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Peptide Modification | Very Important Paper

Tryptophan in Multicomponent Petasis Reactions for Peptide Stapling and Late-Stage Functionalisation

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Dedicated to Hana and Dalimil Dvorak

Abstract: Peptide stapling is a robust strategy for generating enzymatically stable, macrocyclic peptides. The incorporation of biologically relevant tags (such as cell-penetrating motifs or fluorescent dyes) into peptides, while preserving their binding interactions and enhancing their stability, is highly sought after. Despite the unique opportunities offered by tryptophan's indole scaffold for targeted functionalisation, its utilisation in peptide stapling has been limited as compared to other amino acids. Herein, we present an approach for peptide stapling using the tryptophan-mediated Petasis reaction. This method enables the synthesis of both stapled and labelled peptides and is applicable to both solution and solid-phase synthesis. Importantly, the use of the Petasis reaction in combination with tryptophan facilitates the formation of stapled peptides in a straightforward, multicomponent fashion, while circumventing the formation of undesired by-products. Furthermore, this approach allows for efficient and diverse late-stage peptide modifications, thereby enabling rapid production of numerous conjugates for biological and medicinal applications.

Introduction

Peptides have gained increasing attention in recent years due to their ability to mimic the endogenous portion of interacting proteins, low toxicity profiles and synthetic availability.[1] However, peptide drugs are often plagued by pharmacokinetic challenges, particularly poor stability against circulating proteolytic enzymes.[2] To bypass these intrinsic problems, an effective strategy is the use of conformationally constrained peptides.[3] Peptide stapling (PS) is a widely used technique that forces the peptide structure typically into an α-helical conformation, thereby enhancing its stability.[4] While PS is divided into one-component (1C), two-component (2C), and multicomponent methods, the last two mentioned have the advantage that they not only enhance peptide stability, but also allow for divergent modification of the staple moiety (Figure 1, blue star) by introducing useful therapeutic functionalities (e.g., cell-penetrating motifs, biotin or fluorescent tags).[1,5–8]

Cysteine, lysine and aspartic acid are among the most commonly used canonical amino acids (AAs) in two-

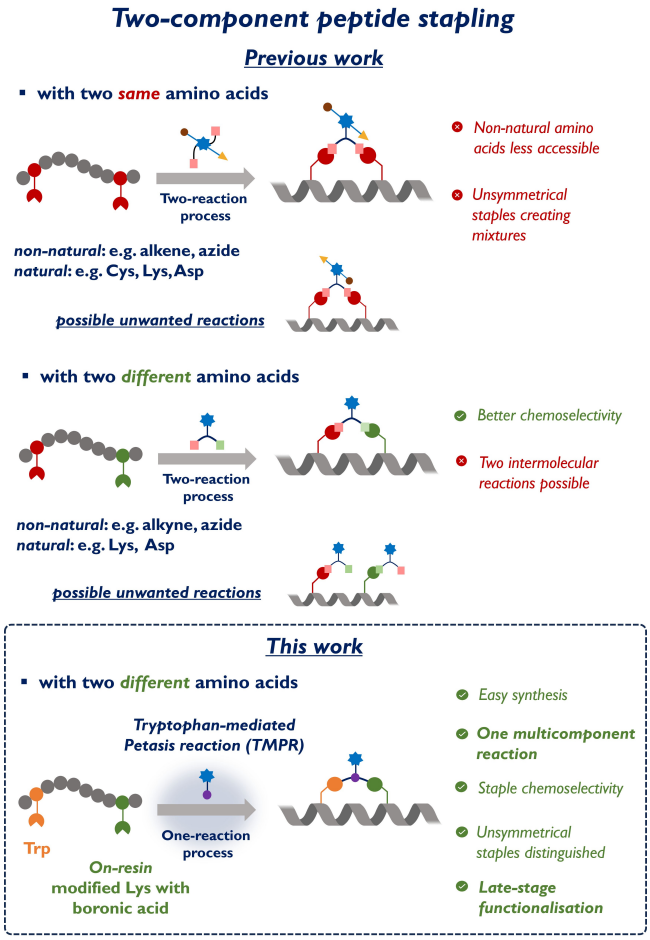

Figure 1. Two-component peptide stapling.

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component peptide stapling (2C-PS), while non-canonical AAs bearing azides, alkenes, or alkynes are frequently used as unnatural modifications (Figure 1).[4,9–12] However, a common burden during peptide stapling is formation of unwanted by-products, caused by undesired side-chain reactivity (e.g. oxidation of Cys) or by formation of regioisomers,[13] in case of unsymmetrical staples. Moreover, all of the mentioned methods require a two-reaction process to form a stapled peptide, which could lead to two intermolecular, rather than an intramolecular reaction (Figure 1; *possible unwanted reactions*). The symmetrical staples are thus more preferred, which limits the potential of diversely substituted staples that could bear functional handles for orthogonal manipulation. Rather than two sequential reactions, a suitable alternative could be seen in multicomponent reactions, in which three or more components react in one-reaction process to form products with high selectivity.[14]

Peptide diversification through multicomponent approaches is a relatively unexplored area, mostly limited to the four-component Ugi reaction.[4,14,15] However, the possibility for more than one reaction mechanistic pathway operating at the same time in a concurrent manner presents a highly complex challenge for controlling the outcome of the reaction. Therefore, a milder and better controllable option, such as Petasis reaction was expected to be more suitable—it involves aldehydes, amines, and boronic acids and has many attractive features such as excellent functional group tolerance and compatibility with physiological conditions.[16–19]

The Petasis reaction was used previously for peptide diversification, with modified Me-Lys/Orn[20] and *N*-terminal Pro residues[21,22] (Figure 2a, b). Heterocyclic natural amino acids bearing an amine functionality such as tryptophan (Trp) could be potentially used in the Petasis reaction as well; however, this has not been reported to date. The use of tryptophan in 2C-PS has great potential. Its application in stapling of peptides is, however, currently limited mostly to transition metal-catalysed reactions,[23–25] photochemical C–H activation[26,27] or to benzaldehyde bridged condensation at the C^2 position[28] (Figure 2c). Moreover, late-stage functionalisation (LSF) after stapling could be challenging with these current methods, likely due to synthetic severity or difficulties in maintaining orthogonality. To the best of our knowledge a widely applicable method for tryptophan-based stapling and further LSF is still lacking. LSF

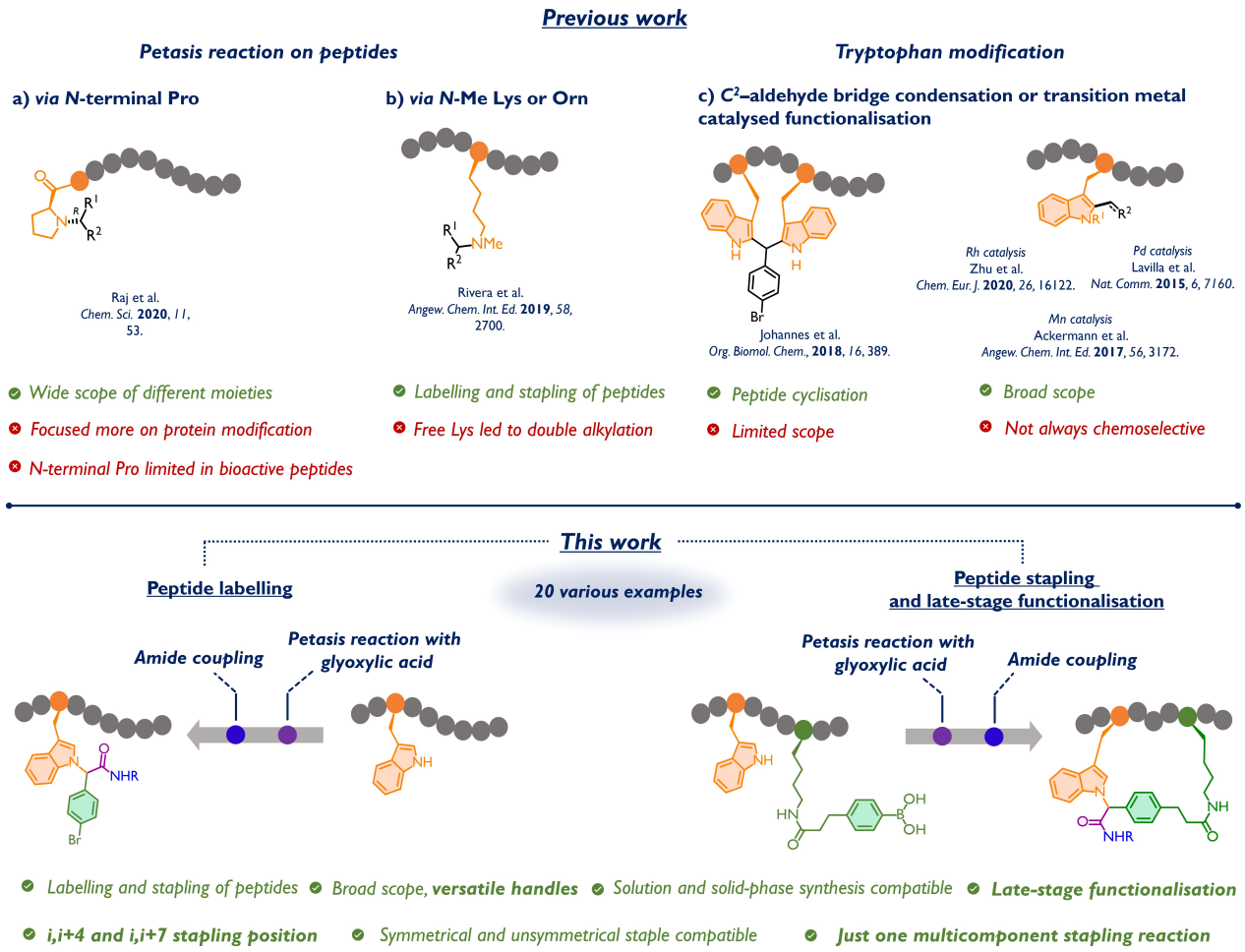


Figure 2. Comparison summary of previous work done in the field of Petasis reactions on peptides (parts a and b) and tryptophan modifications (part c). This work combines the advantages of previous approaches while overcoming the reported obstacles.

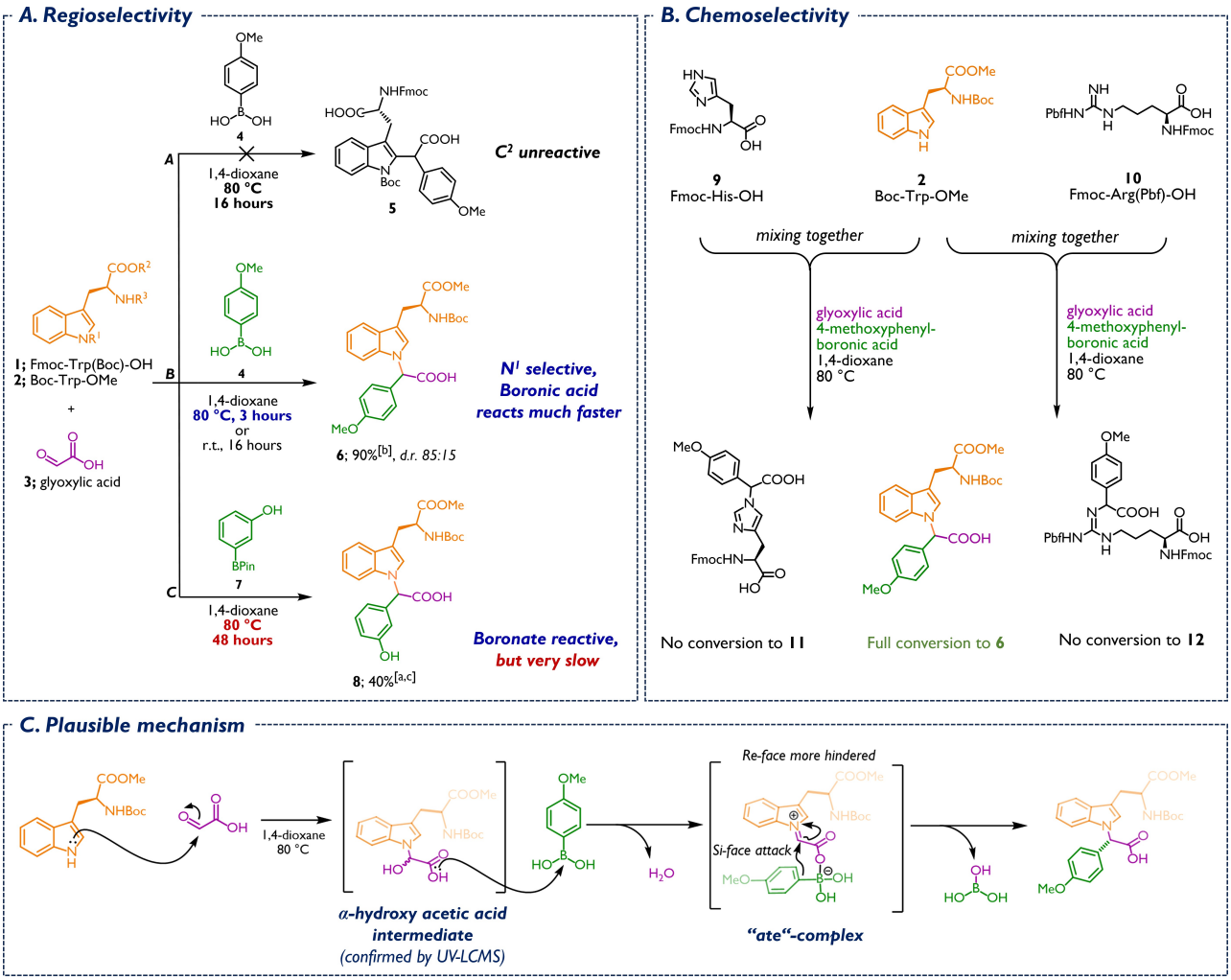
represents a powerful tool for simplifying the preparation of compound libraries, as one precursor molecule can yield several modified derivatives. However, careful design of the stapling method is essential to overcome the later challenges with cross-reactivity of multiple functional groups in peptides.

Taking all these aspects into consideration, a general and novel synthetic platform was developed that utilises tryptophan as an effective tool for labelling, stapling and further late-stage functionalisation of peptides (Figure 2; *This work*). For the first time, the multicomponent Petasis reaction was used in combination with tryptophan, enabling the formation of peptides with fluorescent, affinity, or other biologically useful tags. The method allows for the use of solid-phase synthesis (SPS) as well as standard solution-phase synthesis, using either protected or unprotected peptides, with a *C*-terminal amide or acid, on various stapling positions and with robust functional group tolerance. For the peptide-incorporated boronic acid required for the Petasis reaction, Lys(Alloc) was used as a precursor.[29] Furthermore, within this work we demonstrate that late-stage manipulation can be applied to a stapled, protected peptide, enabling fast and chemoselective incorporation of functional handles without the need for resynthesis and without undesired cross-reactivity.

Results and Discussion

Initial optimisation of the Petasis conditions on tryptophan

The first key step was finding the most suitable Petasis reaction conditions, due to the possible tryptophan reactivity on two different positions (C^2 vs N^1H). For this Fmoc-Trp(Boc)-OH **1** and Boc-Trp-OMe **2** were used as model substrates. First, both amino acids were subjected to reaction with glyoxylic acid **3** and 4-methoxyphenyl boronic acid **4** as other model components of the studied reaction (Scheme 1A; *Regioselectivity, reactions A and B*). In contrast to the published C^2 aldehyde-bridged condensation,[28] the



Scheme 1. Demonstration of A) regio- and B) chemoselective outcomes of the Petasis reaction on model compounds. C) A plausible mechanism to give **6** is also show. (Abbreviations: Boc = *tert*-Butyloxycarbonyl, Fmoc = Fluorenylmethyloxycarbonyl, Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl). [a] d.r. not determined. [b] Combined isolated yield for both diastereomers. [c] UV–LCMS conversion.

reaction with Fmoc-Trp(Boc)-OH **1** did not proceed to product **5**, whereas full conversion of **2** to product **6** after 3 h at 80 °C and 16 h at ambient temperature was observed. Such results suggested that the most reactive part of the tryptophan is the N^1 functional group. The theory was also supported by NMR experiments, where we could observe C^2 proton correlation, but not the indole N^1H proton (see the Supporting Information).

As boronic esters also react in the Petasis reaction,[16] the reaction with model phenyl boronic ester **7** under the identical reaction conditions was performed. Interestingly, the full conversion to **8** was not observed even after 48 h in refluxing 1,4-dioxane (Scheme 1A; *Regioselectivity, reaction C*). Thus, for the further studies only boronic acids were used, as they reacted significantly faster and with a cleaner reaction profile. The mechanism of the Petasis reaction is not yet fully elucidated; however, based on the reaction analysis initial formation of the α-hydroxy acetic acid (glycolic acid) intermediate was observed, followed by coordination of boron and aryl transfer from the boronic acid (Scheme 1C; *Plausible mechanism*). Although the Petasis reaction is known to be stereoselective, with preference for *anti*-attack during aryl transfer,[16] formation of two diastereomers was observed, albeit with strong preference for one of them (confirmed by NMR analysis). Assumption of *Si*-face, rather than *Re*-face attack during aryl transfer was made, likely due to the more sterically hindered *Re*-face caused by a presence of the branched C^3 substitution of the indole. Interestingly, for smaller groups on the boronic acid counterpart (e.g. vinyl), the diastereomeric ratio was strongly pushed to almost one diastereomer (compound **S1**, d.r. 96:4). Although the aim was not to fully elucidate the stereochemical outcome, the diastereoselectivity was attempted to be increased with use of chiral 3,3′-Br_2-BINOLs[16,30] (see the Supporting Information) that had been known as effective catalysts in the Petasis reaction. Although the diastereomeric ratio was slightly improved for compound **6** (from 85:15 to 91:9), the formation of a second diastereomer was not suppressed fully. However, this should not be a problem for the purpose of a stapling.

The next step was the investigation of the role of the boron component. Several boron-containing species were trialled as model substrates under various reaction conditions (Table S1, entries 14–26). The most promising outcomes were observed with electron-donating substituents on the aryl boronic acid and with potassium vinyl trifluoroborate, where the desired products have been obtained in 3 h at 80 °C. Unfortunately, no conversion under the photoredox-catalysed conditions inspired by Molander et al.[31] was detected, which could be due to a fast decarboxylation of glyoxylic acid under such conditions. Another interesting observation was the role played by solvent. In a methanolic mixture (1,4-dioxane/MeOH or THF/MeOH) the reaction was either very slow or did not proceed at all (Table S1, entries 3–6), even though the Petasis reaction is known to be compatible with the use of methanolic mixtures.[16] Interestingly, in acidic buffer (results not shown) the reaction proceeded without problem to the desired products.

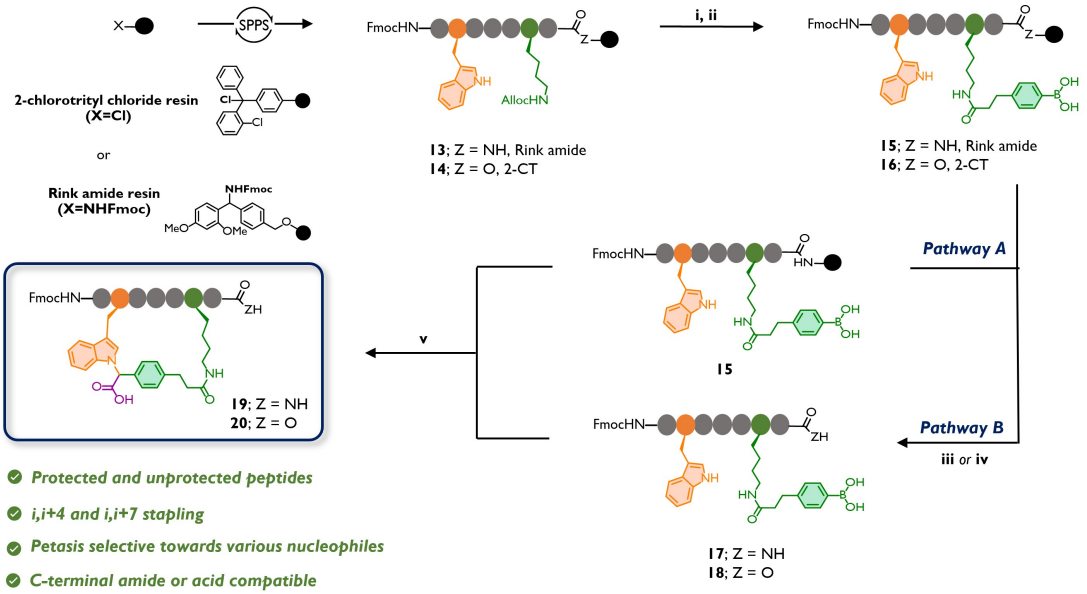
Finally, the role of aldehyde was investigated, using aldehydes such as ethyl glyoxalate, oxoacetamide, 1-octanal and 4-hydroxynicotinaldehyde (Table S1, entries 1–13). The presence of a directing group adjacent to the aldehyde to coordinate boron plays a crucial role in obtaining desired products, as is apparent from the proposed mechanism. In the case of 1-octanal or ethyl glyoxalate no conversion to the desired products was observed. Interestingly, even the 1-oxoacetamide derivative did not lead to desired products, even though formation of the α-hydroxy acetamide intermediate was observed. This could be caused by the stronger preference of boron complexation to oxygen, compared to nitrogen. Due to its broad reactivity, glyoxylic acid **3** was finally chosen as the ideal aldehyde substrate for this work.

Selectivity towards different sp^2-hybridised amines

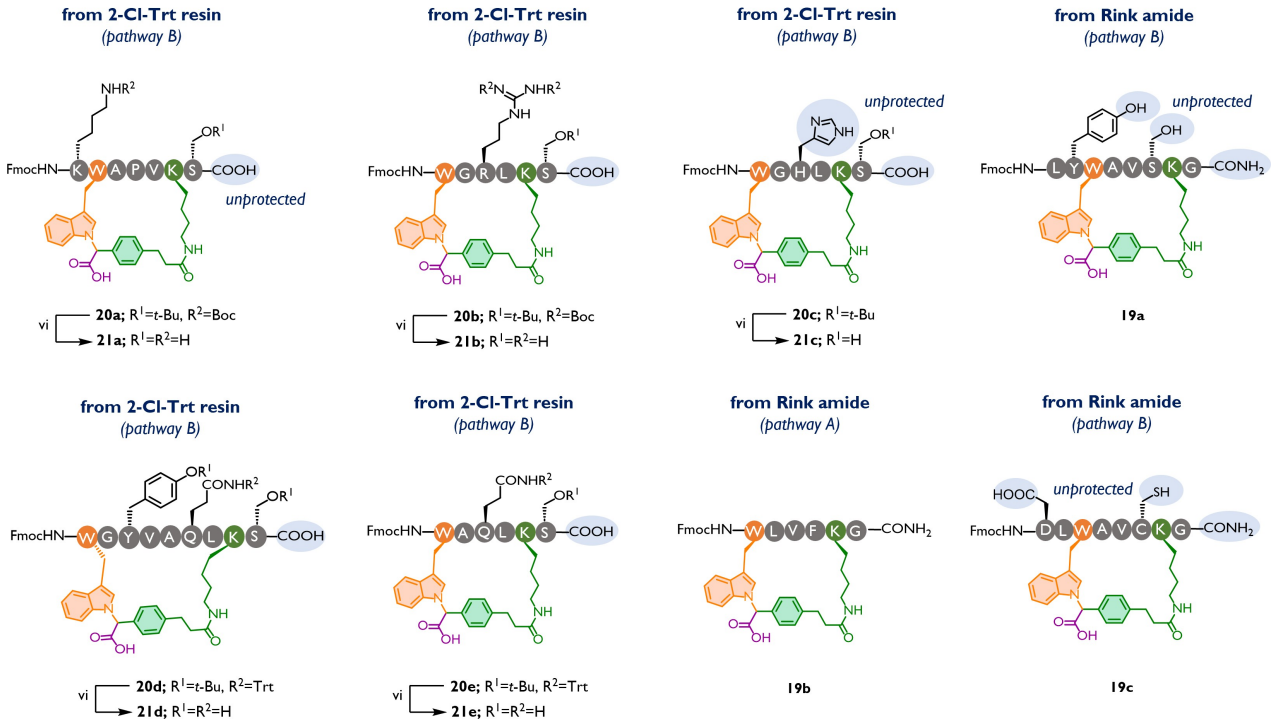
The Petasis reaction was reported to work well in the presence of sp^3 hybridised amines (specifically lysine and proline side-chains).[20,21] However, the selectivity between Trp and other similarly nucleophilic sp^2 amines in peptides has not been elucidated fully. Therefore, the reactivity preference was tested in a competition experiment for tryptophan versus histidine and (protected) arginine (Scheme 1B; *Chemoselectivity*), as both contain sp^2-hybridised secondary amines that could react in a similar way as Trp. Boc-Trp-OMe **2** was mixed with Fmoc-His-OH **9** and Fmoc-Arg(Pbf)-OH **10**, respectively, and subjected to the Petasis conditions. Complete chemoselectivity was observed as neither of the competing products could be detected (**11** or **12**, Scheme 1B). Noteworthy, during a reaction with **10**, the formation of the imino acetic acid by-product of arginine was observed on UV–LCMS (see the Supporting Information), however, this obstacle could be easily overcome with a choice of different protection on the arginine side-chain (e.g. Arg(Boc_2)) or by hydrolysis.

Application of the method to peptide stapling

To implement the tryptophan-mediated Petasis reaction (TMPR) conditions for macrocyclisation and stapling of peptides, several peptides with different amino acid residues were prepared to explore the scope and limitations of the reaction (Scheme 2). To broaden the applicability of the method, aspects such as *C*-terminal substitution (carboxylic acid vs amide), nucleophilic side chain selectivity and potential for post-stapling modifications of peptides were investigated. Peptides **13** and **14** were prepared on either Rink amide resin or 2-chlorotrityl resin using automated peptide synthesis with three degrees of orthogonality. For the model peptides *N*-terminal Fmoc protection was preserved to enable easy monitoring of the stapling reaction using UV–LCMS. Next, the Alloc protecting group was removed using $Pd(PPh_3)_4$ and phenylsilane, followed by amide coupling with commercially available 3-(4-boronophenyl)propanoic acid and HATU/DIPEA coupling reagents, to obtain **15** and **16**, respectively.[32]



Applied TMPR conditions on model substrates



Scheme 2. Substrate scope elucidation of TMPR on model peptides. Reagents and conditions: i) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, r.t., 3 h; ii) 4-(2-carboxyethyl)benzeneboronic acid, HATU, DIPEA, DMF, r.t., 16 h; iii) only peptides bound on Rink amide resin: CH_2Cl_2/TFA 1:1, r.t., 3 h; iv) only peptides bound on 2-chlorotrityl resin: CH_2Cl_2/HFIP 4:1, r.t., 2×2 h. v) glyoxylic acid monohydrate, 1,4-dioxane, 80 °C, 3–16 h; vi) neat TFA, r.t., 2 h (TIPS added to scavenge Trt cation for **21 d** and **21 e**). Yields and purities: **15 b**: 99 %;[c] **17 a**: overall yield 35 %, 99 %;[c] **17 c**: overall yield 25 %, 98 %;[c] **18 a**: overall yield 25 %, 99 %;[c] **18 b**: overall yield 30 %, 92 %;[c] **18 c**: overall yield 41 %, 98 %;[c] **18 d**: overall yield 25 %, 99 %;[c] **18 e**: overall yield 55 %, 99 %;[c] **19 a**: 15 %,[a,b] 98 %;[c] **19 b**: overall yield 14 %,[a,b] 93 %;[c] **19 c**: 35 %,[b] 85 %;[c] **20 a**: 70 %,[b] 95 %;[c] **20 b**: 30 %,[b] 90 %;[c] **20 c**: 23 %,[a,b] 98 %;[c] **20 d**: 18 %,[a,b] 98 %;[c] **20 e**: 25 %,[b] 99 %;[c] **21 a**: 93 %,[c] **21 b**: 97 %;[c] **21 c**: 96 %;[c] **21 d**: 85 %;[c] **21 e**: 90 %.[c] (Abbreviations: Alloc = allyloxycarbonyl, DIPEA = *N,N*-diisopropylethylamine, HATU = hexafluorophosphate azabenzotriazole tetramethyl uronium, HFIP = 1,1,1,3,3,3-hexafluoroisopropanol, SPPS = solid-phase peptide synthesis, TFA = trifluoroacetic acid, TIPS = triisopropyl silane, Trt = trityl.) [a] d.r. not determined. [b] Combined yield of both diastereomers after HPLC. [c] UV–LCMS or HPLC purity.

Interestingly, the unprotected indole in the Fmoc-Trp-OH precursor did not produce any side reactions during automatic peptide synthesis, making it directly usable. This presents an undisputed advantage when there is no need to incorporate additional degrees of orthogonality into the peptide. The commercially available Lys(Alloc) amino acid is the most suitable AA for orthogonal manipulation on resin due to the full orthogonality of the Alloc protecting group with Fmoc and acid-sensitive protecting groups (such as Boc or *t*-Bu), and mild deprotection conditions. Additionally, synthesising advanced intermediates **15** and **16** directly on-resin has proven to be highly advantageous as it eliminates the need for purification, allows for easy removal of excess reagents and enables rapid upscaling with parallel synthesis of multiple batches.

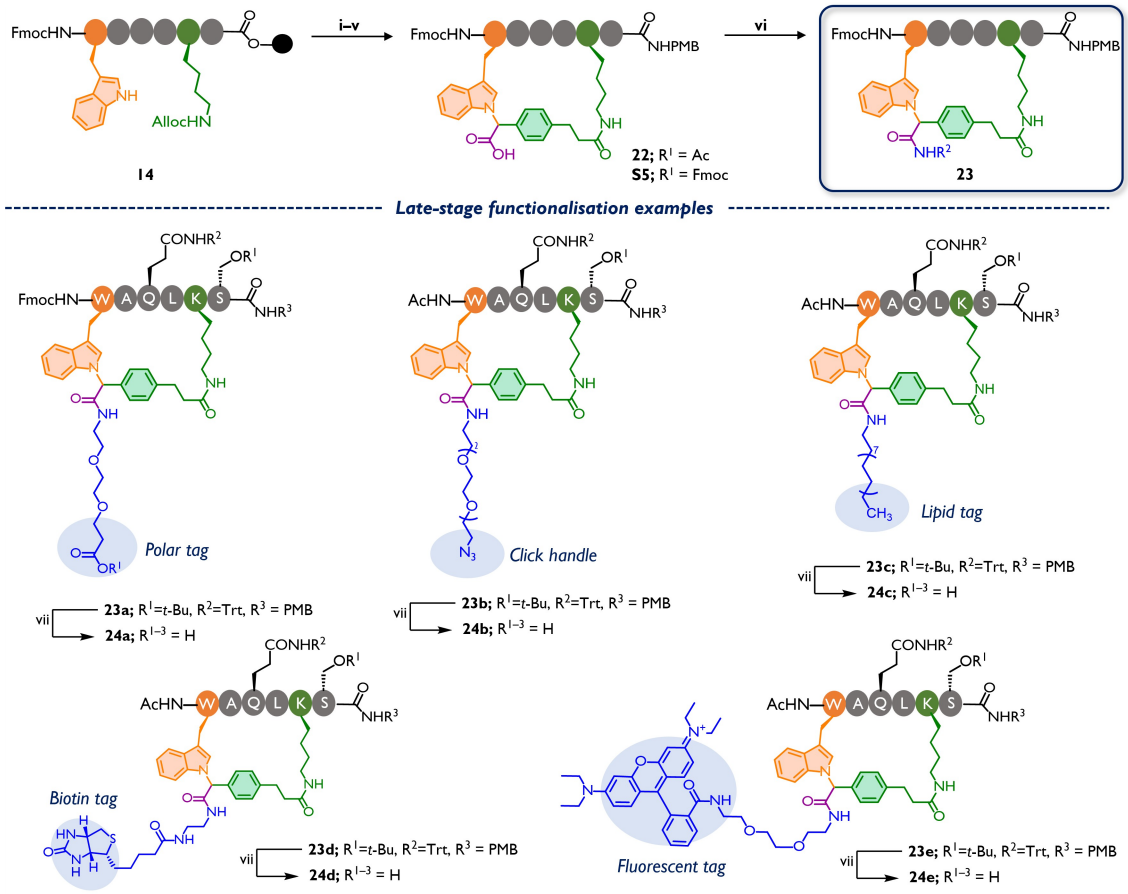
In the case of 2-chlorotrityl resin supported compounds, cleavage from the resin in the presence of a CH_2Cl_2/HFIP mixture provided linear peptides **18** without deprotection of their side chains (Scheme 2, *Pathway B*). In the case of Rink amide resin synthesised substrates **17**, standard cleavage conditions were applied. The Petasis reaction was also tested directly on substrate **15** attached to Rink amide resin (Scheme 2, *Pathway A*). Applying the optimised TMPR conditions on peptides **15**, **17** and **18**, respectively, led to cyclised compounds **19a–c** and **20a–e** (Scheme 2).[33] Each reaction proceeded smoothly under the optimised conditions. Model peptide **20e** was elucidated fully by advanced NMR experiments to prove the preference for the N^1 position for the Petasis reaction on tryptophan.

Global deprotection conditions were tested in the case of peptides **20a–e** to ensure that the cyclised compounds were stable in an acidic environment. Gratifyingly, all peptides proceeded to their deprotected variants **21a–e** with excellent conversion and high crude purity. The Petasis reaction proceeded selectively on tryptophan in the presence of primary amides (*C*-terminal, Gln), carboxylic acids (*C*-terminal, Asp) primary or secondary alcohols (Ser, Thr), phenol (Tyr), thiol (Cys) or imidazole (His) residues, which could act as potential nucleophiles.

For the further, late-stage modification of stapled peptides, *N*-terminal acetylation rather than Fmoc protection was used since this functionality is common in bioactive peptides. Model peptide **14** (Fmoc-WAQ^{Trt}LK^{Alloc}S^{tBu}-OH) was prepared by automated peptide synthesis and subjected to on-resin Fmoc deprotection followed by acetylation. The same sequence of steps—namely, orthogonal deprotection of Alloc in presence of $Pd(PPh_3)_4$, amide coupling with 3-(4-boronophenyl)propanoic acid and HFIP-mediated cleavage from the resin resulted in *N*-Ac peptide **18** (Ac-WAQ^{Trt}LK^{BA}S^{tBu}-OH; BA = phenylboronic acid). To ensure selectivity between the newly formed carboxylic acid upon stapling and the *C*-terminus, it was protected with a *para*-methoxybenzyl (PMB) protecting group to afford compound **S4** (Scheme S9). PMB group was chosen as a sensible alternative that would be stable during Petasis reaction and could be cleaved during global deprotection. Moreover, it enabled easy conversion of on-resin prepared, protected peptide with a *C*-terminal carboxylic acid to the requisite *C*-terminal amide in two straightforward steps in case the *C*-terminal amide moiety is required for the bioactive peptide. The successful Petasis reaction gave protected model peptides **22** (Ac-W^{X}AQ^{Trt}LK^{X}S^{tBu}-NHPMB; X = indicates stapling with glyoxylic acid) and **S5** (Fmoc-W^{X}AQ^{Trt}LK^{X}S^{tBu}-NHPMB). The latter was then subjected to a myriad of different amide coupling conditions to optimise the conversion to **23**. The best outcome was observed when pre-activating the carboxylic acid with *N*-hydroxysuccinimide, followed by the one-pot addition of the corresponding amine (Scheme 3). With the optimised protocol in hands, peptides **23a–e** were obtained in good to excellent yields. The choice of amine-containing substituents was envisaged to incorporate various biologically useful tags into peptides without affecting their *N*- or *C*-terminal ends. Hence, various polyethylene glycol (PEG) tags, containing a polar moiety **23a**, an azido functional group **23b**, or a fluorescent dye **23d** were incorporated. These could be used for fluorescent visualisation of peptides within cells or for further attachment of small molecules drugs via acylation or click chemistry (CuAAC or SPAAC), respectively. Similarly, biotin was attached as an affinity tag useful for affinity chromatography, resulting in compound **23d**. A lipid tag **23c** was attached to potentially increase cell permeability and plasma half-life of peptides, which is often a challenge for peptide therapeutics. Gratifyingly, all derivatives were stable under the highly acidic deprotection conditions and proceeded to their deprotected versions **24a–e** (Schemes S10–S14).

Late-stage labelling of peptides on resin

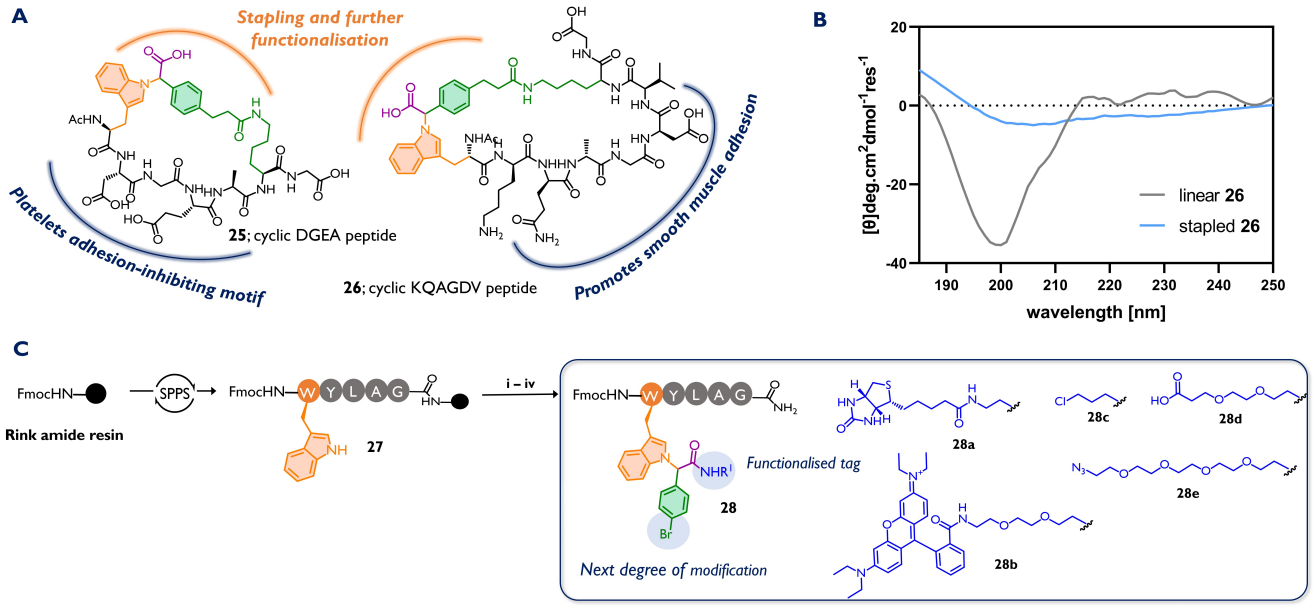
The applicability of the method was also demonstrated by the late-stage functionalisation of peptides. The solid-phase preparation of labelled peptides was aimed for, as it enables rapid preparation, easy manipulation and parallel synthesis of all intermediates, with one final cleavage from the resin. The model peptide **27** (Fmoc-WYLAG-NH_2) was prepared via automatic peptide synthesis on Rink amide resin. It was then treated with glyoxylic acid, where after 16 h (upon cleavage of an analytical sample from the resin) formation of the α-hydroxy acetic acid intermediate was observed (according to UV–LCMS traces). Following addition of the boronic acid led to formation of the Petasis product **S12** (Scheme S17) in excellent conversion and crude purity. The reaction needs to be carried out stepwise, with removal of residual glyoxylic acid from the resin before addition of the boronic acid; otherwise the crude purity dropped significantly. Subsequent amide coupling led to the formation of labelled peptide **28** (Scheme 4C). The same pattern of late-stage diversification described above could be used to incorporate a fluorescent tag (Rhodamine; **28b**), biotin **28a**, short halo tag **28c**, polar **29d**, and azido **29e** PEGylated chains for potential further diversification. Another degree of modification via 4-bromophenylboronic acid was also incorporated. The aryl halogenated motif presented within a peptide scaffold opens up further orthogonal reactivity, e.g. Pd-catalysed reactions, and therefore introduces another degree of modification into peptides.



Scheme 3. Optimisation of Petasis conditions on model tryptophan compounds. Reagents and conditions: i) 2% DBU in CH_2Cl_2, 20 min, r.t., then acetic anhydride, DMAP, CH_2Cl_2, r.t., 16 h; ii) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, r.t., 3 h, then 4-(2-carboxyethyl)benzeneboronic acid, HATU, DIPEA, r.t., 16 h; iii) CH_2Cl_2/HFIP 4:1, r.t.; iv) *para*-methoxybenzyl amine, HOBt, DIC, DMF, r.t., 16 h; v) **3**, 80 °C, 16 h; vi) *N*-hydroxysuccinimide, DIC, DMF, r.t., 5–8 h then corresponding amine, DIPEA, DMF, r.t., 16 h; vii) TFA/DMSO/TIPS 9:1:0.1, 80 °C, 16 h. Yields and purities: **22**: 40%,[a] 98%;[b] **23a**: 60%,[a] 98%;[b] **23b**: 55%,[a] 92%;[b] **23c**: 80%;[b] **23d**: 96%,[a] 98%;[b] **23e**: 65%,[a] 95%;[b] **24a**: 85%;[b] **24b**: 80%;[b] **24c**: 80%;[b] **24d**: 60%;[b] **24e**: 75%.[b] (Abbreviations: DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIC = *N,N'*-diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide, HOBt = hydroxybenzotriazole) [a] Combined yield of both diastereomers after HPLC. [b] UV–LCMS or HPLC purity.

Application of the method to biologically relevant peptides

The methodology was further exemplified on biologically relevant peptides. For a variety of cell types, some cell adhesion ligands interact with limited cell types—the KQAGDV sequence, for instance, promotes smooth muscle cell adhesion.[34] DGEA, a short sequence derived from type I collagen, has the ability to interact with the $\alpha_2\beta_1$ integrin receptor. Moreover, for breast adenocarcinoma cells that use $\alpha_2\beta_1$ as their receptor for collagen and laminin, DGEA-containing peptides were found to be effective in inhibiting adhesion to both collagen and laminin.[34] For both peptides it was found out that their cyclised versions are even more potent, presumably due to their higher stability towards circulating enzymes.[34] Therefore, the incorporation of these peptide motifs was targeted with the Petasis stapling methodology. Both peptides were prepared by the previously described pathways, with additional Fmoc deprotection and *N*-acetylation (Schemes S15 and S16). Subsequent Petasis reaction yielded cyclised conjugates which upon global deprotection yielded the desired bioactive compounds **25** and **26** (Scheme 4A). Although the aim of this research was not to fully test their biological properties, the influence of the cyclisation on their stability in human serum was investigated. Both linear (before TMPR) versions of peptides **25** and **26** showed rapid degradation compared to their stapled counterparts. In the case of peptide **25**, its linear version was almost fully degraded after 45 minutes, whereas 70 % of intact stapled peptide **25** was observed after 6.5 h. Linear **26** showed almost full degradation after 3 h, whereas 85 % of intact stapled peptide **26** was observed after 6.5 h (see the Supporting Information). Moreover, circular dichroism (CD) spectra were obtained for both linear and cyclic peptide **26** to investigate the impact of stapling on its secondary structure. From the obtained results (Scheme 4B) it was apparent that while linear precursor of **26** possessed a typical dip at 200 nm, suggesting random coil, the stapled version could be a mix of α-helix and β-sheet, due to the signals around 205 nm and 225 nm. Therefore, tryptophan mediated stapling had a significant impact on the secondary structure of the peptide, making it less prone to decomposition. These results were very encouraging and indicates



Scheme 4. A) Biologically relevant peptides (for full preparation methods see the Supporting Information). B) Circular dichroism (CD) spectra of peptide **26** (linear and stapled). C) Labelling of a model peptide on resin. Reagents and conditions: i) glyoxylic acid monohydrate, 1,4-dioxane, 80 °C, 16 h; ii) 4-bromophenylboronic acid, 1,4-dioxane, 80 °C, 16 h; iii) corresponding amine, HATU, DIPEA, DMF, r.t., 16 h; iv) CH_2Cl_2/TFA 1:1, r.t., 3 h. Overall yields and purities: **28a**: 7%,[a] 96%;[b] **28b**: 6%,[a] 85%;[b] **28c**: 14%,[a] 99%;[b] **28d**: 13%,[a] 98%;[b] **28e**: 9%,[a] 99%.[b] [a] Combined yield of both diastereomers after HPLC. [b] UV–LCMS or HPLC purity.

how the method could be used widely in a preparation of other biologically active peptides.

Conclusion

In summary, a highly versatile method that is generally applicable for peptide stapling and labelling, involving the naturally occurring amino acid tryptophan, was developed. To the best of our knowledge, this is the first example of tryptophan use in the Petasis reaction for peptide stapling or labelling, thereby illustrating its synthetic versatility and usefulness beyond previous methods. Lys(Alloc) was identified as the second amino acid that could be easily orthogonally deprotected and modified on-resin, thus enabling easy incorporation of a boronic acid counterpart for the Petasis reaction into peptides. The approach was demonstrated on 20 examples of various peptide sequences, including biologically relevant peptides. A straightforward, late-stage functionalisation method upon stapling was demonstrated on a variety of modified peptides with biologically useful tags for cellular fluorescence tracking, affinity chromatography and solubility enhancement (such as a Rhodamine B fluorescent tag, biotin, or PEGylated chains), among others, without alteration of the *C*- or *N*-terminus. Additionally, late-stage functionalisation of one common protected peptide allowed for the rapid preparation of several new derivatives through a simple amide coupling reaction. The Petasis-mediated tryptophan stapling method exhibits robust functional group tolerance and is suitable for peptides bearing a *C*-terminal acid as well as an amide. Rapid and parallel synthesis of advanced intermediates was enabled on resin without the need for intermediate purification and with minimal hands-on time. This practical aspect of synthesis is extremely valuable, especially for pharmaceutically interesting compounds, as it allows for the rapid acceleration and high-throughput investigation of lead molecules. Further application of the method on other biologically relevant peptide and protein targets with the next level of late-stage modification is currently the focus of our research.

Supporting Information

No additional references have been cited within the Supporting Information.

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data supporting this study are included in the paper and provided as Supporting Information.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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