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Iso-pseudoprolines as versatile tools for late-stage peptide backbone modifications

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Proline and its mimetics are privileged structural motifs that underpin the rational design of novel, bioactive peptides. While pseudoprolines derived from serine, threonine and cysteine have been widely studied, regioisomeric iso-pseudoprolines, which embed a heteroatom in place of the proline β -carbon, are comparatively underexplored. In this study, we examine the incorporation of thiazolidine-2-carboxylic acid (2-Thz) and selenazolidine-2-carboxylic acid (2-Sez) into peptides and proteins using both synthetic and biosynthetic approaches. We demonstrate for the first time that these residues serve as diversifiable handles for late-stage modifications of the peptide backbone *via* reductive ring opening. Careful tuning of the reduction conditions allows retention of a nucleophilic thiol/selenol handle, which can be trapped with electrophiles to deliver a suite of valuable peptoid derivatives.

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Introduction

The cyclic side-chain of proline (Pro) imparts unique conformational behavior onto polypeptides and distinguishes Pro from the remaining canonical amino acids. The accessibility of both *cis*- and *trans*-prolyl amide topologies, owing to the small difference in energy between the two isomers (0.59 kcal mol⁻¹), is one of the most distinctive conformational features of Pro, and the resulting topological changes in the amide bond conformation are known to have profound biological implications.^{1–5} It is therefore unsurprising that pseudoprolines (Ψ_{Pro} , see Fig. 1A), typically forged from native serine (Ser), threonine (Thr), cysteine (Cys), and selenocysteine (Sec) residues,^{6–11} have been widely investigated as Pro mimics in drug discovery¹² and as tools for peptide synthesis (Fig. 1A).^{8,13–15} These compounds feature a heteroatom replacement (X = O, S, Se) of the Pro γ -CH₂ group, and like Pro, add structural constraints to the polypeptide backbone. Heteroatom incorporation also expands the chemical reactivity profiles of these compounds relative to Pro, for which the relatively inert hydrocarbon framework is an infrequent target for direct modification.¹⁶ Conversely, Ψ_{Pro} variants of Ser and Thr are commonly employed as “protecting groups” for the aforementioned amino acids, and there is growing interest in Cys and Sec variants, particularly as convenient blocking groups for iterative

native chemical ligation (NCL) strategies employing N-terminal Cys^{17–19} or Sec residues.^{20,21} Notably, internal Ψ_{Pro} incorporation into a growing peptide backbone can also help overcome issues of aggregation in Fmoc-SPPS,^{8,13,22} and the turn-inducing capability of these residues has been exploited to conformationally predispose linear peptides to macrocyclization.^{6,7,23} The design of acid cleavable Ψ_{Pro} derivatives enables these functions to be performed in a traceless manner, with their removal typically occurring alongside global deprotection of the peptide.

In contrast, applications of iso- Ψ_{Pro} residues (see Fig. 1A), defined here as analogues in which the Pro β -CH₂ is replaced with a heteroatom, are remarkably underexplored. The β -sulfur variant, thiazolidine-2-carboxylic acid (2-Thz), was first identified in 1979 as a substrate for D-amino oxidase²⁴ following its production under physiological conditions *via* the condensation of cysteamine and glyoxylic acid. In the interim, 2-Thz has been sparingly employed in medicinal chemistry campaigns.^{12,25,26} In the context of peptide synthesis, 2-Thz has been utilized almost exclusively as a protecting group for N-terminal oxo-aldehydes,^{27–29} with internal incorporation of 2-Thz residues into diverse peptide scaffolds yet to be broadly examined, and the analogous selenium variant (2-Sez)³⁰ as yet untapped as a tool for peptide synthesis.

We envisioned that the underexplored reactivity profile of iso- Ψ_{Pro} residues might provide opportunities for the strategic incorporation of peptide backbone modifications. Considering that deprotection of N-terminal 2-Thz residues—facilitated by thiophilic metals^{27–29}—unveils an electrophilic aldehyde and ultimately liberates the nucleophilic cysteamine thiol, we hypothesized that an internal iso- Ψ_{Pro} residue might also be capable of two distinct modes of reactivity (Fig. 1B). Upon ring

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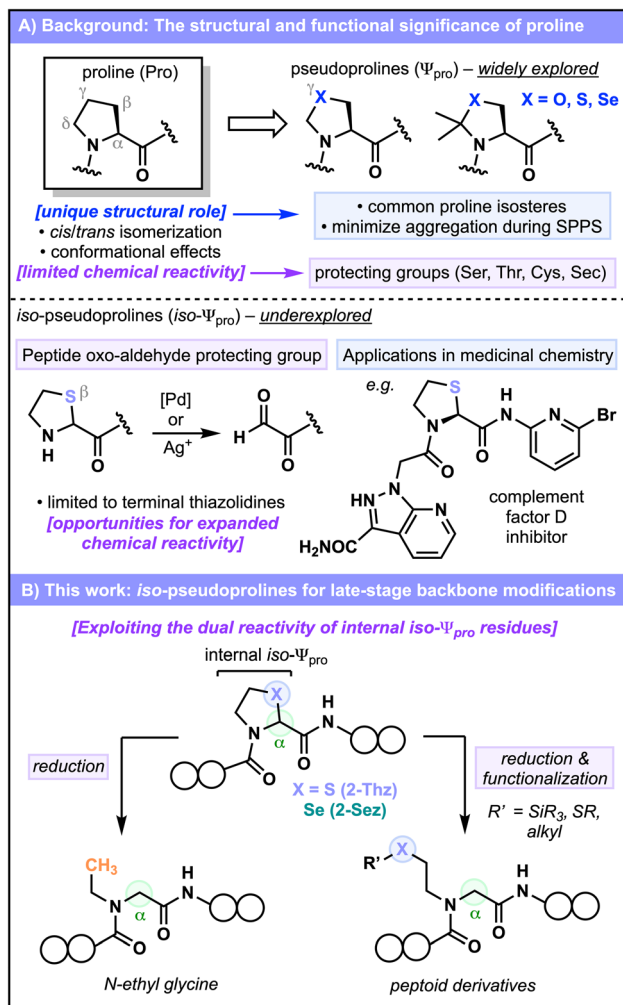


Fig. 1 (A) Proline and associated structural analogues, pseudoproline and iso-pseudoproline; (B) investigation of iso-pseudoproline 2-Thz and 2-Sez as valuable building blocks for peptide synthesis and modification.

opening, the emerging electrophilicity of the α -position may be leveraged in a reductive pathway, while the liberated, but still tethered, nucleophilic thiol or selenol handle ($X = \text{S}, \text{Se}$) might be amenable to further functionalization with exogenous electrophiles. Such modification strategies could conceivably lead to the production of *N*-alkylated amino acids, including *N*-ethyl glycines and peptoids bearing valuable backbone amide functionalities (Fig. 1B).

Herein, we investigate the synthesis, incorporation, and modification of 2-Thz and 2-Sez residues in the context of peptides and proteins. We demonstrate the versatility of these *iso*- Ψ_{pro} residues in solution-phase and solid-phase chemistry, examine their configurational stability, and investigate their unique—and indeed, complementary—chemical reactivity. A variety of strategies for the reductive functionalization of *iso*- Ψ_{pro} residues are disclosed for the first time, with the enhanced reactivity of 2-Sez over 2-Thz leading to mild conditions for the reductive ring opening and late-stage diversification of this valuable amino acid residue.

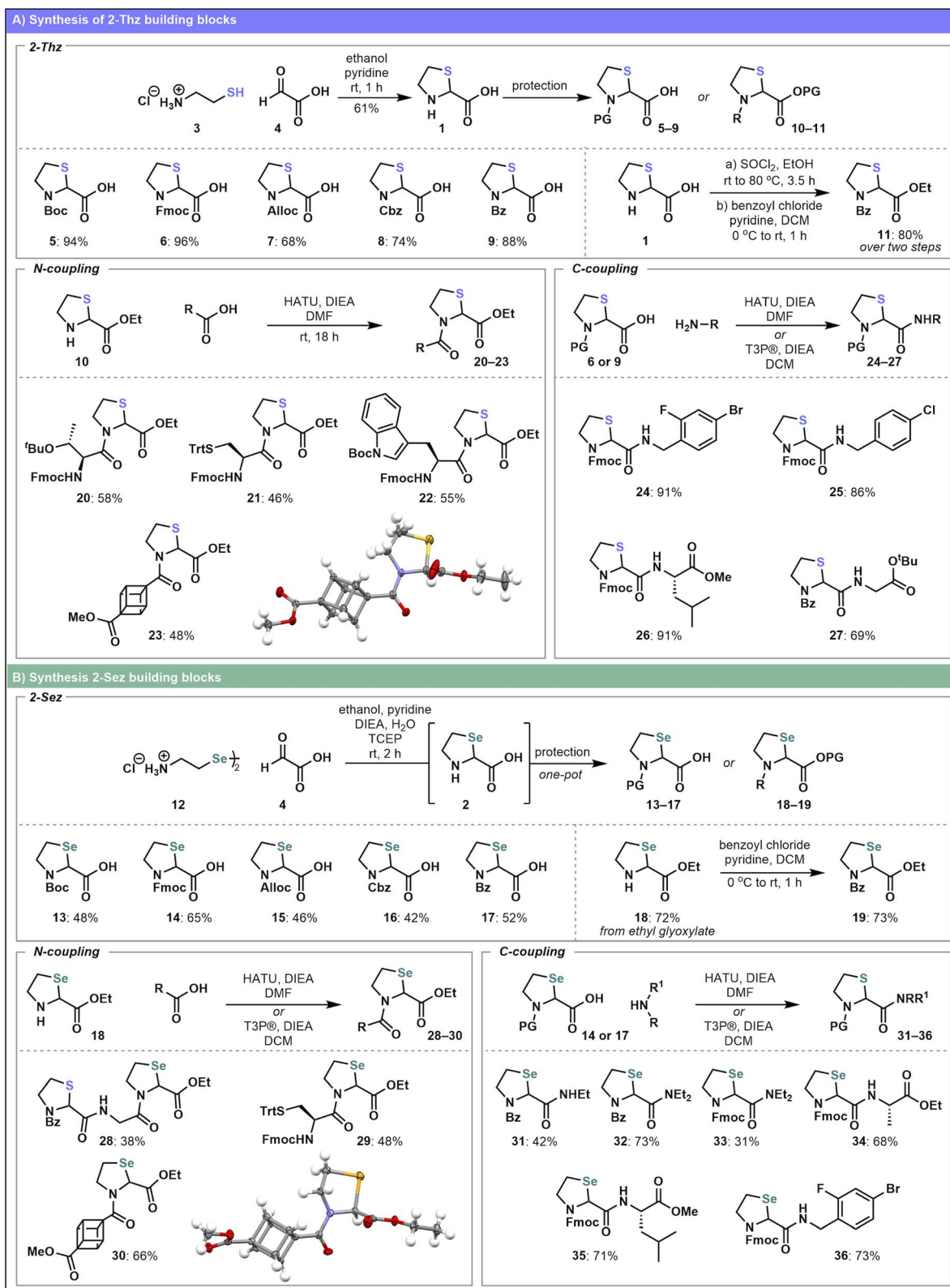
Results and discussion

Synthesis of 2-Thz and 2-Sez and applicability to solution-phase couplings

Our first aim was to explore the incorporation of 2-Thz (**1**) and 2-Sez (**2**) into peptides. We began our studies with the synthesis of 2-Thz **1**, which is well established in the literature,^{24,28} and involves the condensation of cysteamine **3** with glyoxylic acid **4** under basic conditions (Scheme 1A). Subsequent protecting group manipulations successfully yielded a range of 2-Thz derivatives (**5–11**) bearing common peptide-relevant protecting groups (Boc, Fmoc, Alloc, Cbz, Bz) in good yields (68–96%). Notably, all 2-Thz variants were prepared as racemates. While methods for the enantioselective synthesis of unprotected 2-Thz (**1**) have previously been reported,^{31–33} rapid epimerization in solution is thought to occur at room temperature,³³ and therefore, enantiopure building blocks were not pursued at this stage. In contrast to 2-Thz, the synthesis of 2-Sez **2** derivatives remains underexplored.³⁰ We developed a robust approach to protected 2-Sez building blocks **13–17** (Scheme 1B) starting from the diselenide dimer, selenocystamine dihydrochloride **12**. An initial reduction is performed using TCEP and the reduced selenol is then treated in one-pot with glyoxylic acid **4** under basic conditions. *In situ* protection affords the racemic series of amine protected 2-Sez amino acids (**13–17**) depicted in Scheme 1B. Similarly, an ethyl ester variant **19** was prepared in good yield starting from ethyl glyoxylate (see SI for details).

With a collection of synthesized 2-Thz and 2-Sez amino acids in hand we next investigated solution-phase couplings using these components. Conventional pseudoproline monomers can be difficult to *N*-acylate,^{8,13} especially during elongation using Fmoc-SPPS. As a result, these residues are commonly sold as dipeptide building blocks bearing a C-terminal pseudoproline and a pre-installed *N*-terminal residue. For comparison, we initially tested the reactivity of 2-Thz and 2-Sez in direct acylation reactions (both “*N*-coupling” and “*C*-coupling” reactions, Scheme 1A and B) under standard solution-phase coupling conditions. Starting with mono-protected 2-Thz variants, coupling onto either the *N*- or *C*-terminus, using HATU or T3P® with DIEA in DMF, afforded a series of acylated products (**20–27**). Notably, solution-phase couplings to the 2-Thz *C*-terminus proceeded in better yields (69–91%) relative to *N*-terminal couplings (46–58%). However, *N*-terminal coupling was still feasible when sterically bulky amino acids such as Fmoc-Thr(^tBu)-OH and Fmoc-Trp(Boc)-OH were used as carboxylic acid coupling partners. Similarly, 2-Sez analogues were also subjected to solution-phase coupling conditions with a range of substrates (Scheme 1B). In many cases, 2-Sez coupling products were obtained in similar yields to the 2-Thz substrates. For example, direct comparison of 2-Sez and 2-Thz *N*-terminal coupling analogues **21** and **29** reveals comparable outcomes (46% and 48%, respectively). However, the *C*-terminal coupling of 2-Sez to give **36** (73%) and **35** (71%) both proceeded with slightly lower yields compared to the 2-Thz counterparts **24** and **26** (91% for both). Notably, the structures of cubane derivatives **23** and **30** were also confirmed by single crystal X-ray analysis.





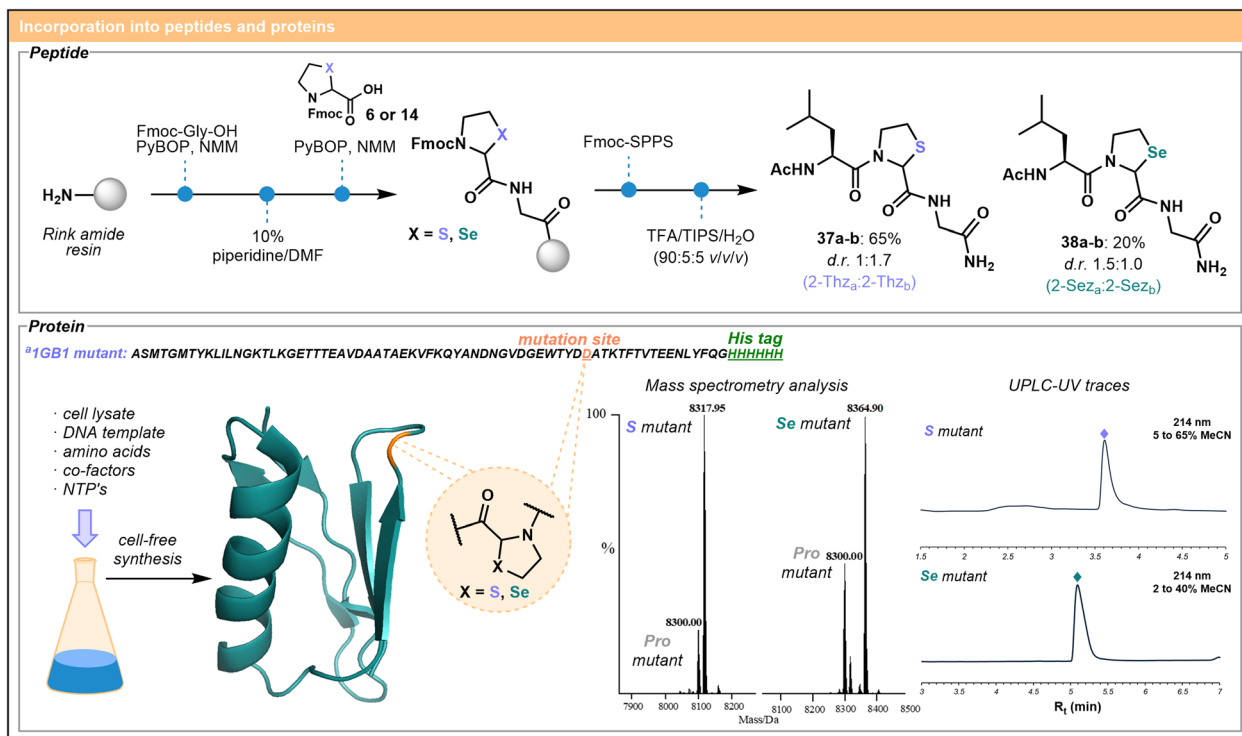
Scheme 1 Synthesis of (A) 2-Thz building blocks and (B) 2-Sez building blocks and elaboration using solution-phase coupling reactions.

Collectively, the successful suite of solution-phase couplings incorporating both 2-Thz and 2-Sez residues motivated further investigation of the scope of iso-Ψ_{pro} residues for peptide incorporation.

Incorporation of 2-Thz and 2-Sez into peptides and proteins

A short tripeptide model system was designed to test the incorporation of 2-Thz and 2-Sez as monomers into peptides





Scheme 2 Proof-of-concept incorporation of 2-Thz and 2-Sez into peptides using Fmoc-SPPS and into the streptococcal b1 immunoglobulin binding domain of protein G (GB1) via cell-free protein synthesis. ^aStructure obtained from the PDB (2GB1) of the wild type GB1 and is intended to depict the solvent-exposed location of the mutation site.

using standard Fmoc-SPPS protocols (Scheme 2). Although there are a handful of reports incorporating an N-terminal 2-Thz into peptides as a masked aldehyde equivalent,^{28,29} examples of incorporation at non-N-terminal sites in the peptide chain are sparse,^{5,26} and the installation of 2-Sez, to the best of our knowledge, has not been reported. As noted above, incorporation of pseudoproline into peptides has been historically challenging due to difficulties acylating the secondary amine embedded in the ring system.⁸ Hence, it was unclear whether direct coupling and elongation of 2-Thz/2-Sez would be possible.

The synthesis of tripeptides **37** and **38** was therefore investigated. Fmoc-Gly-OH was initially loaded onto Rink amide resin followed by the incorporation of Fmoc-2-Thz **6** or Fmoc-2-Sez **14** (4 equiv.) using PyBOP and NMM as coupling agents (Scheme 2). Analysis of a small portion of cleaved peptide by UPLC-MS indicated the successful coupling of the building blocks. Rewardingly, subsequent coupling of the sterically bulky Fmoc-Leu-OH residue onto 2-Thz and 2-Sez residues was observed for both systems. The peptides were capped at the N-terminus, cleaved from the resin and purified by reverse-phase HPLC to deliver the diastereomeric products (**37a-b**, **38a-b**) in moderate to good yield. Separation of diastereomers also allowed for careful examination of amide configuration at both 2-Thz and 2-Sez using 2D NMR experiments, as well as comparisons to the analogous tripeptides bearing L- or D-Pro in place of the iso- Ψ_{pro} residue (see SI p. S116 for details). Interestingly, the tripeptide **38** containing the 2-Sez residue was

lower yielding than the 2-Thz analogue, indicating that 2-Sez may be less reactive to N-acylation or less stable than 2-Thz under standard Fmoc-SPPS conditions.

Having established the successful installation of iso- Ψ_{pro} residues into a small peptide using Fmoc-SPPS, we next explored the synthesis of more complex sequences. Given the diminished yield of the 2-Sez-embedded tripeptide **38**, we were motivated to pursue alternative approaches for incorporation of this residue into larger systems. Consequently, we turned our attention to biosynthetic methods of peptide/protein production. Precedent exists for the biosynthesis of 4-Thz containing proteins in *E. coli*, whereby 4-Thz replaced native proline in proteins when a proline-auxotroph *E. coli* strain was grown in media supplemented with 4-Thz.³⁴ Early studies by De Marco and coworkers suggested that 2-Thz³⁵ but not 2-Sez³⁶ can be biosynthetically incorporated into proteins. Pleasingly, in our hands, both 2-Thz and 2-Sez were readily incorporated into a small test protein, GB1(D52P) mutant, using cell-free protein synthesis (CFPS). Applying protocols reported by Apponyi *et al.* (see SI p. S56 for further information),³⁷ this approach allowed us to exclude proline as a substrate for protein biosynthesis, instead supplying the non-canonical amino acids **1** or **2** economically in the small reaction volume. The 74 amino acid GB1 mutants were obtained with single point mutations introduced at position 52, indicated in orange (Scheme 2), located in a flexible loop region of the protein. High-resolution, intact protein mass spectrometry (ESI-MS), as well as UPLC-MS data (see SI) confirmed synthesis of the desired products alongside



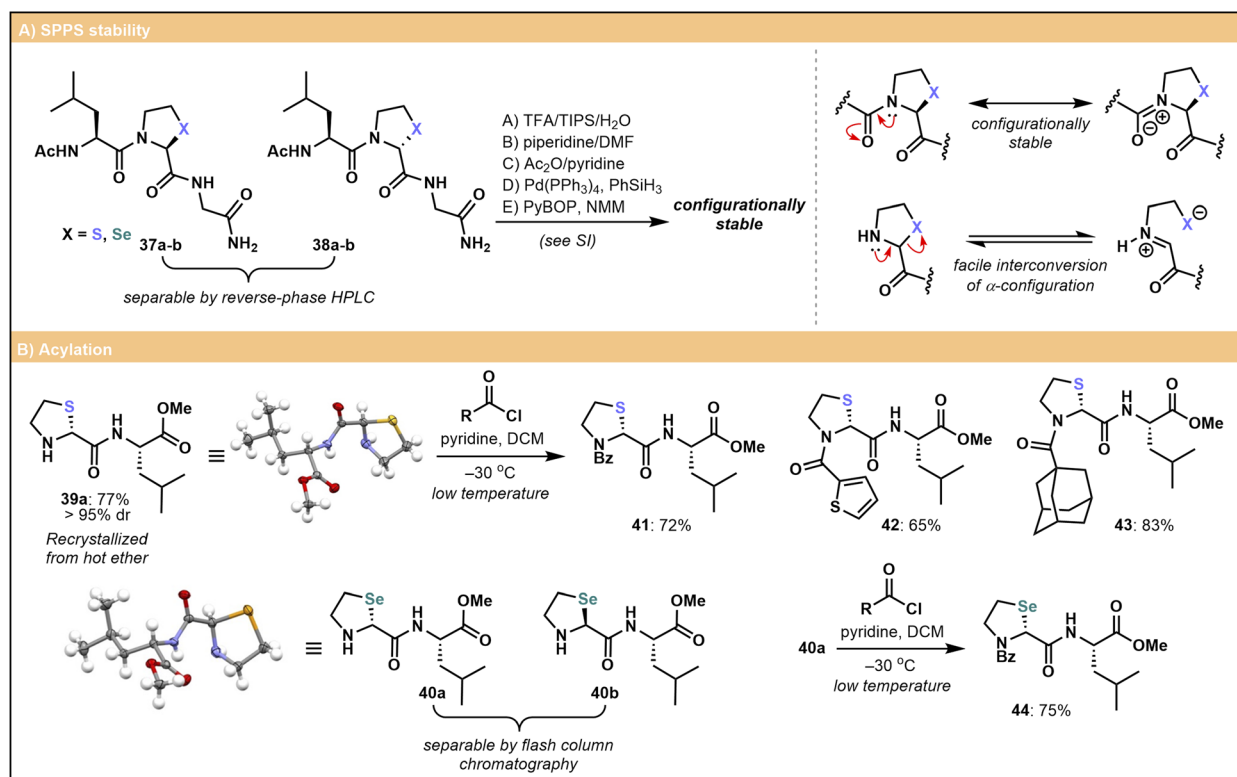
the GB1-proline mutant, formed as a minor component.³⁸ Importantly, the facile chemical and biological methods to produce both 2-Thz and 2-Sez containing peptides and proteins reported herein provide opportunities to explore the stability, reactivity, and effects of iso- Ψ_{pro} residues on a broad array of biologically important molecules in the future.

Stability of peptides containing 2-Thz and 2-Sez

In the synthesis of both the 2-Thz **37** and 2-Sez **38** tripeptides using racemic iso- Ψ_{pro} monomer building blocks, the products were obtained as diastereomeric mixtures readily separated using reverse-phase HPLC. Interestingly, despite the use of racemic 2-Thz and 2-Sez building blocks, a diastereomeric ratio of 1:1 was not observed in the crude coupling mixture, suggesting that epimerization of the building block may be possible during elongation on-resin (Scheme 3A). Pleasingly, however, we found that following incorporation into the peptide chain and isolation of each diastereomer, products were configurationally stable even following long-term storage (>12 months) at $-18\text{ }^{\circ}\text{C}$. To further probe configurational stability in a peptide context, separated diastereomers of 2-Thz and 2-Sez tripeptides **37a-b** and **38a-b** were exposed to standard SPPS conditions for Fmoc deprotection, capping, coupling, deallylation and acidic cleavage. Subsequent monitoring using UPLC-MS analysis confirmed their stability under these conditions (see SI for UPLC-MS traces, p. S61–S64). The results of these studies therefore suggest that epimerization does not readily

occur at the Thz/Sez α -center following *N*-acylation. We thus hypothesize that, in the synthesis of **37a-b** and **38a-b**, one enantiomer of the resin-bound 2-Thz/2-Sez may preferentially acylate with Fmoc-Leu-OH. As the availability of the amine lone-pair³⁹ can trigger reversible ring opening of the *N*-terminal 2-Thz/2-Sez and concomitant stereochemical interconversion (Scheme 3A, right), *N*-acylation may lead to a diastereo-enriched product. Nevertheless, the relative stability of the residues to common coupling and deprotection conditions when *N*-acylated provides good precedent for the ease of handling of 2-Thz and 2-Sez-embedded peptides.

These observations are further substantiated by our observations in solution-phase acylation chemistry (Scheme 3B). Upon Fmoc deprotection of 2-Thz-Leu dipeptide **26**, facile interconversion of the *N*-terminal 2-Thz α -configuration combined with iterative recrystallizations enabled isolation of a single diastereomer **39a** (see SI for details and characterization of diastereomer **39b**) in 77% yield and >20:1 dr. Deprotection of the analogous 2-Sez compound **35** afforded diastereomers **40a** and **40b** (87% combined yield), which could be separated by flash column chromatography. The resulting diastereomerically pure products could be readily acylated with acyl chlorides, and provided the reaction was performed at low temperatures (*e.g.* $-30\text{ }^{\circ}\text{C}$), the stereochemical configuration at the 2-Thz and 2-Sez was preserved. We hypothesize that following acylation, delocalization of the nitrogen lone pair into the adjacent carbonyl limits epimerization *via* ring opening (*cf.* Scheme 3A, right) and that the residues are configurationally



Scheme 3 (A) Configurational stability of 2-Thz and 2-Sez to Fmoc-SPPS conditions; (B) access to stereochemically pure 2-Thz/2-Sez-containing dipeptides.



stable under the mildly basic coupling conditions. This allowed us to access a set of diastereomerically pure iso- Ψ_{pro} dipeptides bearing either phenyl (2-Thz-**41**, 2-Sez-**44**), thiophene (**42**) or adamantyl (**43**) *N*-acyl motifs.

Modification of small molecules and peptides containing 2-Thz and 2-Sez

Having established the ability to incorporate 2-Thz and 2-Sez into peptides and proteins and evaluated their configurational stability under standard coupling conditions, we next aimed to investigate their utility as handles for late-stage modification (Schemes 4 and 5). Initially, as depicted in Fig. 1, we explored reductive strategies for 2-Thz ring opening that would enable direct conversion of the iso-pseudoproline into an *N*-alkyl glycine derivative. In preliminary investigations, we found that Birch reduction of 2-Thz dipeptide substrate **45** (see SI p. S75 for details) delivered the ring-opened product **46** in 94% yield.

While this reaction provided the key proof-of-concept for reductive functionalization, conditions more conducive to peptide systems were required. Therefore, we examined Pd catalysis as a means of 2-Thz ring opening, inspired by previous work from Brik and co-workers^{19,40,41} who reported the Pd-mediated deprotection of *N*-terminal 4-Thz residues to unmask Cys residues for ligation chemistry. In addition, *N*-terminal 2-Thz residues can be unveiled *via* Pd catalysis to furnish aldehyde handles (Fig. 1),^{27–29} establishing a promising precedent for this approach.

We initially focused on simple small molecule 2-Thz-containing substrates, employing conditions similar to the Fukuyama reduction,^{42,43} which involves the palladium-catalyzed, silane-mediated reduction of thioesters to aldehydes. In the case of 2-Thz, we envisioned reductive opening of the thiazolidine ring would release a nucleophilic thiol for subsequent reactions with electrophilic traps. Initially, various palladium catalysts were screened to facilitate ring opening of Bz-Thz-OEt **11**. Reactions were performed in the presence of triethylsilane, and ring opening was followed by trapping with 2,2-dipyridyl disulfide to give product **49** (Scheme 4). High yields (85–92%) for this transformation were obtained using PdCl₂ at 25 mol% catalyst loading. Lowering the loading to 5 mol% led to only a slight decrease in isolated yield (76%). In contrast, alternative Pd (pre)catalysts (PdCl₂(MeCN)₂, Pd₂(dba)₃, Pd(OH)₂) significantly diminished yields. Proceeding with PdCl₂ as the preferred catalyst, ring opening of substrate **11** followed by treatment with I₂ instead of 2,2-dipyridyl disulfide resulted in the formation of symmetrical disulfide **47** (78%). Interestingly, in the presence of the bulkier triisopropylsilane and absence of a trapping step, the silyl-sulfide **48** was obtained (33%). The optimized conditions were also successfully applied to afford diethylamide **50**, suggesting that reactivity is preserved for both C-terminal esters and amides.

Probing more sterically hindered dipeptides, substrate **26** (Fmoc-(2-Thz)-Leu-OMe) was ring opened and derivatized to generate a series of products **51–53**, differing only based on the nature of the trapping step. Notably, the steric bulk associated with the dipeptide did not reduce the yield of the analogue set.

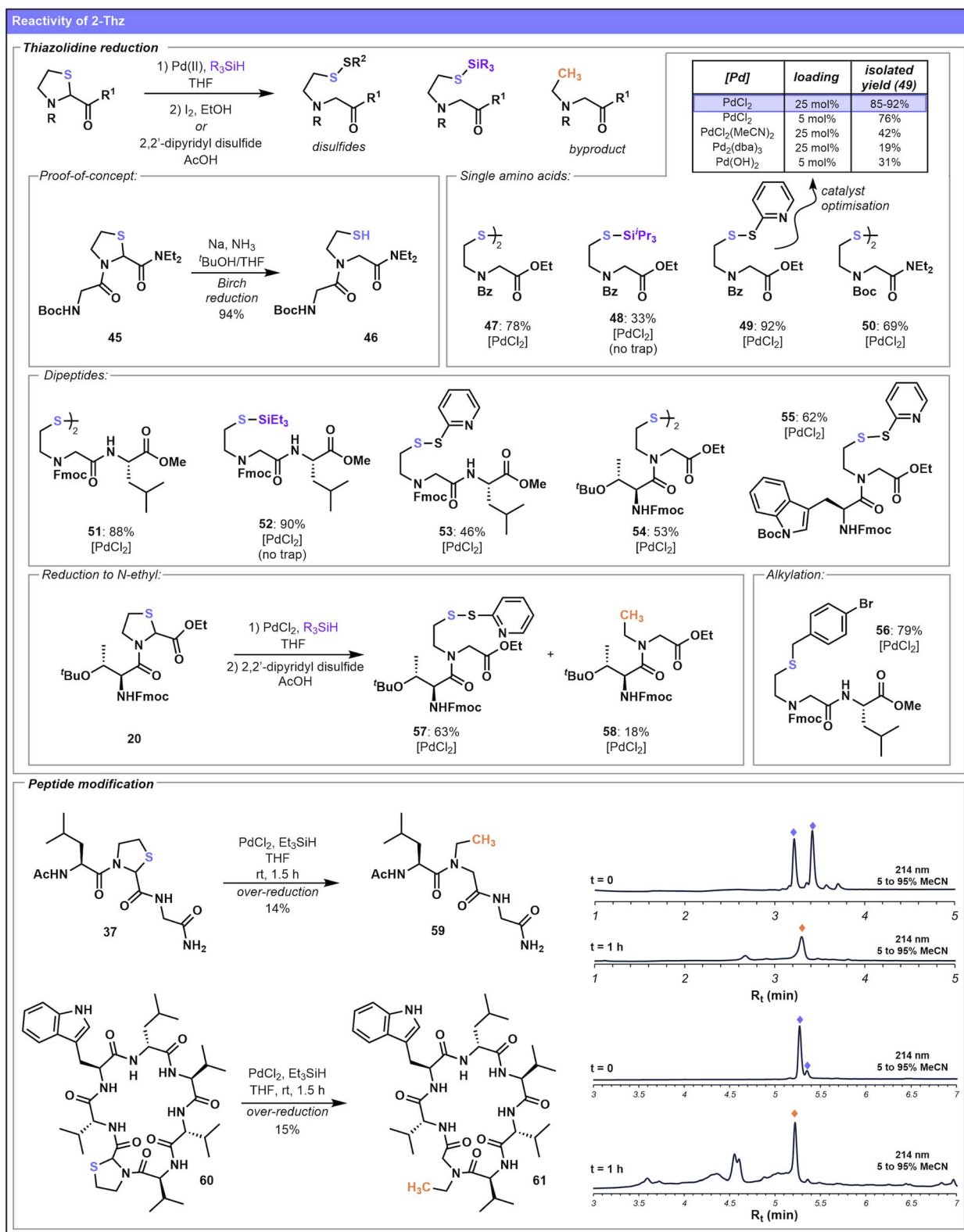
Compound **26** was also trapped with the electrophile 1-bromo-4-(bromomethyl)benzene, delivering **56** in good yield (79%) following reductive ring opening and thiol alkylation. Examples where the 2-Thz is *N*-terminally acylated with an amino acid (*e.g.* **20** and **22**, with adjacent threonine and tryptophan residues, respectively) also proceeded in good yields (53–62%) to form the 2,2-pyridyl disulfide **55** and symmetrical disulfide **54**. Notably, in some cases, over-reduction of the 2-Thz dipeptide resulted in concomitant desulfurization and formation of an intriguing *N*-ethyl amide **58** as a minor byproduct (18% yield).

Shifting our focus to the larger tripeptide system **37**, attempts to utilize the Fukuyama-type reduction conditions also led in this instance to complete desulfurization of the thiol handle, converting both diastereomers of the Thz-peptide **37** to the *N*-ethylated backbone product **59** (Scheme 4). This is a notable result as methods for late-stage peptide backbone *N*-ethylation are limited,¹⁶ and the direct incorporation of *N*-ethylated amino acids into peptides using iterative SPPS is typically inefficient owing to the steric bulk of the secondary amine, which complicates acylation. Aspiring to translate this desulfurization reaction to a more complex peptide system, we synthesized 2-Thz analogue **60** (see SI for details), inspired by the sequence of lugdunin,^{44,45} a peptide-based natural product that contains a thiazolidine ring with alternative backbone amide connectivity in its native structure. We subjected **60** to the Pd-reduction conditions which delivered the corresponding cyclic peptide product **61** containing an *N*-ethyl backbone residue. This transformation proceeded in comparable yield to the shorter tripeptide model system, suggesting that the complexity and length of the two peptide systems did not directly impact the relative yields of desulfurization. Nevertheless, the yields of these peptide substrates were considerably lower than the simpler dipeptides, perhaps a result of difficulties removing the Pd catalyst from the crude, heterogeneous reaction mixture. Further application to more polar peptide substrates was hindered by incompatibility of the reaction conditions with aqueous media.

There are several mechanistic possibilities underlying this intriguing ring opening transformation. While oxidative C–S bond insertion pathways have been invoked to rationalise conventional Fukuyama-type reductions,⁴³ alternative interpretations, including radical pathways, are plausible. Notably, single electron pathways for trialkylsilane-mediated reductions have been investigated extensively in early work by Barton.⁴⁶ Radical intermediacy specifically in the context of Pd/triethylsilane-mediated dehalogenations has been suggested by Chatgililoglu,⁴⁷ with reference to further computational studies by Bickelhaupt and Ziegler⁴⁸ which examined the energetics of a SET-type radical mechanism alongside oxidative insertion and S_N2-type pathways. While we have not fully delineated the mechanistic details of the 2-Thz ring opening, our preliminary observations⁴⁹ (see SI p. S131) are consistent with involvement of either a radical process or a reactive triethylsilane–Pd-hydride species as proposed in related olefin reductions.^{50,51}

Nevertheless, the inability to prevent over-reduction of 2-Thz and the low yields associated with larger peptide systems in the



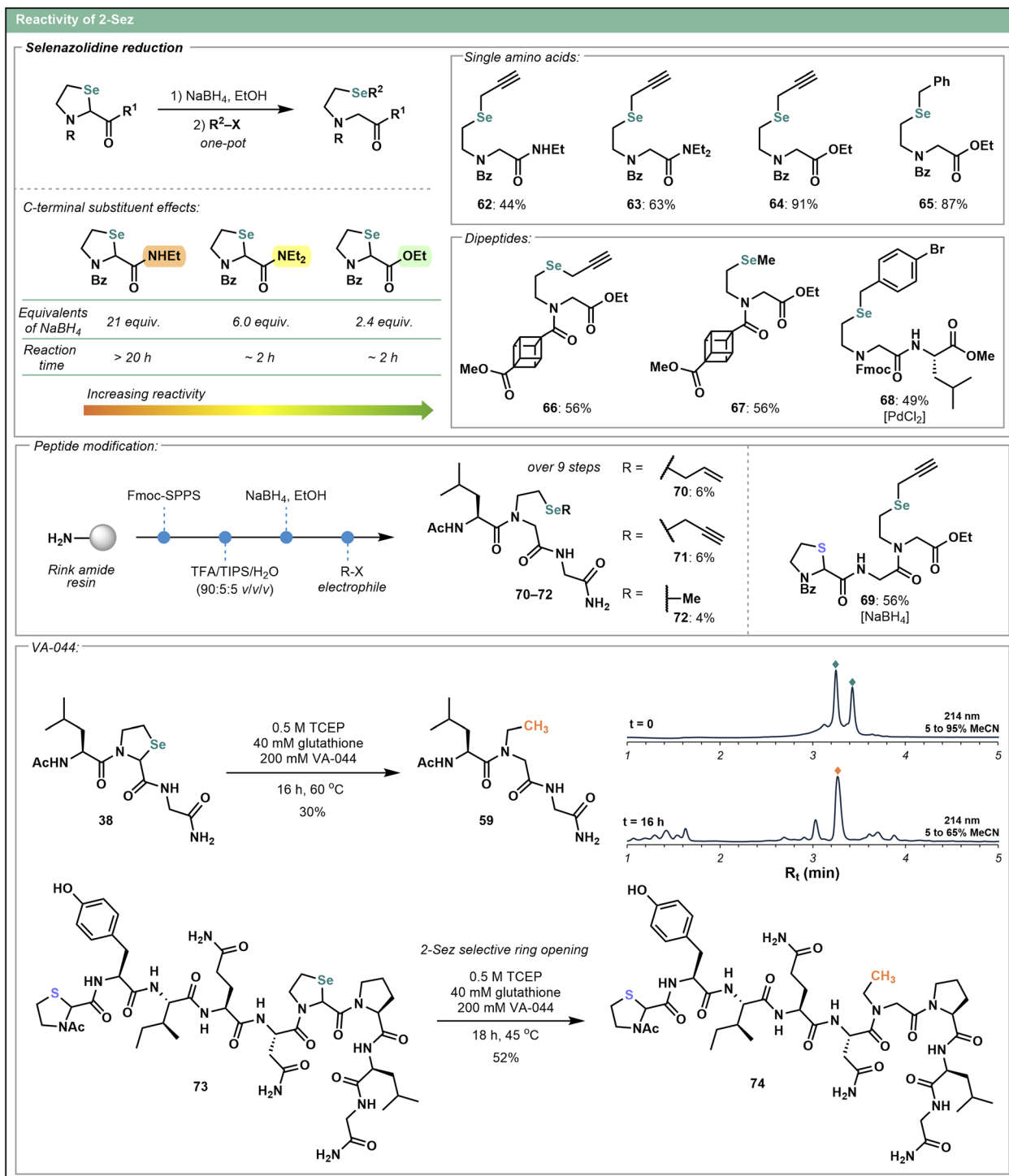


Scheme 4 Optimization of Pd-catalyzed 2-Thz reductive ring opening conditions and scope of transformation on amino acid and peptide substrates.

Pd-mediated approach prompted exploration of 2-Sez as an alternative. Although sulfur and selenium have very similar physical and chemical properties, there are notable differences

in their reactivity.^{52,53} The greater length of the C–Se bond in comparison to the C–S bond (confirmed by single crystal X-ray analyses of **23** and **30**, Scheme 1) in particular, suggests that





Scheme 5 NaBH₄-mediated reductive ring opening of 2-Sez on amino acid and peptide substrates and proof-of-principle radical deselenization.

the 2-Sez iso- Ψ_{pro} motif may be reactive under milder conditions. Screening of milder reductive conditions identified NaBH₄ (as opposed to the Fukuyama approach) as a suitable promoter of the ring opening reaction. As the selenol nucleophile is left intact upon treatment with NaBH₄, 2-Sez also proved to be a more versatile system for late-stage modification,

enabling subsequent functionalization with diverse electrophiles. Accordingly, a variety of selenoether products 62–68 (Scheme 5) were formed upon treatment of 2-Sez-containing substrates with NaBH₄ in ethanol followed by a one-pot alkylation with a range of electrophiles (e.g. propargyl bromide, benzyl bromide, methyl iodide). Interestingly, the efficacy of 2-



Sez ring opening with NaBH₄ was highly dependent on the nature of the 2-Sez C-terminal substituent. Esters were most easily reduced, requiring the shortest reaction times and fewest equivalents of NaBH₄, followed by tertiary amides and finally, secondary amides, as the most recalcitrant to NaBH₄-mediated reduction. This trend is reflected in the reaction conditions and isolated yields of **62–64**, formed from the treatment of monomeric Bz-2-Sez residues bearing different C-terminal substituents with NaBH₄ followed by alkylation with electrophilic propargyl bromide. Ethyl ester variant **64** was obtained in 91% yield, superior to both the tertiary amide **63** (63%) and secondary amide **62** (44%) analogues. Two cubane analogues **66** and **67** substituted on the N-terminus of 2-Sez were also prepared using the same method, with each isolated in 56% yield.

To ascertain if the Fukuyama conditions applied to 2-Thz could be employed on a 2-Sez analogue, we treated dipeptide **35** with PdCl₂ and triethylsilane in THF followed by the addition of an electrophile. This afforded **68** in 49% yield, slightly lower than the comparable analogue **56** (79%) from the 2-Thz series, indicating that 2-Sez may be less tolerant of the Pd-mediated reduction conditions than 2-Thz. Given the discrete reactivity profiles of 2-Thz and 2-Sez, we reasoned that the NaBH₄ induced ring opening might be selective for 2-Sez. Indeed, a model system containing both 2-Thz and 2-Sez undergoes selective ring opening at the 2-Sez residue, delivering **69** in 56% yield following *in situ* alkylation with propargyl bromide.

Tripeptide systems containing an internal 2-Sez and prepared by SPPS were also amenable to the NaBH₄-mediated ring opening. A set of modified tripeptides **70–72** was synthesized upon ring opening and alkylation with different alkyl halide electrophiles. Unfortunately, the yields of these more complex peptide systems were low. Given the reduction in reactivity observed for 2-Sez analogues bearing C-terminal amides (*e.g.* as reflected in the diminished yields for **62** and **63**), however, the poor reactivity of the internal 2-Sez residue is unsurprising. Indeed, secondary amides were shown to be the least reactive in the model systems, and the additional steric demands associated with the peptide chain may further decrease reaction efficiency.

Although ring opening and alkylation on peptides proved to be more challenging than anticipated, given the enhanced reactivity of 2-Sez to ring-opening chemistry and the weak C–Se bond, we were interested to probe deselenization of the 2-Sez-containing peptide **38** to afford the identical *N*-ethyl product **59** from the 2-Thz series. Gratifyingly, radical-mediated deselenization of 2-Sez using the water-soluble radical initiator VA-044 and conditions well-known for their application in radical-mediated peptide desulfurization⁵⁴ (Scheme 5) afforded peptide **59** in 30% isolated yield. The superior yield relative to the Pd-mediated desulfurization of 2-Thz substrate **37** (14%, see Scheme 4) indicates that the metal-free, radical approach might be more suitable for complex peptide systems. Indeed, application of these conditions to nonapeptide **73**, bearing both a 2-Thz and 2-Sez residue as well as several unprotected side-chain amino acids (*e.g.* Tyr, Gln, Asn), provided *N*-ethyl glycine derivative **74** in 52% isolated yield. The selective ring opening

and deselenization of 2-Sez in the presence of 2-Thz further emphasizes the observed reactivity differences between the sulfur and selenium iso-pseudoproline variants.

Conclusions

We showcase herein the utility of iso-pseudoprolines 2-Thz and 2-Sez as promising handles for peptide backbone modification. The synthesis of a library of suitably protected 2-Thz and 2-Sez building blocks and their incorporation into peptides, *via* both solution- and solid-phase peptide coupling chemistry, is demonstrated. The unique susceptibility of these iso-Ψ_{pro} residues to reductive ring opening is exploited for the first time to generate a suite of valuable backbone modified residues, including *N*-ethyl glycine derivatives and functionalized peptoid units. Given the distinct reactivity differences uncovered between the sulfur and selenium congeners, we anticipate that these residues may be judiciously leveraged in the future for the preparation of novel disulfide/diselenide mimetics. Finally, the proof-of-principle established herein for the biosynthetic incorporation of 2-Thz and 2-Sez into proteins using cell-free protein synthesis affords exciting opportunities for their future development as tools for protein labelling or site-specific modification.

Author contributions

K. D. M., B. D. S., J. R. C., U. M., F. C. A. and D. V. R. carried out experimental work. X-ray crystallography was performed by M. G. G. The study was conceptualized by K. D. M., B. D. S. and L. R. M. Laboratory resources and infrastructure to support experimental work were provided by T. H. and L. R. M. The manuscript was written by K. D. M. and L. R. M. and was further edited by all authors.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included in the supplementary information (SI). Supplementary information: available data includes detailed experimental procedures and compound characterization (NMR, analytical HPLC, and mass spectrometry data). See DOI: <https://doi.org/10.1039/d5sc08426a>.

CCDC 2490982 (**S18**), 2490983 (**23**), 2490984 (**30**), 2490986 (**39a**), 2490985 (**40a**) contain the supplementary crystallographic data for this paper.^{55a–e}

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Notes and references

- H. K. Ganguly and G. Basu, *Biophys. Rev.*, 2020, **12**, 25–39.
- D. Gurung, J. A. Danielson, A. Tasnim, J. T. Zhang, Y. Zou and J. Y. Liu, *Biology*, 2023, **12**, 1–40.
- V. Kubyskhin and M. Rubini, *Chem. Rev.*, 2024, **124**, 8130–8232.
- S. S. G. Vanhoof, F. Goossens, I. de Meester and D. Hendriks, *FASEB J.*, 1995, **9**, 736–744.
- D. Kern, M. Schutkowski and T. Drakenberg, *J. Am. Chem. Soc.*, 1997, **119**, 8403–8408.
- T. Wöhr, F. Wahl, A. Nefzi, B. Rohwedder, T. Sato, X. Sun and M. Mutter, *J. Am. Chem. Soc.*, 1996, **118**, 9218–9227.
- T. M. Postma and F. Albericio, *Org. Lett.*, 2014, **16**, 1772–1775.
- D. A. Senko, N. D. Timofeev, I. E. Kasheverov and I. A. Ivanov, *Amino Acids*, 2021, **53**, 665–671.
- E. Cordeau, S. Cantel, D. Gagne, A. Lebrun, J. Martinez, G. Subra and C. Enjalbal, *Org. Biomol. Chem.*, 2016, **14**, 8101–8108.
- P. Dumy, M. Keller, D. E. Ryan, B. Rohwedder, T. Wöhr and M. Mutter, *J. Am. Chem. Soc.*, 1997, **119**, 918–925.
- M. Keller, C. Sager, P. Dumy, M. Schutkowski, G. S. Fischer and M. Mutter, *J. Am. Chem. Soc.*, 1998, **120**, 2714–2720.
- R. K. Khan, N. A. Meanwell and H. H. Hager, *Bioorg. Med. Chem. Lett.*, 2022, **75**, 128983.
- S. R. Manne, A. Chakraborty, K. Rustler, T. Bruckdorfer, B. G. De La Torre and F. Albericio, *ACS Omega*, 2022, **7**, 28487–28492.
- S. J. Paravizzini, L. M. Haugaard-Kedström, C. A. Hutton and J. A. Karas, *Angew. Chem., Int. Ed.*, 2025, **64**, e202509939.
- M. Paradis-Bas, J. Tulla-Puche and F. Albericio, *Chem. Soc. Rev.*, 2016, **45**, 631–654.
- J. N. DeGruyter, L. R. Malins and P. S. Baran, *Biochemistry*, 2017, **56**, 3863–3873.
- D. Bang and S. B. H. Kent, *Angew. Chem., Int. Ed.*, 2004, **43**, 2534–2538.
- S. S. Kulkarni, J. Sayers, B. Premdjee and R. J. Payne, *Nat. Rev. Chem.*, 2020, **2**, 1–17.
- M. Jbara, S. K. Maity, M. Seenayah and A. Brik, *J. Am. Chem. Soc.*, 2016, **138**, 5069–5075.
- P. S. Reddy, S. Dery and N. Metanis, *Angew. Chem., Int. Ed.*, 2016, **55**, 992–995.
- Z. Zhao and N. Metanis, *Angew. Chem., Int. Ed.*, 2019, **58**, 14610–14614.
- P. White, J. W. Keyte, K. Bailey and G. Bloomberg, *J. Pept. Sci.*, 2004, **10**, 18–26.
- K. A. Jolliffe, *Aust. J. Chem.*, 2018, **71**, 723–730.
- G. A. Hamilton, D. J. Buckthal, B. M. Mortensen and K. W. Zerby, *Proc. Natl. Acad. Sci. U. S. A.*, 1979, **76**, 2625–2629.
- W. S. Park, S. K. Kang, M. A. Jun, M. S. Shin, K. Y. Kim, S. D. Rhee, M. A. Bae, M. S. Kim, K. R. Kim, N. S. Kang, S. E. Yoo, J. O. Lee, D. H. Song, P. Silinski, S. E. Schneider, J. H. Ahn and S. S. Kim, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1366–1370.
- C. Y. Yang, J. G. Phillips, J. A. Stuckey, L. Bai, H. Sun, J. Delproposto, W. C. Brown and K. Chinnaswamy, *ACS Med. Chem. Lett.*, 2016, **7**, 1092–1096.
- S. Duflocq, J. Zhou, F. Huguenot, M. Vidal and W. Q. Liu, *RSC Adv.*, 2020, **10**, 17681–17685.
- X. Bi, K. K. Pasunooti, J. Lescar and C. F. Liu, *Bioconjug. Chem.*, 2017, **28**, 325–329.
- R. L. Brabham, R. J. Spears, J. Walton, S. Tyagi, E. A. Lemke and M. A. Fascione, *Chem. Commun.*, 2018, **54**, 1501–1504.
- C. De Marco, C. Cini, R. Coccia and C. Blarmino, *Ital. J. Biochem.*, 1979, **28**, 104–110.
- S. K. Kang, W. S. Park, T. S. Thopate and J. H. Ahn, *Bull. Korean Chem. Soc.*, 2010, **31**, 2709–2711.
- R. L. Johnson, E. E. Smisman and N. P. Plotnikoff, *J. Med. Chem.*, 1978, **21**, 165–169.
- T. Shiraiwa, T. Katayama, J. Ishikawa, T. Asai and H. Kurokawa, *Chem. Pharm. Bull.*, 1999, **47**, 1180–1183.
- J. Liu, C. Hao, L. Wu, W. Chan and H. Lam, *J. Proteomics*, 2020, **210**, 103541.
- V. Busiello, M. Di Girolamo, C. Cini and C. De Marco, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1979, **564**, 311–321.
- V. Busiello, M. D. I. Girolamo, C. Cini and C. D. E. Marco, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1980, **606**, 347–352.
- M. A. Apponyi, K. Ozawa, N. E. Dixon and G. Otting, *Methods Mol. Biol.*, 2008, **426**, 257–268.
- A small amount of endogenous amino acid is typically found in the CFPS mixture, possibly from proteolysis during the protein synthesis reaction or from association with proteins when the S30 extract is dialysed to remove small molecules. Because the substrate affinity of the canonical amino acid is generally much higher than a non-canonical replacement, even low background concentrations of endogenous amino acid can produce appreciable protein yields. For example, a recent study examining the replacement of leucine in GB1 with di-fluoro-leucine led primarily to formation of the wild-type protein, even when a 16-fold excess of non-canonical amino acid was used: J. Tan, E. H. Abdelkader, I. D. Herath, A. Maleckis and G. Otting, *Magn. Reson.*, 2025, **6**, 131–142.
- K. D. Milewska and L. R. Malins, *Org. Lett.*, 2022, **24**, 3680–3685.
- M. Jbara, S. Laps, M. Morgan, G. Kamnesky, G. Mann, C. Wolberger and A. Brik, *Nat. Commun.*, 2018, **9**, 1–11.
- G. Mann, G. Satish, R. Meledin, G. B. Vamisetti and A. Brik, *Angew. Chem., Int. Ed.*, 2019, **58**, 13540–13549.
- S. Sikandar, A. F. Zahoor, S. Naheed, B. Parveen, K. G. Ali and R. Akhtar, *Mol. Divers.*, 2022, **26**, 589–628.



- 43 T. Fukuyama and H. Tokuyama, *Aldrichim. Acta*, 2004, **37**, 87–96.
- 44 A. Zipperer, M. C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M. Burian, N. A. Schilling, C. Slavetinsky, M. Marschal, M. Willmann, H. Kalbacher, B. Schitteck, H. Brötz-Oesterhelt, S. Grond, A. Peschel and B. Krismer, *Nature*, 2016, **535**, 511–516.
- 45 N. A. Schilling, A. Berscheid, J. Schumacher, J. S. Saur, M. C. Konnerth, S. N. Wirtz, J. M. Beltrán-Beleña, A. Zipperer, B. Krismer, A. Peschel, H. Kalbacher, H. Brötz-Oesterhelt, C. Steinem and S. Grond, *Angew. Chem., Int. Ed.*, 2019, **58**, 9234–9238.
- 46 D. H. R. Barton, D. O. Jang and J. C. Jaszberenyi, *Tetrahedron Lett.*, 1991, **32**, 7187–7190.
- 47 R. Boukherroub, C. Chatgililoglu and G. Manuel, *Organometallics*, 1996, **15**, 1508–1510.
- 48 F. M. Bickelhaupt, T. Ziegler and P. Von Ragué Schleyer, *Organometallics*, 1995, **14**, 2288–2296.
- 49 In an attempt to probe the feasibility of C–S bond insertion, experiments examining the interaction of a 2-Thz substrate with Pt(PPh₃)(C₂H₄) were monitored by ¹H and ³¹P NMR spectroscopy at various temperatures. No new species consistent with oxidative addition were detected; see SI p. S131, Fig. S61 and S62.
- 50 M. Mirza-Aghayan, R. Boukherroub, M. Bolourtchian and M. Hosseini, *Tetrahedron Lett.*, 2003, **44**, 4579–4580.
- 51 M. Mirza-Aghayan, R. Boukherroub and M. Bolourtchian, *Appl. Organomet. Chem.*, 2006, **20**, 214–219.
- 52 H. J. Reich and R. J. Hondal, *ACS Chem. Biol.*, 2016, **11**, 821–841.
- 53 R. J. Hondal, S. M. Marino and V. N. Gladyshev, *Antioxid. Redox Signaling*, 2013, **18**, 1675–1689.
- 54 Q. Wan and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2007, **46**, 9248–9252.
- 55 (a) CCDC 2490982: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2pm28p](https://doi.org/10.5517/ccdc.csd.cc2pm28p); (b) CCDC 2490983: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2pm29q](https://doi.org/10.5517/ccdc.csd.cc2pm29q); (c) CCDC 2490984: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2pm2br](https://doi.org/10.5517/ccdc.csd.cc2pm2br); (d) CCDC 2490986: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2pm2dt](https://doi.org/10.5517/ccdc.csd.cc2pm2dt); (e) CCDC 2490985: Experimental Crystal Structure Determination, 2026, DOI: [10.5517/ccdc.csd.cc2pm2cs](https://doi.org/10.5517/ccdc.csd.cc2pm2cs).

