Hz, CH_{val}, 1H), 5.69 (d, J = 7.5 Hz, $NH_{urethane}$, 1H), 6.88 (d, J = 8.7 Hz, NH, 1H), 7.27–7.35 (m, Ar-H_{Fmoc}, 2H), 7.36–7.44 (m, Ar-H_{Fmoc}, 2H), 7.60 (d, J = 7.8 Hz, Ar-H_{Fmoc}, 2H), 7.77 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.71, 18.98, 31.27, 47.04, 50.49, 52.20, 57.04, 67.17, 119.98, 125.08, 127.07, 127.72, 141.26, 143.71, 155.91, 172.25, 172.39. Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60%. Found: C, 67.70; H, 6.67; N, 6.63%.

***N*-Fmoc-*Ot*Bu-D-serinyl-L-alanine methyl ester (7n).** Yield: 82%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (s, $C(CH_3)_3$, 9H), 1.45 (d, J = 7.2 Hz, $CHCH_3$, 3H), 3.34–3.42 (m, CH_{2ser}, 1H), 3.77 (s, OCH_3, 3H), 3.80–3.87 (m, CH_{2ser}, 1H), 4.21–4.32 (m, CH_{Fmoc}, CH_{ser}, 2H), 4.41 (d, J = 6.9 Hz, CH_{2Fmoc}, 2H), 4.60 (app quin, J = 7.2 Hz, CH_{ala}, 1H), 5.81 (d, J = 6.3 Hz, $NH_{urethane}$, 1H), 7.29–7.45 (m, Ar-H_{Fmoc}, NH, 5H), 7.62 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.46, 27.39, 47.11, 48.27, 52.49, 54.02, 61.70, 67.21, 74.29, 119.99, 125.11, 127.06, 127.72, 141.28, 143.71, 156.09, 169.73, 173.00,. Anal. Calcd for $C_{26}H_{32}N_2O_6$ (468.54): C, 66.65; H, 6.88; N, 5.98%. Found: C, 66.85; H, 6.91; N, 6.02%.

Deprotection of *N*-Fmoc-L-isoleucinyl-4-nitrobenzenesulfonamide (4j)

N-Fmoc-L-isoleucinyl-4-nitrobenzenesulfonamide (**4j**) (1 mmol) was dissolved in 10 mL of dry DMF in a 100 mL round-bottomed flask. Piperidine (5 mmol) was added and the mixture was left to stir at room temperature. The reaction, monitored by TLC (Et_2O–CH_3OH 90 : 10, v/v), was completed after 1 h. 10 mL of a 0.2 N aqueous solution of HCl were added to the reaction mixture and the resulting solution extracted with dichloromethane (3 × 10 mL). The aqueous phase was basified to pH 9 with a 0.2 N solution of NaOH and extracted with DCM (3 × 10 mL). The obtained L-isoleucinyl 4-nitrobenzenesulfonamide free on amino function was directly coupled with the *N*-Fmoc-L-phenylalanine.

Synthesis of *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide (8j)

To a magnetically stirred solution of L-isoleucinyl-4-nitrobenzenesulfonamide (1 mmol) in dry dichloromethane (5 mL) and DIPEA (0.8 mmol) was added dropwise a solution of *N*-Fmoc-L-phenylalanine chloride (0.8 mmol) in dry dichloromethane (5 mL). The resulting mixture was stirred at room temperature under an inert atmosphere for 1 h, monitoring the conversion of *N*-Fmoc-L-phenylalanine chloride by TLC (Et_2O–CH_3OH, 90 : 10, v/v). The reaction mixture was washed with 1 N HCl (3 × 10 mL), with a 5% aqueous $NaHCO_3$ solution (2 × 10 mL) and with brine (1 × 5 mL), dried (Na_2SO_4) and evaporated to dryness to give *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide (**8j**) in 72% yield.

Yield: 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.55–0.77 (m, CHCH_3, CH_2CH_3, 6H), 0.78–0.90 (m, CH_{2Ile}, 1H), 0.91–1.20 (m, CH_{2Ile}, 1H), 1.68–1.81 (m, C$H$$CH_3$, 1H), 2.79–3.13 (m, CH_{2Phe}, 2H), 3.90–4.26 (m, α-CH_{Ile}, α-CH_{Phe}, CH_{Fmoc}, CH_{2Fmoc}, 5H), 7.14–7.44 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, NH, 10H), 7.57–7.71 (m, Ar-H_{Fmoc}, 2H), 7.84–7.91 (m, Ar-H_{Fmoc}, 2H), 7.94 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H), 8.19 (d, J = 8.7 Hz, Ar-H_{Ns}, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.32, 15.98, 24.85, 37.88, 38.85, 48.31, 56.21, 65.98, 120.21, 123.12, 125.78, 127.40, 127.48, 127.62, 128.75, 128.09, 129.68, 142.70, 143.58, 147.37, 148.47, 158.15, 160.12, 172.46, 180.81. Anal. Calcd for $C_{36}H_{36}N_4O_8S$ (684.76): C, 63.14; H, 5.30; N, 8.18%. Found: C, 63.33; H, 5.32; N, 8.15%.

Synthesis of *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-*N*-methyl-4-nitrobenzenesulfonamide (9j)

N-Fmoc-L-phenylalanyl-L-isoleucinyl-4-nitrobenzenesulfonamide (**8j**, 1 mmol) was dissolved in 10 mL of dry DMF in a 50 mL round-bottomed flask. Methyl iodide (5 mmol) and DIPEA (5 mmol) were added and the mixture was stirred at room temperature under an inert atmosphere and monitored by TLC (Et_2O–CH_3OH, 90 : 10, v/v). The reaction was completed after 1 h. To the reaction mixture, 10 mL of 5% $NaHSO_4$ aqueous solution were added and the resulting solution was extracted with ethyl acetate (3 × 10 mL). The organic layers were collected and washed with a 5% $NaHCO_3$ aqueous solution (3 × 10 mL) and with a brine solution (1 × 10 mL) and then dried (Na_2SO_4), filtered and evaporated to dryness. The corresponding *N*-Fmoc-L-phenylalanyl-L-isoleucine-*N*-methyl-4-nitrobenzenesulfonamide (**9j**) was recovered in very good yield (78%).

Yield: 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.64–0.89 (m, CHCH_3, CH_2CH_3, 6H), 1.17–1.48 (m, CH_{2Ile}, C$H$$CH_3$, 3H), 2.68–2.81 (m, CH_{2Phe}, 1H), 3.01–3.11 (m, CH_{2Phe}, 1H), 3.62 (s, NCH_3, 3H), 4.08–4.31 (m, α-CH_{Ile}, α-CH_{Phe}, CH_{Fmoc}, CH_{2Fmoc}, 5H), 7.11–7.48 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, NH, 10H), 7.54–7.72 (m, Ar-H_{Fmoc}, 2H), 7.81–7.93 (m, Ar-H_{Fmoc}, 2H), 8.22 (d, J = 8.8 Hz, Ar-H_{Ns}, 2H), 8.42 (d, J = 8.8 Hz, Ar-H_{Ns}, 2H). Anal. Calcd for $C_{37}H_{38}N_4O_8S$ (698.78): C, 63.60; H, 5.48; N, 8.02%. Found: C, 63.84; H, 5.45; N, 8.05%.

Synthesis of *N*-Fmoc-L-phenylalanyl-L-isoleucine (10j)

N-Fmoc-L-phenylalanyl-L-isoleucinyl-*N*-methyl-4-nitrobenzenesulfonamide (**9j**) was dissolved in dry DCM (10 mL) in a 100 mL flask. A 0.5 M aqueous solution of NaOH was added (10 mL) and the reaction mixture was left to stir at room temperature. The reaction, monitored by TLC (Et_2O–CH_3OH, 90 : 10, v/v), was complete after 2 h. The organic solvent was evaporated and the reaction mixture was acidified with a 1 N HCl solution and extracted with ethyl acetate (3 × 10 mL). The organic layer was collected, dried (Na_2SO_4), filtered and evaporated to dryness. *N*-Fmoc-L-phenylalanyl-L-isoleucine (**10j**) was recovered in very good yield (82%).

Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.74–0.95 (m, CHCH_3, CH_2CH_3, 6H), 1.11–1.28 (m, CH_{2Ile}, 1H), 1.34–1.51 (m, CH_{2Ile}, 1H), 1.72–1.88 (m, CH_{Ile}, 1H), 2.70–2.83 (m, CH_{2Phe}, 1H), 2.92–3.07 (m, CH_{2Phe}, 1H), 4.05–4.25 (m, α-CH_{Ile}, CH_{2Fmoc}, CH_{Fmoc}, 4H), 4.31–4.42 (m, α-CH_{Phe}, 1H), 7.19 (d, J = 7.5 Hz, $NH_{urethane}$, 1H), 7.20–7.48 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, 7H), 7.57–7.70 (m, Ar-H_{Fmoc}, 4H), 7.88 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H), 8.07 (d, J = 8.7 Hz, NH, 1H), 12.65 (br s, COOH, 1H). Anal. Calcd for $C_{30}H_{32}N_2O_5$ (500.59): C, 71.98; H, 6.44; N, 5.60%. Found: C, 71.69; H, 6.46; N, 5.62%.

Synthesis of *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-L-alanine methyl ester (11j)

N-Fmoc-L-phenylalanyl-L-isoleucinyl-*N*-methyl-4-nitrobenzenesulfonamide (1 mmol, **9j**) was dissolved in 10 mL of dry dichloromethane in a 100 mL two-necked round-bottomed flask under a nitrogen inert atmosphere. L-alanine methyl ester (1 mmol) and DMAP (2 mmol) and 1 mL of DMF were added. The reaction mixture was stirred at reflux and the reaction was monitored by TLC (Et_2O–CH_3OH, 90 : 10, v/v). After about 1 h the reaction was complete and the organic phase was washed with a 9% aqueous solution of Na_2CO_3 (2 × 10 mL), then with a 1 N HCl solution (2 × 10 mL) and finally with a brine solution (1 × 10 mL). The organic layer was dried (Na_2SO_4), paper-filtered and evaporated under reduced pressure. The crude product was purified by column chromatography (Et_2O–CH_3OH, 90 : 10, v/v) and *N*-Fmoc-L-phenylalanyl-L-isoleucinyl-L-alanine methyl ester (**11j**) was recovered in good yield (75%).

Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80–0.95 (m, $CHCH_3$, CH_2CH_3, 6H), 1.01–1.11 (m, CH_{2Ile}, 2H), 1.24–1.30 (m, CH_{Ile}, 1H), 1.40 (d, J = 6.0 Hz, CH_{3ala}, 3H), 3.03–3.15 (m, CH_{2Phe}, 2H), 3.75 (s, OCH_3, 3H), 4.16–4.37 (m, CH_{2Fmoc}, CH_{Fmoc}, 3H), 4.41–4.57 (m, α-CH_{Phe}, α-CH_{ala}, α-CH_{ile}, 3H), 5.40 (d, J = 7.5 Hz, $NH_{urethane}$, 1H), 6.42 (d, J = 7.5 Hz, NH, 1H), 6.51 (d, J = 7.5 Hz, NH, 1H), 7.15–7.45 (m, Ar-H_{Fmoc}, Ar-H_{Phe}, 9H), 7.51–7.57 (m, Ar-H_{Fmoc}, 2H), 7.78 (d, J = 7.5 Hz, Ar-H_{Fmoc}, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.29, 15.23, 18.20, 25.01, 37.03, 41.45, 45.34, 48.05, 52.43, 57.88, 65.78, 66.95, 120.00, 124.99, 127.09, 127.76, 128.97, 129.31, 139.98, 141.02, 143.62, 154.97, 170.04, 170.98, 172.98. Anal. Calcd for $C_{34}H_{39}N_3O_6$ (585.69): C, 69.72; H, 6.71; N, 7.17%. Found: C, 69.79; H, 6.68; N, 7.15%.

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