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Site-Specific Incorporation of Multiple Thioamide Substitutions into a Peptide Backbone via Solid Phase Peptide Synthesis

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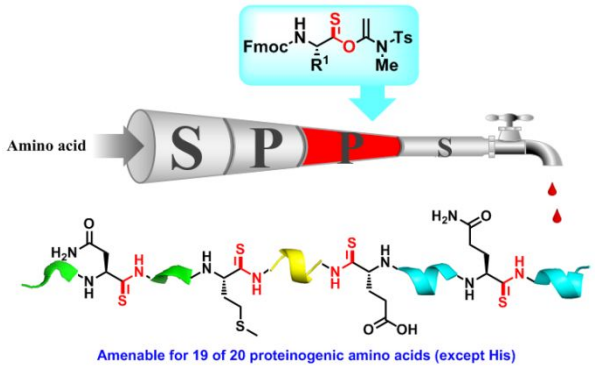
Supporting Information

ABSTRACT: Among various peptide modification strategies, thioamide substitution by replacing the carbonyl oxygen atom of an amide bond by a sulfur atom, constitutes an invaluable tool for chemical biology including peptide drug discovery and protein structure–function studies. However, thioamide substitution effect has not been well studied because of the lack of synthetic methods to site-specifically incorporating a thioamide bond into a peptide backbone, particularly introducing multi thioamide substitutions to peptide on a solid support. Herein, we reported a highly efficient method to incorporate a thioamide bond to the peptide backbone in a site-specific manner by employing α-thioacyloxyenamides, which are formed from the addition of N-protected monothioamino acids and ynamides, as a kind of novel thioacylating reagents in solid phase peptide synthesis. This method is amenable for 19 of 20 proteinogenic amino acids except for the His. Mono to multiple thioamide substitutions could be incorporated into a growing peptide with no or low level of epimerization. By using this method, fully thioamide substituted hexapeptide containing up to five continuous thioamide bonds could be synthesized smoothly. This synthetic methodology will spur the application of the thioamide substitution tool for protein engineering and peptide drug discovery.

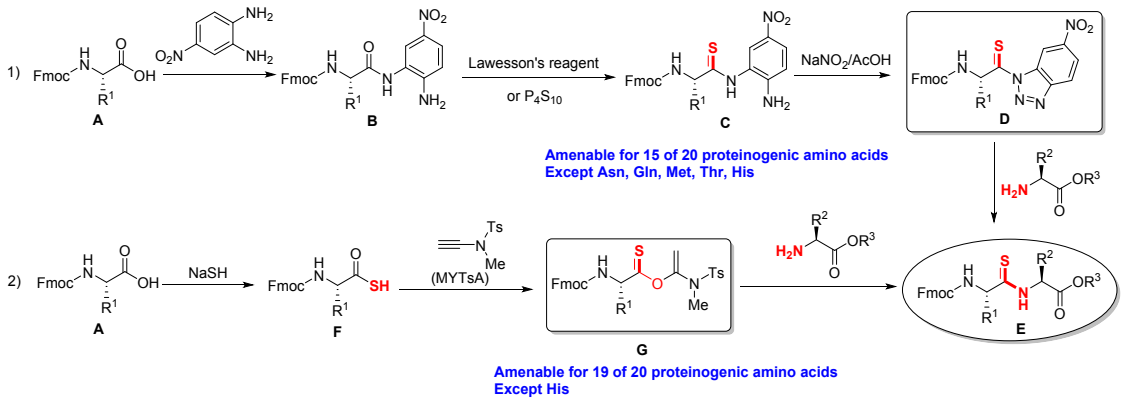


■ INTRODUCTION

Site-specific modification and functionalization of peptides and proteins can adjust their properties and functionalities and thus evolves into an invaluable tool for peptide drug discovery and protein structure–function studies.[1] Compared to the extensively studied post translation sidechain modifications, little attention has been paid to the peptide backbone modifications.[2] Replacing the amide bond with other isosteric functional groups is a straightforward strategy for peptide backbone modification. Among various amide bond surrogates, thioamide substitution, in which the amide bond oxygen atom is replaced with a sulfur atom, is the simplest modification that causes minimal perturbation.[3] Although numerous studies have revealed that the thioamide is isosteric to an amide bond with the same or similar geometry conformations,[3b] both groups retain many intrinsic different chemical and physical properties.[4] For example, the resistance of peptide to enzymatic degradation and bioactive can be dramatically enhanced by replacing the canonical peptide bond with a thioamide linkage.[5] Thioamide exhibits higher nucleophilicity and electrophilicity,[6] altered hydrogen-bonding propensity,[7] unique spectroscopic properties[8] and greater metal binding affinity.[9] Owing to these features, thioamide has been employed as probes for studying the dynamics of H-bond formation in β-sheets,[10] α-helices,[11, 10c] interaction of peptide bonds,[12] *cis/trans* photoswitches,[13] fluorescence quenchers,[14] and metal-binding substrates.[15] Although several intriguing features of thioamides have been recruited for studying protein folding, stability, function, and dynamics, the biological behavior of thioamide substitution on peptides and proteins of interest still is unpredictable. Extensive study of the physical and biological effect of thioamide substitution is impeded mainly by the lack of efficient synthetic strategies to site-specific incorporating thioamide bond into peptide backbone.

A broad range of synthetic methodologies for thioamide have been developed over the past decades.[16] However, only few of them are amenable for incorporating a thioamide bond into a growing peptide because of the difficult availability of chiral starting material and the highly prone to racemization of the α-chiral center of the thioamide residue. There are two strategies, by employing thionating reagents and thioacylating reagents, respectively, for synthesis of thioamide substituted peptide. Thionating reagents such as P_4S_{10},[17] Lawesson's reagent,[18] and other sulfur reagents[19] have been used to selectively convert the carbonyl oxygen atom of an amide bond to an sp^2-hybridized sulfur atom in the presence of ester functionality. However, this method is limited to simple amides and dipeptides because there is no selectivity for substrates containing more than one amide bonds. In this regard, the thioacylating reagents are more flexible because the modular nature enables them to incorporate a thioamide bond in a site-specific manner. Alkyl dithioesters,[20] thiobenzimidazolones,[21] and thioacylnitrobenzotriazoles[22] have been employed as active thioacylating reagents for thioamide substituted peptide synthesis. Thioacylnitrobenzotriazoles prepared via three steps transformations from natural α-amino acids (Figure 1, eq. 1) are the only one class of thioacylating reagents that could be used to introduce a thioamide bond in SPPS.[4, 23] However, this method is limited to the 15 of the 20 common proteinogenic α-amino acids.[2] In addition, the preparation of thioacylnitrobenzotriazole involves the use of toxic and malo-

Figure 1. Thioacylating Reagents Effective for Incorporating Thioamide Substitution in SPPS.



dour thionating reagents. Meanwhile, monothioamino acids have been used as thioacyl donor for thioamide incorporation with the assistance of phosphorus coupling reagents.[24] However, the co-formation of significant amount of corresponding oxo-amide byproduct rendered it not feasible for SPPS. Recently, we disclosed that ynamide could be used as a racemization/epimerization-free coupling reagent for peptide bond formation.[25] Interestingly, thioamide substituted peptide could be obtained in a racemization/epimerization-free manner when monothioamino acid was employed as the acyl donor with α-thioacyloxyenamide formed from the addition of monothioamino acid to ynamide as an efficient thioacylating reagent (Figure 1, eq. 2).[26] Owing to the optimal balance between the reactivity and stability of α-thioacyloxyenamides, they can also be used to incorporate a thioamide bond site-specifically in SPPS. Herein, we provided a systematic study about the use of α-thioacyloxyenamide as thioacylating reagents in SPPS.

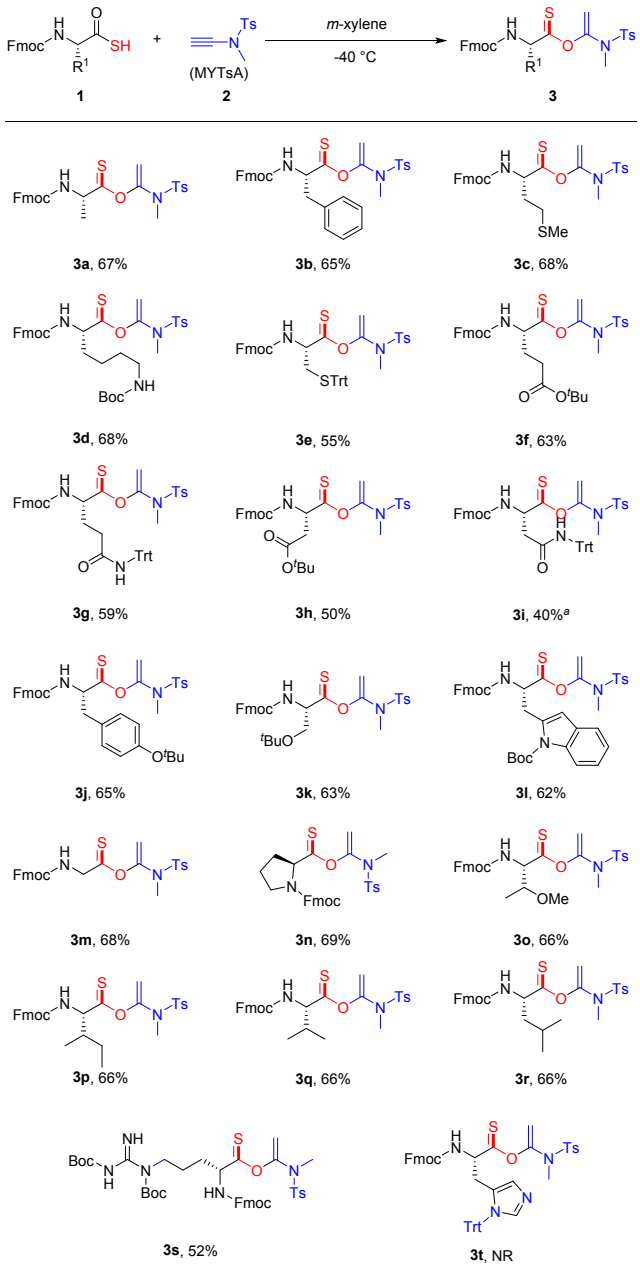
■ RESULTS AND DISCUSSION

In our previous work, we have demonstrated that thioacylating reagents α-thioacyloxyenamides could be obtained as the major product when the addition reaction of monothioamino acids and ynamide (MYTsA) were performed under -40 °C with *m*-xylene as the solvent.[26] To systematically evaluate the potency of α-thioacyloxyenamides to incorporate thioamide substitutions site-specifically to a growing peptide backbone on a solid support, all of the α-thioacyloxyenamides (**3a-s**) originated from 19 of 20 Fmoc-protected proteinogenic α-amino acids were prepared under the optimzed reaction conditions. As shown in Scheme 1, all of the target α-thioacyloxyenamides except that of His could be obtained in good yields under the optimized reaction conditions (Scheme 1).[26] It should be noted that all of these α-thioacyloxyenamides are stable and can be purified and characterized. No deterioration can be detected after they have been kept in fridge for two months.

With these thioacylating reagents in hand, the systematic and comprehensive optimization of the reaction conditions to incorporate one thioamide unit to the N-terminal of a growing peptide on a solid support was conducted. Initial experiments were carried out with the coupling of α-thioacyloxyenamide derived from Fmoc-protected alanine with 0.03 mmol of tripeptide VGF loaded on trityl chloride resin (2-CTC, which can be cleaved under more milder condition to avoid the side chain reaction and Edman degradation during cleavage of the thioamide substituted peptide from resin[23d, 27a]) as the model reaction. The effect of solvent, amount of α-thioacyloxyenamide, and coupling time on the reaction efficiency have been studied systematically (Table 1). Investigation on solvent disclosed that dimethylformamide (DMF) is the optimal choice (Table 1, entry 6), which is beneficial to suppress epimerization (Table 1, entries 2-4) and the co-formation of oxoamide byproduct efficiently (Table 1, entry 1). Interestingly, decreasing the amount of α-thioacyloxyenamide and shortening reaction time from 1 h to 0.5 h have no significant influence on the conversion. On the contrary, prolonged reaction time has deleterious effect on the coupling and resulted more oxoamide byproduct formed. The combination of 3 equiv of α-thioacyloxyenamide, DMF as the solvent, and 1 h coupling time were identified to be the best reaction conditions to incorporate a thioamide substitution to the N-terminal of a growing peptide in SPPS.

Next, the efficiency of incorporating one thioamide substitution to the N-terminal of a tetrapeptide with the tripeptide VGF bearing a bulky Val at the coupling site was evaluated for α-thioacyloxyenamides derived from 19 Fmoc-protected proteinogenic α-amino acids. As shown in Table 2, all of them proceeded smoothly to offer the target thioamide substituted tetrapeptide in quantitative conversion. It's notable that amino acids such as Met, Gln, Asn, and Thr, which can not be used for thioacylnitrobenzotriazole method work well in our case (Table 2, entries **3**, **7**, **9**, **13** and **15**).[2] The bulky side-chain protecting groups, such as Boc, ^{t}Bu, and Trt, have no deleterious effect on the conversion (Table 2, entries **4-11**). The protected hydrophilic and polar amino acids such as Arg, Asn, Ser, Lys, and Tyr are also compatible with this method (Table 2, entries **20, 21** and Table 3, entries **13-15**). Small amount of oxopeptide was formed as the byproduct during the coupling of thiocarbonyl esters of sterically hindered Val and Ile (Table 2, entries **16-17**). This might be attributed to the steric hindrance between these bulky residues and bulky Val at the N terminal (data not shown). This phenomenon could be avoided once the bulky Val was replaced by Ala (Table 2, entries **16-17**). Low level of epimerization was observed for the thiocarbonyl esters derived from Gln, Asp, Asn, and Ser, which are highly prone to racemization upon activation, under the room temperature conditions (Table 2, entries **7-9**, and **11**). As shown previously, such slight epimerization could be suppressed by lowering the

Scheme 1. Synthesis of *α*-thioacyloxyenamides from N-Fmoc Protected Monothioamino Acids

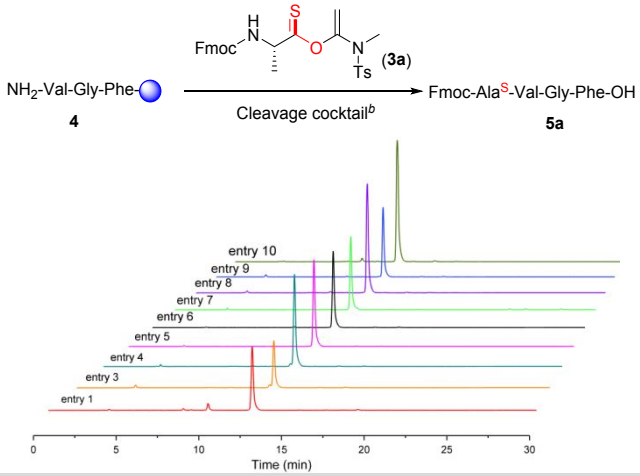


[a]These reactions were carried out with **1** (0.15 mmol, 1.5 equiv), **2** (MYTsA, 0.1 mmol), *m*-xylene (1.0 mL), -40 °C, 8 h, Fmoc = fluorenylmethoxy, Boc = tert-butoxycarbonyl, Trt = trityl, ^{t}Bu = tert-butyl, [a]Isolated yield. [b]CH_2Cl_2, -40 °C, 8 h. [c]NR = No reaction.

reaction temperature to 0 °C.[26] Scaling up the reaction to 0.4 mmol scale did not alter the coupling efficiency, which showcase the viability of this method (Table 2, entry 1).

The success on the incorporation of monothioamide substitution to the N-terminal of a growing peptide promoted us to challenge the synthesis of multiple thioamide substituted peptides, which involves iterative Fmoc deprotection of endothiopeptide. It is noted that the *α*-proton of the thioamide (pKa = ca. 13) residue is more acidic than that of the oxoamide and thus it is highly prone to racemization for thioamide residue under the

Table 1. Optimization of the Reaction Conditions[a]

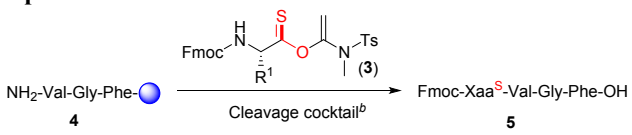


entry	equiv	time (h)	solvent	conversion (**4**/**5a**)[c]	dr[d]
1[e]	3	1	NMP	1:99	> 99:1
2	3	1	MeOH	69:31	98:2
3	3	1	CH_2Cl_2	3:97	95:5
4	3	1	THF	1:99	98:2
5[f]	3	1	DMF/CH_2Cl_2	1:99	> 99:1
6	**3**	**1**	**DMF**	**< 1:99**	**> 99:1**
7	1	1	DMF	2:98	> 99:1
8	2	1	DMF	1:99	> 99:1
9	3	0.5	DMF	1:99	> 99:1
10[g]	3	2	DMF	< 1:99	> 99:1

[a]Reactions were carried out with 0.03 mmol of **4** at rt. [b]Cleavage cocktail: TFE:AcOH:CH_2Cl_2 = 1:3:6. [c]Conversion (**4**/**5a** ratio) was calculated based on HPLC at 254 nm. [d]Product diastereomeric purity was determined by HPLC. [e]8% (oxoamide: thioamide) oxoamide was detected. [f] DMF/CH_2Cl_2 (v/v) = 1:1. [g]2% (oxoamide:thioamide) oxoamide was detected.

conventional Fmoc deprotection conditions (piperidine pKa = ca. 10 in DMF).[4, 27] Petersson and co-works observed that a more sterically hindered and non-nucleophilic base,1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), in DMF is beneficial to suppress epimerization during the removal of Fmoc group of thioamide-containing peptides.[21a, 27a, 28] However, in our hand, iterative exposure of thioamide-containing peptide to such Fmoc deprotection conditions caused racemization during the peptide elongation on a solid support.[27a, 28a] Fortunately, slight modification of Petersson's deprotection conditions by employing a cocktail of DBU (1%)/*n*-$C_6CH_{13}SH$ (25%) (v/v in DMF) offered better results. To further illustrate the robustness of our method, the synthesis of a series of dithioamide substituted peptides, including *i*+1, *i*+2, and *i*+3, were studied (Table 3).[23d, 29] Interestingly, the excellent reaction efficiency could be retained albeit a little longer reaction time was required for the incorporation of the second thioamide substitution. This might be at- tributed to the stronger electron-withdrawing effect of the thio-amide bond than that of oxoamide and thus decreasing the nucleophilicity of the N-terminal amino group of the thioamide-containing peptide. It is found that this electron-withdrawing

Table 2. Synthesis of Mono-Thioamide Substituted Peptides[a]

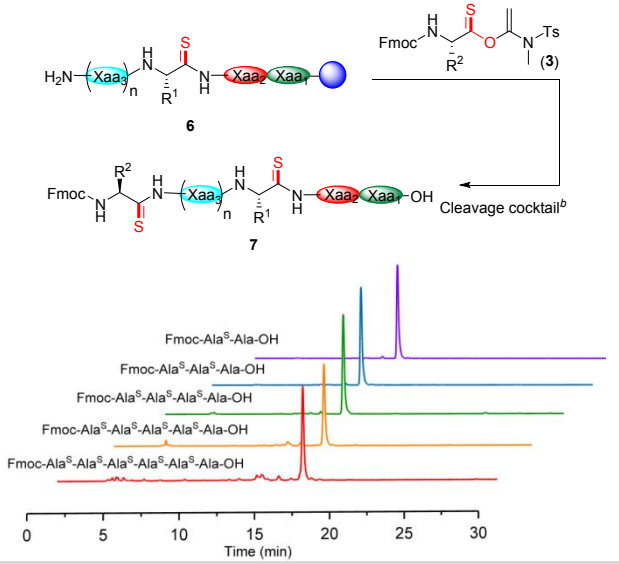


entry	Xaa (**5**)	**4**/**5**[c]	dr[d]
1	Ala (**5a**)[e]	<1:99	>99:1
2	Phe (**5b**)	1:99	99:1
3	Met (**5c**)	1:99	>99:1
4	Lys(Boc) (**5d**)	1:99	>99:1
5	Cys(Trt) (**5e**)	1:99	>99:1
6	Glu(^{t}Bu) (**5f**)	1:99	>99:1
7	Gln(Trt) (**5g**)	1:99	98:2
8	Asp(^{t}Bu) (**5h**)	<1:99	90:10
9	Asn(Trt) (**5i**)	<1:99	95:5
10	Tyr(^{t}Bu) (**5j**)	1:99	99:1
11	Ser(^{t}Bu) (**5k**)	<1:99	97:3
12	Trp(Boc) (**5l**)	1:99	>99:1
13	Gly (**5m**)	<1:99	>99:1
14	Pro (**5n**)	1:99	>99:1
15	Thr(Me) (**5o**)	2:98	>99:1
16	Ile (**5p**)[f]	2:98	>99:1
17	Val (**5q**)[f]	2:98	>99:1
18	Leu (**5r**)[f]	<1:99	>99:1
19	Arg(Boc)$_2$ (**5s**)	2:98	95:5
20	Ala (**5t**)[g]	2:98	>99:1
21	Glu (**5u**)[h,i]	<1:99	>99:1
22	Ala (**5v**)[j,i]	<1:99	>99:1

[a]Reactions were carried with 0.03 mmol of **4** at rt for 1 h. [b]Cleavage cocktail: TFE:AcOH:CH_2Cl_2 = 1:3:6. [c]Conversion (**4**/**5** ratio) was calculated based on HPLC at 254 nm. [d]Product diastereomeric purity was determined by HPLC. [e]**4** (0.4 mmol) was used. [f]NH_2-A-G-F-resin was used. [g]NH_2-A-R(Pbf)-G-resin was used. [h]NH_2-A-Q(Trt)- W(Boc)-P-K(Boc)-resin was used. [i]The product peptide was fully deprotected. [j]NH_2-A-Y(^{t}Bu)-N(Trt)-F-resin was used.

induction effect could be interrupted by inserting of the normal amide bond between the two thioamide bonds (Table 3, entries **1, 4, 6, 9, 12** and **13**). The reaction time for the introduction of the second thioamide moiety of the *i*+3 (2 h) was significantly shorter than that of *i*+1 (4 h), *i*+2 (3 h). It is expected that dithiopeptide would be highly prone to epimerization and to cause other side reactions. Even though, our protocols gave the required dithiopeptides in excellent conversion (93-99%), and purity (different HPLC methods and HRMS were used to assess the purity of final peptides **7a, 7f,** and **7g**, for details see SI). No or low level of epimerization was observed for some special amino acid residues. This promising result prompted us to attempt the synthesis of multiple thioamide substituted peptides, the properties of which have not been studied because of their

Table 3. Synthesis of Di- to Multi-Thioamide Bonds Substituted Peptide Sequence[a]



entry	sequence	time (h)	**6**/**7**[c]	dr[d]
1	A^{S}A^{S}VGF (**7a**)	1	1:99	>99:1
2	E(^{t}Bu)^{S}A^{S}VGF (**7b**)	3	3:97	>99:1
3	S(^{t}Bu)^{S}A^{S}VGF (**7c**)	1	1:99	92:8
4	M^{S}A^{S}VGF (**7d**)	4	4:96	>99:1
5	K(Boc)^{S}A^{S}VGF (**7e**)	4	7:93	>99:1
6	A^{S}LA^{S}VGF (**7f**)	3	<1:99	>99:1
7	F^{S}LA^{S}VGF (**7g**)	1	2:98	99:1
8	K(Boc)^{S}LA^{S}VGF (**7h**)	3	6:94	>99:1
9	M^{S}LA^{S}VGF (**7i**)	3	4:96	>99:1
10	E(^{t}Bu)^{S}LA^{S}VGF (**7j**)	5	7:93	>99:1
11	S(^{t}Bu)^{S}LA^{S}VGF (**7k**)	3	1:99	99:1
12	A^{S}LAA^{S}VF (**7l**)	2	<1:99	>99:1
13	M^{S}AMY(^{t}Bu)^{S}AL (**7m**)	2	1:99	96:4
14	K(Boc)^{S}LK(Boc)A^{S}AGF (**7n**)	2	2:98	>99:1
15	E(^{t}Bu)^{S}S(^{t}Bu)VE(^{t}Bu)^{S}APFN (Trt)GK(Boc) (**7o**)	2	4:96	96:4
16	M^{S}A^{S}A^{S}A^{S}A (**7p**)	10	1:99	>99:1
17	A^{S}A^{S}A^{S}A^{S}A^{S}A (**7q**)	5	2:98	>99:1

[a]Reactions were carried out with 0.03 mmol of **6** at rt for 1-10 h. [b]Cleavage cocktail: TFE:AcOH:CH_2Cl_2 = 1:3:6. [c]Conversion (**6**/**7** ratio) was calculated based on HPLC at 254 nm. [d]Product diastereomeric purity was determined by HPLC.

difficult availability. Multiple thioamide substituted pep tides are frequently observed in natural products such as thioviridamide family, which contains five continuous thioamide bonds and possesses highly potent pro-apoptotic activities and potent antiproliferative.[30] However, chemical synthesis of multiple thioamide substituted peptides on a solid support have never been reported yet. To our delight, *α*-thioacyloxyenamides proved to be effective thioacylating

reagents to incorporate multiple thioamide substitutions into the peptide backbone in SPPS. As shown in Table 3, tetra-thioamide substituted peptide could be easily synthesized by using our method (Table 3, entry 16). Even the fully thioamide substituted homooligo-hexapeptides containing five continuous thioamide bonds, the oxoamide congener of which is highly hydrophobic and has a high tendency to aggregate could also be synthesized on a solid support in an excellent yield by iterative using of *α*-thioacyloxyenamide derived from Fmoc-protected monothio-Alanine as the thioacylating reagent (Table 3, entry 17).[28c, 31]

■ CONCLUSION

In conclusion, we have provided a highly efficient method for site-specific incorporating thioamide substitutions to the backbone of a growing peptide on a solid support. Thiocarbonyl esters of 19 of 20 proteinogenic *α*-amino acids except His worked well to offer the target thioamide substituted peptides with no or low level of epimerization. Coupling conditions, a modified Fmoc deprotection method and the feasibility of incorporation of multiple thioamide substitutions have been systematically studied. The thioamide substituted hexapeptide containing up to five continuous thioamide bonds could also be synthesized by using this method. This study paves the way for the application of *α*-thioacyloxyenamides as a kind of thioacylating reagents to incorporate a thioamide substitution to a peptide backbone in a site-specific manner. No doubt, the novel thioacylating strategy reported herein will spur the further application of thioamide substitution tool in peptide and protein chemical biology.

■ EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded on a Bruker (400 and 100 MHz for ^{1}H and ^{13}C, respectively) instrument, and are internally referenced to residual solvent signals, $CDCl_3$ referenced at δ 7.26 and 77.06 ppm. Data for ^{1}H is reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), broad peaks (br), coupling constant (Hz) and assignment. Data for ^{13}C NMR are reported in terms of chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz) and no special nomenclature is used for equivalent carbons. HRMS (ESI) spectra were obtained by the electrospray ionization time-of-flight (ESI-TOF) mass spectrometry. The diastereomeric ratio (dr) was determined by HPLC with C18 column with H_2O and acetonitrile (ACN) as eluent. Flash column chromatography purification of compound was carried out by gradient elution using ethyl acetate (EA) in light petroleum ether (PE). Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Analytical RP-HPLC was performed on an UltiMate 3000 HPLC system using commercially available columns (Phenomenex C18, 250 mm x 4.6 mm, 5 μm) at a flow rate of 1.0 mL/min, detection at 220 or 254 nm. Mobile phase: solvent A was 0.045% TFA (v/v) in H_2O, solvent B was 10% H_2O and 0.039% TFA (v/v) in ACN. Gradient: a suitably adjusted gradient of 20% B to 100% B was used.

General Procedure 1 for *α*-thioacyloxyenamides (3a-s).

To a stirred solution of the appropriate Fmoc protected amino acid (5.0 mmol, 1.0 equiv) and *N*-hydroxysuccinimide (HOSu, 5.5 mmol, 1.1 equiv) in CH_2Cl_2 (20.0 mL), dicyclohexylcarbodiimide (DCC, 5.5 mmol, 1.1 equiv) was added at 25 ˚C under N_2. Then the mixture was stirred at 25 ˚C for 2 h. The suspension was filtered to remove the resulting white solid which was washed with 20.0 mL of CH_2Cl_2 repeatedly. The filtrate was concentrated about one-fifth of its original volume and left to stand in the refrigerator for about 2 h. Then the mixture was filtered again and concentrated to give the corresponding activated ester as a colorless foam, which was immediately dissolved in CH_2Cl_2 (20.0 mL), followed by addition of NaHS (15 mmol, 3.0 equiv, 70% purity) and 15-crown-5 (1.5 mmol, 0.3 equiv). Then the reaction mixture was stirred at rt for 3 h, diluted with CH_2Cl_2 and poured into ice water. The pH was adjusted to ~3 by careful addition of 1 M HCl and extracted by CH_2Cl_2 (3 × 20.0 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude products **2** were used for the next reaction without further purification.[25a, 26a, 32]

A 5.0 mL round-bottomed flask was charged with MYTsA (0.1 mmol, 1.0 equiv), *m*-xylene (1.0 mL) and **2** (0.15 mmol, 1.5 equiv). The reaction mixture was stirred at -40 ˚C under air until starting material MYTsA was fully consumed. The reaction mixture was concentrated and purified by silica gel chromatography to afford the *α*-thioacyloxyenamides **3**. The products **3a, 3b, 3m, 3p, 3q** and **3r** have been synthesized and characterized in the previous work.[25] The data given here are provided for convenience.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(methylthio) butanethioate (***3c***). Yellow solid (41 mg, 68%); mp 46-50 ℃; R_f = 0.4 (PE/EA = 5:1); [α]25 *D*= + 40.8 (c = 0.87, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 2H), 7.66 – 7.59 (m, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 4H), 5.67 (d, *J* = 9.0 Hz, 1H), 4.89 (d, *J* = 3.0 Hz, 1H), 4.83 – 4.71 (m, 1H), 4.61 (d, *J* = 3.0 Hz, 1H), 4.48 – 4.42 (m, 1H), 4.43 – 4.33 (m, 1H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.01 (s, 3H), 2.67 – 2.50 (m, 2H), 2.43 (s, 3H), 2.38 – 2.23 (m, 1H), 2.13 (s, 3H), 2.03 – 1.89 (m, 1H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 218.4, 155.8, 150.0, 144.5, 143.9, 143.8, 141.3, 132.7, 129.6, 128.2, 127.7, 127.1, 125.2, 120.0, 101.7, 67.1, 61.4, 47.2, 38.3, 34.1, 30.0, 21.6, 15.4 ppm; IR (KBr) ν̃ 3381, 2920, 1723, 1357, 1162, 743 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{30}H_{33}N_2O_5S_3$ $(M + H)^+$: 597.1546, found: 597.1540.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl) amino)hexanethioate (***3d***). Yellow solid (44 mg, 62%); mp 55-58 ℃; R_f = 0.4 (PE/EA = 3:1); [α]25 *D*= + 29.0 (c = 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.62 (t, *J* = 5.4 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 4H), 5.69 (d, *J* = 9.0 Hz, 1H), 4.88 (d, *J* = 3.1 Hz, 1H), 4.74 (s, 1H), 4.67 – 4.55 (m, 2H), 4.45 (dd, *J* = 10.4, 7.0 Hz, 1H), 4.33 (dd, *J* = 10.6, 7.2 Hz, 1H), 4.23 (t, *J* = 7.1 Hz, 1H), 3.17 – 3.06 (m, 2H), 3.00 (s, 3H), 2.41 (s, 3H), 1.97 – 1.88 (m, 1H), 1.82 – 1.68 (m, 1H), 1.57 – 1.37 (m, 13H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 219.2, 156.2, 155.8, 150.0, 144.5, 143.9, 143.8, 141.3, 132.6, 129.6, 128.2, 127.7, 127.1, 125.2, 120.0, 101.8, 79.0, 67.0, 61.9, 47.2, 40.1, 38.3, 34.2, 29.6, 28.5, 22.4, 21.6 ppm; IR (KBr) ν̃ 3399, 2933, 1717, 1514, 1357, 1156, 737 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{36}H_{43}N_3NaO_7S_2$ $(M + Na)^+$: 716.2435, found: 716.2429.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(trityl thio) propanethioate (***3e***). Yellow solid (45 mg, 55%); mp 69-73 ℃; R_f = 0.4 (PE/EA = 5:1); [α]25 *D*= + 40.2 (c = 0.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 2H), 7.69 – 7.58 (m, 4H), 7.45 – 7.35 (m, 8H), 7.32 – 7.16 (m, 13H), 5.36 (d, *J* = 8.9 Hz, 1H), 4.85 (d, *J* = 2.9 Hz, 1H), 4.75 (d, *J* = 3.0 Hz, 1H), 4.53 – 4.39 (m, 2H), 4.38 – 4.28 (m, 1H), 4.23 (t, *J* = 7.0 Hz, 1H), 2.94 (s, 3H), 2.76 – 2.60 (m, 2H), 2.37 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 215.8, 155.3, 149.3, 144.4, 144.3, 143.9, 143.8, 141.3, 129.6, 129.6, 128.1, 128.1, 127.7, 127.1, 126.9, 125.2, 120.0, 101.9, 67.3, 67.0, 60.7, 47.2, 37.4, 35.6, 21.6 ppm; IR (KBr) ν̃ 3405, 3051, 1728, 1498, 1357, 1163, 743 cm^{-1}; MS (ESI-TOF) calcd for $C_{47}H_{43}N_2O_5S_3$ $(M + H)^+$: 811.2329, found: 811.2318.

tert-butyl (S)-4-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)-5-((1-((N,4-dimethylphenyl)sulfonamido)vinyl)oxy) -5-thioxopentanoate (***3f***). Yellow solid (42 mg, 63%); mp 38-43 ℃; R_f = 0.4 (PE/EA = 5:1); [α]25 *D*= + 50.7 (c = 0.56, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.62 (t, *J* = 6.7 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.34 – 7.26 (m, 4H), 5.79 (d, *J* = 8.9 Hz, 1H), 4.88 (d, *J* = 3.0 Hz, 1H), 4.65 (d, *J* = 3.0 Hz, 1H), 4.64 – 4.57 (m, 1H), 4.45 (dd, *J* = 10.4, 7.0 Hz, 1H), 4.32 (dd, *J* = 10.5, 7.3 Hz, 1H), 4.22 (t, *J* = 7.1 Hz, 1H), 2.99 (s, 3H), 2.46 – 2.32 (m, 5H), 2.31 – 2.20 (m, 1H), 2.07 – 1.93 (m, 1H), 1.46 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 218.2, 172.2, 155.8, 149.8, 144.5, 144.0, 143.8, 141.3, 132.9, 129.6, 128.2, 127.7, 127.1, 125.2, 120.0, 101.7, 80.8, 67.1, 61.8, 47.2, 38.0, 31.6, 29.4, 28.1, 21.6 ppm; IR (KBr) ν̃ 3375, 2915, 1717, 1363, 1079, 743 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{34}H_{38}N_2NaO_7S_2$ (M + Na)$^+$: 673.2013, found: 673.2009.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-(tritylamino)pentanethioate (***3g***). Yellow solid (49 mg, 59%); mp 82-86 ℃; R_f = 0.3 (PE/EA = 3:1); [α]25 *D*= + 58.6 (c = 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.55 (m, 4H), 7.40 – 7.33 (m, 2H), 7.30 – 7.26 (m, 4H), 7.25 – 7.21 (m, 11H), 7.20 – 7.15 (m, 4H), 5.91 (d, *J* = 8.4 Hz, 1H), 4.88 (d, *J* = 3.1 Hz, 1H), 4.67 (q, *J* = 6.9 Hz, 1H), 4.47 (d, *J* = 3.1 Hz, 1H), 4.42 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.29 (dd, *J* = 10.4, 7.3 Hz, 1H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.00 (s, 3H), 2.52 – 2.35 (m, 5H), 2.25 (t, *J* = 7.4 Hz, 2H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 218.2, 171.2, 155.9, 150.5, 144.7, 144.7, 144.0, 143.8, 141.3, 129.7, 128.8, 128.3, 127.9, 127.7, 127.2, 126.9, 125.3, 120.0, 101.5, 70.6, 67.2, 61.5, 47.2, 38.8, 33.0, 30.1, 21.7 ppm; IR (KBr) ν̃ 3387, 3063, 1723, 1498, 1351, 1156, 737 cm^{-1}; MS (ESI-TOF) calcd for $C_{49}H_{46}N_3O_6S_2$ (M + H)$^+$: 836.2823, found: 836.2836.

tert-butyl (S)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-((1-((N,4-dimethylphenyl)sulfonamido)vinyl)oxy)-4-thioxobutanoate (***3h***). Yellow solid (32 mg, 50%); mp 40-45 ℃; R_f = 0.2 (PE/EA = 10:1); [α]25 *D*= + 26.8 (c = 1.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.64 (t, *J* = 8.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 4H), 5.99 (d, *J* = 9.3 Hz, 1H), 4.87 (d, *J* = 2.9 Hz, 1H), 4.85 – 4.77 (m, 1H), 4.68 (d, *J* = 3.1 Hz, 1H), 4.46 (dd, *J* = 10.2, 7.0 Hz, 1H), 4.33 (dd, *J* = 10.3, 7.4 Hz, 1H), 4.26 (t, *J* = 7.1 Hz, 1H), 3.01 (s, 3H), 2.89 (d, *J* = 5.6 Hz, 2H), 2.42 (s, 3H), 1.46 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 216.4, 169.2, 155.7, 149.8, 144.4, 143.9, 143.8, 141.3, 129.6, 128.2, 127.7, 127.1, 125.2, 120.0, 101.6, 81.9, 67.3, 58.6, 47.2, 39.6, 37.9, 28.1, 21.6 ppm; IR (KBr) ν̃ 3381, 3063, 2974, 1734, 1357, 1162, 737 cm^{-1}; MS (ESI-TOF) calcd for $C_{33}H_{36}N_2NaO_7S_2$ (M +Na)$^+$: 659.1856, found: 659.1865.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino) butanethioate (***3i***). Yellow solid (33 mg, 40%); mp 81-83 ℃; R_f = 0.2 (PE/EA = 3:1); [α]25 *D*= + 70.0 (c = 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.5 Hz, 2H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.61 (t, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.24 (m, 20H), 6.98 (d, *J* = 8.7 Hz, 1H), 4.81 (m, 2H), 4.46 – 4.31 (m, 2H), 4.18 (m, 2H), 3.25 (dd, *J* = 15.1, 4.4 Hz, 1H), 3.06 – 2.91 (m, 3H), 2.87 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.40 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 216.2, 168.8, 156.1, 150.1, 144.6, 144.3, 144.0, 143.9, 141.2, 132.0, 129.6, 128.8, 128.2, 127.9, 127.6, 127.1, 127.0, 125.4, 119.9, 101.1, 71.0, 67.3, 59.1, 47.1, 39.0, 38.5, 21.6 ppm; IR (KBr) ν̃ 3381, 2927, 2850, 1663, 1462, 1376, 1161, 739 cm^{-1}; MS (ESI-TOF) calcd for $C_{48}H_{44}N_3O_6S_2$ (M + H)$^+$: 822.2666, found: 822.2660.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(tert-butoxy)phenyl) propanethioate (***3j***). Yellow solid (36 mg, 65%); mp 54-57 ℃; R_f = 0.4 (PE/EA = 5:1); [α]25 *D*= + 40.4 (c = 0.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.66 (m, 5H), 7.59 (t, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.36 – 7.28 (m, 5H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.92 (d, *J* = 8.3 Hz, 2H), 5.52 (d, *J* = 8.9 Hz, 1H), 4.87 (q, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 2.6 Hz, 1H), 4.68 (d, *J* = 2.6 Hz, 1H), 4.43 – 4.32 (m, 2H), 4.21 (t, *J* = 7.1 Hz, 1H), 3.21 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.07 – 2.98 (m, 4H), 2.41 (s, 3H), 1.32 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 217.7, 155.3, 154.4, 149.7, 144.5, 143.8, 141.3, 133.0, 130.5, 130.0, 129.6, 128.2, 127.7, 127.1, 125.2, 124.1, 120.0, 102.0, 78.4, 67.0, 62.7, 47.2, 39.9, 38.0, 28.9, 21.6 ppm; IR (KBr) ν̃ 3375, 2986, 1728, 1363, 1168, 743 cm^{-1}; MS (ESI-TOF) calcd for $C_{38}H_{40}N_2NaO_6S_2$ (M + Na)$^+$: 707.2220, found: 707.2233.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propane thioate (***3k***). Yellow grass solid (40 mg, 63%); R_f = 0.4 (PE/EA = 5:1); [α]25 *D*= + 10.5 (c = 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.66 (dd, *J* = 11.1, 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.37 – 7.27 (m, 4H), 5.83 (d, *J* = 9.1 Hz, 1H), 4.89 (d, *J* = 2.8 Hz, 1H), 4.82 (d, *J* = 2.8 Hz, 1H), 4.73 – 4.59 (m, 1H), 4.48 (dd, *J* = 10.6, 7.0 Hz, 1H), 4.36 (dd, *J* = 10.5, 7.3 Hz, 1H), 4.28 (t, *J* = 7.2 Hz, 1H), 3.83 (dd, *J* = 9.1, 3.6 Hz, 1H), 3.66 (dd, *J* = 9.1, 4.0 Hz, 1H), 3.01 (s, 3H), 2.41 (s, 3H), 1.16 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 216.9, 155.8, 149.4, 144.3, 144.0, 143.9, 141.3, 133.6, 129.6, 128.2, 127.7, 127.1, 125.2, 120.0, 102.2, 73.6, 67.2, 63.5, 62.3, 47.2, 37.3, 27.4, 21.6 ppm; IR (KBr) ν̃ 3381, 2969, 1728, 1363, 1162, 737 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{32}H_{36}N_2NaO_6S_2$ (M + Na)$^+$: 631.1907, found: 631.1908.

tert-butyl (S)-2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)-3-((1-((N,4-dimethylphenyl)sulfonamido)vinyl)oxy)-3-thioxopropyl)-1H-indole-1-carboxylate (***3l***). Yellow solid (48 mg, 62%); mp 55-59 ℃; R_f = 0.5 (PE/EA = 5:1); [α]25 *D*= + 40.9 (c = 0.77, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.2 Hz, 1H), 7.78 – 7.67 (m, 4H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.58 – 7.46 (m, 3H), 7.42 – 7.34 (m, 2H), 7.33 – 7.22 (m, 6H), 5.61 (d, *J* = 8.9 Hz, 1H), 4.99 (q, *J* = 7.0 Hz, 1H), 4.80 (d, *J* = 3.0 Hz, 1H), 4.63 (d, *J* = 3.0 Hz, 1H), 4.32 (q, *J* = 10.3, 8.9 Hz, 2H), 4.19 (t, *J* = 7.3 Hz, 1H), 3.41 (dd, *J* = 14.8, 5.7 Hz, 1H), 3.20 (dd, *J* = 14.6, 7.2 Hz, 1H), 3.01 (s, 3H), 2.39 (s, 3H), 1.65 (s, 9H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 217.6, 155.5, 149.9, 149.6, 144.5, 143.8, 141.3, 135.5, 132.8, 130.6, 129.6, 128.2, 127.7, 127.1, 125.2, 124.6, 124.5, 122.7, 119.9, 119.0, 115.3, 114.8, 101.7, 83.7, 67.2, 62.0, 47.1, 38.1, 30.1, 28.2, 21.6 ppm; IR (KBr) ν̃ 3387, 2920, 1734, 1357, 1168, 731 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{41}H_{41}N_3NaO_7S_2$ (M + Na)$^+$: 774.2278, found: 774.2274.

(9H-fluoren-9-yl)methyl (S)-2-(((1-((N,4-dimethylphenyl) sulfonamido)vinyl)oxy) carbonothioyl)pyrrolidine-1-carboxylate (***3n***). Yellow grass solid (40 mg, 69%); R_f = 0.5 (PE/EA = 5:1); [α]25 *D*= + 12.9 (c = 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.50 (m, 6H), 7.44 – 7.05 (m, 6H), 4.90 (d, *J* = 2.6 Hz, 0.5H), 4.82 – 4.74 (m, 1H), 4.72 (d, *J* = 2.7 Hz, 0.5H), 4.57 (d, *J* = 2.7 Hz, 0.5H), 4.54 – 4.39 (m, 1.5H), 4.36 – 4.07 (m, 2H), 3.80 – 3.63 (m, 1H), 3.63 – 3.48 (m, 1H), 2.98 (m, 3H), 2.38 (m, 3H), 2.24 (m, 2H), 2.07 (m, 1H), 1.98 – 1.82 (m, 1H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 219.2, 219.0, 154.7, 154.4, 150.4, 150.0, 144.5, 144.4, 144.3, 143.9, 143.8, 141.3, 141.3, 141.2, 132.7, 132.4, 129.6, 129.6, 128.3, 128.3, 127.7, 127.7, 127.1, 127.1, 125.3, 125.2, 125.2, 125.1, 120.0, 120.0, 101.7, 101.5, 68.2, 67.9, 67.5, 67.4, 47.4, 47.3, 47.2, 46.7, 38.5, 38.2, 33.0, 31.8, 23.8, 22.8, 21.6, 21.6 ppm; IR (KBr) ν̃ 3068, 2927, 1705, 1363, 1162, 743 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{30}H_{30}N_2NaO_5S_2$ (M + Na)$^+$: 585.1488, found: 585.1484.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (2S,3R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxybutane thioate (***3o***). Yellow solid (40 mg, 66%); mp 48-50 ℃; R_f = 0.4 (PE/EA = 8:1); [α]25 *D*= + 47.8 (c = 0.88, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 7.4 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.45 – 7.37 (m,

2H), 7.36 – 7.29 (m, 4H), 5.76 (d, *J* = 9.9 Hz, 1H), 4.92 (d, *J* = 2.9 Hz, 1H), 4.67 (d, *J* = 2.9 Hz, 1H), 4.54 – 4.43 (m, 2H), 4.37 – 4.24 (m, 2H), 4.13 (qd, *J* = 6.2, 2.1 Hz, 1H), 3.33 (s, 3H), 3.01 (s, 3H), 2.43 (s, 3H), 1.25 (d, *J* = 6.3 Hz, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 217.2, 156.6, 150.0, 144.4, 144.0, 143.8, 141.3, 132.9, 129.6, 128.3, 127.7, 127.1, 125.3, 120.0, 102.5, 76.9, 67.2, 66.2, 57.2, 47.2, 38.1, 21.6, 16.6 ppm; IR (KBr) $\tilde{\nu}$ 3428, 3057, 2933, 1723, 1357, 1085, 731 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{30}H_{32}N_2NaO_6S_2$ $(M + Na)^+$: 603.1594, found: 603.1591.

O-(1-((N,4-dimethylphenyl)sulfonamido)vinyl) (S)-2-((((9H-fluoren-9-yl)methoxy) carbonyl)amino)-5-(1,3-bis(tert-butoxy carbonyl)guanidino)pentanethioate (***3s***). Yellow solid (45 mg, 55%); mp 66-69 ℃; R_f = 0.4 (PE/EA = 3:1); [α]25 *D*= + 41.5 (c = 0.48, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 11.52 (s, 1H), 8.36 (t, *J* = 5.6 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.71 (d, *J* = 7.9 Hz, 2H), 7.64 (t, *J* = 9.0 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.27 (m, 4H), 5.92 (d, *J* = 9.0 Hz, 1H), 4.87 (d, *J* = 3.1 Hz, 1H), 4.68 (d, *J* = 3.0 Hz, 1H), 4.64 – 4.53 (m, 1H), 4.50 – 4.33 (m, 2H), 4.23 (t, *J* = 6.9 Hz, 1H), 3.51 (dd, *J* = 12.8, 6.2 Hz, 1H), 3.39 (dd, *J* = 12.7, 6.1 Hz, 1H), 2.99 (s, 3H), 2.41 (s, 3H), 2.03 – 1.91 (m, 1H), 1.79 – 1.59 (m, 3H), 1.50 (s, 9H), 1.48 (s, 9H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 218.6, 163.6, 156.3, 155.8, 153.3, 149.7, 144.4, 144.0, 143.8, 141.3, 133.0, 129.6, 128.2, 127.7, 127.1, 125.2, 119.9, 101.8, 83.1, 79.2, 67.0, 62.1, 47.2, 40.3, 37.9, 31.4, 28.3, 28.1, 25.6, 21.6 ppm; IR (KBr) $\tilde{\nu}$ 3328, 2974, 1728, 1640, 1357, 1126, 743 cm^{-1}; MS (ESI-TOF) calcd for $C_{41}H_{52}N_5O_9S_2$ $(M + H)^+$: 822.3201, found: 822.3195.

General Procedure 2 for Incorporation of the Monothioamide on a Solid Support.

Normal peptide assembly by Fmoc based SPPS (*4*). 2-CTC resin (39 mg, 0.03 mmol) with a loading of 0.77 mmol/g, was placed into 5.0 mL fritted syringes, the resin was swelled in CH_2Cl_2 (3.0 mL, 30 min). The syringe was then drained, Fmoc-Phe-OH (35 mg, 3.0 equiv), N,N-Diisopropyl-ethylamin (DIPEA, 10.0 µL, 2.0 equiv) and 3.0 mL DMF/CH_2Cl_2 (v/v 1:1) were added into the fritted syringe. The loading reaction was left to react 2 h at rt before being drained. Then, the resin was washed with DMF (3.0 mL x 4) and CH_2Cl_2 (3.0 mL x 4). The solution of 20% piperidine in DMF (3.0 mL) was added to the fritted syringes to react 2 min before being drained. A further 3.0 mL of 20% piperidine was added to react 18 min. Then, the resin was again carefully washed with DMF (3.0 mL x 4) and CH_2Cl_2 (3.0 mL x 4). The peptide elongation was carried out by adding a solution of Fmoc protected amino acid (3.0 equiv), HBTU (35 mg, 3.0 equiv) and DIPEA (30.0 µL, 6.0 equiv) in 3.0 mL DMF/CH_2Cl_2 (1:1). After 1 h, the resin was washed with DMF (3.0 mL x 4) and CH_2Cl_2 (3.0 mL x 4). The resin-bound peptide **4** was obtained by using standard SPPS protocols as described above.[23d, 26a, 28a]

Synthesis of the Thioamide Substituted Tetrapeptide (5). Prior to the thioamide coupling, the **4** (0.03 mmol) was washed with DMF. The solution of 3a (3.0 equiv) in DMF (3.0 mL) was taken into the fritted syringes. The reaction was shaken at rt for 1 h. Then, the solvent was drained off and the resin was thoroughly washed with DMF (3.0 mL x 4) and CH_2Cl_2 (3.0 mL x 4). The peptide 5a was obtained after treat 10 mg resin with 100.0 µL of cleavage cocktail (2,2,2-Trifluoroethanol (TFE)/AcOH/CH_2Cl_2 - 1:3:6) for 30 min. The CH_2Cl_2 was removed from the cocktail. Then the solution of water/acetonitrile (v/v 1:1, 200.0 µL) was added to the residue. The resin was filtered off and the filtrates was analysed by HPLC and MS. Peptide **5u** and **5v** were obtained after treat 30 mg resin with 100.0 µL of cleavage cocktail (TFA/TIPS/Thioanisole/H_2O = 95:2.5:2.5:2.5) for 1 h. The cleavage mixture was evaporated to an oil and then cold ether was added. The precipitate was collected and analysed by HPLC and MS.

Synthesis of Fmoc-Ala^S-Val-Gly-Phe-OH (***5a***). Peptide **5a** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.85% conversion of crude HPLC traces; t_R = 12.513 min; MS (ESI-TOF) calcd for $C_{34}H_{39}N_4O_6S$ $(M + H)^+$: 631.26, found 630.81.

Synthesis of Fmoc-Phe^S-Val-Gly-Phe-OH (***5b***). Peptide **5b** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.60% conversion of crude HPLC traces; t_R = 12.091 min (major); MS (ESI-TOF) calcd for $C_{40}H_{43}N_4O_6S$ $(M + H)^+$: 707.29, found 706.97.

Synthesis of Fmoc-Met^S-Val-Gly-Phe-OH (***5c***). Peptide **5c** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.00% conversion of crude HPLC traces; t_R = 10.260 min (major); MS (ESI-TOF) calcd for $C_{36}H_{43}N_4O_6S_2$ $(M + H)^+$: 691.26, found 690.96.

Synthesis of Fmoc-Lys(Boc)^S-Val-Gly-Phe-OH (***5d***). Peptide **5d** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.03% conversion of crude HPLC traces; t_R = 11.918 min; MS (ESI-TOF) calcd for $C_{42}H_{54}N_5O_8S$ $(M + H)^+$: 788.37, found 788.18.

Synthesis of Fmoc-Cys(Trt)^S-Val-Gly-Phe-OH (***5e***). Peptide **5e** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.02% conversion of crude HPLC traces; t_R = 24.000 min; MS (ESI-TOF) calcd for $C_{53}H_{51}N_4O_6S_2$ $(M - H)^-$: 903.33, found 903.14.

Synthesis of Fmoc-Glu(^{t}Bu)^S-Val-Gly-Phe-OH (***5f***). Peptide **5f** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.80% conversion of crude HPLC traces; t_R = 13.185 min; MS (ESI-TOF) calcd for $C_{40}H_{47}N_4O_8S$ $(M - H)^-$: 743.31, found 743.45.

Synthesis of Fmoc-Gln(Trt)^S-Val-Gly-Phe-OH (***5g***). Peptide **5g** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.70% conversion of crude HPLC traces; t_R = 14.013 min (major); MS (ESI-TOF) calcd for $C_{55}H_{56}N_5O_7S$ $(M + H)^+$: 930.39, found 930.23.

Synthesis of Fmoc-Asp(^{t}Bu)^S-Val-Gly-Phe-OH (***5h***). Peptide **5h** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.23% conversion of crude HPLC traces; t_R = 18.360 min (major); MS (ESI-TOF) calcd for $C_{39}H_{47}N_4O_8S$ $(M + H)^+$: 731.31, found 730.91.

Synthesis of Fmoc-Asn(Trt)^S-Val-Gly-Phe-OH (***5i***). Peptide **5i** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.19% conversion of crude HPLC traces; t_R = 18.318 min (major); MS (ESI-TOF) calcd for $C_{54}H_{53}N_5O_7S$ $(M + H)^+$: 916.37, found 916.01.

Synthesis of Fmoc-Tyr(^{t}Bu)^S-Val-Gly-Phe-OH (***5j***). Peptide **5j** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 40-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.78% conversion of crude HPLC traces; t_R = 24.581 min (major); MS (ESI-TOF) calcd for $C_{44}H_{49}N_4O_7S$ $(M - H)^-$: 777.33, found 777.45.

Synthesis of Fmoc-Ser(^{t}Bu)^S-Val-Gly-Phe-OH (***5k***). Peptide **5k** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.65% conversion of crude HPLC traces; t_R = 13.370 min (major); MS (ESI-TOF) calcd for $C_{38}H_{47}N_4O_7S$ $(M + H)^+$: 703.32, found 703.12.

Synthesis of Fmoc-Trp(Boc)^S-Val-Gly-Phe-OH (***5l***). Peptide **5l** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 70-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.17% conversion of crude HPLC traces; t_R = 14.834 min; MS (ESI-TOF) calcd for $C_{47}H_{52}N_5O_8S$ $(M + H)^+$: 846.35, found 846.33.

Synthesis of Fmoc-Gly^S-Val-Gly-Phe-OH (***5m***). Peptide **5m** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 40-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.58% conversion of crude HPLC traces; t_R = 15.706 min; MS (ESI-TOF) calcd for $C_{33}H_{37}N_4O_6S$ $(M - H)^-$: 615.23, found 615.67.

Synthesis of Fmoc-Pro^S-Val-Gly-Phe-OH (***5n***). Peptide **5n** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.85% conversion of crude HPLC traces; t_R = 18.054 min; MS (ESI-TOF) calcd for $C_{35}H_{39}N_4O_6S$ $(M - H)^-$: 655.26, found 655.24.

Synthesis of Fmoc-Thr(Me)^S-Val-Gly-Phe-OH (***5o***). Peptide **5o** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 40-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.72% conversion of crude HPLC traces; t_R = 18.833 min; MS (ESI-TOF) calcd for $C_{36}H_{43}N_4O_7S$ $(M + H)^+$: 673.27, found 673.20.

Synthesis of Fmoc-Ile^S-Ala-Gly-Phe-OH (***5p***). Peptide **5p** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 97.22% conversion of crude HPLC traces; t_R = 14.701 min; MS (ESI-TOF) calcd for $C_{35}H_{39}N_4O_6S$ $(M - H)^-$: 643.26, found 643.36.

Synthesis of Fmoc-Val^S-Ala-Gly-Phe-OH (***5q***). Peptide **5q** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 97.94% conversion of crude HPLC traces; t_R = 13.036 min; MS (ESI-TOF) calcd for $C_{34}H_{36}N_4O_6S$ $(M - H)^-$: 629.24, found 629.20.

Synthesis of Fmoc-Leu^S-Ala-Gly-Phe-OH (***5r***). Peptide **5r** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.88% conversion of crude HPLC traces; t_R = 15.873 min; MS (ESI-TOF) calcd for $C_{35}H_{39}N_4O_6S$ $(M - H)^-$: 643.26, found 643.38.

Synthesis of Fmoc-Arg(Boc)$_2^S$-Val-Gly-Phe-OH (***5s***). Peptide **5s** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.43% conversion of crude HPLC traces; t_R = 19.433 min (major); MS (ESI-TOF) calcd for $C_{47}H_{60}N_7O_{10}S$ $(M - H)^-$: 914.41, found 914.47.

Synthesis of Fmoc-Ala^S-Val-Arg(pbf)-Gly-OH (***5t***). Peptide **5t** was obtained using the procedure 2. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-80% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.01% conversion of crude HPLC traces; t_R = 17.550 min. MS (ESI-TOF) calcd for $C_{44}H_{58}N_7O_9S_2^+$ $(M + H)^+$: 892.37, found 892.26.

Synthesis of NH_2-Glu^S-Ala-Gln-Trp-Pro-Lys-OH (**5u**). HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 5-50% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 100% conversion of crude HPLC traces; t_R = 14.357 min. MS (ESI-TOF) calcd for $C_{35}H_{52}N_9O_9S^+$ $(M + H)^+$: 774.36, found 774.30.

Synthesis of NH_2-Aal^S-Ala-Tyr-Asn-Phe-OH (**5v**). HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 5-50% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 100% conversion of crude HPLC traces; t_R = 18.433 min. MS (ESI-TOF) calcd for $C_{28}H_{37}N_6O_7S^+$ $(M + H)^+$: 601.24, found 601.15.

General Procedure 3 for the Incorporation of Multi-thioamide on a Solid Support.

Fmoc Removal. After the first thioamide incorporation, the **6** (0.03 mmol) was washed with DMF. A solution of DBU/*n*-$C_6CH_{13}SH$ (1%/25%, v/v in DMF, 3.0 mL) was added to the fritted syringes to react 2 min before being drained. A further 3.0 mL of DBU/*n*-$C_6CH_{13}SH$ was added to react 2 min. Then, the resin was carefully washed with DMF (3.0 mL x 4) and CH_2Cl_2 (3.0 mL x 4).

Subsequent Thioamide Bonds Incorporation. After the first thioamide was incorporated into the peptide sequence, the subsequent Fmoc deprotections and peptide elongation were treated with DBU/*n*-$C_6CH_{13}SH$ and HBTU/DIPEA conditions. The second thioamide incorporation proceeded as described above general procedure 1 to get target **7** on the resin. The peptide **7** was obtained after treat 10 mg resin with 100.0 μL of cleavage cocktail (TFE/AcOH/CH_2Cl_2 - 1:3:6) for 30 min. The CH_2Cl_2 was removed from the cocktail. Then the solution of water/acetonitrile (v/v 1:1, 200.0 μL) was added to the residue. The resin was filtered off and the filtrates was analysed by HPLC and MS.

Synthesis of Fmoc-A^{S}A^{S}VGF-OH (***7a***). Peptide **7a** was obtained using the procedure 3 after 1 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.42% conversion of crude HPLC traces; t_R = 14.520 min; HRMS (ESI-TOF) calcd for $C_{37}H_{42}N_5O_6S_2$ $(M - H)^-$: 716.2582, found 716.2593.

Synthesis of Fmoc-E(^{t}Bu)^{S}A^{S}VGF-OH (***7b***). Peptide **7b** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 96.66% conversion of crude HPLC traces; t_R = 14.316 min; MS (ESI-TOF) calcd for $C_{43}H_{54}N_5O_8S_2$ $(M + H)^+$: 832.34, found 831.97.

Synthesis of Fmoc-S(^{t}Bu)^{S}A^{S}VGF-OH (***7c***). Peptide **7c** was obtained using the procedure 3 after 1 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.25% conversion of crude HPLC traces; t_R = 13.095 min (major); MS (ESI-TOF) calcd for $C_{41}H_{52}N_5O_7S_2$ $(M + H)^+$: 790.33, found 790.01.

Synthesis of Fmoc-M^{S}A^{S}VGF-OH (***7d***). Peptide **7d** was obtained using the procedure 3 after 4 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 95.51% conversion of crude HPLC traces; t_R = 11.121 min; MS (ESI-TOF) calcd for $C_{39}H_{48}N_5O_6S_3$ $(M + H)^+$: 778.28, found 777.92.

Synthesis of Fmoc-K(Boc)^{S}A^{S}VGF-OH (***7e***). Peptide **7e** was obtained using the procedure 3 after 4 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 92.92% conversion of crude

HPLC traces; t_R = 12.843 min; MS (ESI-TOF) calcd for $C_{45}H_{59}N_6O_8S_2$ $(M + H)^+$: 875.38, found 875.25.

Synthesis of Fmoc-A^{S}LA^{S}VGF-OH (***7f***). Peptide **7f** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.63% conversion of crude HPLC traces; t_R = 11.883 min; HRMS (ESI-TOF) calcd for $C_{43}H_{53}N_6O_7S_2$ $(M - H)^-$: 829.3423, found 829.3425.

Synthesis of Fmoc-F^{S}LA^{S}VGF-OH (***7g***). Peptide **7g** was obtained using the procedure 3 after 1 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.10% conversion of crude HPLC traces; t_R = 15.975 min (major); HRMS (ESI-TOF) calcd for $C_{49}H_{55}N_6O_7S_2$ $(M -H)^-$: 905.3736, found 905.3750.

Synthesis of Fmoc-K(Boc)^{S}LA^{S}VGF-OH (***7h***). Peptide **7h** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 93.64% conversion of crude HPLC traces; t_R = 16.006 min; MS (ESI-TOF) calcd for $C_{51}H_{70}N_7O_9S_2$ $(M + H)^+$: 988.47, found 988.39.

Synthesis of Fmoc-M^{S}LA^{S}VGF-OH (***7i***). Peptide **7i** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 95.62% conversion of crude HPLC traces; t_R = 13.833 min; MS (ESI-TOF) calcd for $C_{45}H_{57}N_6O_7S_3$ $(M - H)^-$: 889.26, found 889.35.

Synthesis of Fmoc-E(^{t}Bu)^{S}LA^{S}VGF-OH (***7j***). Peptide **7j** was obtained using the procedure 3 after 5 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 93.34% conversion of crude HPLC traces; t_R = 17.450 min; MS (ESI-TOF) calcd for $C_{49}H_{65}N_6O_9S_2$ $(M + H)^+$: 945.42, found 945.04.

Synthesis of Fmoc-S(^{t}Bu)^{S}LA^{S}VGF-OH (***7k***). Peptide **7k** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.54% conversion of crude HPLC traces; t_R = 17.791 min (major); MS (ESI-TOF) calcd for $C_{47}H_{63}N_6O_8S_2$ $(M + H)^+$: 903.41, found 902.99.

Synthesis of Fmoc-A^{S}LAA^{S}VF-OH (***7l***). Peptide **7l** was obtained using the procedure 3 after 2 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 100% conversion of crude HPLC traces; t_R = 13.330 min; MS (ESI-TOF) calcd for $C_{44}H_{55}N_6O_7S_2$ $(M - H)^-$: 843.36, found 843.44.

Synthesis of Fmoc-M^{S}AMY(^{t}Bu)^{S}AL-OH (***7m***). Peptide **7m** was obtained using the procedure 3 after 2 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.89% conversion of crude HPLC traces; t_R = 24.743 min (major); MS (ESI-TOF) calcd for $C_{50}H_{67}N_6O_8S_4$ $(M - H)^-$: 1007.39, found 1007.66.

Synthesis of Fmoc-K(Boc)^{S}LK(Boc)A^{S}AGF-OH (***7n***). Peptide **7n** was obtained using the procedure 3 after 2 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 97.68% conversion of crude HPLC traces; t_R = 23.400 min; MS (ESI-TOF) calcd for $C_{60}H_{84}N_9O_{12}S_2$ $(M - H)^-$: 1186.57, found 1186.50.

Synthesis of Fmoc-E(^{t}Bu)^{S}S(^{t}Bu)VE(^{t}Bu)^{S}APFN(Trt)GK(Boc)-OH (***7o***). Peptide **7o** was obtained using the procedure 3 after 3 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 80-100% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 24.133 min (major). MS (ESI-TOF) calcd for $C_{98}H_{127}N_{12}O_{19}S_2^-$ $(M - H)^-$: 1839.88, found 1839.75.

Synthesis of Fmoc-M^{S}A^{S}A^{S}A^{S}A-OH (***7p***). Peptide **7p** was obtained using the procedure 3 after 10 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 70-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.24% conversion of crude HPLC traces; t_R = 8.151 min; MS (ESI-TOF) calcd for $C_{32}H_{40}N_5O_4S_5$ $(M - H)^-$: 718.17, found 717.94.

Synthesis of Fmoc-A^{S}A^{S}A^{S}A^{S}A^{S}A-OH (***7q***). Peptide **7q** was obtained using the procedure 3 after 5 h. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 97.75% conversion of crude HPLC traces; t_R = 16.620 min; MS (ESI-TOF) calcd for $C_{33}H_{41}N_6O_4S_5$ $(M - H)^-$: 745.18, found 745.25.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Optimization of reaction conditions; ^{1}H- and ^{13}C NMR spectra; HPLC data; ESI-MS spectra; additional experimental procedures (PDF)

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Notes

The authors declare no competing financial interests.

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