Chemistry–A European Journal
Research Article
doi.org/10.1002/chem.202201339
Chemistry Europe
European Chemical Societies Publishing
www.chemeurj.org

# Late-Stage Functionalisation of Peptides on the Solid Phase by an Iodination-Substitution Approach

Marius Werner,[a, b, c] Julius Pampel,[d] Truc Lam Pham,*[a, b] and Franziska Thomas*[a, b]

**Abstract:** The functionalisation of peptides at a late synthesis stage holds great potential, for example, for the synthesis of peptide pharmaceuticals, fluorescent biosensors or peptidomimetics. Here we describe an on-resin iodination-substitution reaction sequence on homoserine that is also suitable for peptide modification in a combinatorial format. The reaction sequence is accessible to a wide range of sulfur nucleophiles with various functional groups including boronic acids, hydroxy groups or aromatic amines. In this way, methionine-like thioethers or thioesters and thiosulfonates are accessible. Next to sulfur nucleophiles, selenols, pyridines and carboxylic acids were successfully used as nucleophiles, whereas phenols did not react. The late-stage iodination-substitution approach is not only applicable to short peptides but also to the more complex 34-amino-acid WW domains. We applied this strategy to introduce 7-mercapto-4-methylcoumarin into a switchable $Zn^{II}$ responsive WW domain to design an iFRET-based $Zn^{II}$ sensor.

## Introduction

Powerful methods for introducing natural or unnatural modifications into a peptide are of great importance, as this is a possibility to either modulate peptides' function or to label them in order to elucidate their function.[1] For example, pharmaceutically relevant peptides require modifications that modulate their pharmacokinetic profiles such as the half-life or bioavailability.[2] Modifications can be introduced either during peptide chain assembly or at a later synthesis stage, ideally post-synthetic, by biorthogonal chemical means. The introduction of modifications during solid-phase peptide synthesis (SPPS) usually requires specific amino acid building blocks, which often have to be prepared in advance by chemical synthesis. As this is time-consuming and tedious, chemical methods that allow late-stage functionalisation (LSF) are very attractive.

These methods usually provide rapid and easy diversification of peptides from a single peptide precursor synthesised by SPPS.[3]

Among the available methods, bioconjugations that do not rely on the protection of functional groups, such as the copper(I)-catalysed azide–alkyne cycloaddition,[4] the tetrazine click reaction[5] or the recently established arylation chemistries,[6] certainly appear highly useful, as they also can be applied for biomolecules that are not accessible by chemical synthesis. However, many biorthogonal reactions often suffer from the need for additional unnatural and bulky linkers between biomolecule and modification, which limits their use for the functionalisation of peptides. Alternatively, synthetic peptides can be easily modified at a late synthesis stage on the solid phase after peptide chain assembly. Typically, orthogonal protection of a lysine or glutamic acid side chain allows selective deprotection and modification, for instance, by amide bond formation. However, to expand the availability of different linkages that also affect the function of the introduced modification, several new LSF methods have recently been developed. These include C–H activation on aromatic amino acid side chains or other transition metal-catalysed reactions such as the Suzuki–Miyaura cross-coupling reaction or Ni-catalysed decarboxylative coupling.[7] Furthermore, a transition-metal-free method, the on-resin Petasis reaction, was recently applied for LSF by Ricardo et al.[8] and Openy et al. performed the functionalisation of lysine residues by a Katritzky salt formation and subsequent Giese reaction.[9] Surprisingly, less complex chemical transformations like on-resin nucleophilic substitutions are rare. Very few examples describe the introduction of halogenated amino acids such as bromo- or chlorohomoalanine[10] that were only used to integrate stable

[a] *M. Werner, T. L. Pham, Prof. Dr. F. Thomas*
*Institute of Organic Chemistry, Heidelberg University*
*Im Neuenheimer Feld 270, 69120 Heidelberg (Germany)*
*E-mail: truclam.pham@uni-heidelberg.de*
*franziska.thomas@oci.uni-heidelberg.de*

[b] *M. Werner, T. L. Pham, Prof. Dr. F. Thomas*
*Centre for Advanced Materials (CAM)*
*Heidelberg University*
*Im Neuenheimer Feld 225, 69120 Heidelberg (Germany)*

[c] *M. Werner*
*Department of Medicinal Chemistry*
*Institute of Pharmacy and Molecular Biotechnology (IPMB)*
*Heidelberg University*
*Im Neuenheimer Feld 364, 69120 Heidelberg (Germany)*

[d] *J. Pampel*
*Department of Chemical Biology*
*Max Planck Institute for Medical Research*
*Jahnstraße 29, 69120 Heidelberg (Germany)*

*Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202201339*

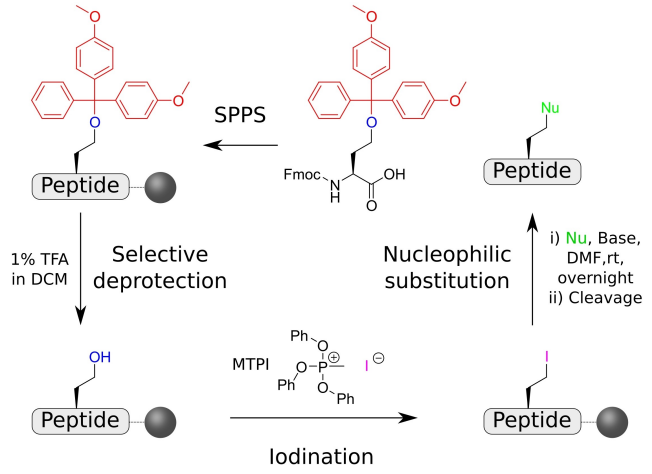
cystathione as disulfide mimetics.[10b,11] However, iodohomoalanine was not used in this regard, and the potential of halogenated homoalanine for LSF and consequently peptide diversification was also not demonstrated. Here we present an easy-to-use iodination-substitution LSF that takes advantage of the on-resin conversion of homoserine to iodohomoalanine, which is then susceptible to subsequent functionalisation by nucleophiles, and in a combinatorial manner, if desired. Our method is suitable for a range of aromatic and aliphatic thiols, as well as pyridine derivatives and benzoic acids. We were able to successfully apply this method to 34 amino acid WW domains. By using our method in the fluorescent labelling of a designed $Zn^{II}$ mini-receptor prototype, we developed an intrinsic FRET (iFRET)-based $Zn^{II}$ sensor.

## Results and Discussion

### Optimisation of the iodination-substitution LSF

The concept of our LSF strategy relies on the on-resin iodination of homoserine (Hse) followed by nucleophilic substitution (Scheme 1). We chose homoserine rather than the more obvious serine because the serine-derived iodoalanine is prone to side reactions such as 2-oxazoline formation[12] or elimination to dehydroalanine.[13] To allow selective modification of the Hse side chain, the hydroxy group of the Fmoc-Hse building block was protected with the hyper acid-sensitive 4,4′-dimethoxytrityl (Dmt) group, which can be removed with 1% trifluoroacetic acid (TFA) in dichloromethane, leaving all other protecting groups within the peptide sequence intact.[14] To establish the on-resin iodination-substitution reaction sequence, we chose the simple test peptide **P1** (Ac-GFXFGG-$NH_2$), in which Hse (X) was the only amino acid that carried an additional functional group in its side chain. ChemMatrix was selected as the resin for the SPPS because of its excellent swelling properties in diverse solvents.[15] After microwave-assisted SPPS, the Dmt group was removed under mild acidic conditions, using the orange colour of the trityl cation to monitor the reaction.[16] Accordingly, the resin was washed with 1% TFA in $CH_2Cl_2$ until the filtrate was colourless. Hse was then iodinated with methyltriphenoxyphosphonium iodide (MTPI), a reagent that was previously used successfully in DNA functionalisation,[17] to yield iodohomoalanine, which was then susceptible to nucleophilic substitution.

During the optimisation process, peptide synthesis, iodination and nucleophilic substitution were followed by acidic cleavage of the respective peptide from the resin to allow HPLC and mass spectrometry (MS) analysis of the peptide species formed (Figure 1). Synthesis of **P1** was almost quantitative, which was expected for a peptide of such small size (Figure 1A). Interestingly, iodination with MTPI followed by TFA cleavage resulted in the formation of several by-products (Figure 1B). After detailed analysis, we found that some of these by-products were short peptide species from nucleophilic frag-




**Scheme 1.** Concept of the on-resin iodination-substitution late-stage peptide functionalisation.

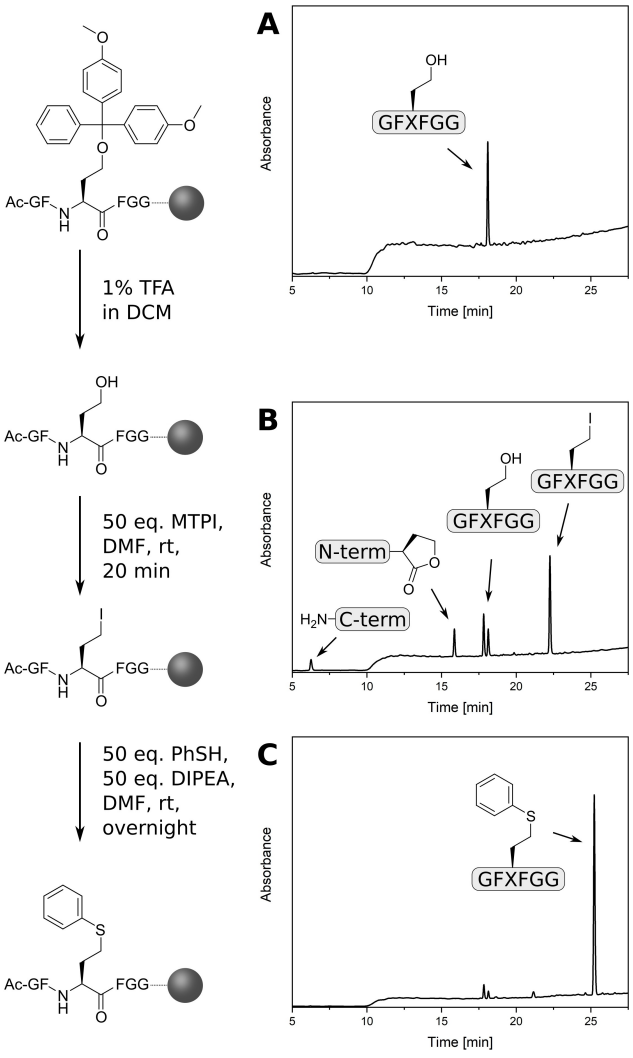


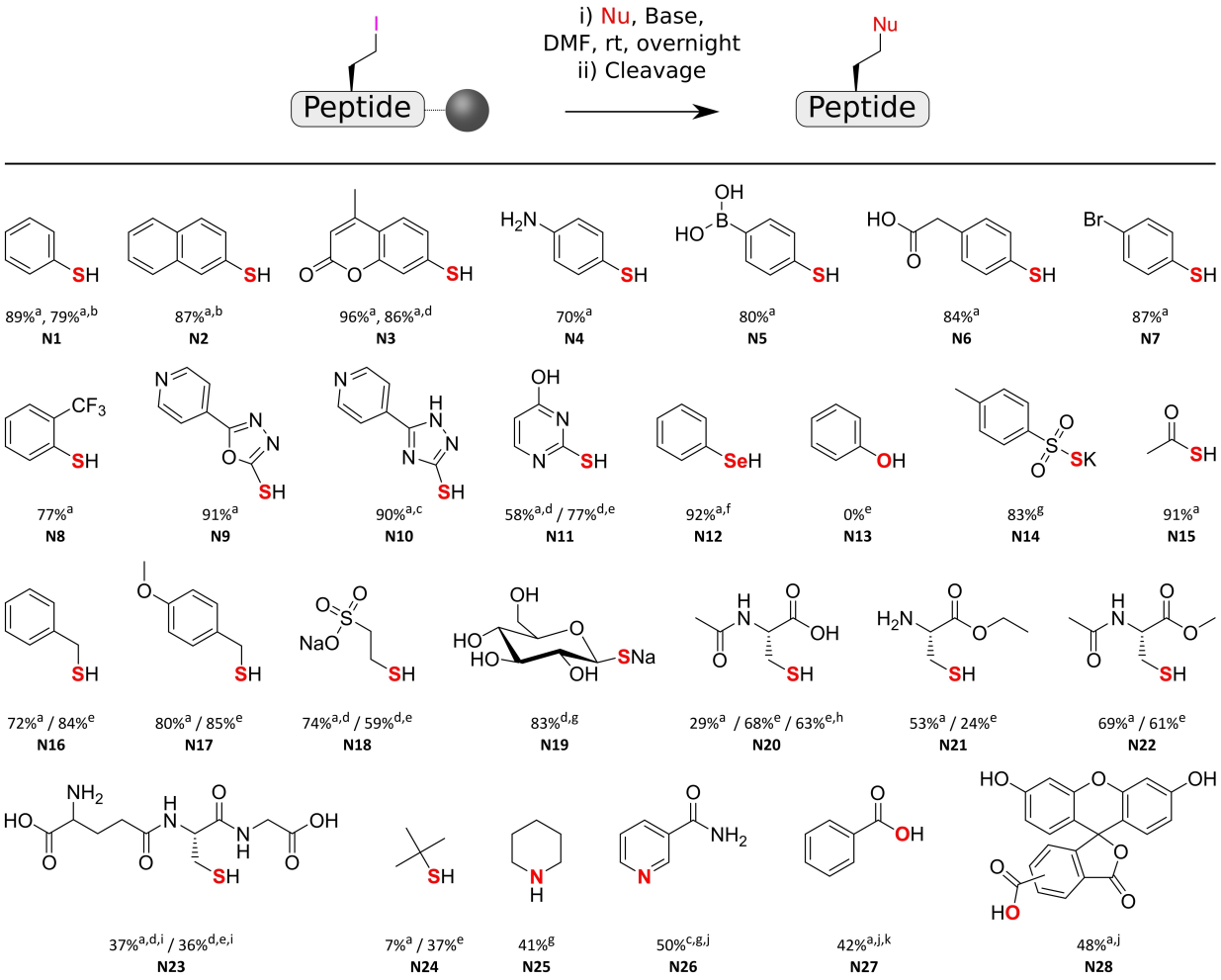

**Figure 1.** Monitoring of the iodination-substitution LSF in **P1**. HPLC chromatogram of cleaved peptide GFXFGG after A) peptide synthesis, B) iodination and C) nucleophilic substitution.

mentation and lactone formation (Figures S1 and S2 in the Supporting Information).

Such side reactions have previously been reported for similar compounds.[18] Furthermore, the Hse species was detected, which could result from either incomplete iodination or hydrolysis. However, as our studies progressed (see below), we found that all these by-products were formed during the acidic cleavage of the reactive iodinated peptide and not during the iodination reaction. Another by-product found mainly after prolonged storage of the Hse peptide resin was the depsipeptide, the formation of which may have been promoted by traces of TFA on the resin after Dmt deprotection (Figure S3).[19] This side reaction lowered the iodination efficiency but could be minimised by immediate iodination after Dmt deprotection. To test the on-resin nucleophilic substitution we chose the excellent nucleophile thiophenol (**N1**). The reaction was performed in the presence of *N*,*N*-diisopropylethylamine (DIPEA). HPLC and MS analyses showed near quantitative formation of the thioether, thus, also confirming near quantitative yields for the earlier reaction steps. It should be noted, however, that satisfactory conversion was achieved only with an excess of the iodinating reagent or nucleophile.

### Scope and limitations of the iodination-substitution LSF

We tested a range of nucleophiles potentially suitable for our iodination-substitution LSF protocol (Scheme 2). In general, polar aprotic solvents such as DMF, DMSO, NMP, and THF were used as they promote $S_N2$ reactions, and support excellent swelling of the ChemMatrix® resin.[15] Variations in the choice of solvent mainly depended on the solubility of the nucleophile of interest. However, in few cases (e.g., **N1** or **N3**) we performed the $S_N2$ reaction comparatively in DMF and THF or DMSO, respectively, and found that DMF gave the better yield. Therefore, DMF was generally our first choice.




**Scheme 2.** Scope and limitations of iodination-substitution LSF of test peptide **P1**. Yields were calculated from HPLC traces. In the case of **N17**, the yield refers to homocysteine-containing peptide after acidic cleavage of the p-methoxybenzyl group at 40 °C (Figures S19, S20). a) DIPEA was used as base. b) THF was used as solvent instead of DMF. c) HPLC traces of the by-product and product overlap. d) DMSO was used as solvent instead of DMF. e) Caesium carbonate was used as base. f) Benzeneselenol was obtained in situ from the diselenide by reduction with tris(2-carboxyethyl)phosphine (TCEP). g) No additional base was added. h) NMP was used as solvent instead of DMF. i) 25 equiv. were used instead of 50 equiv. j) Reaction time was increased to 3 days. k) 200 equiv. were used instead of 50 equiv. Detailed reaction conditions can be found in Table S19.

Aromatic thiols and selenols reacted very well in the nucleophilic substitution of iodohomoalanine, giving methionine-like thioethers in yields ranging from 70 to 96%. The reaction was selective for the aromatic thiol even when other potential nucleophiles such as aromatic amines, pyridines, and other heterocyclic residues or carboxylic acids were present (Figures S4–S15). The compounds tested included 2-naphthalene thiol (**N2**) and 7-mercapto-4-methylcoumarin (**N3**), which gave fluorescently labelled peptides. Both fluorophores have already been integrated as thioethers into an amino acid structure and incorporated into peptides by Navo et al.[20] Our LSF approach now allows more efficient and inexpensive access to such labelled peptides. In addition to aromatic thiols, we also tested other sulfur nucleophiles. Thioacids and thiosulfonates as well as sterically undemanding aliphatic thiols reacted quantitatively (Figures S16–S22). Substitution of iodide by unprotected thioglucose (**N19**) highly selectively afforded the corresponding thioglycoside in very good yield. This highlights the potential of our approach for the selective late-stage synthesis of thioglycosylated peptides, which represent a highly interesting class of compounds due to the higher hydrolytic stability of the *S*-compared to the *O*-glyosidic bond.[21]

Cysteine derivatives, on the other hand, showed reduced reactivity when aliphatic amines or carboxylic acids were present, as was the case with cysteine ethyl ester (**N21**) or glutathione (**N23**) (Figure S23–S26). In addition, glutathione could only be used in 25 equivalents, due its comparatively low solubility in DMF. For *N*-acetylcysteine (**N20**), as well as other nucleophiles, the yield was improved by using the stronger base caesium carbonate instead of DIPEA (**N11**, **N16**, **N17**, **N20**, **N24**), while changing the solvent from DMF to the slightly less polar NMP[22] (**N20**) had no effect. In particular, in the case of the sterically very demanding *tert*-butyl mercaptan (**N24**) the yield of the corresponding thioether was increased from 7 to 37% when caesium carbonate was used (Figure S27). However, caesium carbonate also promoted the competing elimination reaction to vinylglycine. For instance, for **N21**, which was used as hydrochloride, the hydrochlorination product was found in significant amounts, which we assume resulted from the initial $Cs_2CO_3$-promoted elimination and subsequent addition (Figure S24).

In addition to sulfur nucleophiles, we also investigated *N*- and *O*-nucleophiles, which were less reactive than *S*-nucleophiles and therefore required longer reaction times and/or usage in higher excess. The aliphatic amine piperidine (**N25**) could be used without additional base and gave the desired product in 41% yield; however, a considerable amount of the elimination product vinylglycine was formed (Figures S28, S29). Nicotinamide (**N26**) reacted in almost 50% yield and gave the corresponding pyridinium compound (Figure S30). Such peptide derivatives can be used as $NAD^+$ mimetics and are of interest for enantioselective reductions.[23] Carboxylic acids such as the fluorescent dye carboxyfluorescein (**N28**) reacted in moderate yield (Figure S32), and phenols were unreactive even when caesium carbonate was used as a base.

## Fluorescent labelling of peptides

Having shown that different modifications can be introduced into the test peptide **P1**, we applied our method to fluorescently label functional peptides (for sequences, see Table 1) using 7-mercapto-4-methylcoumarin (**N3**), for example the recognition motif of the WW domain of hPin1 ($hPin1_{WW}$) within the C-terminal domain of RNA polymerase II (CTD peptide, **P2**) and the $hPin1_{WW}$ itself. The CTD peptide contains a phosphorylated amino acid, phosphoserine, whose phosphate group is only partially protected during SPPS and therefore can function equally as a nucleophile or leaving group. This usually requires careful consideration of peptide synthesis conditions, such as reduced temperatures during microwave-assisted SPPS. Application of our iodination-substitution protocol led to complete conversion to the thioether and yielded **P2_N3** in excellent quality (Figure S33). We then increased the complexity and labelled $hPin1_{WW}$ at various positions. $hPin1_{WW}$ is an independently folding triple-stranded β-sheet peptide of 34 amino acids and contains all canonical amino acids except cysteine, aspartate, and methionine, with methionine replaced by norleucine for ease of handling. Five labelled $hPin1_{WW}$ variants were synthesised: K13coum (**P3_N3**), S19coum (**P4_N3**), Y24coum (**P5_N3**), Q33coum (**P6_N3**), and W34coum (**P7_N3**). In all cases, electrophilic LSF followed by nucleophilic substitution with **N3** worked excellently (for HPLC and MS analysis, see Figures S34–S38), demonstrating the power of this approach even in the context of complex peptides. Because the introduction of a fluorescent label can disrupt the folding of $hPin1_{WW}$, we investigated the structure and thermodynamic stability of **P3_N3** to **P7_N3** using circular dichroism (CD) spectroscopy (Figure 2A). The CD spectra showed correct folding, indicated by the characteristic exciton signal at 227 nm

**Table 1.** Sequences of late-stage functionalised peptides with 7-mercapto-4-methylcoumarin (**N3**). **X**: Position of Fmoc-Hse(Dmt)-OH.

| Peptide | Sequence | Yield [%][a] |
|---|---|---|
| **P2_N3** | Ac-SPTpSPS**X**-$NH_2$ | 10 |
| **P3_N3** | Ac-KLPPGWE**X**R*Nle*SRSSGRVYYFNHITNASQWERPSG-$NH_2$ | 15 |
| **P4_N3** | Ac-KLPPGWEKR*Nle*SRS**X**GRVYYFNHITNASQWERPSG-$NH_2$ | 21 |
| **P5_N3** | Ac-KLPPGWEKR*Nle*SRSSGRVY**X**FNHITNASQWERPSG-$NH_2$ | 21 |
| **P6_N3** | Ac-KLPPGWEKR*Nle*SRSSGRVYYFNHITNAS**X**WERPSG-$NH_2$ | 17 |
| **P7_N3** | Ac-KLPPGWEKR*Nle*SRSSGRVYYFNHITNASQ**X**ERPSG-$NH_2$ | 23 |
| **P8_N3** | Ac-KLPPGWEKH*Nle*SRSSGQVHYHNSITNASQ**X**ERPSG-$NH_2$ | 28 |

[a] Isolated yields are given and were determined by weight. Based on the HPLC analysis of the crude peptides, the iodination-substitution protocol proceeded in almost quantitative yields (Figures S33–S38, S41).

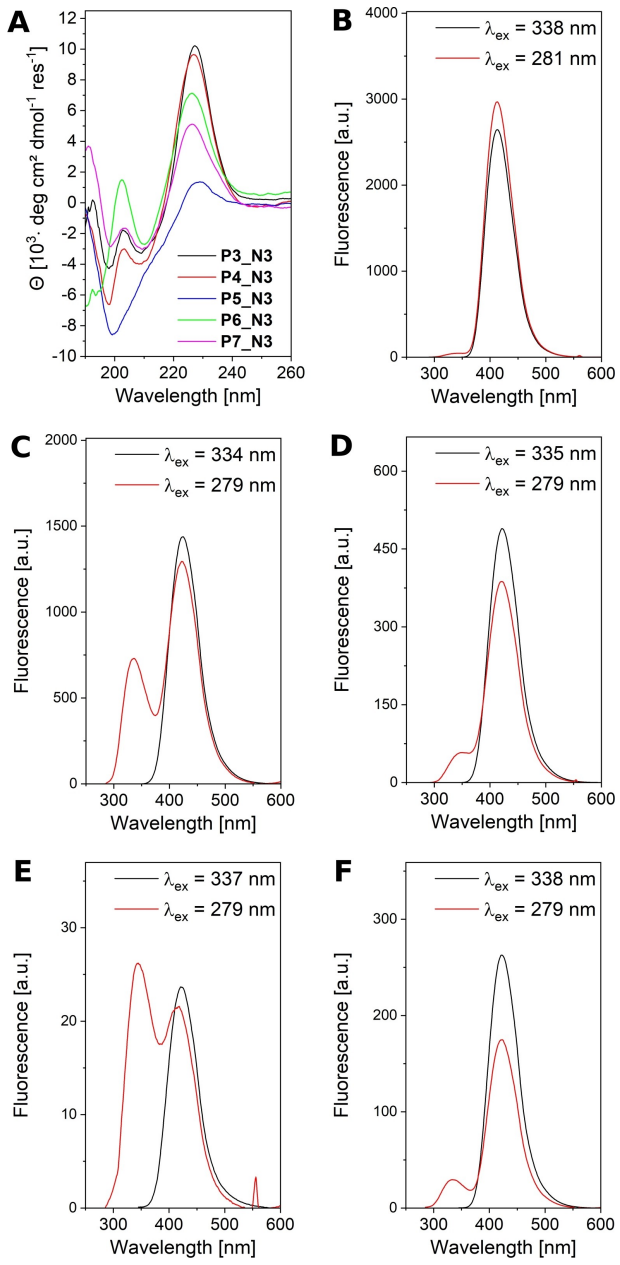



**Figure 2.** Biophysical characterisation of labelled $hPin1_{WW}$ variants. A) CD spectra of coumarin-labelled $hPin1_{WW}$ peptides. Fluorescence spectra of B) **P3_N3**, C) **P4_N3**, D) **P5_N3**, E) **P6_N3**, and F) **P7_N3**. Conditions for CD spectroscopy: 20 °C, 50 μM peptide in phosphate buffer (10 mM, pH 7). Conditions for fluorescence spectroscopy: 10 μM peptide in phosphate buffer (10 mM, pH 7). The excitation wavelength corresponds to the absorbance maxima in the UV-VIS spectra (Figure S40).

for all $hPin1_{WW}$ variants. Y24coum, however, was only weakly folded, which we attributed to the fact that the highly conserved Y24 is a key residue of the inner hydrophobic core and is therefore important for the structural integrity of the peptide.[24] Mutation of W34 also resulted in decreased intensity of the exciton signal, because W34 usually contributes to the exciton coupling in WW domains.[25] Thermodynamic stability was investigated by recording the thermal denaturation curves at 227 nm, which showed cooperative folding-to-unfolding transitions for all peptides except of Y24coum, consistent with the results of CD spectra measurements (Figures S39 and 2A). Interestingly, the coumarin-labelled $hPin1_{WW}$ variants proved to be more stable than the wildtype $hPin1_{WW}$,[24] with the exception of Y24coum, as melting temperatures of 54.6–64.6 °C were determined, an increase of up to 5 °C.

## Design of a $Zn^{II}$ sensor

7-Mercapto-4-methylcoumarin (**N3**) is weakly fluorescent in the mercapto form but emits strongly between 413 and 427 nm, depending on the hydrophobicity of the environment, when converted to a thioether.[20b] In addition, **N3** is a FRET acceptor for intrinsically fluorescent tryptophan and can therefore be used, for example, to study conformational changes in peptides or proteins. We sought to exploit the unique properties of 7-mercapto-4-methylcoumarin (**N3**) to develop a $Zn^{II}$ sensor based on the WW-domain scaffold. Previously, we reported an engineered WW domain, WW-CA, that was able to bind $Zn^{II}$ via a $His_3$-site on its surface. The binding of $Zn^{II}$ resulted in a significant conformational change from a molten globule to a folded WW domain.[26] Such a change should be detectable by iFRET, as it is strongly distance dependent.

However, before starting the synthesis of a WW-CA variant labelled with **N3**, we examined the labelled $hPin1_{WW}$ variants **P3_N3** to **P7_N3** with respect to iFRET efficiency in dependence on the positioning of the coumarin dye. As mentioned earlier, the fluorescence measurements showed a strong distance dependence of the iFRET. When the iFRET acceptor was positioned directly adjacent to the hydrophobic core and tryptophans, as is the case of K13coum (**P3_N3**), the tryptophan fluorescence is completely quenched (Figure 2B). In contrast, S19coum (**P4_N3**), in which coumarin is positioned in a loop, showed significantly lower iFRET efficiency (Figure 2C). The lowest iFRET efficiency was observed for Q33coum (**P6_N3**) (Figure 2E), whereas W34coum (**P7_N3**) gave a good iFRET signal but no complete quenching of tryptophan fluorescence (Figure 2F). In addition, environment sensitivity of coumarin was observed, with a more hydrophobic environment leading to a blue shift in wavelength emission. In the case of K13coum (**P3_N3**), the emission maximum was at 413 nm, whereas for S19coum (**P4_N3**) it was shifted to 422 nm. An efficient iFRET-based sensor should allow detection of both decreasing and increasing distance between iFRET donor and acceptor, that is, a good iFRET efficiency with residual donor fluorescence remaining at the sensory start point is desirable. In addition, the donor and acceptor should be in close proximity in the folded peptide, but ideally far apart in the unfolded peptide. After reviewing the results of iFRET measurements of the labelled $hPin1_{WW}$ variants, only W34coum (**P7_N3**) met these two criteria. We therefore synthesised WW-CA(W34coum) (**P8_N3**) in which the coumarin label was reintroduced through the late-stage iodination-substitution reaction sequence (Figure S41). The synthesis proceeded smoothly, and CD measurements revealed $Zn^{II}$-dependent changes in structure and thermodynamic stability. The exciton signal at 227 nm was enhanced by

the addition of $Zn^{II}$, and the melting temperature for holo WW-CA(W34coum) increased from 17.6 to 47.0 °C (Figure 3B, C). We then investigated the suitability of WW-CA(W34coum) (**P8_N3**) as an iFRET-based $Zn^{II}$ sensor. In the absence of $Zn^{II}$, the residual fluorescence of tryptophan was clearly detectable, but was almost completely quenched after the addition of $Zn^{II}$, while the iFRET efficiency was increased by a factor of two (Figure 3D).

## Conclusion

We have presented a new approach for late-stage functionalisation that combines on-resin iodination of homoserine with subsequent nucleophilic substitution. Our approach is straightforward, metal-free, and yields a functionalisation product that features a short, sterically undemanding linker compared to other methods. The iodination-substitution approach is suitable for a broad range of nucleophiles, for example, aromatic and aliphatic thiols, yielding methionine-like thioethers that display a range of functional groups, including aromatic amines, boronic acids, phenols, or hydroxy groups. Similarly, selenoethers, thioesters, or thiosulfonates are accessible. In addition to sulfur nucleophiles, iodohomoalanine was successfully reacted with pyridines and carboxylic acids. Aliphatic amines also provided the desired substitution product, though elimination was a significant side reaction. Furthermore, the easy-to-use synthesis protocol makes this method very attractive for combinatorial synthesis approaches.

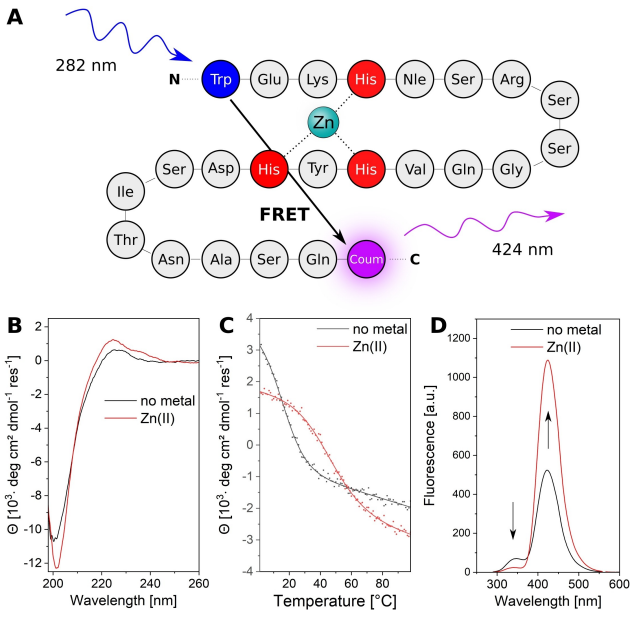



**Figure 3.** Design of a $Zn^{II}$ sensor. A) Design of WW-CA(W34coum) (**P8_N3**). B) CD spectra in the absence and presence of $Zn^{II}$, C) thermal denaturation profiles in the absence and presence of $Zn^{II}$, and D) fluorescence spectra of **P8_N3** in the absence of $Zn^{II}$ and in a 1:1 molar ratio with $Zn^{II}$. Conditions for CD spectroscopy: 20 °C, 50 μM peptide, 10 mM MOPS, pH 7.2, 150 mM NaCl. Conditions for fluorescence spectroscopy: 10 μM peptide, 10 mM MOPS, pH 7.2, 150 mM NaCl, $\lambda_{ex}$ = 282 nm.

With this approach, highly relevant components such as nicotinamide, thiouracil, fluorescent dyes, and even small peptides such as glutathione have been introduced. Of particular interest, for example, for the production of novel vaccines,[21,27] is the synthesis of an *S*-linked thioglycopeptide, as they have the same biological function as *O*-linked glycopeptides but are stable to enzymatic degradation. Therefore, several synthetic strategies have been developed, which are now nicely complemented by our iodination-substitution protocol.[21,28] Moreover, we demonstrated that our LSF is applicable not only to small peptides but also to complex peptides such as a phosphorylated peptide or WW domains with 34 amino acids. We used our LSF approach to design and synthesise an iFRET-based $Zn^{II}$ sensor derived from $Zn^{II}$-binding WW-CA, which showed $Zn^{II}$-dependent iFRET enhancement.

To date, on-resin halogenation of homoserine-containing peptides has had a niche existence, being used exclusively for the synthesis of disulfide mimetics,[11b,29] thus significantly underusing its potential for LSF. In the past, on-resin halogenation of serine followed by nucleophilic substitution has been attempted, however, the formation of dehydroalanine and subsequent stereorandom Michael-addition leading to epimerised products has been a major problem,[13] as has the formation of 2-oxazoline,[12] thus making halogenation of homoserine a far better option in this regard. With the development of our approach, we have introduced the reagent MTPI, previously known only in nucleic acid chemistry,[17] into the peptide field. Taken together, our approach of iodination-substitution of homoserine is a powerful method for late-stage diversification and functionalisation of peptides on a solid support and therefore very promising for combinatorial synthesis approaches. In this report, we have shown only a fraction of the possible modifications that can be introduced by nucleophilic substitution, but we are convinced that this can be easily extended in future applications.

## Author Contributions

F.T. and T.L.P. designed the project. M.W., J.P. and T.L.P. synthesised the Fmoc-Hse(Dmt)-OH building block and the peptides. M.W. and J.P. optimised the late-stage functionalisation. J.P. investigated the side reactions. M.W. prepared the scope and performed fluorescence and CD spectroscopy. The data were analysed and discussed by all authors. M.W., T.L.P. and F.T. wrote the manuscript.

## *Acknowledgements*

T.L.P. is grateful to the Fonds der Chemischen Industrie for financial support through a Kekulé Fellowship. This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) 414261058 and by the DFG under Germany's Excellence Strategy 2082/1 390761711. We are grateful to Prof. Dr. Christian Klein (Heidelberg University) for

providing access to a CD spectrometer. Open Access funding enabled and organized by Projekt DEAL.

## Conflict of Interest

The authors declare no conflict of interests.

## Data Availability Statement

The experimental data associated with this article can be found in the Supporting Information.

**Keywords:** solid-phase synthesis · peptides · late-stage functionalisation · WW domains · biosensors

[1] a) Z. Cheng, E. Kuru, A. Sachdeva, M. Vendrell, *Nat. Chem. Rev.* **2020**, *4*, 275–290; b) W. Wang, M. M. Lorion, O. Martinazzoli, L. Ackermann, *Angew. Chem. Int. Ed.* **2018**, *57*, 10554–10558; *Angew. Chem.* **2018**, *130*, 10714–10718; c) P. J. Stahl, J. C. Cruz, Y. Li, S. M. Yu, K. Hristova, *Anal. Biochem.* **2012**, *424*, 137–139; d) Y. Wu, W.-S. Tam, H.-F. Chau, S. Kaur, W. Thor, W. S. Aik, W.-L. Chan, M. Zweckstetter, K.-L. Wong, *Chem. Sci.* **2020**, *11*, 11266–11273.
[2] a) M. Erak, K. Bellmann-Sickert, S. Els-Heindl, A. G. Beck-Sickinger, *Bioorg. Med. Chem.* **2018**, *26*, 2759–2765; b) D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson, A. Wood, *Nat. Chem.* **2018**, *10*, 383–394.
[3] J. N. deGruyter, L. R. Malins, P. S. Baran, *Biochemistry* **2017**, *56*, 3863–3873.
[4] Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
[5] a) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519; b) N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem.* **2008**, *19*, 2297–2299.
[6] C. Zhang, E. V. Vinogradova, A. M. Spokoyny, S. L. Buchwald, B. L. Pentelute, *Angew. Chem. Int. Ed.* **2019**, *58*, 4810–4839; *Angew. Chem.* **2019**, *131*, 4860–4892.
[7] a) W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi, L. Ackermann, *Angew. Chem. Int. Ed.* **2018**, *57*, 14700–14717; *Angew. Chem.* **2018**, *130*, 14912–14930; b) N.-D. Doan, S. Bourgault, M. Létourneau, A. Fournier, *J. Comb. Chem.* **2008**, *10*, 44–51; c) L. R. Malins, *Pept. Sci.* **2018**, *110*, e24049; d) L. R. Malins, *Curr. Opin. Chem. Biol.* **2018**, *46*, 25–32.
[8] M. G. Ricardo, D. Llanes, L. A. Wessjohann, D. G. Rivera, *Angew. Chem. Int. Ed.* **2019**, *58*, 2700–2704; *Angew. Chem.* **2019**, *131*, 2726–2730.
[9] J. Openy, G. Amrahova, J.-Y. Chang, A. Noisier, P. 't Hart, *Chem. Eur. J.* **2022**, e202201121.
[10] a) E. Remond, J. Bayardon, M.-J. L. Ondel-Eymin, S. Jugé, *J. Org. Chem.* **2012**, *77*, 7579–7587; b) J. R. Harjani, B. K. Yap, R. S. Norton, J. B. Baell, *Tetrahedron* **2016**, *72*, 3256–3261; c) P. J. Knerr, A. Tzekou, D. Ricklin, H. Qu, H. Chen, W. A. Van Der Donk, J. D. Lambris, *ACS Chem. Biol.* **2011**, *6*, 753–760.
[11] a) J. P. Mayer, J. R. Heil, J. Zhang, M. C. Munson, *Tetrahedron Lett.* **1995**, *36*, 7387–7390; b) L. Yu, Y. Lai, J. V. Wade, S. M. Coutts, *Tetrahedron Lett.* **1998**, *39*, 6633–6636; c) J. A. Karas, N. A. Patil, J. Tailhades, M.-A. Sani, D. B. Scanlon, B. E. Forbes, J. Gardiner, F. Separovic, J. D. Wade, M. A. Hossain, *Angew. Chem. Int. Ed.* **2016**, *55*, 14743–14747; *Angew. Chem.* **2016**, *128*, 14963–14967.
[12] J. M. Benito, C. A. Christensen, M. Meldal, *Org. Lett.* **2005**, *7*, 581–584.
[13] a) J. M. Benito, M. Meldal, *QSAR Comb. Sci.* **2004**, *23*, 117–129; b) J. P. Mayer, J. Zhang, S. Groeger, C. F. Liu, M. A. Jarosinski, *J. Pept. Res.* **1998**, *51*, 432–436; c) A. B. Tabor, *Org. Biomol. Chem.* **2011**, *9*, 7606–7628.
[14] J. Nielsen, S. Brenner, K. D. Janda, *J. Am. Chem. Soc.* **1993**, *115*, 9812–9813.
[15] F. García-Martín, M. Quintanar-Audelo, Y. García-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Côté, J. Tulla-Puche, F. Albericio, *J. Comb. Chem.* **2006**, *8*, 213–220.
[16] C. Höbartner, F. Wachowius, in *The Chemical Biology of Nucleic Acids* (Ed.: G. Mayer), Wiley, Chichester, **2010**, pp. 1–37.
[17] G. P. Miller, E. T. Kool, *Org. Lett.* **2002**, *4*, 3599–3601.
[18] a) B. Wang, K. C. Brown, M. Lodder, C. S. Craik, S. M. Hecht, *Biochemistry* **2002**, *41*, 2805–2813; b) J. W. Back, O. David, G. Kramer, G. Masson, P. T. Kasper, L. J. de Koning, L. de Jong, J. H. van Maarseveen, C. G. de Koster, *Angew. Chem. Int. Ed.* **2005**, *44*, 7946–7950; *Angew. Chem.* **2005**, *117*, 8160–8164; c) E. Gross, B. Witkop, *J. Biol. Chem.* **1962**, *237*, 1856–1860.
[19] a) L. A. Carpino, E. Krause, C. D. Sferdean, M. Bienert, M. Beyermann, *Tetrahedron Lett.* **2005**, *46*, 1361–1364; b) H. Eberhard, O. Seitz, *Org. Biomol. Chem.* **2008**, *6*, 1349–1355.
[20] a) C. D. Navo, A. Asín, E. Gómez-Orte, M. I. Gutiérrez-Jiménez, I. Compañón, B. Ezcurra, A. Avenoza, J. H. Busto, F. Corzana, M. M. Zurbano, *Chem. Eur. J.* **2018**, *24*, 7991–8000; b) A. E. Lanterna, M. González-Béjar, M. Frenette, J. C. Scaiano, *Photochem. Photobiol. Sci.* **2017**, *16*, 1284–1289.
[21] D. Specker, V. Wittmann, in *Glycopeptides and Glycoproteins* (Ed.: V. Wittmann), Springer, Berlin, Heidelberg, **2006**, pp. 65–107.
[22] C. Reichardt, *Chem. Rev.* **1994**, *94*, 2319–2358.
[23] J. Bédat, N. Plé, G. Dupas, J. Bourguignon, G. Quéguiner, *Tetrahedron: Asymmetry* **1995**, *6*, 923–932.
[24] M. Jäger, M. Dendle, J. W. Kelly, *Protein Sci.* **2009**, *18*, 1806–1813.
[25] C. M. Davis, R. B. Dyer, *Biochemistry* **2014**, *53*, 5476–5484.
[26] T. L. Pham, M. Kovermann, F. Thomas, *ACS Synth. Biol.* **2022**, *11*, 254–264.
[27] D. Montoir, M. Amoura, Z. E. A. Ababsa, T. Vishwanatha, E. Yen-Pon, V. Robert, M. Beltramo, V. Piller, M. Alami, V. Aucagne, *Chem. Sci.* **2018**, *9*, 8753–8759.
[28] a) G.-L. Zhang, M. R. Gadi, X. Cui, D. Liu, J. Zhang, V. Saikam, C. Gibbons, P. G. Wang, L. Li, *Green Chem.* **2021**, *23*, 2907–2912; b) D. A. Thayer, H. N. Yu, M. C. Galan, C. H. Wong, *Angew. Chem. Int. Ed.* **2005**, *44*, 4596–4599; *Angew. Chem.* **2005**, *117*, 4672–4675; c) S. Knapp, D. S. Myers, *J. Org. Chem.* **2001**, *66*, 3636–3638; d) D. P. Galonić, W. A. Van Der Donk, D. Y. Gin, *J. Am. Chem. Soc.* **2004**, *126*, 12712–12713; e) X. Zhu, R. R. Schmidt, *Chem. Eur. J.* **2004**, *10*, 875–887; f) A. Novoa, S. Barluenga, C. Serba, N. Winssinger, *Chem. Commun.* **2013**, *49*, 7608–7610.
[29] A. D. D. Araujo, M. Mobli, J. Castro, A. M. Harrington, I. Vetter, Z. Dekan, M. Muttenthaler, J. Wan, R. J. Lewis, G. F. King, *Nat. Commun.* **2014**, *5*, 3165.

Manuscript received: April 30, 2022
Accepted manuscript online: June 14, 2022
Version of record online: July 20, 2022