



Cite this: *Org. Biomol. Chem.*, 2026, **24**, 861

Use of homoserinyl γ -aldehyde-containing peptides in solid-phase reductive amination

Dimitrios Tolis, Dimitra Magkafa and Spyridon Mourtas  *

Reduced peptide bonds of the methyleneamino $\Psi[\text{CH}_2-\text{NH}]$ type are widely recognized as peptide bond isosteres with significant value in drug discovery and development. Solid-Phase Synthesis (SPS) enables Solid-Phase Fragment Condensation (SPFC), a key strategy for the construction of complex peptides. In this study, we report the SPS of peptides containing selectively side-chain deprotected homoserine (Hse) residues, followed by solution-phase oxidation of the liberated Hse side-chain hydroxyl group to the corresponding γ -aldehydes. The intrinsic instability of these intermediates, primarily due to intramolecular cyclization to γ -hydroxy lactam or lactone products, is systematically examined, and stabilization strategies to overcome these limitations are developed. The resulting stabilized homoserinyl γ -aldehyde peptides were subsequently employed, as proof of concept, in solid-phase reductive amination with the N-terminus of resin-bound peptides. Overall, this approach enables the efficient formation of Hse- β - $\Psi[\text{CH}_2-\text{NH}]$ reduced peptide bonds and establishes a versatile platform for broader peptide ligation and modification strategies.

Received 1st November 2025,
Accepted 19th December 2025

DOI: 10.1039/d5ob01718a

rsc.li/obc

1. Introduction

Peptides are well known for their high biological specificity and favorable safety profile, which are major factors contributing to their extensive use as therapeutic agents. However, the clinical application of native peptides has been limited primarily due to their poor metabolic stability and short half-life.¹ Various strategies have been proposed to overcome these limitations, including chemical modifications and novel drug delivery approaches, or combinations thereof, to enable intact peptides to reach their targets.¹ In the development of chemically modified peptides and peptide conjugates, several synthetic strategies have been employed, such as lipidation, incorporation of non-natural amino acids into the peptide backbone, cyclization, replacement of amide bonds with pseudopeptide bonds, and prodrug synthesis, among others.²⁻⁸

Pseudopeptides are peptidomimetics in which one or more peptide bonds are replaced by an isostere (also known as isosteric surrogate). The most straightforward examples of isostere replacements are summarized in Fig. 1. As shown, the replacement of amide bond [CO-NH] **1** by the general formula bond [CH₂-Y], where Y can be CH₂, NH, O, S, leads to the formation of the corresponding $\Psi[\text{CH}_2-\text{CH}_2]$ (**2a**), $\Psi[\text{CH}_2-\text{NH}]$ (**2b**), $\Psi[\text{CH}_2-\text{O}]$ (**2c**), and $\Psi[\text{CH}_2-\text{S}]$ (**2d**) isosteres. This replacement represents a viable strategy for the rational design of peptidomimetics, not only because it increases the metabolic stability

of the resulting compounds, but also due to enhanced potency and biological activity they often exhibit.^{9,10}

Solid-Phase Synthesis (SPS) and Solid-Phase Fragment Condensation (SPFC) strategies have been extensively employed in the synthesis of peptides and their analogues, including chemically modified peptides. These methodologies have enabled the production of numerous commercially available peptides and continue to shape the future of peptide-based therapeutics.^{3,7,11-14} Among the various types of resins developed over the years, the acid labile 2-chlorotriptyl chloride (CLTR) resin is widely used in Solid-Phase Peptide Synthesis (SPPS) due to its unique advantages. For example, it prevents racemization during the loading of the first amino acid, inhibits diketopiperazine formation, and allows cleavage under

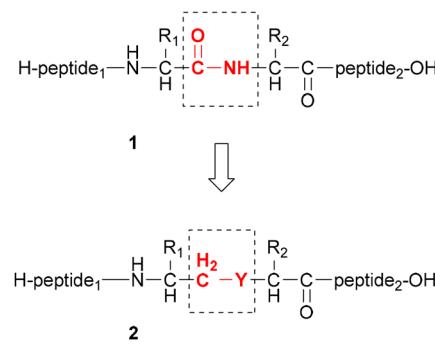


Fig. 1 Peptide bond isostere representation of $\Psi[\text{CH}_2-\text{Y}]$ type: Y = CH₂ (a), NH (b), O (c), S (d).



relatively mild acidic conditions. Additionally, the use of the Fmoc/^tBu strategy combined with different side-chain protecting groups enables an orthogonal protection approach, facilitating selective deprotection during synthesis.^{13–16}

As part of our ongoing efforts to develop SPS methods and strategies for drug design and development, we have established procedures for replacing peptide bonds with the [CH₂–S] isostere,¹⁷ and for synthesizing thiol-containing peptoids by incorporating *N*-mercaptoalkylglycine residues into peptide chains, effectively substituting amino acids with their *N*-mercaptoalkylglycine counterparts.¹⁸

In this work, we rationalized the SPS of homoserine (Hse)-containing peptides synthesized on CLTR resin 3 using the Fmoc/^tBu methodology, with the acid-labile Trt group serving as the side-chain protecting group for Hse. This orthogonal protection strategy enables the synthesis of Hse(Trt)-containing peptides, which upon cleavage from the resin and subsequent selective detritylation, could yield homoserine-containing peptides 4. Controlled oxidation of the liberated γ -hydroxyl side-chain of Hse could then allow formation of the corresponding homoserinyl γ -aldehyde peptides 5. These aldehyde peptides could be further employed in solid-phase reductive amination with the N-terminus of a second solid-phase synthesized peptide 6, to afford a side-chain-elongated peptide 7, wherein a linkage between the two peptides (peptide₁–peptide₂) would be based on a newly formed pseudopeptide bond of Hse- β - Ψ [CH₂–NH] type (Scheme 1).

While the formation of reduced amide bonds of Ψ [CH₂–NH] type in peptide-based drug design has been proposed as a strategy to enhance metabolic stability, potency and biological activity,^{9,19} the solid-phase reductive amination between homoserinyl peptide aldehydes and the N-terminus of resin-bound peptides has not been reported. This paper presents the challenges encountered in the synthesis of homoserinyl γ -aldehyde peptides and describes problem-solving approaches aimed at their efficient synthesis and subsequent application

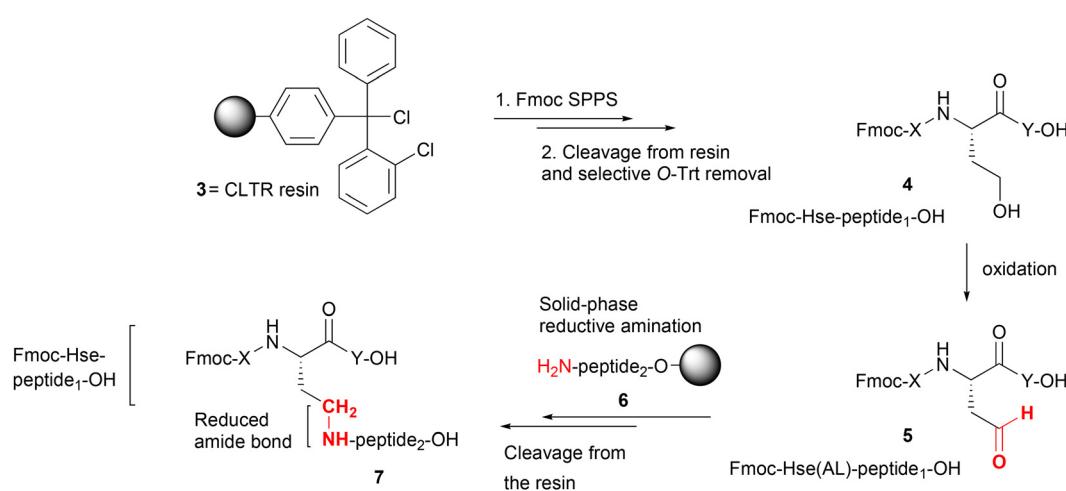
in solid-phase fragment condensation under reductive amination conditions.

2. Results and discussion

2.1. Methods to prepare homoserinyl γ -aldehyde peptides – challenges

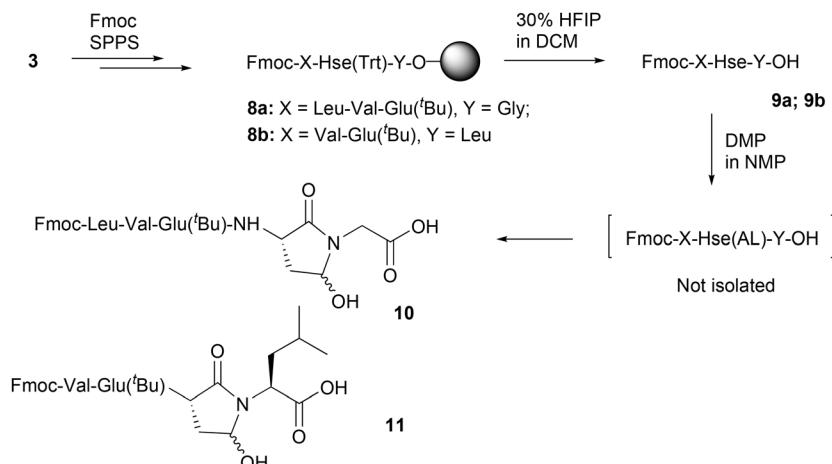
In order to investigate the synthesis of homoserinyl γ -aldehyde peptides and their further use in SPS, we initially considered the solid-phase synthesis (SPS) of selectively side-chain deprotected homoserine peptides. For this, we applied standard solid-phase peptide synthesis (SPPS) methods, by using 2-chlorotriptyl chloride resin (CLTR) 3 and Fmoc/^tBu methodology. The orthogonal protection of Hse was achieved by using the trityl (Trt) group as the side-chain protecting group, which allows selective removal of O-Trt group without affecting the ^tBu-type protecting groups.^{20–22} Thus, we synthesized small peptides containing both Hse(Trt) and Glu(^tBu) (as a representative ^tBu-protected amino acid). The coupling of the Fmoc-amino acids during the peptide assembly was performed using *N*-hydroxybenzotriazole (HOBT) and *N,N*-diisopropylcarbodiimide (DIC) as the condensing agents, while removal of the Fmoc group was achieved by using 25% piperidine in NMP. By this method, the Hse(Trt)-containing peptidyl-resin 8a/b (Y = Gly; Leu respectively) was obtained (Scheme 2).

Treatment of resin 8a with 30% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in dichloromethane (DCM), simultaneously cleaved the peptide from the resin and removed the Trt-type protecting group, to afford the selectively Hse deprotected peptide, 9a (Fig. S1) which was further subjected to standard Dess–Martin oxidation.^{23–25} The reaction process was followed by HPLC analysis, where a clear consumption of the starting material (9a) and the formation of a new product was evidenced, which was isolated. However, the isolated product was not identified as the expected Hse aldehyde peptide. In fact,



Scheme 1 Initial synthetic plan for the formation of Hse- β - Ψ [CH₂–NH] peptide isosteres; X represents the peptide segment preceding the homoserine (Hse) residue, and Y represents the segment following it.



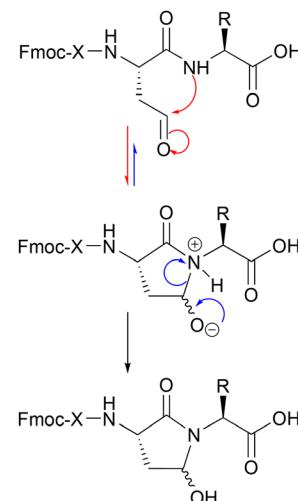


Scheme 2 First attempt to synthesize homoserinyl γ -aldehyde peptides by DMP oxidation of Fmoc-Leu-Val-Glu(Bu)-Hse-Gly-OH (**9a**) and Fmoc-Val-Glu(Bu)-Hse-Leu-OH (**9b**) which resulted to the synthesis of the corresponding γ -hydroxy lactams **10** and **11**; X represents the peptide segment preceding the Hse residue, and Y represents the residue next to the Hse ($n + 1$ residue).

although the isolated product had the expected mass, the ^1H -NMR and ^{13}C -NMR analysis revealed the absence of the expected aldehyde-H/C. In addition, when this product was subjected to standard reductive amination process (as it is analytically described in section 2.3), this was not able to react. Thus, we concluded that the isolated product corresponds to γ -hydroxy lactam peptide **10** (Fig. S2). In order to further investigate this finding, we replaced Gly with Leu, by synthesizing the Hse-containing resin-bound peptide **8b** and the corresponding **9b** (Fig. S3), which was also oxidized with DMP reagent. The oxidation reaction was followed by HPLC analysis, showing the successful oxidation of **9b**. However, also in this case, we did not obtain the expected Hse aldehyde peptide, but instead, we obtained a product with the expected mass, but no aldehyde-H/C in ^1H and ^{13}C -NMR analysis. In addition, this product was unable to further react under standard reductive amination conditions (as analytically described in section 2.3). Thus, we concluded that the isolated product corresponds to the γ -hydroxy lactam peptide **11** (Fig. S4).

As an explanation for this finding, we propose the mechanism of Scheme 3. According to this, the initially formed aldehyde (after DMP oxidation) is transformed to the corresponding γ -hydroxy lactam through an intramolecular nucleophilic attack of the nitrogen of the neighboring amide bond (of the $n + 1$ residue relative to Hse) to an initially formed intermediate, which upon proton migration affords the finally formed γ -hydroxy lactam. The results indicate that the final step of this transformation is not an equilibrium, as no aldehyde-H/C were evidenced in ^1H / ^{13}C -NMR of the isolated product, nor was the isolated product able to react under reductive amination conditions (as it is analytically discussed in section 2.3).

A similar behavior has been proposed for homoserinyl peptides during the conversion of Hse to Asp by oxidizing the side-chain γ -hydroxyl group to the corresponding carboxylic acid group. In that case, the rapid oxidation of homoserine to



Scheme 3 Plausible mechanism for the formation of γ -hydroxy lactams by intramolecular nucleophilic attack of the nitrogen of the amide bond at the $n + 1$ position of a homoserinyl γ -aldehyde peptide; X represents the peptide segment preceding the Hse residue.

Asp reduced the formation of γ -hydroxy lactam.²⁶ In our case, where the aldehyde was the desired product, γ -hydroxy lactam was finally formed. A clear difficulty was therefore presented in our attempt to propose a protocol for the solid-phase synthesis of homoserinyl γ -aldehyde peptides and their use in solid-phase reductive amination. Strategies to overcome these difficulties/limitations were adopted, as described in the following section.

2.2. Methods to prepare homoserinyl γ -aldehyde peptides – effective approaches

In order to overcome the aforementioned difficulties, due to the formation of homoserinyl γ -hydroxy lactams instead of



γ -aldehydes, we investigated the possibility to stabilize the synthesized homoserinyl γ -aldehyde peptides. For this, two strategies were investigated: (a) the replacement of the $n + 1$ residue of the peptide chain (relative to the Hse residue) with an *N*-alkyl- α -amino acid, and (b) the use of C-terminal homoserinyl γ -aldehyde peptides. Both approaches are analytically presented and discussed in sections 2.2.1 and 2.2.2, respectively.

2.2.1. Replacing the $n + 1$ residue with an *N*-alkyl- α -amino acid. In this approach, the replacement of the amide hydrogen by an *N*-alkyl group, at the $n + 1$ position of the Hse residue, was considered (Scheme 4A). We rationalized that this replacement in the peptide sequence of an Hse-containing peptide would enable the formation of the corresponding *N*-alkylated peptide aldehyde, which would not allow cyclization to the corresponding γ -hydroxy lactam, as in this case, this transformation is not favored. This approach is illustrated in Scheme 4A. As shown, this method could yield the *N*-alkylated homoserinyl γ -aldehyde peptide (*N*-alkylated at the $n + 1$ position relative to the Hse residue), as the nucleophilic attack by the amino group of the preceding residue ($n + 1$ residue) has been blocked.

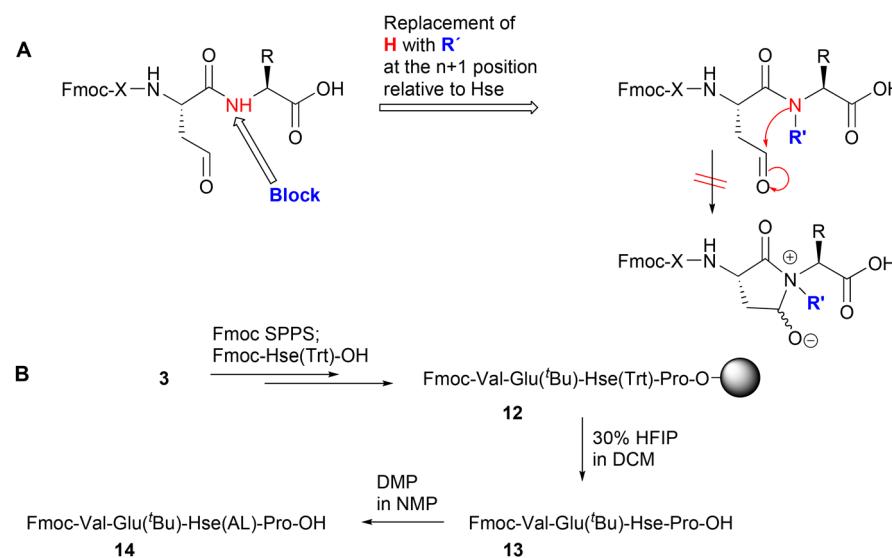
The experimental part for this approach confirmed our hypothesis, as the solid-phase synthesis of the resin-bound peptide **12**, where the $n + 1$ residue was replaced by proline (Pro), a naturally occurring *N*-alkyl- α -amino acid, afforded, after selective detritylation with 30% HFIP, the homoserinyl γ -aldehyde peptide **13** (Hse selectively deprotected), which, after DMP oxidation afforded **14** (Scheme 4B). The reaction progress was followed by HPLC analysis, where a clear oxidation of **13** to form **14** was evidenced (Fig. S5 and S6). In contrary to the non-alkylated Hse-containing peptide aldehydes, ^1H and ^{13}C -NMR analysis of the isolated **14** revealed the presence of the expected aldehyde-H/C, which provides evidence of

our hypothesis (Fig. S11–S13). In addition, peptide **14** was effectively used in solid-phase reductive amination processes (as it is analytically described in section 2.3).

2.2.2. The use of C-terminal homoserinyl γ -aldehyde peptides. In the second approach, we considered the formation of C-terminal homoserinyl γ -aldehyde peptides, such as those illustrated in Scheme 5A. Using this approach, we expected that the possible nucleophilic attack of the hydroxyl group of the C-terminal α -carboxylic acid group to the neighboring side chain aldehyde group, to form the corresponding γ -hydroxy lactone, would be an equilibrium, thereby allowing the desired utilization of the C-terminal homoserine peptide aldehydes.

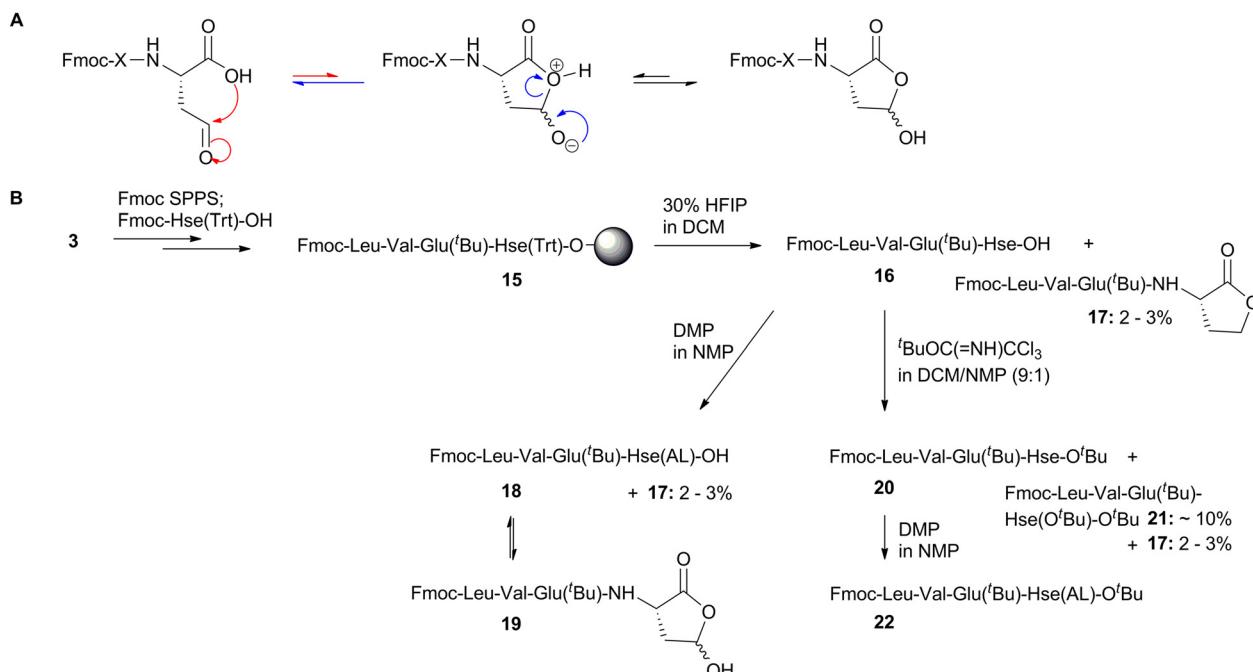
Thus, we initially synthesized the resin-bound C-terminal Hse(Trt) peptide **15** and the corresponding C-terminal Hse peptide **16**, using standard SPPS and 30% HFIP, which enables cleavage from the resin and selective removal of the Hse(Trt) protecting group (Scheme 5B). This treatment also produced a small amount (2–3%) of lactone **17** (Fig. S7). Given the low percentage of **17** and the fact that this byproduct would not participate in the subsequent chemical reactions, we proceeded to the next step with this mixture, which was subjected to DMP oxidation, allowing conversion of **16** to the C-terminal homoserinyl γ -aldehyde peptide **18** (**17** was not affected during this treatment) (Fig. S8).

In addition to this approach, we *tert*-butylated **16**, by using *tert*-butyl 2,2,2-trichloroacetimidate (TBTA).^{27–29} The preferable *tert*-butylation of carboxylic acids over hydroxyl groups has also been reported,^{29–31} and therefore, we used this reagent to selectively *tert*-butylate the C-terminal α -carboxylic acid group over Hse hydroxyl group. This reaction, although relatively slow, finally afforded the C-terminal homoserinyl *a*-*tert*-butylated γ -hydroxy peptide **20** (Scheme 5B). Besides **20**, the bis-*tert*-butylated **21** was also formed at approximately 10% (inte-



Scheme 4 (A) Formation of *N*-alkylated peptides at the $n + 1$ position (relative to Hse) as a first approach to stabilize homoserinyl γ -aldehyde peptides, avoiding intramolecular cyclization of homoserinyl γ -aldehyde peptides to the corresponding γ -hydroxy lactams; X represents the peptide segment preceding the Hse residue; (B) solid-phase synthesis of a stabilized homoserinyl γ -aldehyde peptide.





Scheme 5 (A) Formation of C-terminal homoserinyl γ -aldehydes as a second approach to stabilize homoserinyl γ -aldehyde peptides; X represents the peptide segment preceding the Hse residue; (B) solid-phase synthesis of the C-terminal homoserinyl γ -aldehyde peptides **18** (in equilibrium with the C-terminal γ -hydroxy lactone peptide **19**), and the α -*tert*-butylated C-terminal homoserinyl γ -aldehyde peptide **22**.

grated by HPLC analysis), while lactone **17** (approx. 2–3%) was also identified in the final mixture (as remnant of the previous step) (Fig. S9). Nevertheless, this mixture was effectively subjected to DMP oxidation, to afford the C-terminal homoserinyl α -*tert*-butylated γ -aldehyde **22** (no participation of both **17** or **21** was evidenced in this reaction) (Fig. S10). Since the presence of both **17** and **21** in the final product was not considered as a problem for the reductive amination reaction, we used this mixture directly to the next step.

It should be noted that the progress and purity of the reactions and products were monitored by HPLC analysis, and the products and byproducts were identified by ESI-MS analysis. To further support our findings, we also performed ^1H and ^{13}C -NMR analysis (Fig. S14 and S15). No aldehyde-H/C was observed in the case of **18/19**, whereas **22** showed clear signals for both aldehyde-H/C. These results provide evidence for the cyclization of peptide aldehyde **18** to the corresponding γ -hydroxy lactone **19**, while no such cyclization occurred in the case of **22**.

It is worth noting that the C-terminal homoserine γ -hydroxy **16** was found to be prone to lactonization (forming the corresponding peptide lactone **17**) during storage. In fact, **16** cyclized to **17** at a percentage of 13–15% within 15 days at 4 °C, as determined by HPLC analysis. However, **16** remained stable even after 30 days at –20 °C.

2.3. Solid-phase reductive amination of Hse(AL) containing peptides

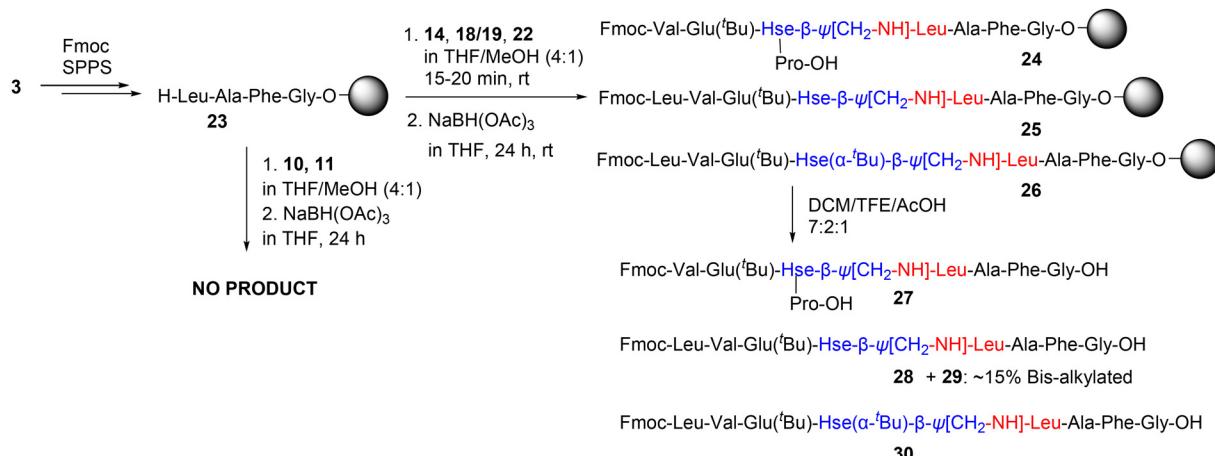
In this part of the work, products **10**, **11**, **14**, **18/19** and **22** were subjected to the solid-phase reductive amination reaction. As a

model substrate, a tetrapeptide was initially synthesized on CLTR resin **3** by using standard SPPS (Scheme 6). Additionally, Phe was incorporated into the peptide sequence to facilitate monitoring of reaction completion by HPLC analysis. In the final step of the peptide synthesis, the N-terminal Fmoc group was removed to yield the corresponding peptidyl-resin **23**.

For the solid-phase reductive amination, our protocol was based on a two-step procedure: first, the formation of the corresponding imine between the N-terminus of the resin-bound peptide **23** and the aldehyde group of the Hse-containing peptides followed by washing of the unreacted peptide; second, reduction of the imine using the mild reducing agent sodium triacetoxyborohydride $[\text{NaBH}(\text{OAc})_3]$.^{32–34} This procedure enabled the formation of the resin-bound pseudopeptides **24–26**, which were subsequently treated with a mixture of DCM/2,2,2-trifluoroethanol (TFE)/AcOH (7:2:1) to finally yield the corresponding Hse- β - Ψ [CH₂-NH] containing **27**, **28** and **30** (Scheme 6).

It should be noted that, although in case of **10** and **11**, no reaction was noticed (Fig. S16), the use of the Hse(AL)-Pro containing peptide **14** was efficiently subjected to solid-phase reductive amination to form the peptidyl-resin **24** and the corresponding **27** (Fig. S17). In case of **18/19**, where the Hse residue was introduced at the C-terminus of the peptide chain, the γ -aldehyde group of Hse, in equilibrium with the corresponding γ -hydroxy lactone ring (as discussed in section 2.2.2), allowed the solid-phase reductive amination, obviously by the open form of the peptide, finally resulting to the peptidyl-resin **25** and the corresponding pseudopeptide **28**. In this case, a



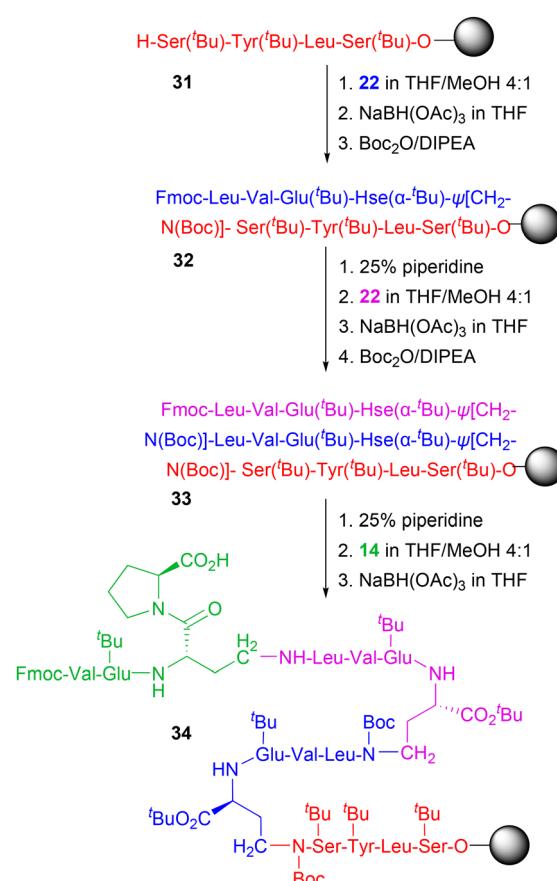


Scheme 6 Solid-phase reductive amination of the Hse-containing peptide aldehydes **14, 18/19** and **22**.

small percentage (approx. 15%) of the bis-alkylated peptide **29** was also formed (a well-known by-product of reductive amination processes) (Fig. S18).³⁵ In the case of peptide **22**, where the C-terminal α -carboxylic acid group of the peptide was *tert*-butylated, this was also effectively reacted with resin **23**, allowing the formation of the corresponding resin-bound **26** and finally the formation of **30** (Fig. S19). For this set of experiments (solid-phase peptide synthesis, solid-phase reductive amination processes) the reaction progress and completion, as well as the purity of the reaction products, were monitored by HPLC analysis and the products/byproducts were identified by ESI-MS analysis where the expected masses were identified.

The incapability of the Hse-containing peptides **10** and **11** to react with resin **23** (under the same reductive conditions that were applied for peptides **14, 18/19** and **22**), is clearly due to the chemical stability of the γ -hydroxy lactam ring and its inability to equilibrate with its open form. This indicates that, while the synthesis of the C-terminal Hse-containing peptide aldehydes could be an effective approach for preparing γ -hydroxy lactam peptides, these could not serve as alkylating agents in reductive amination processes, at least under the described protocol.

Among the many opportunities for peptide modification and conjugation strategies enabled by the stabilized Hse peptide aldehydes developed in this work, as well as by the proposed solid-phase reductive amination protocol, we provide as an illustrative example the Solid-Phase Fragment Condensation (SPFC) of peptide **34** (Scheme 7), which contains three sequentially formed Hse- β - Ψ [CH₂-NH] bonds. This peptide was assembled on resin **31** by initial reductive amination with fragment aldehyde **22** (using the previously reported protocol). The resulting Hse- β - Ψ [CH₂-NH] bond was then protected with the Boc group to afford the resin-bound fragment **32**. Subsequent Fmoc removal with 25% piperidine enabled a second imine formation and reduction step with fragment **22** allowing the formation of the second Hse- β - Ψ [CH₂-NH] bond, which was also protected with Boc group to afford **33**. After another Fmoc removal, fragment aldehyde **14** was reacted



Scheme 7 Example of a longer peptide containing three Hse- β - Ψ [CH₂-NH] peptide isostere bonds, synthesized by using the Hse peptide fragments **14** and **22**. The different fragments were obtained by treatment of the resins **32–34** (as well as their non-Boc-protected precursors) with DCM/TFE/AcOH (7:2:1) (Fig. S20).

using the same imine-amine protocol to introduce the third Hse- β - Ψ [CH₂-NH] bond. The reaction progress was monitored by HPLC analysis after treatment of resin samples with DCM/



TFE/AcOH (7:2:1), which afforded the corresponding fully protected (C-terminal deprotected) resin-cleaved fragments of **32–34** (as well as their non-Boc-protected precursors) (Fig. S20). This example demonstrates that the methodology enables the efficient formation of Hse- β - Ψ [CH₂-NH] bonds and offers significant advantages for fragment preparation suitable for further use/modification in SPFC strategies.

2.4. Investigating O-Trt cleavage from Hse(Trt)-containing peptides – lactone formation

As already presented in sections 2.2.2 and 2.3, the use of C-terminal Hse peptides in solid-phase reductive amination processes is a viable method. In one of the methods applied herein, treatment of **15** with 30% HFIP afforded the Hse peptide **16** and a small percentage (2–3%) of lactone **17** (as previously described in section 2.2.2).

To further investigate this issue, we treated resin **15** with 1% TFA in DCM at rt, where the formation of lactone **17** was confirmed within 15–20 min (Scheme 8). The same product was obtained by the treatment of resin **15** with 30% HFIP in DCM for 45 min to initially afford the liberated O-Trt deprotected peptide **16** followed by treatment with only 0.1% TFA in DCM (or 1% TFA in DCM), which afforded lactone **17** within 15 min (Fig. S21–S23).

No racemization was measured during lactone formation (as evidenced by ¹H-NMR analysis of H-Leu-Val-Glu(^tBu)-L-Hse-lactone and H-Leu-Val-Glu(^tBu)-D-Hse-lactone) (Fig. S24). Thus, we concluded that treatment of **15** with 30% HFIP followed by treatment with only 0.1% TFA or treatment of **15** with 1% TFA, could be used for the synthesis of the corresponding lactone **17**, but these cleavage protocols cannot be used for the formation of the C-terminal Hse(OL) peptides (as they would rapidly cyclize to lactones).

Further studies on the preparation of C-terminal Hse O-Trt deprotected peptides from the resin, by replacing HFIP with TFE, showed that treatment of resin **15** with 30% TFE in DCM

did not afford any of the desired O-Trt deprotected peptide, in agreement with previous studies.²¹ However, treatment of the resin **15** with TFE/DCM/TES (30/65/5) afforded the O-Trt deprotected peptide alcohol **16** within 4 h, through an initial fast release of the Hse O-Trt protected peptide **34** followed by O-Trt deprotection, with limited lactone formation (2–3%) within 4 h, as determined by HPLC analysis (Fig. S25 and 26).

Summarizing this section, treatment of CLTR resin-bound C-terminal Hse(Trt) peptides with TFE/DCM/TES 30/65/5 represents an alternative method for peptide cleavage from the resin and removal of the O-Trt protecting group(s), allowing the synthesis of C-terminal Hse-containing peptides, which worked well for the Glu(^tBu)/Hse(Trt)-containing substrate tested.

In addition, the use of TFA for the cleavage of Hse(Trt)-containing peptides from CLTR resin, besides the well-known limitation of trifluoroacetylation of the liberated hydroxy groups,^{36,37} has a second limitation in the case of the C-terminal Hse peptides: the rapid formation of lactones. Nevertheless, this method, *i.e.* treatment of the resin-bound C-terminal Hse(Trt) peptides, or the initially cleaved C-terminal Hse peptides with TFA, could be used for the easy and efficient cyclization to the corresponding C-terminal peptide lactones.

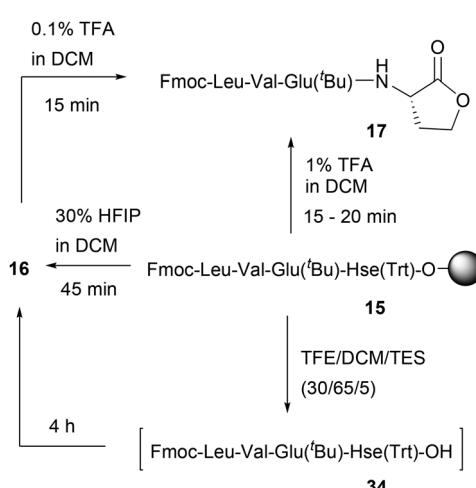
3. Conclusions

In this work, we propose two complementary approaches for solid-phase reductive amination using homoserinyl (Hse) γ -aldehyde containing peptides. Both strategies rely on the initial synthesis of peptides bearing side-chain selectively deprotected Hse residues, followed by oxidation of the Hse side-chain hydroxyl group to the corresponding aldehyde.

In the first approach, replacement of the amide hydrogen at the *n* + 1 position relative to the Hse residue with an *N*-alkyl group, effectively prevents intramolecular cyclization to the corresponding γ -hydroxy lactam, thereby stabilizing the peptide aldehyde, enabling its use in subsequent solid-phase reductive amination. In the second approach, a C-terminal Hse γ -aldehyde peptide is generated. In this case, although cyclization to the corresponding γ -hydroxy lactone may occur, the lactone remains reactive under reductive amination condition, presumably *via* its open-chain form. Additional stabilization strategies for C-terminal Hse γ -aldehyde peptides, through *tert*-butylation of the α -COOH group of the C-terminal Hse residue, are also demonstrated.

As proof of concept, all three types of Hse-derived peptide aldehydes, *i.e.* *N*-alkylated at the *n* + 1 position, or present as the C-terminal aldehyde (in equilibrium with the γ -hydroxy lactone) or as its stabilized precursor, were successfully employed in solid-phase reductive amination, leading to ligated peptides *via* formation of a methyleneamino Hse- β - Ψ [CH₂-NH] isosteric bond.

Beyond this specific demonstration, the methodology offers broad applicability to a wide range of peptide ligation and modification strategies that exploit Hse γ -aldehyde peptide



Scheme 8 Protocols tested for the SPS of C-terminal Hse-containing peptides and C-terminal peptide lactones.

intermediates and the incorporation of the Hse- β - Ψ [CH₂-NH] isostere, thus providing a versatile platform for modern peptide chemistry and conjugate development.

4. Experimental

4.1. Materials

2-Chlorotritiy chloride resin (CLTR) 100–200 mesh, 1% divinylbenzene (DVB) (loading capacity 1.2–2.0 mmol g⁻¹) and Fmoc-protected amino acids were kindly provided by CBL Patras S.A. (Industrial area of Patras, Building block 1, GR-25018, Patras, Greece). Plastic reactors for Peptide Synthesis (polypropylene syringes equipped with porous polyethylene frits at the bottom) with a pore size of 25 μ m were obtained from Carl Roth GmbH + Co. KG, Karlsruhe, Germany. Tetrahydrofuran (THF) (ACS reagent, Reag. Ph. Eur., \geq 99.9%, containing 250 ppm BHT as inhibitor) was purchased from Honeywell Riedel de Haen (Germany). THF was dried over molecular sieves type 4A (beads, diameter 1.7–2.4 mm) 48 h prior to use (Sigma-Aldrich O.M. Ltd, Athens, Greece). Methanol (MeOH) (HPLC grade, \geq 99.8%) was purchased from Fischer Chemicals (Thermo Fisher Scientific, Geel, Belgium). All other chemicals were purchased from Sigma-Aldrich O.M. Ltd (Athens, Greece). All chemicals were used without further purification.

4.2. Analytical methods

High Performance Liquid Chromatography (HPLC) analysis was performed on a Waters 2695 multisolvent delivery system (Milford, MA, USA), combined with Waters 996 photodiode array detector. The following columns were used: (A) Column: Lichrospher RP-18, 5 μ m, 125–4 mm; (B) Column: Purospher RP-8e, 5 μ m, 125–4 mm; (C) Column: Purospher RP-8e, 5 μ m, 250–4 mm. ESI-MS spectra were recorded on a Waters Micromass ZQ 4000 mass detector (positive mode), controlled by MassLynx 4.1 software, by direct infusion, using a syringe pump at a flow rate of 5 mL min⁻¹. Cone voltage was set at 30 V and scan time at 1 s, with the interscan delay at 0.1 s. NMR spectra were recorded on a Brucker DPX 600 MHz instrument (Peoria, IL, USA). The sample spectra were recorded at 25 °C. Chemical shifts (δ) were referenced to the corresponding solvent peaks and are reported in parts per million (ppm).

5. Experimental procedures

5.1. Solid-phase synthesis – general protocol

Solid-phase peptide synthesis was carried out manually, either in plastic peptide synthesis reactors attached to a Visiprep Solid Phase Extraction Vacuum Manifold, or in Eppendorf tubes, where the reaction mixture was transferred to either microfilters or polypropylene syringes for washing.

5.1.1. Attachment of the first amino acid on 2-CLTR resin. CLTR (1.5–2.0 mmol g⁻¹ resin) was suspended in dichloromethane (DCM) (10 mL g⁻¹ resin) for 10 min. *N,N'*-Diisopropylethylamine (DIPEA) (4 mmol g⁻¹ resin) was then

added and the resin was shaken for 1 min. The required Fmoc-amino acid (1.5 mmol g⁻¹ resin), dissolved in the minimum volume of DCM, was subsequently added, and the resin was gently agitated for 1.5 h at rt. Next, methanol (MeOH) (1 mL g⁻¹ resin) and DIPEA (2 mmol g⁻¹ resin) were added, and the resin was agitated for an additional 30 min at rt. The resin was then filtered and sequentially washed with DCM (2 \times 20 mL g⁻¹ resin), DCM/MeOH/DIPEA 85 : 10 : 5 (3 \times 20 mL g⁻¹ resin for 5, 10, 15 min, respectively), *N*-methyl-2-pyrrolidone (NMP) (\times 5), isopropyl alcohol (iPrOH) (\times 3), diethyl ether (DEE) (\times 2) and dried *in vacuo*.

Removal of the Fmoc group for the first amino acid attachment was performed directly after attachment of the Fmoc-amino acid, as described in protocol: “*Fmoc removal during solid-phase peptide assembly*”.

Resin loading was estimated on the N-deprotected amino acid resin by measuring the resin weight gain, using the following formula: $S(\text{wt}) = [\text{Wt}(\text{g}) \times 1000]/[\text{Wt}(\text{add}) \times \text{Wt}(\text{t})]$, where $S(\text{wt})$: weight gain substitution (mmol g⁻¹); $\text{Wt}(\text{g})$: weight gained by resin (g); $\text{Wt}(\text{add})$: molecular weight added to the resin = MW of amino acid minus MW of leaving group (g mol⁻¹); $\text{Wt}(\text{t})$: total weight gain of the resin after loading.

5.1.2. Coupling of Fmoc-amino acids – peptide assembly. The appropriate Fmoc-amino acid (3 mmol) and *N*-hydroxybenzotriazole (HOBT) (4.5 mmol) were dissolved in NMP (5 mL) and the mixture was cooled at 4 °C for 15 min. *N,N'*-diisopropylcarbodiimide (DIC) (3.6 mmol) was then added, and the solution was agitated for 15 min at 4 °C before being added to the resin-bound amino acid or peptide (1.0 mmol g⁻¹ resin), and the resin was agitated for 3 h at rt.

Completion of the coupling reaction was monitored using the Kaiser test. Briefly, a small sample of the resin was taken and washed sequentially with NMP, iPrOH, DEE several times. The sample was then transferred to a small glass tube, and two drops of each Kaiser reagent were added. The tube was heated at 110–120 °C for 4–5 min. A positive test (blue resin beads) indicated incomplete coupling, while a negative test (colorless or yellowish beads) indicated complete coupling. In the case of incomplete coupling, recoupling was carried out using a freshly prepared solution of the activated Fmoc-amino acid.

Finally, the resin was filtered and washed with NMP (\times 5), iPrOH (\times 3), DEE (\times 2) and dried *in vacuo*.

5.1.3. Fmoc removal during solid-phase peptide assembly. The resin-bound Fmoc-protected amino acid or Fmoc-protected peptide was initially washed with NMP (6 mL g⁻¹ resin) (\times 5) and then treated twice with 25% piperidine in NMP (6 mL g⁻¹ resin) for 15 and 30 min at rt, respectively. To confirm complete removal of the Fmoc group, two tests were performed. First, a positive Kaiser test indicated successful removal of the Fmoc group. To ensure complete deprotection, a second test was carried out: a resin sample (approx. 2 mg) was treated with 25% piperidine in NMP (20 μ L) and heated at 100 °C for 5 min. From the resulting solution, 10 μ L were spotted on a TLC plate, which was developed a few centimeters and examined under UV lamp for any UV-absorbing material. If Fmoc absorbance was detected, deprotection was repeated



for an additional 30 min. Finally, the resin was filtered, washed with NMP ($\times 5$), iPrOH ($\times 3$), DEE ($\times 2$) and dried *in vacuo*.

5.2. General procedures for acidic cleavage of resin-bound peptides – preparation of peptide alcohols 9a, 9b, 13, 16

5.2.1. t Bu-protected/Hse(O-Trt)-protected peptides. The resin-bound peptides were initially treated twice with 0.1% trifluoroacetic acid (TFA) in DCM to remove residual solvents, followed by two washes with DCM. The resin was then treated with a cleavage mixture consisting of DCM/2,2,2-trifluoroethanol (TFE)/acetic acid (AcOH) (7:2:1) for 15 min at rt (10–15 mL g⁻¹ resin), to cleave the fully protected peptides from the resin. The cleavage solution was filtered, and the resin was washed with an additional portion of the cleavage mixture, followed by DCM ($\times 2$). The combined filtrates were concentrated under reduced pressure on a rotary evaporator until an oily residue was obtained. DEE was then added to precipitate the peptide as a white solid, which was collected by filtration and washed with DEE ($\times 3$). The crude peptide was finally dried *in vacuo*.

5.2.2. t Bu-protected/Hse-deprotected peptides. The resin-bound peptides were initially treated twice with 0.1% TFA in DCM to remove residual solvents, followed by two washes with DCM. This resin was then treated with one of the following cleavage mixtures: (a) 30% hexafluoro-2-propanol (HFIP) in DCM for 45 min at rt (10–15 mL g⁻¹ resin); or (b) TFE/DCM/triethylsilane (TES) (30:65:5) for 4–5 h at rt (10 mL g⁻¹ resin). The cleavage solution was filtered, and the resin was washed with an additional portion of the cleavage mixture, followed by DCM ($\times 2$). The combined filtrates were concentrated under reduced pressure on a rotary evaporator until an oily residue was obtained. DEE was added to precipitate the peptide as a white solid, which was collected by filtration and washed with DEE ($\times 3$). The resulting side-chain deprotected peptide alcohols were obtained as white solids and further dried *in vacuo*.

Yields from 1.0 g resin (resin 3) were as follows: Fmoc-Leu-Val-Glu(t Bu)-Hse-Gly-OH (**9a**): 0.70 g (yield: 88.0%); Fmoc-Val-Glu(t Bu)-Hse-Leu-OH (**9b**): 0.68 g (yield: 92.0%); Fmoc-Val-Glu(t Bu)-Hse-Pro-OH (**13**): 0.75 g (yield: 89.7%); Fmoc-Leu-Val-Glu(t Bu)-Hse-OH (**16**): 0.65 g (yield: 88.0%) [both cleavage methods gave comparable results].

5.3. *tert*-Butylation

5.3.1. *tert*-Butylation of C-terminal Hse peptide **16 to afford the C-terminal homoserinyl *a*-*tert*-butylated γ -hydroxy peptide **20**.** Fmoc-Leu-Val-Glu(t Bu)-Hse-OH (**16**) (0.22 mmol; 163.2 mg) (containing 2–3% lactone) was dissolved in a mixture of DCM/NMP (9:1) (2.2 mL) (both solvents were dried over molecular sieves 4A for at least 48 h prior to use). To this solution, *tert*-butyl 2,2,2-trichloroacetimidate (TBTA) (0.44 mmol; 96.53 mg) was added, and the reaction mixture was stirred at rt. Reaction progress was monitored by HPLC analysis. After 24 h and 48 h, fresh portions of TBTA (0.44 mmol; 96.53 mg) were added, and the reaction was terminated at 72 h. The reaction mixture was diluted with DCM,

and the organic layer was sequentially extracted with 10% aqueous sodium hydrogen carbonate (NaHCO₃) ($\times 2$) and water ($\times 3$). The organic layer was then dried over magnesium sulfate (MgSO₄), filtered, and concentrated under reduced pressure to yield an oily residue. This residue was treated with DEE to precipitate a white solid, which was collected by filtration and washed with DEE.

This procedure afforded the desired Fmoc-Leu-Val-Glu(t Bu)-Hse- t Bu (**20**) (115.0 mg; yield: 65.5%). The final product contained 2–3% lactone **17** and 11.0% of the di-*tert* butylated derivative **21**.

5.4. DMP oxidation

5.4.1. DMP oxidation of **9a, **9b**, **13**, **20**.** The Fmoc- t Bu-protected, Hse-deprotected peptide alcohols **9a,b** and **13** (prepared as described in protocol *General procedures for acidic cleavage of resin-bound peptides – preparation of peptide alcohols **9a**, **9b**, **13**, **16***), and compound **20** (prepared as described in protocols *General procedures for acidic cleavage of resin-bound peptides – preparation of peptide alcohols **9a**, **9b**, **13**, **16*** and *tert-Butylation of C-terminal Hse peptide **16** to afford the C-terminal homoserinyl *a*-*tert*-butylated γ -hydroxy peptide **20***), were dissolved in NMP at a concentration of 0.1–0.2 M. Dess–Martin periodinane (DMP) (1.2 eq.) was added as a solid, and the reaction mixture was stirred at rt. Reaction progress was monitored by TLC. Oxidation was typically complete within 2–3 h. If additional time was required, a further 10% of the initial calculated amount of DMP was added, resulting in completion within the next hour.

Upon completion, DCM (twice the volume of NMP) was added, and the mixture was cooled in an ice-bath for 10 min. Water (twice the volume of DCM) was then added, and the mixture was stirred for 10 min. The aqueous and organic layers were separated, and the aqueous phase was washed once more with DCM. The combined organic layers were treated with an aqueous 10% sodium thiosulfate (Na₂S₂O₃) (approx. of equal volume to the combined DCM and NMP fractions), under vigorous stirring in an ice bath for 15 min. After phase separation, the organic layer was washed with a 10% aqueous NaHCO₃ ($\times 2$) and water ($\times 1$), then acidified to pH 3–4 using 10% aqueous citric acid and finally washed with water ($\times 3$). The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure to yield an oily residue. This was treated with DEE to precipitate the desired γ -hydroxy lactam peptides as white solids.

Yields were as follows: Fmoc-Leu-Val-Glu(t Bu)-Hse-Gly-OH (**9a**) (0.251 mmol; 200 mg) afforded γ -hydroxy lactam of Fmoc-Leu-Val-Glu(t Bu)-Hse(AL)-Gly-OH (**10**) (169.57 mg; yield: 85%); Fmoc-Val-Glu(t Bu)-Hse-Leu-OH (**9b**) (0.406 mmol; 300 mg) afforded γ -hydroxy lactam of Fmoc-Val-Glu(t Bu)-Hse-Leu-OH (**11**) (263.45 mg; yield: 88%); Fmoc-Val-Glu(t Bu)-Hse-Pro-OH (**13**) (0.277 mmol; 200 mg) afforded Fmoc-Val-Glu(t Bu)-Hse(AL)-Pro-OH (**14**) (163.54 mg; yield: 82%); Fmoc-Leu-Val-Glu(t Bu)-Hse-O- t Bu (**20**) (0.12 mmol; 95 mg) afforded Fmoc-Leu-Val-Glu(t Bu)-Hse(AL)-O- t Bu (**22**) (84.33 mg; yield: 89%).



5.4.2. DMP oxidation of 16. The Fmoc-^tBu-protected peptide alcohol **16**, bearing a side-chain O-Trt-deprotected Hse residue (prepared as described in protocol *General procedures for acidic cleavage of resin-bound peptides – Preparation of peptide alcohols 9a, 9b, 13, 16*), was dissolved in NMP at a concentration of 0.1–0.2 M. DMP (1.2 eq.) was added, and the reaction mixture was stirred at rt. Reaction progress was monitored by TLC, and oxidation was typically complete within 2–3 h. If additional time was required, 10% of the initially calculated amount of DMP was added, which led to completion within 1 additional hour.

Upon completion, DCM (twice the volume of NMP) was added, followed by an aqueous solution of 10% Na₂S₂O₃ (approx. equal in volume to the combined DCM and NMP). The mixture was stirred vigorously for 15 min. The organic and aqueous layers were then separated, and the aqueous phase was washed once more with DCM. The combined organic layers were subsequently treated with water, resulting in the formation of a white solid at the interface. The phases were separated, and this washing procedure with water was repeated multiple times. After four washings, interfacial precipitation consistently yielded a white solid (formed by combining all collected solids formed at the interface during extractions). The combined solids were further washed with a mixture of DCM and water (1 : 1) (×2), then dried over MgSO₄ to afford **18**.

Fmoc-Leu-Val-Glu(^tBu)-Hse-OH (**16**) (0.135 mmol; 100 mg) afforded Fmoc-Leu-Val-Glu(^tBu)-Hse(AL)-OH (**18**) as a white solid (84.77 mg; yield: 85%).

5.5. Solid-phase reductive amination

5.5.1. Solid-phase reductive amination between 10, 11 and Fmoc-peptide aldehydes (14, 18/19, 22) with resin 23. The peptide resin **23** (H-Leu-Ala-Phe-Gly-O-CLTR) (100 mg; 0.1 mmol based on a resin loading 0.9–1.0 mmol g⁻¹), synthesized according to the protocols described in *Solid-phase synthesis – general protocol*, was initially washed with anhydrous THF (×3), and the dry resin was transferred to an Eppendorf tube. A solution of anhydrous THF/MeOH (4 : 1) (5 mL g⁻¹ resin) was added, followed by the addition of **10**, **11** (1.2 eq.; 0.12 mmol) or the peptide aldehydes (**14**, **18/19**, **22**) (1.2 eq.; 0.12 mmol). The suspension was agitated for 15–20 min at rt. The resin was then filtered and washed with anhydrous THF (×3) to remove any excess unreacted peptide aldehyde.

Next, the resin was transferred to a new Eppendorf tube, and anhydrous THF (5 mL g⁻¹ resin) was added, followed by solid sodium triacetoxyborohydride NaBH(OAc)₃ (2.2 eq.; 0.22 mmol). The reaction mixture was stirred at rt for 24 h. Reaction completion was monitored by HPLC analysis of the crude cleavage mixture of a resin sample. In general, the reaction was completed within 24 h. If incomplete conversion was observed by HPLC analysis, the resin was washed as described below and the imine–amine procedure was repeated to ensure full transformation.

After the reduction step, the reaction mixture was removed from the resin and discarded. The resin was then washed by addition and decantation of THF (3 × 4–5 volumes), and the resin was subsequently transferred to a filter and washed with THF, THF/MeOH (4 : 1) (4 × 15 min), THF (×5), NMP (×5), iPrOH (×3), and DEE (×2).

The final cleavage from the resin was performed by treating the resin with DCM/TFE/AcOH (7 : 2 : 1) (15 min), followed by DCM (×2). The combined filtrates were concentrated under reduced pressure to afford an oily residue, which was treated with DEE to precipitate the product. The resulting white solid was filtered and washed with DEE to yield the desired Hse-β-Ψ[CH₂-NH] peptides.

Products and yields for a typical resin loading (0.9–1.0 mmol g⁻¹) were as follows: Fmoc-Val-Glu(^tBu)-Hse (Pro-OH)-β-Ψ[CH₂NH]-Leu-Ala-Phe-Gly-OH (**27**), obtained by reductive amination between peptide aldehyde **14** and resin **23** (78.6 mg; yield: 78.6%); Fmoc-Leu-Val-Glu(^tBu)-Hse-β-Ψ[CH₂NH]-Leu-Ala-Phe-Gly-OH (**28**) and bis-alkylated (~15%) (**29**), obtained by reductive amination between peptide aldehyde **18/19** and resin **23** (70.5 mg; yield: 69.5%); Fmoc-Leu-Val-Glu(^tBu)-Hse(α-^tBu)-β-Ψ[CH₂NH]-Leu-Ala-Phe-Gly-OH (**30**), obtained by reductive amination between peptide aldehyde **22** and resin **23** (75.2 mg; yield: 70.6%); no products were identified for the reductive amination between **10**, **11** and resin **23**.

5.5.2. Solid-phase synthesis of resin-bound peptides 32, 33, 34. Peptide resin **31** was synthesized according to the procedures described in *Solid-phase synthesis – general protocol*. Peptide fragments **14** and **22** were introduced using the procedures described in *Solid-phase reductive amination protocol*.

Boc protection of each Hse-β-Ψ[CH₂-NH] bond was performed by first washing the resin with NMP to remove residual solvents. The resin was then suspended in a mixture of DCM/NMP (1 : 1) (5 mL g⁻¹ resin). DIPEA (10 eq.) and di-*tert*-butyl dicarbonate Boc₂O (10 eq.) were added sequentially, and the resin was agitated for 2 × 24 h at rt. HPLC analysis indicated that approximately 15–20% of the Hse-β-Ψ[CH₂-NH] bonds remained unreacted after 24 h; therefore, a second protection cycle was required to achieve complete Boc protection. The resin was then washed with NMP (×5), iPrOH (×3), and DEE (×2) to afford the resin-bound Hse-β-Ψ[CH₂-N(Boc)]-containing peptides.

5.6. C-terminal peptide lactone formation (17)

5.6.1. Procedure A: lactone formation via 30% HFIP cleavage followed by mild acid treatment. The C-terminal Hse-containing peptide, Fmoc-Leu-Val-Glu(^tBu)-Hse-OH (**16**) (^tBu-protected, O-Trt deprotected), was prepared as described in protocol *^tBu-protected/Hse-deprotected peptides*. Briefly, the resin-bound peptide **15** (^tBu-protected, O-Trt protected) (100 mg resin; 0.5 mmol g⁻¹) was treated with 30% HFIP in DCM (1.5 mL; 15 mL g⁻¹ resin) for 45 min at rt. The resin was then filtered and washed with DCM (×2). The combined filtrates were concentrated using a rotary evaporator to afford an oily residue.



This residue was directly treated with 0.1% TFA in DCM (1.5 mL), and the cyclization to the corresponding C-terminal peptide lactone **17** was completed within 15–20 min (as monitored by HPLC analysis). The reaction mixture was concentrated under reduced pressure, and the oily residue was treated with DEE to induce precipitation. The resulting solid was filtered, washed with DEE ($\times 2$), and dried *in vacuo* to afford lactone **17** (34.8 mg; yield: 96.6%).

5.6.2. Procedure B/C: direct lactonization *via* 1% TFA in DCM. Resin-bound peptide **15** (t Bu-protected, *O*-Trt protected) (100 mg resin; 0.5 mmol g⁻¹) was treated with 1% TFA in DCM (15 mL g⁻¹ resin) for 15 min at rt. This single-step treatment enabled simultaneous cleavage and intramolecular cyclization of the C-terminal Hse to its corresponding lactone (the successful cleavage/cyclization was effectively monitored by HPLC analysis).

The reaction mixture was concentrated under reduced pressure, and the oily residue was treated with DEE to precipitate the product. The resulting white solid was filtered, washed with DEE ($\times 2$), and dried *in vacuo* to yield lactone **17** (34.1 mg; yield: 94.6%).

Author contributions

Conceptualization, S. M.; methodology, S. M.; validation, S. M.; formal analysis, S. M., D. T., D. M.; investigation, D. T., S. M., D. M.; resources, S. M.; data curation, S. M.; writing – original draft preparation, S. M.; writing – review and editing, S. M.; supervision, S. M.; project administration, S. M.; funding acquisition, S. M. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5ob01718a>.

Acknowledgements

This paper has been partly financed by the funding programme “MEDICUS” of the University of Patras—Research Committee, Code 81762; The publication of the article in OA mode was financially supported by HEAL-Link; The authors acknowledge CBL Patras S.A., Patras, Greece and Emeritus Prof. K. Barlos for kindly providing 2-chlorotritly chloride resin and Fmoc-amino acids; The authors acknowledge Prof. Dimitrios Gatos for access to HPLC equipment.

References

- O. Al Musaimi, L. Lombardi, D. R. Williams and F. Albericio, *Pharmaceutics*, 2022, **15**, 1283.
- L. Gentilucci, R. De Marco and L. Cerisoli, *Curr. Pharm. Des.*, 2010, **16**, 3185.
- C. Lamers, *Future Drug Discovery*, 2022, **4**(2), FDD75.
- I. Avan, C. D. Hall and A. R. Katritzky, *Chem. Soc. Rev.*, 2014, **43**, 3575.
- N. Qvit, S. J. S. Rubin, T. J. Urban, D. Mochly-Rosen and E. R. Gross, *Drug Discovery Today*, 2017, **22**, 454.
- J. M. Ahn, N. A. Boyle, M. T. MacDonald and K. D. Janda, *Mini-Rev. Med. Chem.*, 2002, **2**, 463.
- L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G. Shao, X. Wang, R. Wang and C. Fu, *Signal Transduction Targeted Ther.*, 2022, **7**, 48.
- X. Zhang, X. Li, Q. You and X. Zhang, *Eur. J. Med. Chem.*, 2017, **139**, 542.
- P. L. Scognamiglio, G. Morelli and D. Marasco, *Methods Mol. Biol.*, 2015, **1268**, 159.
- A. F. Spatola, *Methods Neurosci.*, 1993, **13**, 19.
- P. Barman, S. Joshi, S. Sharma, S. Preet, S. Sharma and A. Saini, *Int. J. Pept. Res. Ther.*, 2023, **29**, 61.
- L. Otvos Jr and J. D. Wade, *Front. Chem.*, 2014, **2**, 62.
- K. Barlos and D. Gatos, in *Fmoc Solid Phase Peptide Synthesis - A Practical Approach*, ed. W. C. Chan and P. D. White, Oxford University Press, 2000, pp. 215–228.
- J. M. Palomo, *RSC Adv.*, 2014, **4**, 32658.
- F. Albericio, *Biopolymers*, 2000, **55**, 123.
- M. Amblard, J. A. Fehrentz, J. Martinez and G. Subra, *Mol. Biotechnol.*, 2006, **33**, 239.
- S. Mourtas, C. Katakalou, D. Gatos and K. Barlos, *Molecules*, 2020, **25**, 218.
- S. Mourtas, D. Gatos and K. Barlos, *Molecules*, 2019, **24**, 4261.
- J.-M. Ahn, K. Kassee, T.-K. Lee, B. Manandhar and A. M. Yousif, in *Comprehensive Medicinal Chemistry III*, Elsevier, 2017, vol. 6, DOI: [10.1016/B978-0-12-409547-2.12413-8](https://doi.org/10.1016/B978-0-12-409547-2.12413-8).
- K. Barlos, D. Gatos and S. Koutsogianni, *J. Pept. Res.*, 1998, **51**, 194.
- A. Wester, A. M. Hansen, P. R. Hansen and H. Franzky, *Amino Acids*, 2021, **53**, 1455.
- A. Isidro-Llobet, M. Alvarez and F. Albericio, *Chem. Rev.*, 2009, **109**, 2455.
- AG. Myers, B. Zhong, M. Movassaghi, D. W. Kung, B. A. Lanman and S. Kwon, *Tetrahedron Lett.*, 2000, **41**, 1359.
- S. I. Al-Gharabli, S. T. A. Shah, S. Weik, M. F. Schmidt, J. R. Mesters, D. Kuhn, G. Klebe, R. Hilgenfeld and J. Rademann, *ChemBioChem*, 2006, **7**, 1048.
- R. Colombo, Z. Wang, J. Han, R. Balachandran, H. N. Daghestani, D. P. Camarco, A. Vogt, B. W. Day, D. Mendel and P. Wipf, *J. Org. Chem.*, 2016, **81**, 10302.
- W. Yang, S. Ramadan, B. Yang, K. Yoshida and X. Huang, *J. Org. Chem.*, 2016, **81**, 12052.
- A. Armstrong, I. Brackenridge, R. F. W. Jackson and J. M. Kirk, *Tetrahedron Lett.*, 1988, **29**, 2483.



28 N. S. Mahajani, R. I. L. Meador, T. J. Smith, S. E. Canarelli, A. A. Adhikari, J. P. Shah, C. M. Russo, D. R. Wallach, K. T. Howard, A. M. Millimaci and J. D. Chisholm, *J. Org. Chem.*, 2019, **84**, 7871.

29 Y. Tian, D. Wang, J. Li, C. Shi, H. Zhao, X. Niu and Z. Li, *Chem. Commun.*, 2016, **52**, 9275.

30 H.-K. Cui, Y. Guo, Y. He, F.-L. Wang, H.-N. Chang, Y.-J. Wang, F.-M. Wu, C.-L. Tian and L. Liu, *Angew. Chem., Int. Ed.*, 2013, **52**, 9558.

31 L. J. Dowman, S. M. Agten, J. Ripoll-Rozada, B. M. Calisto, P. J. B. Pereira and R. J. Payne, *Chem. Commun.*, 2021, **57**, 10923.

32 AF. Abdel-Magid, C. A. Mayano and K. G. Carson, *Tetrahedron Lett.*, 1990, **31**, 5595.

33 AF. Abdel-Magid, K. G. Carson, B. D. Harris, C. A. Maryanoff and R. D. Shah, *J. Org. Chem.*, 1996, **61**, 3849.

34 AF. Abdel-Magid and S. J. Mehrman, *Org. Process Res. Dev.*, 2006, **10**, 971.

35 AF. Abdel-Magid, B. D. Harris and C. A. Maryanoff, *Synlett*, 1994, 81.

36 L. Kocsis, F. Ruff and G. Orosz, *J. Pept. Sci.*, 2006, **12**, 428.

37 S. B. H. Kent, A. R. Mitchell, M. Engelhard and R. B. Merrifield, *Proc. Natl. Acad. Sci. U. S. A.*, 1979, **76**, 2180.

