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Methionine-Containing Peptides: Avoiding Secondary Reactions in the Final Global Deprotection

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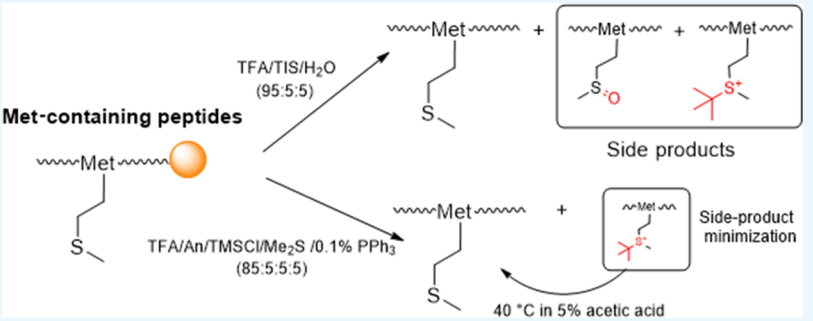

ABSTRACT: The solid-phase synthesis of Met-containing peptides using a fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu) protection scheme is inevitably accompanied by two stubborn side reactions, namely, oxidation and *S*-alkylation (*tert*-butylation), which result in the formation of Met(O) and sulfonium salt impurities of the target peptide, respectively. These two reactions are acid-catalyzed, and they occur during the final trifluoroacetic (TFA)-based acidolytic cleavage step. Herein, we developed two new cleavage solutions that eradicate the oxidation and reduce *S*-alkylation. TFA-anisole-trimethylsilyl chloride (TMSCl)-Me_2S-triisopropylsilane (TIS) containing 1 mg of triphenyl phosphine per mL of solution was the optimal mixture for Cys-containing peptides, while for the remaining peptides, TIS was not required. Both cleavage solutions proved to be excellent when sensitive amino acids such as Cys and Trp were involved. TMSCl did not affect either of these sensitive amino acids. Reversing the sulfonium salt to free Met-containing peptide was achieved by heating the peptide at 40 °C for 24 h using 5% acetic acid.

INTRODUCTION

Methionine (Met) is an essential amino acid present in proteins, and it plays various biological roles, including translation initiation.[1] Having an *S*-methyl thioether side chain, Met is one of two sulfur-containing proteinogenic amino acids, the other being cysteine (Cys). Given the nature of the side chain, Met is classified as a non-polar aliphatic residue. However, its hydrophobic side chain becomes highly polar because of the ease of oxidization. Thus, in peptides and proteins, the thioether group of Met residues can be readily oxidized, either chemically or biologically, into Met sulfoxide residues (Met(O)), leading to two diastereomers. To revert Met oxidation, organisms produce the enzymes methionine sulfoxide reductase (MSR) A and B, which are specific for each diastereomer.[2] Oxidation is not the only chemical change that Met residues can undergo. In biological systems, these residues are often present as sulfonium derivatives after being alkylated. The most abundant alkylated derivative found in animals is *S*-adenosyl-L-methionine, which plays a critical role as a cosubstrate for many methyltransferases.[3] Furthermore, *S*-methylmethionine is found in plants, sometimes at even higher concentrations than Met.[4]

During chemical peptide synthesis, the formation of Met(O) and *S*-*tert*-butylated Met is an important side reaction. Both take place during acidic treatments, in contrast to amino acid residues such as Lys, Cys, and His, which are highly reactive at neutral pH and above but lose their reactivity at acidic pH due to the protonation of their functional groups. However, Met thioether is highly resistant to protonation and is a reactive nucleophile at pH < 3. Thus, in *tert*-butoxycarbonyl (Boc)/benzyl (Bzl) SPPS, *tert*-butylation is a greater issue than in fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu) SPPS, since *tert*-butylation can occur during the removal of the Boc group in each synthetic cycle in addition to the final cleavage from the resin. Oxidation in both strategies takes place during the final cleavage, although in Boc/Bz, it can also occur in some extension during the Boc-removal. In long peptides, the

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removal of these impurities is not straightforward and it can hinder the purity of the final product.

These two side reactions can be minimized by the inclusion of appropriate additives (commonly named scavengers) in the acidic solution used for cleavage and final deprotection. Such molecules can scavenge the *t*Bu cation,[5] prevent oxidation, or reduce the already formed sulfoxide. With respect to Boc/Bzl chemistry, Tam et al.[6,7] studied the minimization of these side reactions extensively and Nicolás et al.[8] proposed the use of TFA-NH_4I-Me_2S for the reduction of Met(O), without disrupting disulfide bridges. In the Fmoc/*t*Bu strategy, unspecific cleavage using TFA-based reagents has mostly been described,[9] with reagent H (TFA/phenol/thioanisole/1,2-ethanedithiol/H_2O/Me_2S/NH_4I (81:5:5:2.5:3:2:1.5)[10] being the only one specifically designed for preventing Met oxidation. Furthermore, the addition of NH_4I to other reagents reduces the formation of sulfoxide.[11,12] However, this salt shows poor solubility in TFA and high solubility in H_2O and thus its use makes workup extremely difficult.

Herein, we studied the development of a reagent for minimizing these two side reactions. This reagent is compatible with the presence of protecting groups that are difficult to remove (Trt from Cys) and in the presence of sensitive residues such as Cys and Trp.

EXPERIMENTAL PART

Materials and Methods. All reagents and solvents were from commercial suppliers and used without further purification. Fmoc amino acids and 2-CTC resin (loading 0.86 mmol/g) were from Purolite (UK). DIC and OxymaPure were gifts from Luxembourg Biotech (Ness Ziona, Israel), and *N*,*N*-diisopropylethylamine (DIEA), acetic acid, and piperidine were supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Organic solvents [DMF, CH_2Cl_2 (DCM)] and HPLC quality acetonitrile (MeCN) were purchased from Merck (Kenilworth, New Jersey, USA). Milli-Q water was used. LC−MS was performed on an UltiMate 3000, Aeris 3.6 μm-wide pore column, Phenomenex C18 (150 × 4.6 mm), over a 5−95% gradient of MeCN (0.1% HCOOH)/H_2O (0.1% HCOOH) for 15 min, if not stated otherwise.

Peptide Synthesis. All peptides were manually synthesized following the standard Fmoc/*t*Bu protocol in a syringe fitted with a porous polyethylene disc. The syntheses were carried out on 2-CTC resin, which was activated with 50% thionyl chloride in DCM for 2 h. Then, resin was washed properly with DCM and first coupling was done with Fmoc-Asp(*t*Bu)-OH (3 eq) in DCM in the presence of DIEA (10 eq) for 2 h, and then MeOH (50 μL) was added for capping the unreacted Cl groups for 30 min at room temperature (loading 0.86 mmol/g). For Fmoc removal, treatment with 20% piperidine in DMF for 7 min was done at each cycle. The remaining couplings were done with a threefold excess of Fmoc-amino acid, *N*,*N*′-diisopropylcarbodiimide (DIC), and OxymaPure in a 1:1:1 ratio for 45 min. At the end of the chain elongation, peptides were acetylated at the end terminus using acetic anhydride (10 eq) and DIEA (20 eq) in DMF for 45 min.

Ac-Met-Glu(*t*Bu)-Glu(*t*Bu)-Pro-Asp(*t*Bu)-CTC resin was dried under vacuum aliquoted in 10 mg portions, which were used for testing the different cleavage conditions and mixtures described in Tables 1 and 2. In all cases, at the end of the cleavage reaction, chilled ether was added to precipitate the peptides, and after centrifugation and decantation, the peptides were taken up in water. The crude products obtained were analyzed by LC−MS (SI, Figure S1−S28).

Peptide Cleavage. The peptides were cleaved from the resin using the conditions described in Tables 1 and 2 in a ratio resin/cleavage mixture 1:10 (W:V). The best conditions were achieved by treatment at RT for 1 h with the mixture TFA/An/TMSCl/Me2S/0.1% PPh3 (85:5:5:5) except in the case of the Cys-containing peptide, in which TIS has to be added to the mixture to entrap Trt carbocation; thus, the final cocktail has to be TFA/An/TMSCl/TIS/Me2S/0.1% PPh3 (80:5:5:5:5).

Total Procedure to Obtain Ac-MEEPD-OH Free of Both Side Products [Met(O) and M(*t*Bu)]. Ac-Met-Glu(*t*Bu)-Glu(*t*Bu)-Pro-Asp(*t*Bu)-CTC resin was synthesized in a 0.1 mmol scale following the same procedure described above. The cleavage and total deprotection was carried out using best-performing cleavage conditions found (Table 2, #11). Then, the peptide was precipitated using chilled ether, followed by centrifugation and decantation of the ether mixture. The dried crude was then dissolved in water and lyophilized. Then, the lyophilized peptide was dissolved in 10 mL of 5% acetic acid solution and heated in a water bath at 40 °C. A sample (5 μL of the solution) was taken at different times, diluted with H_2O (45 μL), and injected into the LC−MS to monitor the evolution of the conversion of Ac-M(*t*Bu)EEPD-OH into Ac-MEEPD-OH.

RESULTS AND DISCUSSION

The peptide sequence target for this study, Ac-Met-Glu-Glu-Pro-Asp-OH, was prepared on 2-chlorotrityl chloride (2-CTC) resin following the standard Fmoc/*t*Bu strategy. Initially, an aliquot of the peptide resin was cleaved under standard conditions, i.e., TFA/TIS/H_2O (95:2.5:2.5), for 1 h. This result was used as a reference for the other conditions tested. The HPLC trace from the crude peptide obtained showed two side products (Figure 1A,B). The most polar compound showed an *m*/*z* of 718.52, corresponding to the alkylated Met peptide [Ac-M(*t*Bu)EEPD-OH, **3**], which accounted for 23.9% of *S*-*tert*-butylated Met by UV integration. The second peak of *m*/*z* 677.68 was identified as the oxidized Met peptide [Ac-M(O)EEPD-OH, **2**], accounting for 1.4%. The main peak (74.7%) corresponded to the target peptide [Ac-MEEPD-OH (**1**)], which had an *m*/z of 662.45 (Figure 1 and Figures S1−S4).

The first point to be clarified was whether the *N*-terminal position of the Met residue favored the formation of the alkyl derivative since a slight change in the structure often has an important impact on the formation of side reactions.[13] Thus, the sequence Ac-Glu-Glu-**Met**-Pro-Asp-OH was synthesized and cleaved under the same conditions as described above. The crude peptide was analyzed by HPLC (Figure 1C). As before, the most polar compound **3′** (*m*/*z* 718.52) corresponded to the alkylated Met peptide (Ac-EEM(*t*Bu)-PD-OH), accounting for 24.4% by UV integration. The second peak (*m*/*z* 677.68) was the oxidized Met peptide [Ac-EEM(*O*)PD-OH, **2′**], representing 2.9%. The main peak (*m*/z 662.45), the target peptide [Ac-EEMPD-OH, **1′**], accounted for 72.7%. These results indicated that the extension of the side reactions is not determined by the order of the amino acids in the sequence but by the amino acid content itself.

Searching for a different set of scavengers to minimize or prevent the Met side reactions, the effect of the cleavage reaction time and the temperature was studied (Table 1,

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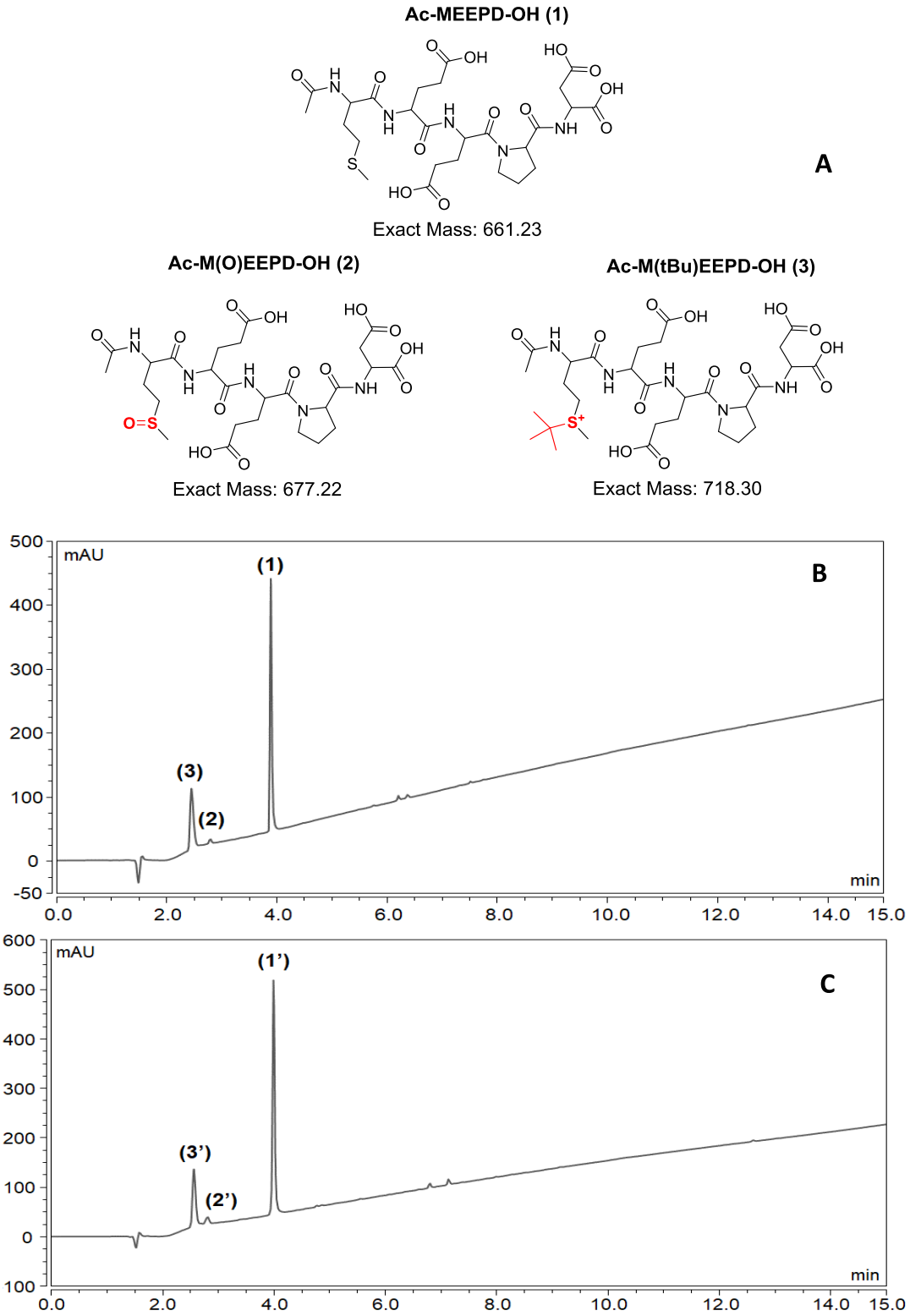

Figure 1. Chemical structure and expected mass of the side products and the target peptide (A). HPLC trace after standard cleavage with TFA/TIS/H_2O (95:2.5:2.5) for 1 h at RT of crude Ac-Met-Glu-Glu-Pro-Asp-OH (B) and HPLC Ac-Glu-Glu-Met-Pro-Asp-OH (C).

Figures S5−S8). Two samples of peptide resin were cleaved again using TFA/TIS/H_2O (95:2.5:2.5), one for 30 min and the other for 2 h. The HPLC analyses of the crude mixtures obtained were compared with the profile of the 1 h reaction time (Table 1, nos. 1, 2, 3). These data indicated that the alkylated by-product was reduced when the time was shortened from 1 to 0.5 h (Table 1, no. 2 vs 1). On the other hand, when the cleavage was extended from 1 to 2 h, the increase in the formation of the alkylated compound was less than expected. This result can be explained by the fact that alkylation can be reverted in acidic media. Therefore, the alkylation can even be minimized by a longer reaction time. Regarding the effect of the temperature, two additional cleavage reactions were run, one at 40 °C and the other at 6

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°C, (Table 1 nos. 4, 5), for comparison with that at 25 °C (Table 1, no. 1). As expected, the higher the temperature, the greater the amount of by-products. From the experiment at 40 °C, where the alkylated Met peptide was the main product, we deduced that the alkylation of Met was faster than the oxidation. At 6 °C for 1 h, the by-products were formed in a similar fashion as at 25 °C for 0.5 h. The oxidation of side-product Met(O) merits further discussion. At first glance, it appears that the oxidation did not follow a rational trend but that the extra alkyl group acts as protecting group, preventing oxidation. In this regard, 1 h at 40 °C (Table 2, no. 4) and 2 h at 25 °C (Table 2, no. 3) gave the highest amount of oxidized product with respect to the target peptide.

Considering the previous results, we set as standard condition 1 h of cleavage at 25 °C. We then tested the capacity of different scavengers and/or reducing agents to suppress or minimize the two side reactions (Table 2 and Figures S9−S19).

First, anisole (An), which has been previously demonstrated to trap the *t*Bu carbocation,[14] was used as the sole scavenger (Table 2, no. 2) and in the presence of H_2O (Table 2, no. 3) and TIS (Table 2, no. 4), the latter being a mild reducing agent. In all three cases, the results in terms of reducing the alkylation were slightly better than those achieved under standard conditions (Table 2, no. 1), with ratios of *t*Bu and sulfoxide side product formation, fluctuating slightly. In turn, for the sulfoxide reduction, the best was when An and H_2O were used (Table 2, no. 3). The use of TMSCl (Table 2, no. 5) as the only additive rendered slightly better results for both side reactions, while when used in combination with An (Table 2, no. 6), or An and TIS (Table 2, no. 7), the minimization of both side reactions was more pronounced.

This effect was enhanced when another reducing agent, namely, Me_2S, which reduces sulfoxide to sulfenyl, was used instead of TIS in the An-TMSCl mixture (Table 2, no. 8). The simultaneous presence of both reducing agents, TIS and Me_2S (Table 2, no. 9), did not improve the results in comparison with those achieved in the presence of only Me_2S. In the next round of conditions tested, the addition of a tiny amount of another reducing agent, PPh_3 (0.1%), in addition to TIS (Table 2, no. 10) or Me_2S (Table 2, no. 11), rendered interesting results. While the addition of PPh_3 to the TIS mixture resulted in a moderate setback in the formation of side reactions (Table 2, no. 10), the addition of PPh_3 to Me_2S (Table 2, no. 11) provided the best conditions, with no sulfoxide being detected, alongside a roughly 5% alkylated product. From experiments 7−11 (Table 2), it can be concluded that the combination of Me_2S and PPh_3 minimized the Met side reactions. Finally, in experiment 12 (Table 2), the presence of TIS together with Me_2S and PPh_3 did not confer any additional advantage. In conclusion, the TFA/An/TMSCl/Me_2S/0.1% PPh_3 combination prevented the oxidation reaction and minimized the alkylation side reaction (from approximately 20 down to 5%), thus providing optimal conditions. The presence of TMSCl and PPh_3 plays a crucial role in the mixture, probably through a mechanism (Figure 2) similar to the one based on the reduction of sulfoxides to sulfides with thionyl chloride and triphenylphosphine proposed by Jang et al.[15]

Table 1. Effect of Reaction Time and Temperature on the Formation of Oxidized and Alkylated Met By-Products

cleavage conditions	no.	t (h)	T (°C)	Ac-MEEPD-OH	Ac-M(*t*Bu)-	Ac-M(O)-
TFA/TIS/H_2O	**1**	1	25	74.8	23.6	1.6(2.1)[a]
95:2.5:2.5	**2**	1/2	25	86.8	11.4	1.8(2.0)[a]
	3	2	25	71.7	26.0	2.3(3.2)[a]
	4	1	40	26.2	71.9	1.9(7.3)[a]
	5	1	6	85.9	12.4	1.7(2.0)[a]

[a]With respect to the target peptide.

Table 2. Conditions Developed to Eradicate the Two Major Side Reactions in Cleaving Met-Containing Peptides

no.	cleavage conditions	Ac-MEEPD-OH	Ac-M(*t*Bu)-	Ac-M(O)-
1	TFA/TIS/H_2O (95:2.5:2.5)RT, 1 h	74.8	23.60	1.6
2	TFA/An[1] (95:5)RT, 1 h	78.03	20.20	1.77
3	TFA/An/H_2O (95:2.5:2.5)RT, 1 h	77.92	20.98	1.10
4	TFA/An/TIS (95:2.5:2.5)RT, 1 h	79.50	18.98	1.52
5	TFA/TMSCl (95:5)RT, 1 h	80.70	18.12	1.18
6	TFA/An/TMSCl (95:2.5:2.5)RT, 1 h	87.64	11.47	0.89
7	TFA/An/TMSCl/TIS (85:5:5:5)RT, 1 h	88.54	10.99	0.48
8	TFA/An/TMSCl/Me_2S (85:5:5:5) RT, 1 h	**93.14**	6.72	0.14
9	TFA/An/TMSCl/TIS/Me_2S (85:5:5:5) RT, 1 h	**93.76**	5.81	0.43
10	TFA/An/TMSCl/TIS/0.1% PPh_3(85:5:5:5) RT, 1 h	88.92	10.20	0.88
11	TFA/An/TMSCl/Me_2S /0.1% PPh_3(85:5:5:5) RT, 1 h	**94.58**	5.42	0
12	TFA/An/TMSCl/TIS/Me_2S/0.1% PPh_3(80:5:5:5:5) RT, 1 h	**93.43**	6.57	0

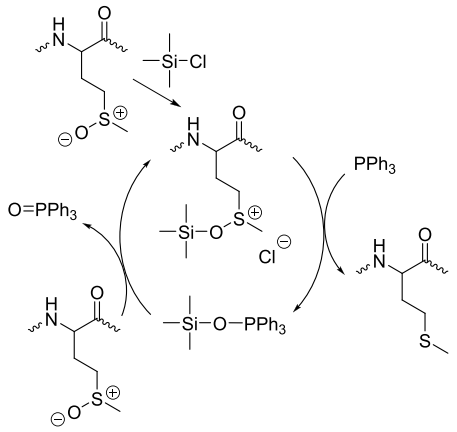

Figure 2. Mechanism proposed for avoiding the formation of Met(O) in the final peptide by using TMSCl and PPh_3.

These optimized conditions were then used for the global deprotection of peptides containing not only Met but also Cys or Trp, the latter two possibly being sensitive to the components of the TFA mixture. For instance, Trp can be reduced if triethylsilane (TES) is used instead of TIS during a normal global deprotection. Thus, two hexapeptides with sequences Ac-Cys-Met-Glu-Glu-Pro-Asp-OH **I** and Ac-Trp-Met-Glu-Glu-Pro-Asp-OH **II** were assembled on 2-CTC resin.

Peptide resin **I** was subjected to standard cleavage condition no. 1 (Table 2) resulting in the two-oxidation (7.6%) and

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alkylation (3.9%) impurities (Figure 3A). The peptide resin was stored for 1 year at +4 °C, and peptide cleavage was then

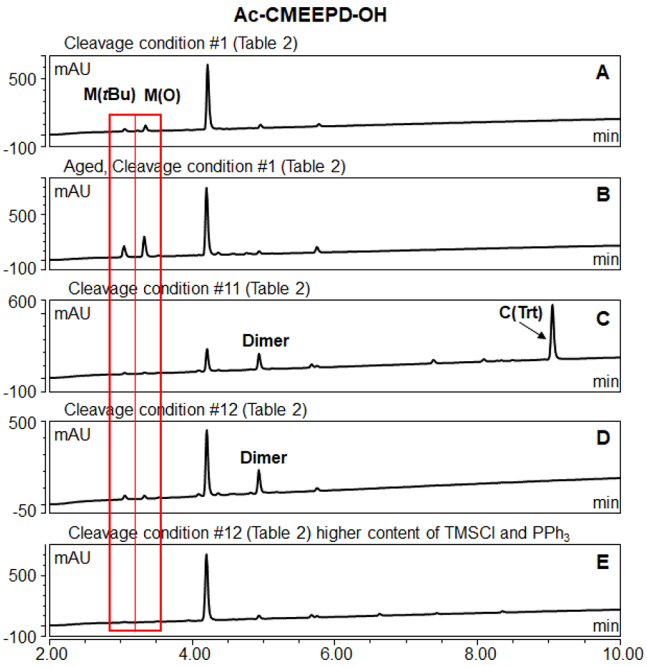

Figure 3. HPLC chromatograms of crude Ac-Cys-Met-Glu-Glu-Pro-Asp-OH. (A) Freshly synthesized peptide after cleavage using condition no. 1 (Table 2); (B) aged peptide after cleavage using condition no. 1 (Table 2); (C) aged peptide after cleavage using condition no. 11 (Table 2); (D) aged peptide after cleavage using condition no. 12 (Table 2); and (E) aged peptide after cleavage using condition no. 12 (Table 2) increasing the amount of TMSCl and PPh_3. For MS spectra, see Figures S20–S25.

repeated under the same conditions (Table 2, no. 1). In the second experiment, an important increase on the impurity's percentage [oxidation (19.8%); alkylation (12.8%)] was observed. Furthermore, a tiny amount of a compound with a mass of +32 amu was observed, presumably as a result of oxidation from sulfoxide to sulfone in the peptide (Figure 3B). To test the scope of our method for minimizing Met-associated side reactions, the "aged" peptide resin was used. Under the optimized conditions (no. 11, Table 2), the main peak (61.3%) corresponded to the peptide containing a Trt moiety (Figure 3C). Moreover, the formation of some disulfide dimer was observed (15.6%) (Figure 3C). These results highlight the importance of an adequate scavenger in the removal of the Trt group from Cys, which is known to be a reversible reaction. Thus, condition no. 12 (Table 2), which is similar to no. 11 (Table 2) plus TIS, proved effective at removing Trt from Cys, although the disulfide dimer was still present (24.1%), as were the two key impurities [oxidation (2.8%); alkylation (4.2%)] (Figure 3D). Finally, condition no. 12 (Table 2), with an increase in the amount of TMSCl and PPh_3 [TFA/An/TMSCl/TIS/Me_2S/0.2% PPh_3 (75:5:10:5:5)], proved optimal because Trt was removed, with no dimer formation, nor the presence of the other impurities (Figure 3E). It is important to note that the Cys residue was unaffected by the presence of TMSCl.

Recently, synthesized peptide resin **II** was subjected to standard cleavage condition no. 1 (Figure 4A), yielding crude peptide Ac-WMEEPD-OH with a purity of 84.6%, while

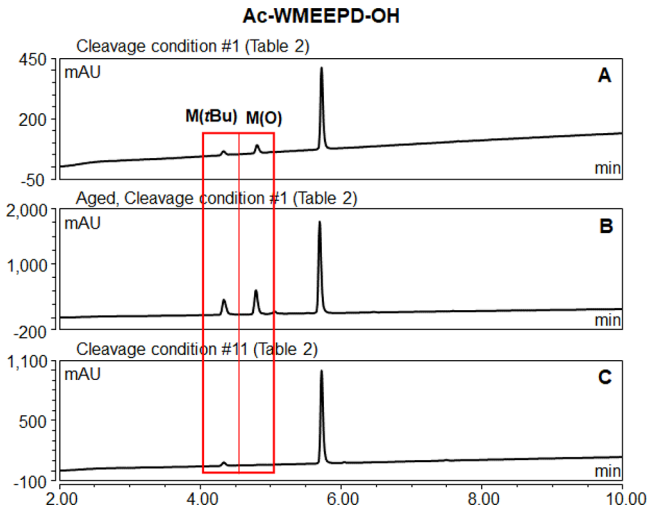

Figure 4. HPLC chromatograms of crude Ac-Trp-Met-Glu-Glu-Pro-Asp-OH. (A) Freshly synthesized peptide after cleavage using condition no. 1 (Table 2); (B) aged peptide after cleavage using condition no. 1 (Table 2); (C) aged peptide after cleavage using condition no. 11 (Table 2). For MS spectra, see Figure S26–S28.

oxidation and alkylation were present at 10.2 and 5.2%, respectively. However, under condition no. 1, cleavage of the same peptide resin stored at +4 °C for 1 year resulted in greater amounts of the two impurities [oxidation (19.8%); alkylation (13.2%), Figure 4B]. Finally, the cleavage of this "aged" peptide resin in condition no. 11 rendered the target peptide with a purity of 95.2%, with the presence of the *t*Bu impurity of 4.8% (Figure 4C). As in the case of Cys, the Trp residue was also stable to TMSCl.

From the previous experiments, we can conclude that the use of either condition no. 11 or no. 12 (with TIS, for Cys-containing peptides, Table 2) practically eradicated the undesired oxidation reaction, but approximately 5% of the *S*-alkylated (sulfonium salt) side product was consistently present. However, it is well known that the alkylation reaction is reversible in a mild acidic medium. Thus, Ac-MEEPD-OH was cleaved from the CTC-resin using the best-performing cleavage condition (Table 2, no. 11). The peptide was obtained at a purity of 96.0% with 4.0% of the *S*-alkylated peptide, and no traces of the sulfoxide side product were detected, as shown by LC–MS (Figure 5, 0 min). The lyophilized peptide was treated with 5% acetic acid at 40 °C for 24 h. The in-between analysis of the same peptide solution by LC-MS showed that *S*-alkylation was eliminated by 24 h, yielding the target peptide with excellent purity (Figure 5).

The same experiment (5% acetic acid at 40 °C) was also repeated using ultrasound giving slightly poorer results. Moreover, the results became worse when 5% TFA (without and with ultrasound) was used (no shown).

CONCLUSIONS

Met-containing peptides are known to produce some inevitable side products during the final acidolytic cleavage step in the Fmoc/*t*Bu SPPS strategy. The two most common side reactions are oxidation and *S*-alkylation, with the formation of Met(O) and sulfonium salt residues, respectively. Although a large number of cleavage conditions have been described in previous literature, they do not fully arrest these side reactions. Here, we have demonstrated that the presence of TMSCl and

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T (h)	Ac-MEEPD-OH	Ac-M(tBu)-
0	94	6.0
1	95.4	4.6
2	96.1	3.9
4	97.3	2.7
6	97.9	2.1
8	98.8	1.2
24	100	-

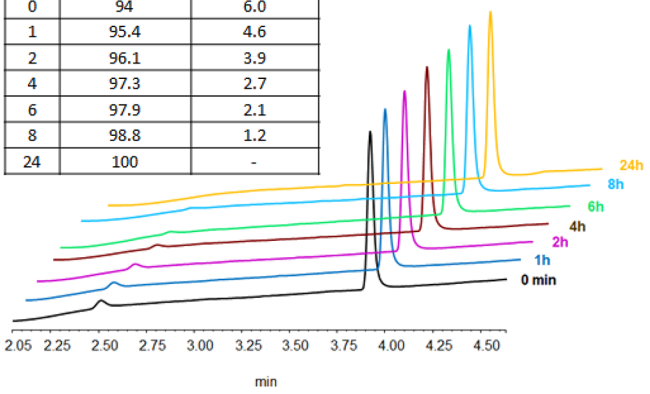

Figure 5. HPLC chromatograms showing the conversion of the lyophilized peptide containing the sulfonium salt into the right peptide by heating at 40 °C in 5% acetic acid.

PPh_3 in the cleavage condition is key for the minimization and even eradication of the oxidation side reaction.

TFA/An/TMSCl/Me_2S (85:5:5:5) containing 1 mg of Ph_3P per mL of reagent (Table 2, no. 11) and TFA/An/TIS/TMSCl/Me_2S (85:5:5:5:5) containing 1 mg of Ph_3P per mL of reagent (no. 12) eradicated Met(O) and reduced *S*-alkylation in all the peptides containing Met. The presence of TIS is imperative for the removal of Trt of the Cys residue. This is a reversible reaction, which is displaced to the free Cys by scavenging the Trt carbocation. Both reagents (Table 2, nos. 11 and 12) gave excellent results for the peptides involving sensitive amino acids such as Cys and Trp. TMSCl did not affect any of these amino acids. These reagents circumvent the need for NH_4I in a late stage of the treatment. This is a considerable advantage as NH_4I is insoluble in organic solvents and soluble in aqueous ones, thus making its removal from the final peptide tedious as well as hindering the workup.

Reversing the sulfonium salt of the peptide to the free thioether peptide after lyophilization was also accomplished by heating the peptide at 40 °C for 24 h in 5% AcOH. Interestingly, Met-containing peptide resins stored at 4 °C for several months rendered larger amounts of the two side products when standard cleavage conditions were used (no. 1, Table 2). This phenomenon should be further studied.

Met(O) has been used in the solid-phase synthesis of hydrophobic protected peptides to facilitate the further assembly by trouble-free management and purification of the peptides.[16] In this regard, reagent developed herein can favor this strategy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01058.

Characterization of the peptides on HPLC and LCMS (PDF)

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Author Contributions

The strategy was designed by all of the authors. Experimental works were executed by K.P.N. and M.A. All of the authors discussed the results and prepared the manuscript. All of the authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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