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Synthesis and Utility of β-Selenophenylalanine and β-Selenoleucine in Diselenide-Selenoester Ligation (DSL).

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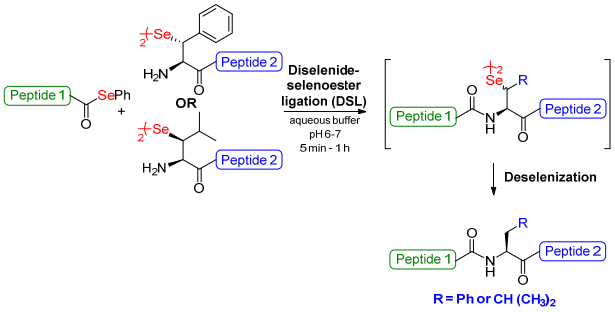


ABSTRACT: The synthesis of suitably protected β-selenophenylalanine and β-selenoleucine amino acids was accomplished from Garner's aldehyde as a common starting point. These selenoamino acids were incorporated into model peptides and shown to facilitate rapid diselenide-selenoester ligation (DSL) with peptide selenoesters which, when coupled with *in situ* deselenization, afforded native peptide products. The utility of one-pot DSL-deselenization chemistry at phenylalanine and leucine was demonstrated through the rapid synthesis of a glycosylated interferon-γ fragment and the chemokine-binding protein UL22A, respectively.

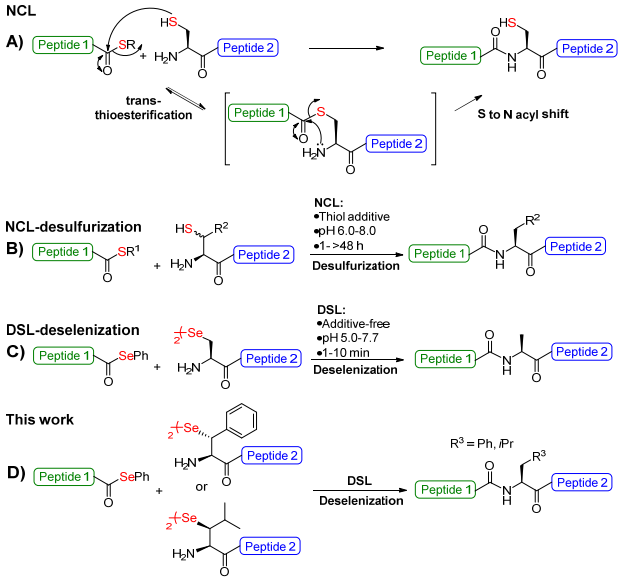
INTRODUCTION

The field of protein science has greatly benefited from the advent of peptide ligation chemistry[1-3] that allows proteins to be chemically synthesized by convergent assembly of smaller polypeptide fragments. The most widely used methodology is native chemical ligation (NCL)[4] which involves the chemoselective reaction between a peptide with an N-terminal cysteine residue and another fragment functionalized at the C-terminus as a thioester. These two species react through a reversible and rate-limiting transthioesterification step, followed by a rapid intramolecular S-to-N acyl shift to afford a native amide bond (Scheme 1A). The method has been significantly expanded by the use of desulfurization chemistry to convert Cys to native Ala residues[5-7] and through the use of synthetic thiol-derived amino acids which can also be desulfurized to native amino acids following the ligation event[8-24] (Scheme 1B). The slow kinetics of NCL reactions at sterically hindered ligation junctions, coupled with the lack of chemoselectivity of desulfurization (that results in the removal of all thiol groups, including those of native Cys residues) has prompted exploration of ligation chemistry at the 21st amino acid selenocysteine (Sec)[25-27]. The weaker C-Se bond strength in Sec, when compared to the C-S bond in Cys residues, enables the chemoselective deselenization of Sec to Ala in the presence of unprotected Cys, a finding first reported by Metanis and Dawson.[28] This chemistry has provided the impetus to perform NCL at selenoamino acids, namely γ-Se-Pro[29] and β-Se-Phe[30], thus enabling access to Xaa-Pro and Xaa-Phe junctions following chemoselective deselenization. Despite the increased nucleophilicity of Sec, which should lead to a significant rate enhancement in NCL, reaction kinetics with peptide thioesters are slow due to the low reduction potential of the diselenide (-381 mV), which limits the steady state concentration of the reactive selenolate under standard NCL conditions that employ thiol reductants.

We recently reported a novel peptide ligation reaction between peptide dimers bearing an N-terminal selenocystine (the oxidized form of Sec) and peptide selenoesters - dubbed the diselenide-selenoester ligation [DSL, (Scheme 1C)].[31-32] The reaction proceeds in aqueous denaturing ligation buffer in the absence of a reductant with reactions proceeding cleanly and to completion within 10 min, even at sterically-encumbered ligation junctions such as Val and Ile (cf. NCL where these reactions take 48 h or longer[33]). Importantly, DSL reactions can also be coupled with *in situ* deselenization to afford alanine at the ligation junction (or serine through an oxidative deselenization reaction[34-35]). The concept of one-pot DSL-deselenization has recently been extended to amino acids other than the 21st amino acid Sec, e.g. γ-Se-Pro[36], β-Se-Asp and γ-Se-Glu[37]. Together, these selenoamino acids have been used to rapidly access native selenoproteins as well as synthetic libraries of small post-translationally modified proteins[38] through one-pot DSL-deselenization. However, the DSL reaction manifold is still

limited to reactions at a select number of proteinogenic amino acids.

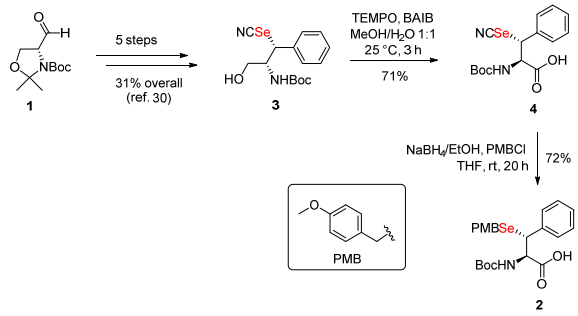
Herein, we report the development of one-pot DSL-deselenization chemistry at *β*-Se-Phe and *β*-Se-Leu to expand the scope of the DSL methodology (Scheme 1D). As part of this work, we also intended to establish a general synthetic route to *β*-Se-Phe and *β*-Se-Leu from a common precursor. The power of the DSL-deselenization chemistry at *β*-Se-Phe and *β*-Se-Leu is highlighted in the synthesis of a fragment of human interferon-γ and a virus-derived chemokine-binding protein, UL22A, respectively.



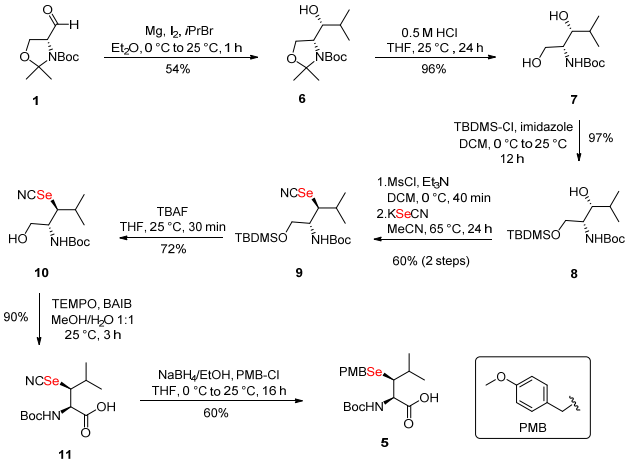
Scheme 1. The evolution of chemical ligation reactions from NCL to DSL

RESULTS AND DISCUSSION

The synthesis of both target selenoamino acids was proposed from Garner's aldehyde **1** as a common starting point. The target *p*-methoxybenzyl (PMB) protected *β*-Se-Phe [Boc-PMB-(*β*-Se)Phe] building block **2** was first synthesized over 9 steps from Garner's aldehyde **1** through slight modifications to our previously reported method[30] (Scheme 2). Specifically, to avoid the tedious work-up associated with the pyridinium dichromate oxidation, the final oxidation of primary alcohol **3** was instead carried out using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and (diacetoxyiodo)benzene (BAIB). The resulting selenocyanated amino acid **4** was reductively alkylated with PMB-Cl to afford the desired Boc-PMB-(*β*-Se)Phe building block **2**. When preparing the corresponding Boc-PMB-(*β*-Se)Leu building block **5** (Scheme 3), it was noted that the initial Grignard addition of *i*PrMgBr to Garner's aldehyde **1** delivered the alcohol intermediate **6** as only the *syn*-diastereomer (vs. 2:3 *syn/anti* for PhMgBr), thereby alleviating the need for a lengthy oxidation and reduction sequence to obtain diastereomerically-pure material. However, attempts to displace the corresponding *syn*-mesylate with KSeCN were unsuccessful due to the unfavourable conformational preference imposed by the oxazolidine (the *syn*-mesylate was also observed to be unreactive during the original Boc-PMB-(*β*-Se)Phe synthesis[30]). Therefore, the isopropylidene moiety was first removed to yield diol **7**. The primary alcohol was then protected as the TBDMS ether **8**, which underwent smooth mesylation and KSeCN displacement at the secondary alcohol to afford the *anti*-selenocyanate **9** in 60% yield over 2 steps. Next, the TBDMS ether was cleaved using TBAF to yield primary alcohol **10**, which was subjected to TEMPO/BAIB oxidation to yield the carboxylic acid **11**. Reduction of the selenocyanate with $NaBH_4$ in the presence of PMBCl then afforded the Boc-PMB-(β-Se)Leu building block **5** with an overall yield of 12% over 8 steps.



Scheme 2. Synthesis of Boc-PMB-(*β*-Se)Phe (2) from Garner's aldehyde.

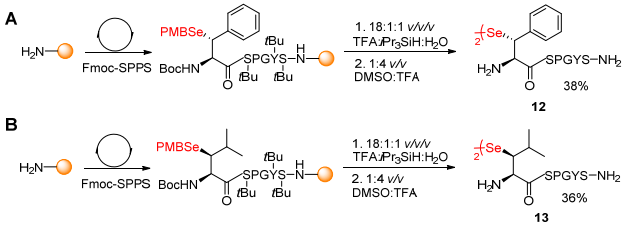


Scheme 3. Synthesis of Boc-PMB-(*β*-Se)Leu (5) from Garner's aldehyde.

In order to evaluate the utility of selenoamino acid building blocks **2** and **5** in DSL chemistry, each was coupled to the N-terminus of the Rink amide resin-bound model pentapeptide SPGYS, which was assembled through Fmoc-strategy solid-phase peptide synthesis (Fmoc-SPPS, Scheme 4). After global cleavage of the acid-labile side chain protecting groups, and subsequent PMB deprotection, the two model peptides were afforded as their corresponding diselenide dimers **12** and **13** in 38% and 36%, respectively, after HPLC purification. The two selenopeptide fragments were next evaluated in the additive-free DSL reaction with a wide range of peptidyl selenoesters LYRANX-SePh (**14a-f**) bearing different C-terminal residues (Tables 1 and 2). The additive-free DSL reactions were conducted by simply dissolving both the peptide diselenide dimer **12** or **13** and selenoester **14a-f** in 6 M Gn·HCl, 0.1 M Na_2HPO_4 aqueous buffer (final pH 6.1-6.4 without adjustment)[32]. In each case, three intermediates were observed in the ligation mixture corresponding to **I**) ligated product bearing an additional equivalent of the C-terminal fragment as a side chain peptidyl selenoester, **II**) the symmetric diselenide of the ligated product, and **III**) the asymmetric diselenide encompassing the ligated product with a side chain phenylselenyl group (**III** was only observed for ligations with *β*-Se-Phe peptide **12**). It was noteworthy that all ligations proceeded on a dramatically reduced timescale compared with the thiolated counterparts, *β*-SH-Phe[11] or

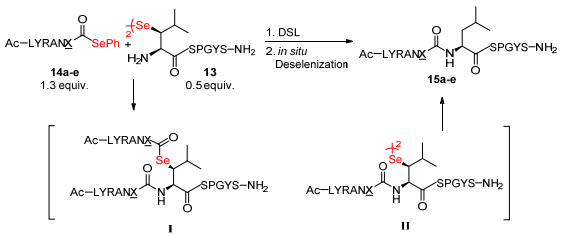
β-SH-Leu[12-13], under NCL conditions. Remarkably, the ligations between diselenide dimer **12** and **13** and the selenoester **14d** and **14e**, containing sterically hindered C-terminal residues Ile and Val, reached completion in 1 h for Phe (Table 2, entries 4 and 5) and 3 h for Leu (Table 1, entries 4 and 5). This compares favorably to ligations of the thiol counterparts, β-SH-Phe (24 h) and β-SH-Leu (8 h), under NCL conditions. Given the similar rates of ligation for the β-Se-Leu and β-Se-Phe peptides (bearing opposite stereochemistry at the β position), it can be inferred that the configuration of the selenium auxiliary does not dramatically affect the rate of ligation. This is in contrast to the corresponding β-SH-Leu amino acid, for which the *S* to *N* acyl shift becomes rate-limiting when the thiol is *anti*-configured.[13]

Without purification, the DSL mixtures were degassed with argon and then subjected to *in situ* deselenization with *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP) and DL-dithiothreitol (DTT) to convert **I-III** into the native peptide products. The deselenization of β-Se-Leu peptides proceeded cleanly in 16 h to yield Xaa-Leu peptides **15a-e** in 55-82% yield over two steps after HPLC purification (Table 1). In contrast, the deselenization of β-Se-Phe, was initially accompanied by two diastereomeric, β-hydroxylated peptide byproducts despite extensive degassing (see Figure S3 in Supporting Information). These β-hydroxylated byproducts were also observed during NCL-deselenization reactions between thioesters and the selenopeptide **12** in previous work[30]. In order to suppress the hydroxylated byproducts, we developed a competing oxygen scavenger *p*-carboxybenzyl diselenide **16** which was synthesized in two steps from 4-bromomethylbenzoic acid (Scheme 5). Diselenide **16** was introduced into the reaction mixture prior to deselenization with the hope that the benzylic radical generated upon deselenization would out-compete that of the Phe side chain for oxygen. The radical initiator VA-044 was also added to the deselenization mixture to accelerate the reaction, which was slowed down by the addition of **16**. Thankfully, under the modified conditions, the unwanted β-hydroxylation side-reaction was suppressed from ~ 35% to < 2% as judged by analytical UPLC-MS, and the reactions were complete within 5 minutes when the reactions were performed at 37 °C. Following HPLC purification native peptide products **17a-f** were afforded in 51-74% over the two steps (Table 2). It should be noted that deselenization of the β-Se-Leu peptides can also pushed to completion within 5 min (instead of 16 h) through addition of VA-044 and incubation at 37 °C. However, these conditions are not selective, and will also lead to desulfurization of Cys residues.



Scheme 4. Synthesis of model selenopeptides containing β-selenol amino acid building blocks (A: β-Se-Phe; B: β-Se-Leu) at N-termini.

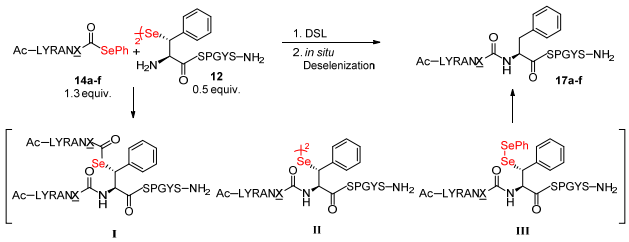
Table 1. Reaction time and yield for one-pot ligation-deselenization at β-Se-Leu.



En-try	Selenoester (**14**)	Ligation Time	Desel. Time*	Yield of **15**
1	**a** X=Ala (A)	10 min	16 h	58%
2	**b** X=Ser (S)	10 min	16 h	69%
3	**c** X=Leu (L)	1 h	16 h	82%
4	**d** X=Ile (I)	3 h	16 h	55%
5	**e** X=Val (V)	3 h	16 h	64%

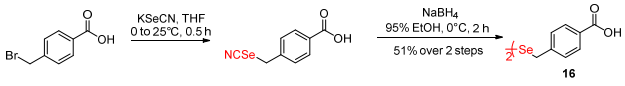
*Deselenization was conducted using TCEP (50 equiv.) and DTT (50 equiv.) at rt.

Table 2. Reaction time and yield for one-pot DSL-deselenization at β-Se-Phe.



Entry	Selenoester (**14**)	Ligation Time	Desel. Time*	Yield of **17**
1	**a** X=Ala (A)	5 min	5 min	68%
2	**b** X=Ser (S)	5 min	5 min	66%
3	**c** X=Leu (L)	5 min	5 min	74%
4	**d** X=Ile (I)	1 h	5 min	62%
5	**e** X=Val (V)	1 h	5 min	51%
6	**f** X=Thr (T)	1 h	5 min	65%

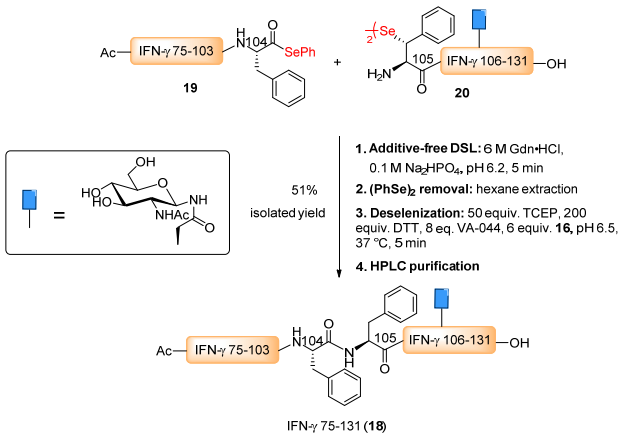
*Deselenization was conducted using TCEP (50 equiv.) and DTT (200 equiv.) in the presence of **16** (4.4 equiv) and VA-044 (1.7 equiv.) at 37°C.



Scheme 5. Synthesis of oxygen scavenger *p*-carboxybenzyl diselenide 16.

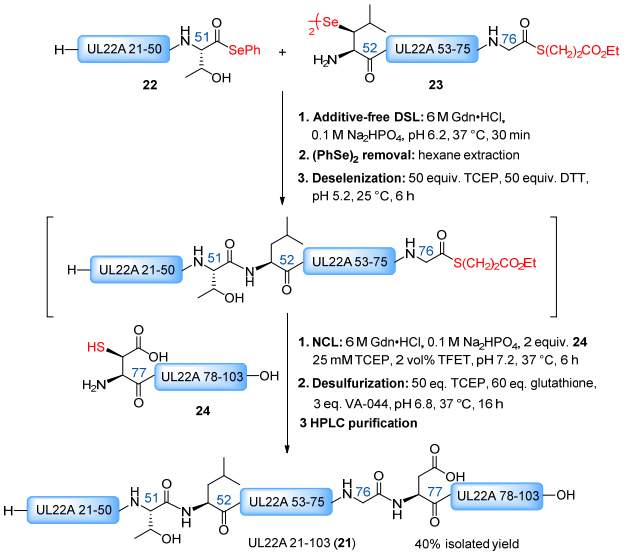
In order to evaluate the robustness of Boc-PMB-(β-Se)Phe building block **2** in ligation towards more complex targets, focus was directed towards the synthesis of a truncated version of human interferon-γ (IFN-γ 75-131, **18**), a cysteine-free 57-mer glycopeptide bearing an *N*-linked β-GlcNAc moiety at

Asn120 (Scheme 6). IFN-γ is a cytokine secreted by T lymphocytes and natural killing cells and imparts crucial antimicrobial and antiviral functions in human innate and acquired immunity. The target IFN-γ 75-131 peptide **18** was disconnected almost evenly into two fragments, selenoester IFN-γ 75-104 **19**, possessing a C-terminal phenylalanine residue, and diselenide dimer IFN-γ 105-131 **20**, containing an N-terminal *β*-Se-Phe. Both IFN-γ fragments were assembled on 2-chlorotrityl chloride resin *via* Fmoc-SPPS, using a per-*O*-acetylated Fmoc-Asn[GlcNAc(OAc)$_3$]-OH building block[39] to install the glycan in diselenide dimer IFN-γ 105-131 **20**. The DSL ligation of selenoester IFN-γ 75-104 **19** and diselenide dimer IFN-γ 105-131 **20** was performed by dissolving peptides **19** and **20** in 6 M Gn·HCl, 0.1 M Na_2HPO_4 aqueous buffer and the final pH adjusted to 6.1-6.4. The DSL reaction proceeded smoothly and to completion within 5 min, as evidenced by the generation of a yellow precipitate of $(PhSe)_2$, and confirmed by UPLC-MS analysis. Upon hexane extraction of $(PhSe)_2$, an aqueous solution of oxygen scavenger **16** was added to the ligation reaction, and the resulting mixture was degassed for 10 minutes under a stream of argon before the addition of radical initiator VA-044. Following treatment with TCEP (50 eq.) and DTT (200 eq.), the deselenization mixture was sealed and incubated at 37 °C, resulting in clean conversion to IFN-γ 75-131 **18** within 5 min without any observed hydroxylated byproducts. The crude reaction mixture was purified by reverse-phase HPLC to afford the final glycopeptide IFN-γ 75-131 **18** in 51% yield over two steps.



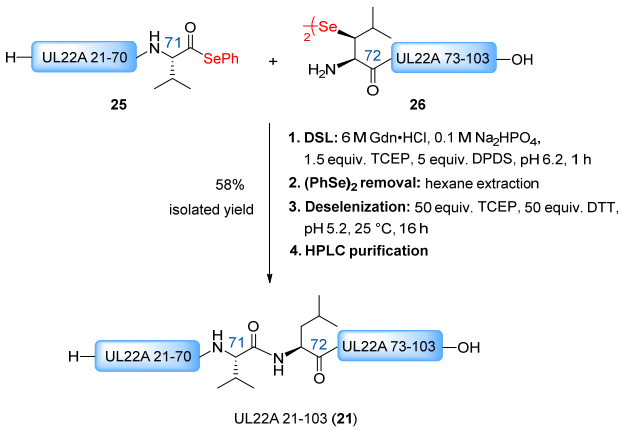
Scheme 6. Assembly of IFN-γ 75-131 (18) *via* one-pot DSL-deselenization chemistry using *β*-Se-Phe.

Next, we investigated the utility of the *β*-Se-Leu building block **5** in the synthesis of a larger target, namely the chemokine-binding protein from human cytomegalovirus called UL22A (**21**) (Scheme 7). UL22A has been shown to bind to RANTES with high affinity[40] and we have recently shown that sulfation of two tyrosine residues leads to significant improvements in affinity[41]. We first attempted to perform a one-pot three-fragment kinetically-controlled ligation starting with DSL (using *β*-Se-Leu) followed by NCL (*β*-SH-Asp ligation[9]). Specifically, the full-length protein was disconnected into N-terminal fragment UL22A 21-51 as a C-terminal phenylselenoester **22**, bifunctional middle fragment UL22A 52-76 as diselenide thioester dimer **23** and C-terminal fragment UL22A 77-103 as thiolated peptide bearing an N-terminal *β*-SH-Asp **24**. UL22A 21-51 selenoester **22** was first ligated to UL22A 52-76 diselenide dimer **23** under DSL conditions (6 M Gn·HCl, 0.1 M Na_2HPO_4 aqueous buffer at a final pH of 6.1-6.4). Incubation of the reaction at 37 °C allowed the ligation to proceed to completion within 30 min (as judged by UPLC-MS analysis); the superior kinetics of DSL meant that no ligation to the thioester at Gly-76 was oberved, despite the steric accessibility of this site. After hexane extraction of $(PhSe)_2$, and deselenization, the UL22A 77-103 fragment **24** was added to the same Eppendorf tube together with TCEP (25 mM) and trifluoroethanethiol (TFET, 2 vol.%) as a thiol additive[42]. Finally, a standard radical desulfurization was performed to convert *β*-SH-Asp into native Asp, which provided UL22A 21-103 (**21**) in 40% yield over 4 steps after HPLC purification.



Scheme 7. One-pot three-component kinetically-controlled assembly of UL22A 21-103 (21) *via* DSL at *β*-Se-Leu followed by NCL at *β*-thiol-Asp.

In order to improve the efficiency and yield for the assembly of UL22A, the target protein was subsequently re-disconnected into two fragments, selenoester UL22A 21-71 **25** and diselenide dimer UL22A 72-103 **26**, which we hoped would enable the synthesis of UL22A *via* a single DSL reaction (Scheme 8). Unfortunately, on this occasion the two fragments did not ligate under standard DSL conditions, which we attributed to steric hinderance at the Val-Leu junction i.e. a combination of a large diselenide dimer fragment and a bulky valine selenoester. In order to test this hypothesis, we performed an 'additive' DSL reaction[41]. Specifically, a combination of $(PhSe)_2$ and TCEP was included in the ligation mixture to reduce diselenide **26.** It is noteworthy that under these conditions, 5-8% of diselenide dimer **26** was deselenized due to the presence of TCEP. However, the ligation proceeded smoothly, providing the target UL22A protein in 58% yield following hexane extraction, deselenization and HPLC purification. The need for additives in this case (and not in the model Val-Leu ligation) highlights the fact that protein ligation reactions are strongly dependent on the specific nature of the fragments, not only on the sterics of the reactive termini.



Scheme 8. Alternative one-pot assembly of UL22A 21-103 (21) through a single DSL reaction at *β*-Se-Leu.

CONCLUSION

In summary, we have demonstrated the feasibility of a general synthetic route towards two suitably protected *β*-selenoamino acids, *β*-Se-Phe and *β*-Se-Leu, from a single commercially available precursor, Garner's aldehyde. We have demonstrated the utility of *β*-Se-Phe and *β*-Se-Leu in rapid DSL with a range of peptidyl aryl selenoesters. Notably, the *β*-hydroxylation by-products previously observed during the deselenization of *β*-Se-Phe were circumvented by the introduction of a novel radical scavenger *p*-carboxybenzyl diselenide. Furthermore, *β*-Se-Phe and *β*-Se-Leu were successfully applied to the one-pot assembly of a fragment of human interferon-γ and the chemokine-binding protein, UL22A. Future work in our group will aim to build on the toolbox of selenoamino acids that can be used in DSL to further expand the scope of this methodology as well as the application of these DSL with these selenoamino acids to larger protein targets.

EXPERIMENTAL SECTION

General Procedures

^{1}H NMR and ^{13}C{^{1}H} NMR spectra were recorded at 300 K using a Bruker Avance DPX 400 spectrometer. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ (δ 7.26 [^{1}H], 77.2 [^{13}C]) and DMSO-d_6 (δ 2.50 [^{1}H], 39.52 [^{13}C]). ^{1}H NMR data is reported as chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets), relative integral, coupling constant (*J* Hz) and assignment where possible. ^{13}C{^{1}H} NMR data is reported as chemical shift (δ). ^{1}H NMR assignments were made on the basis of COSY spectra where applicable. Low-resolution mass spectra were recorded on a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. High resolution ESI mass spectra were measured on a BrukerDaltonics Apex Ultra 7.0 T fourier transform mass spectrometer (FTICR). MALDI-TOF mass spectra were measured on a Bruker autoflex speed MALDI-TOF instrument using a matrix of sinapinic acid in water/acetonitrile (7:3 *v/v*) containing 0.1 vol% TFA in linear mode. Infrared (IR) absorption spectra were recorded on a Bruker ALPHA Spectrometer with Attenuated Total Reflection (ATR) capability, using OPUS 6.5 software. Optical rotations of enantioenriched compounds were recorded on a Perkin–Elmer 341 polarimeter at 589 nm (sodium D line) with a cell path length of 1 dm. Concentrations are reported as g/100 mL.

UPLC-MS was performed on a Shimadzu LC-MS 2020 system equipped with a Nexera X2 LC-30AD pump and a Nexera X2 SPD-M30A diode array detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Peptides were analyzed using an Acquity UPLC BEH 1.7 µm (C18) 2.1 x 50 mm column at a flow rate of 0.6 mL min^{-1} using a mobile phase of 0.1% formic acid (FA) in water (Solvent A) and 0.1% FA in acetonitrile (Solvent B) and a linear gradient of 0-30% B over 8 min or 0-50% B over 8 min or 0-70% B over 8 min.

Analytical reverse-phase UPLC was performed on a Waters Acquity UPLC system equipped with a PDA eλ detector (λ = 210 – 400 nm), Sample Manager FAN and Quaternary Solvent Manager (H-class) modules at 30 °C. Peptides were analyzed using a Waters Acquity UPLC BEH 1.7 µm 2.1 x 50 mm column (C18) at a flow rate of 0.6 mL min^{-1} using a mobile phase composed of 0.1% trifluoroacetic acid (TFA) in H_2O (Solvent A) and 0.1% trifluoroacetic acid (TFA) in acetonitrile (Solvent B) and a linear gradient as specified. Chromatograms were analyzed using Waters Empower software.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 214, 230 and 280 nm. Model peptides, UL22A fragments and Interferon-γ fragments were purified on a Waters Sunfire 5 µm (C18) 19 x 150 mm preparative column operating at a flow rate of 7 mL min^{-1}. UL22A proteins and Interferon-γ proteins were purified on a XBridge Peptide BEH 5 µm (C18) 300 Å 10 × 250 mm semi-preparative column operating at a flow rate of 4 mL min^{-1}. Preparative HPLC used a mobile phase of 0.1% trifluoroacetic acid in water (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B) or 0.1% formic acid (FA) in water (Solvent A) and 0.1% FA in acetonitrile (Solvent B) and a linear gradient as specified. Ligation yields were adjusted to account for the removal of aliquots for reaction monitoring (e.g. UPLC-MS, pH measurement).

Materials

Commercially available materials were purchased from Merck, Sigma Aldrich, AK Scientific Inc or Mimotopes and used without further purification. Amino acids, coupling reagents and resins were obtained from PCAS Biomatrix Inc, Novabiochem or GL Biochem. *N*,*N*-dimethylformamide (DMF) was obtained as peptide synthesis grade from Merck or Labscan. Dichloromethane was purchased from Merck. Thin layer chromatography was performed on Merck TLC silica gel 60 0.25 nm F524 silica plates and visualized by UV light at 254 nm or using vanillin or ninhydrin stain. Flash column chromatography was performed on 230-400 mesh silica gel from Grace. Solid phase peptide synthesis was conducted in polypropylene syringes from Torviq containing Teflon frits. A stirring hotplate with round bottom flask adapter was used for large-scale reactions that required heating. An Eppendorf tube heating block (Benchmark) was used for ligation reactions that required heating.

Synthesis of *β*-Selenoamino acid Building Blocks 2 and 5:

*(2R,3S)-2-((tert-butoxycarbonyl)amino)-3-phenyl-3-selenocyanatopropanoic acid (**4**).* To a solution of **3**[30] (1.88 g, 5.29 mmol) in a mixed solvent of MeCN/H_2O 1:1 (v/v, 40 mL) was added (diacetoxyiodo)benzene (8.52 g, 26.45 mmol) and TEMPO (0.41 g, 2.7 mmol) in one portion. The orange solution was stirred at 25 °C for 3 h and then concentrated under a stream of N_2 followed by lyophilization. The crude product was purified by flash column chromatography (96:2:2 CH_2Cl_2:MeOH:AcOH) to afford carboxylic acid **4** (1.39 g, 71%) as a yellow oil. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.35 (m, 5H, Ar-H),

5.27 (m, 1H, NH), 5.20 (m, 1H, benzylic CH), 4.97-4.85 (m, 1H, CH_α), 1.45 (s, 9H, Boc). The characterization data is in agreement with previously reported literature data by Malins et al. [30]

*(2R,3S)-2-((tert-butoxycarbonyl)amino)-3-((4-methoxybenzyl)selanyl)-3-phenylpropanoic acid (**2**).* To a solution of compound **4** (1.38 g, 3.73 mmol) in THF (26 mL) at 0 °C was added $NaBH_4$ (0.28 g, 7.5 mmol) in 95% EtOH (3.7 mL). The reaction was stirred for 1 h at 0 °C before the addition of PMB-Cl (2.02 mL, 14.92 mmol) and degassed 2 M NaOH solution (10 mL). The reaction mixture was warmed to room temperature overnight and poured into 1 M citric acid (200 mL). The aqueous layer was extracted with EtOAc (3 × 200 mL), and the combined organic layers were washed with water (3 × 200 mL) and brine (300 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified *via* flash column chromatography (3:10 to 100:0 EtOAc/hexane v/v + 0.1% AcOH), affording the final building block **2** (1.25 g, 72%) as a pale-yellow oil. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 7.28-7.26 (m, 5H, Ar-H (phenyl)), 7.11 (d, 2H, J = 9.0 Hz, Ar-H (PMB)), 6.78 (d, 2H, J = 6.0 Hz, Ar-H (PMB)), 5.01-4.98 (m, 1H, NH), 4.83 (m, 1H, CH_α), 4.33 (m, 1H, benzylic CH), 3.78 (s, 3H, OCH_3), 3.73-3.54 (m, 2H, PMB benzylic CH_2), 1.41 (s, 9H, Boc). The characterization data is in agreement with previously reported literature data by Malins et al.[30]

*tert-Butyl (R)-4-((R)-1-hydroxy-2-methylpropyl)-2,2-dimethyloxazolidine-3-carboxylate (**6**).* Magnesium turnings (0.27 g, 11 mmol) were dried in a 3-necked round bottom flask *in vacuo* with heat and stirring for 0.5 h before the addition of dry Et_2O (2 mL). A spatula tip of iodine was quickly added to the above mixture and the resulting brown suspension was stirred vigorously at 25 °C for 30 min. Isopropyl bromide (0.52 mL, 5.5 mmol) in Et_2O (3 mL) was added to the mixture dropwise at 0 °C, and the mixture was subsequently stirred at 40 °C for 1 h. A solution of Garner's aldehyde **1** (970 mg, 4.25 mmol) in Et_2O (5 mL) was added dropwise to the Grignard reagent at 0 °C. The reaction mixture was continually stirred at 0 °C and allowed to warm to 25 °C over 5 h. The reaction was quenched with saturated aq. NH_4Cl solution (8 mL) at 0 °C before extraction with EtOAc (20 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (15:85, EtOAc/Hexane, R_f = 0.32) to afford *syn*-alcohol **6** as a white solid (630 mg, 2.29 mmol, 54%). $[\alpha]_D^{20}$ = +54.5° (c 0.33, CH_2Cl_2); **m.p.** 82-83 °C; **IR** (ν/cm^{-1}, film) 3399, 2963, 2935, 2875, 1657, 1400, 1366, 1248, 1172, 1108, 1058, 1018, 865; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 4.04 (br s, 1H, CHNH), 3.94-3.91 (m, 1H, CH_{2a}), 3.76-3.74 (m, 1H, CH_{2b}), 3.50-3.48 (m, 1H, CHOH), 1.70-1.64 (m, 1H, $CH(CH_3)_2$), 1.59-1.44 (m, 15H, $CH(CH_3)_2$, $C(CH_3)_3$), 1.02 (d, 3H, J = 6.8 Hz, $CH(CH_3)_{2a}$), 0.89 (d, 3H, J = 6.7 Hz, $CH(CH_3)_{2b}$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 75 MHz) δ 155.7, 99.1, 79.4, 77.3, 65.8, 45.4, 29.9, 29.3, 28.5, 19.1, 18.7, 17.6; **HRMS** (ESI$^+$): m/z calcd. For $C_{14}H_{27}NO_4Na$ $[M+Na]^+$ 296.1832, found 296.1833.

*tert-Butyl ((2R,3R)-1,3-dihydroxy-4-methylpentan-2-yl)carbamate (**7**).* 0.5 M aq. HCl (20 mL) was added slowly to a solution of *syn* alcohol **6** (2.54 g, 9.29 mmol) in THF (380 mL). The mixture was stirred at 25 °C for 24 h before being quenched by the addition of $NaHCO_3$ (1.7 g, 20 mmol), followed by co-evaporation with toluene to dryness. The crude material was diluted with EtOAc (100 mL), filtered through cotton wool and concentrated in vacuo. The crude product was purified by flash column chromatography (1:1, v/v, EtOAc/Hexane, R_f = 0.34) to obtain *syn* diol **7** as a yellow oil (2.09 g, 8.95 mmol, 96%). $[\alpha]_D^{20}$ = -15.1° (c 0.37, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 3377, 2961, 2927, 2874, 1685, 1503, 1392, 1366, 1258, 1166, 1064, 1012, 803; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.28 (br s, 1H, NH), 3.77-3.72 (m, 3H, CH_2, CHNH), 3.48-3.46 (m, 1H, CHOH), 3.12 (br s, 2H, OH), 1.77-1.68 (m, 1H, $CH(CH_3)_2$), 1.43 (s, 9H, $C(CH_3)_3$), 0.99 (d, 3H, J = 6.6 Hz, $CH(CH_3)_{2a}$), 0.90 (d, 3H, J = 6.7 Hz, $CH(CH_3)_{2b}$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100MHz) δ 156.6, 79.8, 78.0, 65.4, 52.4, 31.1, 28.5, 19.1, 18.9; **HRMS** (ESI$^+$): m/z calcd. for $C_{11}H_{23}NO_4Na$ $[M+Na]^+$ 256.1523, found 256.1524.

*tert-Butyl-((2R,3R)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxy-4-methylpentan-2-yl)carbamate (**8**).* *syn*-Diol **7** (2.08 g, 8.95 mmol) and imidazole (1.22 g, 17.9 mmol) were dissolved in dry CH_2Cl_2 (8.5 mL) followed by addition of tert-butyldimethylsilyl chloride (1.62 g, 10.7 mmol) in one portion at 0 °C. The mixture was warmed to room temperature and the reaction stirred for 12 h. After complete consumption of the starting material, the reaction was quenched with saturated aq. NH_4Cl solution (20 mL) and extracted with CH_2Cl_2 (60 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (5:95, EtOAc/Hexane, R_f = 0.28) to give TBS-protected *syn* diol **8** as a yellow oil (3.00 g, 8.66 mmol, 97%). $[\alpha]_D^{20}$ = -26.4° (c 0.54, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 3444, 2956, 2929, 2858, 1716, 1692, 1498, 1472, 1390, 1366, 1254, 1168, 1097, 1017, 835, 776; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.19 (d, 1H, J = 8.5 Hz, NH), 3.97 (dd, 1H, J = 3.4 Hz, 10.2 Hz, CH_{2a}), 3.78 (dd, 1H, J = 2.3 Hz, 10.2 Hz, CH_{2b}), 3.70 (m, 1H, CHNH), 3.51 (m, CHOH), 1.74-1.69 (m, 1H, $CH(CH_3)_2$), 1.43 (s, 9H, $C(CH_3)_3$), 0.99 (d, 3H, J = 6.6 Hz, $CH(CH_3)_{2a}$), 0.91-0.87 (m, 12H, $SiC(CH_3)_3$, $CH(CH_3)_{2b}$), 0.07 (s, 6H, $Si(CH_3)_2$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100MHz) δ 156.0, 79.3, 67.1, 51.3, 30.9, 28.5, 26.0, 19.1, 19.0, 18.3, -5.5; **HRMS** (ESI$^+$): m/z calcd. for $C_{17}H_{37}NO_4SiNa$ $[M+Na]^+$ 370.2384, found 370.2386.

*tert-Butyl-((2R,3S)-1-((tert-butyldimethylsilyl)oxy)-4-methyl-3-selenocyanatopentan-2-yl)carbamate (**9**).* Triethylamine (1.80 mL, 13.0 mmol) and mesyl chloride (1.04 mL, 10.4 mmol) were slowly added into a solution of TBS-protected *syn* diol **8** (3.00 g, 8.66 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 40 min before being quenched with saturated aq. NH_4Cl solution (40 mL) followed by extraction with CH_2Cl_2 (60 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the crude mesylate as a pale yellow solid. The crude mesylate and potassium selenocyanate (18.71 g, 129.9 mmol) were dissolved in dry MeCN (40 mL) and stirred at 65 °C for 24 h. The reaction mixture was concentrated in vacuo, diluted with CH_2Cl_2 (50 mL) and poured into water (70 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (70 mL × 2). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (4:96, EtOAc/Hexane, R_f = 0.31) to yield selenocyanate **9** as a yellow oil (2.26 g, 5.19 mmol, 60%). $[\alpha]_D^{20}$ = -52.3° (c 0.34, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 2959, 2930, 2885, 2858, 2149 (SeCN), 1715, 1490, 1391, 1366, 1255, 1170, 1103, 1046, 836, 779; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.27 (d, 1H, J = 8.6 Hz, NH), 4.08-4.07 (m, 1H, CHNH), 3.87 (dd, 1H, J = 2.2 Hz, 10.7 Hz, CH_{2a}), 3.73 (dd, 1H, J = 1.92 Hz, 10.6 Hz, CH_{2b}), 3.51-3.47 (m, 1H, CHSeCN), 2.11-2.02 (m, 1H, $CH(CH_3)_2$), 1.47 (s, 9H, $C(CH_3)_3$), 1.30-1.28 (m, 3H, $CH(CH_3)_{2a}$), 1.13-1.12 (d, 3H, J = 6.7 Hz, $CH(CH_3)_{2b}$) 0.91 (s, 9H, $SiC(CH_3)_3$), 0.10 (s, 3H, $Si(CH_3)_{2a}$), 0.09 (s, 3H, $Si(CH_3)_{2b}$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz) δ 155.1, 106.3(SeCN), 80.4, 64.1, 62.7, 51.2, 33.2, 29.8, 28.5, 26.1, 21.3, 18.6, -5.2, -5.3; **HRMS** (ESI$^+$): m/z calcd. for $C_{18}H_{36}N_2O_3SeSiNa$ $[M+Na]^+$ 459.1552, found 459.1554.

*tert-Butyl ((2R,3S)-1-hydroxy-4-methyl-3-selenocyanatopentan-2-yl)carbamate (**10**)*. A solution of tetrabutylammonium fluoride (5.02 mL, 5.02 mmol, 1 M in THF) was added dropwise to a solution of TBDMS ether **9** (1.82 g, 4.18 mmol) in dry THF (32 mL) and the reaction mixture was stirred at 25 °C for 30 min. $CaCO_3$ (1.06 g), DOWEX® 50W X8 (200-400 mesh, 3.11 g) and MeOH (7.53 mL) were added to the reaction flask and the reaction was stirred for 1 h. The resulting suspension was filtered through celite, washed with MeOH and concentrated *in vacuo*. The crude product was purified by flash column chromatography (3:7, EtOAc/Hexane, R_f = 0.32) to afford **10** as a yellow oil (0.97 g, 3.03 mmol, 72%). $[\alpha]_D^{20}$ = -34.4° (c 0.28, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 3421, 2967, 2932, 2155 (SeCN), 1687, 1505, 1392, 1368, 1250, 1168, 1052, 803; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.33 (d, 1H, J = 7.8 Hz, NH), 4.07 (m, 1H, CHNH), 3.97 (dd, 1H, J = 3.3 Hz, 11.2 Hz, CH_{2a}), 3.83 (dd, 1H, J = 2.4 Hz, 11.3 Hz, CH_{2b}), 3.55-3.52 (m, 1H, CHSeCN), 2.35 (br s, 1H, OH), 2.18-2.11 (m, 1H, $CH(CH_3)_2$), 1.46 (s, 9H, $C(CH_3)_3$), 1.21 (d, 3H, J = 6.6 Hz, $CH(CH_3)_{2a}$), 1.14 (d, 3H, J = 6.6 Hz, $CH(CH_3)_{2b}$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz) δ 155.4, 105.2 (SeCN), 80.5, 63.2, 62.2, 52.2, 31.9, 28.5, 21.6, 21.5; **HRMS** (ESI$^+$): m/z calcd. for $C_{12}H_{22}N_2O_3SeNa$ $[M+Na]^+$ 345.0688, found 345.0688.

*(2R,3S)-2-((tert-butoxycarbonyl)amino)-4-methyl-3-selenocyanatopentanoic acid (**11**)*. Primary alcohol **10** (158 mg, 0.490 mmol) was dissolved in a mixed solvent of MeCN/H_2O 1:1 (v/v, 4 mL) before the addition of (diacetoxyiodo)benzene (0.79 g, 2.5 mmol) and TEMPO (38 mg, 0.25 mmol) in one portion. The orange solution was stirred at 25 °C for 3 h and then concentrated under a stream of N_2 followed by lyophilization. The crude product was purified by flash column chromatography (96:2:2, CH_2Cl_2:MeOH:AcOH, R_f = 0.33) to provide **11** as a yellow oil (149 mg, 0.44 mmol, 90%). $[\alpha]_D^{20}$ = -42.7° (c 0.33, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 2965, 2923, 2852, 2153 (SeCN), 1705, 1503, 1457, 1393, 1369, 1253, 1160, 1057, 1019, 856, 801; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.52 (d, 1H, J = 5.5 Hz, NH), 4.87-4.74 (m, 1H, CHNH), 3.49-3.47 (m, 1H, CHSeCN), 2.30-2.22 (m, 1H, $CH(CH_3)_2$), 1.47 (s, 9H, $C(CH_3)_3$), 1.21 (d, 6H, J = 5.9 Hz, $CH(CH_3)_2$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz) δ 172.5 (COOH), 155.4, 102.8 (SeCN), 81.7, 60.9, 55.2, 29.8, 28.4, 21.2; **HRMS** (ESI$^+$): m/z calcd. For $C_{12}H_{20}N_2O_4SeNa$ $[M+Na]^+$ 359.0481, found 359.0481.

*(2R,3S)-2-((tert-Butoxycarbonyl)amino)-3-((4-methoxybenzyl)selanyl)-4-methylpentanoic acid (**5**)*. To a solution of **11** (209 mg, 0.870 mmol) in THF (6 mL) was added a solution of $NaBH_4$ (65 mg, 1.73 mmol) in 95% EtOH (0.86 mL) at 0 °C under an argon atmosphere. After stirring the reaction mixture at 0 °C for 1 h, p-methoxybenzyl chloride (0.542 g, 3.46 mmol) and aq. 2 M NaOH (degassed, 2.31 mL) were added while maintaining the reaction temperature at 0 °C. The mixture was allowed to warm up to 25 °C and stirred for a further 16 hours. The reaction was quenched with AcOH (2 mL) and co-evaporated with toluene to dryness. The crude product was purified by flash column chromatography (97:1:2, CH_2Cl_2:MeOH:AcOH, R_f = 0.35) to provide *β*-selenoleucine building block **5** as a yellow oil (224 mg, 0.520 mmol, 60%). $[\alpha]_D^{20}$ = -72.7° (c 0.29, CH_2Cl_2); **IR** (ν/cm^{-1}, film) 2960, 2926, 2853, 1715, 1610, 1511, 1458, 1393, 1368, 1248, 1171, 1035, 831; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.19 (d, 2H, J = 8.5 Hz, Ar-H), 6.80 (d, 2H, J = 8.0 Hz, Ar-H), 5.19 (d, 1H, J = 7.1 Hz, NH), 4.66 (s, 1H, CHNH), 3.77 (s, 3H, OCH_3), 3.75-3.74 (m, 2H, CH_2), 2.61 (m, 1H, CHSe), 2.03-2.01 (m, 1H, $CH(CH_3)_2$), 1.45 (s, 9H, $C(CH_3)_3$), 0.97 (d, 6H, $CH(CH_3)_2$); **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz) δ 175.2 (COOH), 158.7, 155.5, 130.3, 114.1, 80.6, 56.2, 55.4, 51.6, 29.8, 28.9, 28.4, 21.4, 21.0; **HRMS** (ESI$^+$): m/z calcd. for $C_{19}H_{29}NO_5SeNa$ $[M+Na]^+$ 454.1103, found 454.1101.

*Synthesis of 4,4'-(diselanediylbis(methylene))dibenzoic acid (**16**)*. 4-(Bromomethyl)benzoic acid (0.510 g, 2.37 mmol) was dissolved in dry THF (6 mL) and the solution was cooled to 0 °C. The resulting solution was treated with potassium selenocyanate (0.510 g, 3.55 mmol) in one portion at 0 °C and stirred at 25 °C for 0.5 h. The reaction mixture was diluted with 100 mL of a mixed solvent (40:60:2, v/v/v, ethyl acetate:hexane:AcOH) and filtered through celite. The filtrate was concentrated, redissolved in 95% EtOH (2 mL) and cooled to 0 °C before the addition of a solution of $NaBH_4$ (0.13 g, 3.6 mmol) in 95% EtOH (2 mL) at 0 °C. The reaction was then stirred at 0 °C under an argon atmosphere for 2 h before being quenched by 1 M HCl until pH 1-2. The yellow precipitate was filtered out, washed with H_2O (3 mL × 2) and then dried *in vacuo* overnight to afford **16** as a pale yellow solid (0.260 g, 1.21 mmol, 51%). **^{1}H NMR** (DMSO-d_6, 400 MHz) δ 12.88 (br s, 2H, COOH), 7.88 (d, 4H, J = 8.4 Hz, Ar-H), 7.33 (d, 4H, J = 8.4 Hz, Ar-H), 4.01 (s, 4H, CH_2); **^{13}C{^{1}H} NMR** (DMSO-d_6, 125 MHz) δ 167.1, 144.4, 129.4, 129.3, 129.1, 30.9; **LRMS** (ESI$^+$): m/z calcd. for $C_{16}H_{14}NaO_4Se_2$ $[M+Na]^+$ 452.9, found 453.1. The ^{1}H NMR data is in agreement with that reported by Lin *et al.*[43].

General procedures for solid phase peptide synthesis (SPPS)

Preloading of 2-chlorotrityl-chloride resin: 2-chlorotrityl-chloride resin (1.5 mmol/g loading capacity) was swollen in dry CH_2Cl_2 for 30 min and then washed thoroughly (CH_2Cl_2 × 5, DMF × 5, CH_2Cl_2 × 5 and DMF × 5, ~ 3 mL per wash). A solution of Fmoc-AA-OH (2 equiv.) and iPr_2NEt (4 equiv.) in dry DMF (final concentration 0.2 M) was added and the resin was agitated for 3-16 h at 25 °C (or 16 h for *β*-branched amino acids). Following a thorough wash (DMF × 5, CH_2Cl_2 × 5, DMF × 5 and CH_2Cl_2 × 5), the resin was treated with a capping solution (17:2:1, v/v/v, CH_2Cl_2/MeOH/iPr_2NEt, 3 mL) for 30 min at 25 °C. The resin was washed again (CH_2Cl_2 × 5, DMF × 5, CH_2Cl_2 × 5 and DMF × 5) and subjected to iterative peptide assembly (Fmoc-SPPS).

Preloading of Chemmatrix® Trityl-OH resin: Chemmatrix® Trityl-OH resin (0.3 mmol/g loading capacity) was swollen in dry CH_2Cl_2 for 30 min and then washed thoroughly (CH_2Cl_2 × 5, DMF × 5 and CH_2Cl_2 × 5). An activating solution (10 vol% $SOCl_2$ in dry CH_2Cl_2, 4 mL) was then added and the resin was shaken for 2 h at 25 °C. After filtration, the resin was washed with dry CH_2Cl_2 (10 × 3 mL) and 10 vol% iPr_2NEt in dry CH_2Cl_2 (10 mL). Then a solution of Fmoc-AA-OH (4 equiv.) and iPr_2NEt (8 equiv.) in dry DMF (final concentration 0.2 M) was added to the resin and the mixture was agitated for 3 h (16 h for *β*-branched amino acids). The resin was washed again (DMF × 5, CH_2Cl_2 × 5, DMF × 5 and CH_2Cl_2 × 5), and treated with a solution of CH_2Cl_2/MeOH/iPr_2NEt (17:2:1 v/v/v, 3 mL) for 30 min at 25 °C. The resin was finally washed (CH_2Cl_2 × 5, DMF × 5, CH_2Cl_2 × 5 and DMF × 5) and subjected to iterative peptide assembly (Fmoc-SPPS).

Preloading of Rink amide resin: Fmoc-Rink amide resin (0.8 mmol/g loading capacity) was swollen in dry CH_2Cl_2 for 30 min and then washed thoroughly (CH_2Cl_2 × 5, DMF × 5 and CH_2Cl_2 × 5). The N-terminal Fmoc group was removed by piperidine (20 vol%) in DMF (3 mL, 2 x 5 min). The resin was washed thoroughly (DMF × 5, CH_2Cl_2 × 5, DMF × 5) before a solution of Fmoc-AA(PG)-OH (4 equiv.), *N,N'*-Diisopropylcarbodiimide (DIC, 4 equiv.), and Oxyma (4 equiv.) in DMF (final concentration 0.3 mmol/mL) was added and the mixture was agitated for 3 h (16 h for *β*-branched amino acids). The loaded resin was

washed again (CH_2Cl_2 × 5, DMF × 5, CH_2Cl_2 × 5 and DMF × 5) and subjected to iterative peptide assembly (Fmoc-SPPS).

Estimation of amino acid loading: The resin was treated with 20% piperidine/DMF (2 × 3 mL, 5 min) and 50 µL of the combined deprotection solution was diluted to 10 mL using 20% piperidine/DMF in a 10 mL volumetric flask. The UV absorbance of the resulting piperidine-fulvene adduct was measured (λ = 301 nm, ε = 7800 M^{-1} cm^{-1}) to estimate the amount of amino acid loaded onto the resin.

Iterative peptide assembly (Fmoc-SPPS): *Deprotection*: The resin was treated with 20% piperidine/DMF (2 × 3 mL, 5 min) and washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). *General amino acid coupling*: A solution of protected amino acid (4 equiv.), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 4 equiv.) and 4-methylmorpholine (NMM, 8 equiv.) in DMF (final concentration 0.1 M) was added to the resin. After 1 h, the resin was washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). *Capping*: Acetic anhydride/pyridine (1:9 v/v) was added to the resin (3 mL). After 3 min the resin was washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL). *Cleavage*: A mixture of TFA, triisopropylsilane (TIS) and water (95:5:5 v/v/v) was added to the resin. After 3 h, the resin was washed with TFA (3 × 2 mL). *Work-up*: The combined cleavage and TFA wash solutions were concentrated under a stream of nitrogen to less than 5 mL. Cold diethyl ether (30 mL) was added to precipitate the peptide, the suspension cooled at -20 °C and centrifuged at 4000 rpm for 5 min. The pellet was then dissolved in water containing 0.1% TFA or FA, filtered, purified by preparative HPLC and analyzed by UPLC-MS and ESI mass spectrometry.

Automated solid-phase peptide synthesis

Automated Fmoc-SPPS was carried out on a Biotage Initiator+ Alstra microwave peptide synthesizer equipped with an inert gas manifold. General synthetic protocols for Fmoc-deprotection and capping were carried out in accordance with the manufacturer's specifications. Standard amino acid couplings were performed for 20 min at 50 °C under microwave irradiation in the presence of amino acid (0.3 M in DMF), Oxyma (0.5 M in DMF) and DIC (0.5 M in DMF). Peptide cleavage and work-up were carried out as described above for manual SPPS.

Coupling conditions for synthetic amino acids:

Coupling of Boc-PMB-(β-Se)Phe (**2**): After the removal of the N-terminal Fmoc group, a solution of PMB-β-Se-Phe **2** (1.2 equiv.), 1-Hydroxy-7-azabenzotriazole (HOAt, 1.2 equiv.) and DIC (1.2 equiv.) in DMF (0.07 M amino acid concentration) was added to the elongated peptide on resin and the reaction was shaken for 16 h at 25 °C. Following filtration, the resin was washed thoroughly (DMF × 5, CH_2Cl_2 × 5, DMF × 5).

Coupling of Boc-PMB-(β-Se)Leu (**5**). A solution of Boc-PMB-(β-Se)Leu **5** (26 mg, 0.06 mmol, 1.2equiv.), HOAt (8 mg, 0.06 mmol, 1.2 equiv.), DIC (9 µL, 0.06 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.1 M amino acid concentration) was added to the resin and the reaction was shaken for 16 h at 25 °C. Following filtration, the resin was washed with DMF (5 × 3 mL), CH_2Cl_2 (5 × 3 mL) and DMF (5 × 3 mL).

Coupling of Fmoc-Asn[GlcNAc(OAc)$_3$]-OH. After the removal of the N-terminal Fmoc group, a solution of peracetylated Fmoc-Asn[GlcNAc(OAc)$_3$]-OH[39] (2 equiv.), (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP, 2 equiv.) and NMM (4 equiv.) in DMF (0.08 M amino acid concentration) was added to the elongated resin-bound peptide and the reaction was shaken for 16 h at 25 °C. Following filtration, the resin was washed thoroughly (DMF × 5, CH_2Cl_2 × 5, DMF × 5).

*Synthesis of H-(β-Se)FSPGYS-NH_2 dimer (**12**)*. A solution of Boc-β-Se(PMB)-Phe-OH **2** (28 mg, 0.060 mmol, 1.2 equiv.), HOAt (8 mg, 0.06 mmol, 1.2 equiv.) and DIC (9 µL, 0.06 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.1 M amino acid concentration) was added to H-SPGYS (0.185 g, 0.05 mmol, 1.0 equiv., side-chain protected) on Rink Amide resin (0.3 mmol/g loading) and the reaction vessel was shaken for 16 h at 25 °C. The PMB-protected selenopeptide was cleaved by treatment with a mixture of TFA, triisopropylsilane (TIS) and water (95:5:5 v/v/v) for 2 h at 25 °C. The resulting peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude peptide was dissolved in TFA (10 mL) and added dropwise into a mixture of DMSO/TFA (1:4 v/v, 10 mL) at 0 °C and the PMB group was cleaved off after 16 h. The crude diselenide peptide solution was concentrated (~ 4 mL), resuspended in cold diethyl ether (0 °C) and centrifuged. The pellet was purified by preparative reverse phase HPLC (10-20% MeCN in H_2O over 40 min, 0.1% TFA, Sunfire prep C18 OBD 5 µm 19 × 150 mm column) and lyophilized to give the desired peptide dimer **12** (13.9 mg, 19 µmol, 38%).

*Synthesis of H-(β-Se)LSPGYS-NH_2 dimer (**13**)*. A solution of Boc-β-Se(PMB)-Leu-OH **5** (26 mg, 0.06 mmol, 1.2 equiv.), HOAt (8 mg, 0.06 mmol, 1.2 equiv.) and DIC (9 µL, 0.06 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.1 M amino acid concentration) was added to H-SPGYS (0.185 g, 0.05 mmol, 1.0 equiv., side-chain protected and resin-bound) on Rink Amide resin (0.3 mmol/g loading) and the reaction was shaken for 16 h at 25 °C. The PMB-selenopeptide was cleaved by treatment with a mixture of TFA, triisopropylsilane (TIS) and water (95:5:5 v/v/v) for 2 h at 25 °C. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude peptide was dissolved in TFA (10 mL) and added dropwise into a mixture of DMSO/TFA (*v/v*, 1:4, 10 mL) at 0 °C and the PMB group was cleaved off after 16 h. The crude diselenide peptide in solution was concentrated (~ 4 mL), precipitated with cold Et_2O and separated by centrifugation. The resulting pellet was purified by preparative reverse phase HPLC (10-20% B over 40 min, 0.1% TFA, Sunfire prep C18 OBD 5 µm 19 × 150 mm column) and lyophilized to give the desired peptide dimer **13** (12.7 mg, 18 µmol, 36%).

*General procedure for one-pot DSL-deselenization of model peptide **12** with an N-terminal β-Se-Phe with **14a-f***: The diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.00 mg, 1.36 µmol, 0.5 equiv.) and selenoester Ac-LYRANX-SePh **14a-f** (X= Ala, Ser , Leu, Ile, Val, Thr, 3.54 µmol, 1.3 equiv.) were dissolved in ligation buffer (545 µL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) at a concentration of 5 mM with respect to the selenopeptide monomer (6.5 mM with respect to the selenoester) and to a final pH of 6.1-6.4 (without adjustment). The reaction was agitated and allowed to stand at 25 °C. 1 µL aliquots were taken from the reaction mixture at various time points and diluted with 40 µL of Milli-Q H_2O for analytical UPLC-MS and HPLC (Sunfire C18 5 µm 2.1 × 150 mm column) [or UPLC (ACQUITY UPLC BEH C18 1.7µm 2.1 × 50 mm column)] analysis until the diselenide peptide was totally consumed and ligation products were generated. The ligation mixture was washed with hexane (10 × 600 µL) to remove $(PhSe)_2$, and treated with a solution of 4,4'-(diselanediylbis(methylene))-dibenzoic acid **16** (60 µL, 200 mM in 6 M Gn·HCl, 0.1 M Na_2HPO_4 ligation buffer, pH= 6.5). The

resulting yellow mixture was degassed with argon for 10 min. Meanwhile, a solution of TCEP (250 mM, 72 mg, 50 equiv.) and DTT (100 mM, 144 mg, 200 equiv.) was prepared by dissolving the two components in ligation buffer (1 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) and adjusting to pH 6-6.8 with 2 M aq. NaOH. The TCEP/DTT solution was degassed with argon for 10 min. Then, radical initiator 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, 1.5 mg, 4.6 μmol) was added as a solid into the ligation mixture under argon before the addition of the degassed TCEP/DTT solution (605 μL) in one portion. The deselenization reaction was sealed, agitated, and incubated at 37 °C for 5 min. 1 μL of aliquots were taken for analytical UPLC-MS and HPLC or UPLC analysis once the deselenization was completed. The crude one-pot ligation-deselenization product was purified by reverse phase preparative HPLC (15-45% MeCN in H_2O over 40 min, 0.1% TFA, Sunfire prep C18 OBD 5 μm 19 × 150 mm column) and lyophilized to afford the native peptide as a white solid.

*Ac-LYRANAFSPGYS-NH_2 **(17a)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.12 mg, 1.44 μmol, 0.5 equiv.) and selenoester Ac-LYRANA-SePh **14a** (3.32 mg, 3.74 μmol, 1.3 equiv.). The crude ligation- deselenization product was purified and lyophilized to obtain Ac-LYRANAFSPGYS-NH_2 **17a** as a white solid (2.72 mg, 1.96 μmol, 68% over 2 steps).

*Ac-LYRANSFSPGYS-NH_2 **(17b)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.08 mg, 1.42 μmol, 0.5 equiv.) and selenoester Ac-LYRANS-SePh **14b** (3.33 mg, 3.69 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANSFSPGYS-NH_2 **17b** as a white solid (2.62 mg, 1.87 μmol, 66% over 2 steps).

*Ac-LYRANLFSPGYS-NH_2 **(17c)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.26 mg, 1.54 μmol, 0.5 equiv.) and selenoester Ac-LYRANL-SePh **14c** (3.72 mg, 4.00 μmol, 1.3 equiv.). The crude ligation- deselenization product was purified and lyophilized to obtain Ac-LYRANLFSPGYS-NH_2 **17c** as a white solid (3.26 mg, 2.28 μmol, 74% over 2 steps).

*Ac-LYRANIFSPGYS-NH_2 **(17d)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (1.92 mg, 1.31 μmol, 0.5 equiv.) and selenoester Ac-LYRANI-SePh **14d** (3.16 mg, 3.40 μmol, 1.3 equiv.). The crude ligation- deselenization product was purified and lyophilized to obtain Ac-LYRANIFSPGYS-NH_2 **17d** as a white solid (2.32 mg, 1.62 μmol, 62% over 2 steps).

*Ac-LYRANVFSPGYS-NH_2 **(17e)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.10 mg, 1.43 μmol, 0.5 equiv.) and selenoester Ac-LYRANV-SePh **14e** (3.41 mg, 3.72 μmol, 1.3 equiv.). The crude ligation- deselenization product was purified and lyophilized to obtain Ac-LYRANVFSPGYS-NH_2 **17e** as a white solid (2.07 mg, 1.46 μmol, 51% over 2 steps).

*Ac-LYRANTFSPGYS-NH_2 **(17f)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)FSPGYS-NH_2 **12** (2.02 mg, 1.38 μmol, 0.5 equiv.) and selenoester Ac-LYRANT-SePh **14f** (3.29 mg, 3.58 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANTFSPGYS-NH_2 **17f** as a white solid (2.54 mg, 1.79 μmol, 65% over 2 steps).

*General procedure for one-pot DSL-deselenization of model peptide **13** with an N-terminal β-Se-Leu with **14a-e***: The diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (2.00 mg, 1.43 μmol, 0.5 equiv.) and selenoester Ac-LYRANX-SePh **14a-e** (X= Ala, Ser , Leu, Ile, Val, 3.72 μmol, 1.3 equiv.) were dissolved in ligation buffer (570 μL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) to the concentration of 5 mM with respect to the selenopeptide monomer (6.5 mM with respect to the selenoester) and to a final pH of 6.1-6.4 (without adjustment). The reaction was agitated and allowed to stand at 25 °C. 1 μL aliquots were taken from the reaction mixture at various time points and diluted with 40 μL of Milli-Q H_2O for analytical UPLC-MS and HPLC (Sunfire C18 5 μm 2.1 × 150 mm column) [or UPLC (ACQUITY UPLC BEH C18 1.7μm 2.1 × 50 mm column)] analysis until the diselenide peptide was totally consumed and ligation products were generated. The ligation mixture was washed with hexane (10 × 600 μL) to remove $(PhSe)_2$ and degassed with argon for 10 min. Meanwhile, a solution of TCEP (250 mM, 72 mg, 50 equiv.) and DTT (250 mM, 36 mg, 50 equiv.) was prepared by dissolving the two components in ligation buffer (1 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) and adjusting to pH 5-5.5 with 2 M aq. NaOH. The TCEP/DTT solution was degassed with argon for 10 min before adding (570 μL) into the ligation mixture in one portion. The deselenization reaction was sealed, agitated, and kept on bench at room temperature for 16 h. 1 μL of aliquots were taken for analytical UPLC-MS and HPLC or UPLC analysis until the completion of deselenization. The crude one-pot ligation-deselenization product was purified by reverse phase preparative HPLC (15-45% B over 40 min, 0.1% TFA, Sunfire prep C18 OBD 5 μm 19 × 150 mm column) and lyophilized to afford the native peptide product as a white solid.

*Ac-LYRANALSPGYS-NH_2 **(15a)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (2.20 mg, 1.57 μmol, 0.5 equiv.) and selenoester Ac-LYRANA-SePh **14a** (3.63 mg, 4.09 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANALSPGYS-NH_2 **15a** as a white solid (2.47 mg, 1.83 μmol, 58% over 2 steps).

*Ac-LYRANSLSPGYS-NH_2 **(15b)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (2.10 mg, 1.50 μmol, 0.5 equiv.) and selenoester Ac-LYRANS-SePh **14b** (3.64 mg, 4.03 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANSLSPGYS-NH_2 **15b** as a white solid (2.82 mg, 2.06 μmol, 69% over 2 steps).

*Ac-LYRANLLSPGYS-NH_2 **(15c)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (2.03 mg, 1.45 μmol, 0.5 equiv.) and selenoester Ac-LYRANL-SePh **14c** (3.51 mg, 3.77 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANLLSPGYS-NH_2 **15c** as a white solid (3.30 mg, 2.34 μmol, 82% over 2 steps).

*Ac-LYRANILSPGYS-NH_2 **(15d)***. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (1.96 mg, 1.40 μmol, 0.5 equiv.) and selenoester Ac-LYRANI-SePh **14d** (3.39 mg, 3.64 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-

LYRANILSPGYS-NH_2 **15d** as a white solid (2.13 mg, 1.53 μmol, 55% over 2 steps).

*Ac-LYRANVLSPGYS-NH_2 (**15e**)*. The title compound was produced *via* the one-pot ligation-deselenization method outlined above using diselenide peptide H-(β-Se)LSPGYS-NH_2 **13** (2.00 mg, 1.43 μmol, 0.5 equiv.) and selenoester Ac-LYRANV-SePh **14e** (3.40 mg, 3.72 μmol, 1.3 equiv.). The crude ligation-deselenization product was purified and lyophilized to obtain Ac-LYRANVLSPGYS-NH_2 **15e** as a white solid (2.53 mg, 1.83 μmol, 64% over 2 steps).

Synthesis of IFN-γ (75-131) glycopeptide (**18**)

*N-terminal fragment IFN-γ (75-104) (**19**)*. The N-terminal fragment IFN-γ (75-104) (**19**) (30 μmol) was prepared by loading Fmoc-Phe-OH on 2-chlorotrityl chloride resin and elongating to the target peptide sequence using automated Fmoc-SPPS as described in the general procedures. The side-chain protected peptide was acetylated at its N-terminus and cleaved from resin *via* treatment with HFIP/CH_2Cl_2 (3:7, v/v, 4 mL × 2, 1.5 h each time) for 3 h and concentrated *in vacuo*. The cleaved side-chain protected peptide and $(PhSe)_2$ (0.31 g, 1 mmol, 50 equiv.) were dissolved in anhydrous DMF (0.5 mL) under argon at 0 °C. Bu_3P (0.24 mL, 1 mmol, 50 equiv.) was added dropwise and the mixture was stirred for 3 h at 0 °C before the removal of solvent under a stream of N_2. The crude peptide was treated with a TFA cleavage cocktail (90:5:5, v/v/v, TFA:TIS:H_2O, 10 mL) at 0 °C and stirred for 3 h at room temperature. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude peptide selenoester was purified by preparative reverse phase HPLC (30-80% MeCN in H_2O over 40 min, 0.1% TFA, Sunfire Prep C18 5 μm 19 × 150 mm column) and lyophilized to give the desired peptide selenoester IFN-γ (75-104) **19** (32.5 mg, 8.33 μmol, 28%).

*C-terminal fragment IFN-γ (105-131) (**20**)*. The C-terminal fragment IFN-γ (105-131) **20** (30 μmol) was prepared by loading Fmoc-Lys(Boc)-OH on to 2-chlorotrityl chloride resin and elongating to the desired peptide sequence using automated Fmoc-SPPS as described in the general procedures. Then a deacetylation solution (10% NH_2NH_2 in DMF, 4 mL) was added to the elongated peptide on resin after its N-terminal Fmoc group was deprotected, and the reaction was shaken for 16 h at 25 °C. After thoroughly washing the resin, a solution of β-Se(PMB)-Phe-OH **2** (20 mg, 0.042 mmol, 1.2 equiv.), HOAt (6 mg, 0.042 mmol, 1.2 equiv.) and DIC (7 μL, 0.042 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.07 M amino acid concentration) was added to the elongated peptide on resin and the reaction was shaken for 16 h at 25 °C. The fully assembled peptide was globally cleaved using a TFA cleavage cocktail (90:5:5, v/v/v, TFA:TIS:H_2O) for 3 h at 25 °C. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude PMB-protected peptide was purified by preparative reverse phase HPLC (20-50% B over 50 min, 0.1% TFA, Sunfire Prep C18 5 μm 19×150 mm column) and lyophilized to yield the PMB-protected selenopeptide (34 mg). The PMB group of the purified peptide was removed after the peptide was agitated in a mixed solution of DMSO/ligation buffer (6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2)/TFA (1:1:3, v/v/v, 3 mL, 16 mg/mL) for 0.5 h at 25 °C. The resulting solution was diluted with 0.1% TFA/H_2O and purified by preparative reverse phase HPLC (20-50% MeCN in H_2O over 50 min, 0.1% TFA, Sunfire Prep C18 5 μm 19 × 150 mm column) to give the desired diselenide peptide **20** (28.1 mg, 3.93 μmol as dimer, 26% from resin loading) after lyophilization.

*Synthesis of IFN-γ (75-131) (**18**)*. The ligation of IFN-γ (75-104) selenoester **19** (2.44 mg, 0.63 μmol, 1.1 equiv.) and IFN-γ (105-131) diselenide dimer **20** (2.03 mg, 0.57 μmol, 1 equiv. with respect to selenopeptide monomer) was performed by dissolving both solids in ligation buffer (100 μL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2, 5 mM with respect to selenopeptide monomer) and the final pH adjusted to 6.1-6.4 by 0.5 M aq. NaOH. The resulting solution was sealed, agitated and incubated at 25 °C for 5 min, after which time UPLC-MS analysis indicated complete conversion into the ligated peptide. The ligation mixture was extracted with hexane (10 × 200 μL) to remove $(PhSe)_2$, an then treated with a solution of 4,4'-(diselanediylbis(methylene))dibenzoic acid **16** (30 μL, 200 mM in 6 M Gn·HCl, 0.1 M Na_2HPO_4 ligation buffer, pH= 6.5). The resulting yellow mixture was degassed with argon for 10 min. Meanwhile, a solution of TCEP (250 mM, 72 mg, 50 equiv.) and DTT (100 mM, 144 mg, 200 equiv.) was prepared by dissolving the two components in ligation buffer (1 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) and adjusting to pH 6-6.8 with 2 M aq. NaOH. The TCEP/DTT solution was degassed with argon for 10 min. Then, radical initiator 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, 1.5 mg, 4.64 μmol) was added as a solid into the ligation mixture under argon before the addition of the degassed TCEP/DTT solution (130 μL) in one portion. The deselenization reaction was sealed, agitated, and incubated at 37 °C for 5 min, at which point the in situ deselenization reaction was shown to have reached completion by UPLC-MS. IFN-γ 75-131 was purified by preparative reverse phase HPLC (20-80% MeCN in H_2O over 60 min, 0.1% TFA, XBridge Peptide BEH Prep C18 300 Å 5 μm 10 × 250 mm column) and lyophilized to give IFN-γ (75-131) **18** (2.13 mg, 0.29 μmol, 51% over 2 steps) as a white solid. Note: Reported isolated yield takes into account the aliquots removed for analytical UPLC monitoring at each step.

Synthesis of UL22A (**21**).

*Synthesis of N-terminal fragment UL22A (21-51) (**22**)*. The N-terminal fragment UL22A 21-50 (50 μmol) (**22**) was prepared by loading Fmoc-Thr(*t*Bu)-OH on 2-chlorotrityl chloride resin and elongating to the desired peptide sequence using automated Fmoc-SPPS as described in the general procedures. The N-terminal alanine residue at position 21 was incorporated as Boc-Ala-OH. The side-chain protected peptide was cleaved from resin *via* treatment with HFIP/CH_2Cl_2 (3:7, v/v, 4 mL, 2 x 1.5 h) and the combined filtrates were concentrated *in vacuo*. The cleaved side-chain protected peptide and $(PhSe)_2$ (0.78 g, 2.5 mmol, 50 equiv.) were dissolved in anhydrous DMF (2.5 mL) under argon at 0 °C. The reaction mixture was treated with Bu_3P (0.62 mL, 2.5 mmol, 50 equiv.) dropwise and stirred for 3 h at 0 °C before the removal of solvent under a stream of N_2. The crude peptide was treated with a TFA cleavage cocktail (90:5:5, v/v/v, TFA:TIS:H_2O, 10 mL) at 0 °C and stirred for 3 h at room temperature. The peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude peptide selenoester was purified by preparative reverse phase HPLC (10-30% MeCN in H_2O over 40 min, 0.1% TFA, XBridge BEH300 Prep C18 5 μm 19 × 150 mm column) and lyophilized to give the desired peptide selenoester **22** (30.8 mg, 9.25 μmol, 19%).

*Synthesis of middle bifunctional fragment of UL22A (52-76) (**23**)*. The middle fragment of UL22A (52-76) (**23**) (50 μmol) was prepared by loading Fmoc-Gly-OH on preactivated trityl-OH ChemMatrix® resin and elongating to the desired peptide sequence using automated Fmoc-SPPS as described in the general procedures. A solution of Boc-β-Se(PMB)-Leu-OH **5** (26 mg, 0.06 mmol, 1.2 equiv.), HOAt (8 mg, 0.06 mmol, 1.2 equiv.) and

DIC (9 μL, 0.06 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.1 M amino acid concentration) was added to the elongated peptide on resin and the reaction was shaken for 16 h at 25 °C. The side-chain protected peptide was cleaved from resin *via* treatment with HFIP/CH_2Cl_2 (3:7, v/v, 4 mL, 2 x 1.5 h) and the combined filtrates were concentrated in vacuo. The cleaved side-chain protected peptide was dissolved in anhydrous DMF (2 mL) under argon at -30 °C followed by the addition of ethyl-mercaptopropionate (0.19 mL, 1.5 mmol, 30 equiv.), iPr_2NEt (0.043 mL, 0.25 mmol, 5 equiv.) and PyBOP (0.13 g, 0.25 mmol, 5 equiv.). The solution was stirred for 5 h at -30 °C before the removal of solvent under a stream of N_2. The crude peptide was treated with a TFA cleavage cocktail (90:5:5, v/v/v, TFA:TIS:H_2O, 10 mL) at 0 °C and stirred for 3 h at room temperature. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude PMB-protected peptide was purified by preparative reverse phase HPLC (0-50% MeCN in H_2O over 30 min, 0.1% TFA, XBridge BEH300 Prep C18 5 μm 19 × 150 mm column) and lyophilised to yield the pure PMB-protected peptide thioester intermediate (12.57 mg, 4.32 μmol, 9 %). The PMB group of the purified peptide thioester (12.57 mg, 4.32 μmol) was removed after being agitated in a mixed solution of DMSO/ ligation buffer (6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2)/TFA (1:1:3, v/v/v, 2 mL, 6 mg/mL) for 3 h at room temperature. The resulting solution was diluted with 0.1% TFA/H_2O and purified by preparative reverse phase HPLC (0-50% MeCN in H_2O over 30 min, 0.1% TFA, XBridge BEH300 Prep C18 5 μm 19 × 150 mm column) to give the desired diselenide peptide thioester **23** (4.32 mg, 1.55 μmol, 3% from resin loading) after lyophilization.

*Synthesis of C-terminal fragment UL22A (77-103) (**24**)*. The C-terminal fragment UL22A (77-103) (**24**) (50 μmol) was prepared by loading Fmoc-Gln(Trt)-OH on 2-chlorotrityl chloride resin and elongating to the desired peptide sequence using automated Fmoc-SPPS as described in the general procedures. A solution of *syn*-Boc-Asp(tBu, STmob)-OH[9] (30 mg, 0.06 mmol, 1.2 equiv.), HOAt (8 mg, 0.06 mmol, 1.2 equiv.) and DIC (9 μL, 0.06 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.1 M amino acid concentration) was added to the elongated peptide on resin and the reaction was shaken for 16 h at 25 °C. The fully assembled peptide was globally cleaved by treating with a TFA cleavage cocktail (90:5:5, v/v/v, TFA:TIS:H_2O) for 3 h at 25 °C. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude β-thio-Asp containing peptide was purified by preparative reverse phase HPLC (0-20% MeCN in H_2O over 30 min, 0.1% TFA, XBridge BEH300 Prep C18 5 μm 19 × 150 mm column) and lyophilised to afford the desired peptide **24** (33.20 mg, 10.26 μmol, 21%).

Synthesis of UL22A protein (**21**) *via* the 3-component strategy:

The ligation of UL22A (21-51) selenoester **22** (4 mg, 1.22 μmol, 2 equiv.) and diselenide dimer UL22A (52-76) **23** (1.7 mg, 0.31 μmol, 0.5 equiv.) was performed by dissolving both solids in ligation buffer (62 μL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH = 7.2, 5 mM respect to diselenide thioester dimer) together and the final pH was adjusted to 6.2 with 1 M aq. NaOH. The resulting solution was agitated and incubated at 37 °C for 0.5 h, after which time UPLC-MS analysis indicated full conversion into the corresponding ligation products. The ligation mixture was washed with hexane (10 × 200 μL) to remove $(PhSe)_2$ and degassed with argon for 10 min. Meanwhile, a solution of TCEP (250 mM, 72 mg, 50 equiv.) and DTT (250 mM, 36 mg, 50 equiv.) was prepared by dissolving the two components in ligation buffer (1 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH = 7.2), adjusting to pH 5.2 with 2 M aq. NaOH and degassing with Argon for 10 min before the addition (125 μL) into the ligation mixture in one portion (1.7 mM respect to diselenide thioester dimer). The deselenization reaction was sealed, agitated, and allowed to stand at room temperature. After 6 h, the *in situ* deselenization reaction was complete according to UPLC-MS.

To the deselenized mixture was added UL22A (77-103) **24** (3.95 mg, 1.22 μmol, 2 equiv.) and TCEP solution (20 μL, 250 mM solution in 6 M Gn·HCl/0.1 M Na_2HPO_4 ligation buffer, pH 7.1, to achieve a final TCEP concentration of 25 mM) and then adjusted to pH 7.2 (1.5 mM respect to diselenide thioester dimer). TFET (4 μL, 2 vol.%) was added and the reaction was incubated at 37 °C. UPLC-MS analysis indicated complete consumption of thioester UL22A (21-76) after 6 h.

A solution of TCEP (150 mM, 21 mg, 50 equiv.) and reduced glutathione (200 mM, 30 mg, 67 equiv.) was prepared by dissolving the two components in ligation buffer (0.5 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH = 7.2) and adjusting to pH 6.8 with 2 M aq. NaOH. 210 μL of this solution was subsequently added into the ligation mixture (0.75 mM respect to diselenide thioester dimer). The resulting mixture was degassed with argon for 10 min followed by the addition of VA-044 (4.5 mM, 0.61 mg, 3 equiv.). The reaction was sealed, agitated and incubated at 37 °C for 16 h. The crude peptide was purified by preparative reverse phase HPLC (0-40% MeCN in H_2O over 45 min, 0.1% TFA, XBridge BEH300 Prep C18 5 μm 10 × 150 mm column) and lyophilized to give native UL22A (21-103) **21** (2.22 mg, 0.25 μmol, 40% over 4 steps) as a white solid. Note: The reported isolated yield takes into account the aliquots removed for analytical UPLC monitoring in each step.

*Synthesis of N-terminal Fragment UL22A (21-71) (**25**)*. The N-terminal fragment UL22A (21-71) (**25**) (20 μmol) was prepared by loading Fmoc-Val-OH on 2-chlorotrityl chloride resin and elongating to the target peptide sequence using automated Fmoc-SPPS as described in the general procedures. The side-chain protected peptide was cleaved from resin *via* treatment with HFIP/CH_2Cl_2 (3:7, *v/v*, 4 mL, 2 x 1.5 h) and the combined filtrates were concentrated *in vacuo*. The cleaved side-chain protected peptide and $(PhSe)_2$ (0.31 g, 1 mmol, 50 equiv.) were dissolved in anhydrous DMF (0.5 mL) under argon at 0 °C. Bu_3P (0.24 mL, 1 mmol, 50 equiv.) was added dropwise and the mixture was stirred for 3 h at 0 °C before the removal of solvent under a stream of N_2. The crude peptide was treated with a TFA cleavage cocktail (90:5:5, *v/v/v*, TFA:TIS:H_2O, 10 mL) at 0 °C and stirred for 3 h at room temperature. The cleaved peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude peptide selenoester was purified by preparative reverse phase HPLC (15-45% MeCN in H_2O over 30 min, 0.1% TFA, Sunfire Prep C18 5 μm 19 × 150 mm column) and lyophilized to give the desired peptide selenoester UL22A (21-71) (**25**) (27.7 mg, 4.63 μmol, 23%).

*Synthesis of C-terminal fragment UL22A (72-103) (**26**)*. The C-terminal fragment UL22A (72-103) **26** (30 μmol) was prepared by loading Fmoc-Gln(Trt)-OH on 2-chlorotrityl chloride resin and elongating to the desired peptide sequence using automated Fmoc-SPPS as described in the general procedures. A solution of Boc-β-Se(PMB)-Leu-OH **5** (18 mg, 0.042 mmol, 1.2equiv.), HOAt (6 mg, 0.042 mmol, 1.2 equiv.) and DIC (7 μL, 0.042 mmol, 1.2 equiv.) in DMF (0.6 mL, 0.07 M amino acid concentration) was added to the elongated peptide on resin and the reaction was shaken for 16 h at 25 °C. The fully assembled peptide was globally cleaved using a TFA cleavage cocktail (90:5:5, *v/v/v*, TFA:TIS:H_2O) for 3 h at 25 °C. The cleaved

peptide solution was concentrated under a stream of N_2, and the peptide was precipitated from cold Et_2O and separated *via* centrifugation. The crude PMB-protected peptide was purified by preparative reverse phase HPLC (0-20% B over 30 min, 0.1% TFA, Sunfire Prep C18 5 μm 19 × 150 mm column) and lyophilized to yield PMB-protected selenopeptide (48 mg). The PMB group of the purified peptide was removed after being agitated in a mixed solution of DMSO/ligation buffer (6 M Gn·HCl, 0.1 M Na_2HPO_4, pH = 7.2)/TFA (1:1:3, *v/v/v*, 3 mL, 16 mg/mL) for 0.5 h at 25 °C. The resulting solution was diluted with 0.1% TFA/H_2O and purified by preparative reverse phase HPLC (0-30% MeCN in H_2O over 40 min, 0.1% TFA, Sunfire Prep C18 5 μm 19 × 150 mm column) to give the desired diselenide peptide **26** (41 mg, 4.32 μmol as dimer, 29% from resin loading) after lyophilization.

*Synthesis of UL22A protein (**21**) via one-pot DSL-deselenization with additives.* The ligation of UL22A (21-71) selenoester **25** (3.09 mg, 0.52 μmol, 1.2 equiv.) and UL22A (72-103) diselenide dimer **26** (2.01 mg, 0.43 μmol, 1 equiv. with respect to selenopeptide monomer) was performed by dissolving both solids in ligation buffer (43 μL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH = 7.2, 10 mM with respect to selenopeptide monomer) and the final pH adjusted to 6.1-6.4 with 0.5 M aq. NaOH. A solution of TCEP (15 mM, 4.3 mg, 1.5 equiv.) and $(PhSe)_2$ (50 mM, 15.6 mg, 5 equiv.) was prepared by dissolving the two components in ligation buffer (1 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2) and adjusting to pH 6.2-6.5 with 2 M aq. NaOH. 43 μL of this solution was subsequently added into the ligation mixture. The resulting solution was sealed, agitated and incubated at 25 °C for 1 h, after which time UPLC-MS analysis indicated complete conversion into the corresponding ligation products.

The ligation mixture was extracted with hexane (10 × 200 μL) to remove $(PhSe)_2$ and degassed with argon for 5 min. Meanwhile, a solution of TCEP (250 mM, 36 mg, 50 equiv.) and DTT (250 mM, 19 mg, 50 equiv.) was prepared in ligation buffer (0.5 mL, 6 M Gn·HCl, 0.1 M Na_2HPO_4, pH= 7.2), adjusted to pH 5.1-5.5 with 2 M aq. NaOH and degassed with argon for 5 min before the addition (86 μL) into the ligation mixture in one portion (2.5 mM with respect to selenopeptide monomer). The deselenization reaction was sealed, agitated, and incubated at 25 °C for 16 h, at which point the *in situ* deselenization reaction was shown to have reached completion by UPLC-MS. The UL22A protein was purified by preparative reverse phase HPLC (0-60% MeCN in H_2O over 60 min, 0.1% TFA, XBridge Peptide BEH Prep C18 300 Å 5 μm 10 × 250 mm column) and lyophilized to give UL22A (21-103) (**21**) (2.57 mg, 0.25 μmol, 58% over 2 steps) as a white solid. Note: The reported isolated yield takes into account the aliquots removed for analytical UPLC monitoring in each step.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Analytical UPLC traces and Low-resolution MS data (ESI+ or MALDI) for all ligation reactions and purified peptides; ^1H and $^{13}C\{^1H\}$ NMR spectra for small molecules (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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REFERENCES

1. Kulkarni, S. S.; Sayers, J.; Premdjee, B.; Payne, R. J., Rapid and Efficient Protein Synthesis through Expansion of the Native Chemical Ligation Concept. *Nat. Rev. Chem.* **2018**, *2*, 0122.
2. Agouridas, V.; El Mahdi, O. a.; Diemer, V.; Cargoët, M.; Monbaliu, J.-C. M.; Melnyk, O., Native Chemical Ligation and Extended Methods: Mechanisms, Catalysis, Scope, and Limitations. *Chem. Rev.* **2019**, *119*, 7328-7443.
3. Wang, S.; Thopate, Y. A.; Zhou, Q.; Wang, P., Chemical Protein Synthesis by Native Chemical Ligation and Variations Thereof. *Chin. J. Chem.* **2019**, *37*, 1181-1193.
4. Dawson, P.; Muir, T.; Clark-Lewis, I.; Kent, S., Synthesis of Proteins by Native Chemical Ligation. *Science* **1994**, *266*, 776-779.
5. Dawson, P. E., Native Chemical Ligation Combined with Desulfurization and Deselenization: A General Strategy for Chemical Protein Synthesis. *Isr. J. Chem.* **2011**, *51*, 862-867.
6. Yan, L. Z.; Dawson, P. E., Synthesis of Peptides and Proteins without Cysteine Residues by Native Chemical Ligation Combined with Desulfurization. *J. Am. Chem. Soc.* **2001**, *123*, 526-533.
7. Wan, Q.; Danishefsky, S. J., Free-Radical-Based, Specific Desulfurization of Cysteine: A Powerful Advance in the Synthesis of Polypeptides and Glycopolypeptides. *Angew. Chem. Int. Ed.* **2007**, *46*, 9248-52.
8. Crich, D.; Banerjee, A., Native Chemical Ligation at Phenylalanine. *J. Am. Chem. Soc.* **2007**, *129*, 10064-5.
9. Thompson, R. E.; Chan, B.; Radom, L.; Jolliffe, K. A.; Payne, R. J., Chemoselective Peptide Ligation–Desulfurization at Aspartate. *Angew. Chem. Int. Ed.* **2013**, *52*, 9723-9727.
10. Malins, L. R.; Cergol, K. M.; Payne, R. J., Peptide Ligation-Desulfurization Chemistry at Arginine. *ChemBioChem* **2013**, *14*, 559-63.
11. Malins, L. R.; Giltrap, A. M.; Dowman, L. J.; Payne, R. J., Synthesis of *β*-Thiol Phenylalanine for Applications in One-Pot Ligation–Desulfurization Chemistry. *Org. Lett.* **2015**, *17*, 2070-2073.
12. Harpaz, Z.; Siman, P.; Kumar, K. S. A.; Brik, A., Protein Synthesis Assisted by Native Chemical Ligation at Leucine. *ChemBioChem* **2010**, *11*, 1232-1235.
13. Tan, Z.; Shang, S.; Danishefsky, S. J., Insights into the Finer Issues of Native Chemical Ligation: An Approach to Cascade Ligations. *Angew. Chem. Int. Ed.* **2010**, *49*, 9500-9503.
14. Chen, J.; Wang, P.; Zhu, J.; Wan, Q.; Danishefsky, S. J., A Program for Ligation at Threonine Sites: Application to the Controlled Total Synthesis of Glycopeptides. *Tetrahedron* **2010**, *66*, 2277-2283.
15. Haase, C.; Rohde, H.; Seitz, O., Native Chemical Ligation at Valine. *Angew. Chem. Int. Ed.* **2008**, *47*, 6807-10.
16. Chen, J.; Wan, Q.; Yuan, Y.; Zhu, J.; Danishefsky, S. J., Native Chemical Ligation at Valine: A Contribution to Peptide and Glycopeptide Synthesis. *Angew. Chem. Int. Ed.* **2008**, *47*, 8521-4.
17. Yang, R.; Pasunooti, K. K.; Li, F.; Liu, X.-W.; Liu, C.-F., Dual Native Chemical Ligation at Lysine. *J. Am. Chem. Soc.* **2009**, *131*, 13592-13593.
18. Malins, L. R.; Cergol, K. M.; Payne, R. J., Chemoselective Sulfenylation and Peptide Ligation at Tryptophan. *Chem. Sci.* **2014**, *5*, 260-266.
19. Cergol, K. M.; Thompson, R. E.; Malins, L. R.; Turner, P.; Payne, R. J., One-Pot Peptide Ligation–Desulfurization at Glutamate. *Org. Lett.* **2014**, *16*, 290-293.
20. Sayers, J.; Thompson, R. E.; Perry, K. J.; Malins, L. R.; Payne, R. J., Thiazolidine-Protected *β*-Thiol Asparagine: Applications in One-Pot Ligation–Desulfurization Chemistry. *Org. Lett.* **2015**, *17*, 4902-4905.

21. Ajish Kumar, K. S.; Haj-Yahya, M.; Olschewski, D.; Lashuel, H. A.; Brik, A., Highly Efficient and Chemoselective Peptide Ubiquitylation. *Angew. Chem. Int. Ed.* **2009**, *48*, 8090-8094.
22. Merkx, R.; de Bruin, G.; Kruithof, A.; van den Bergh, T.; Snip, E.; Lutz, M.; El Oualid, F.; Ovaa, H., Scalable Synthesis of Γ-Thiolysine Starting from Lysine and a Side by Side Comparison with δ-Thiolysine in Non-Enzymatic Ubiquitination. *Chem. Sci.* **2013**, *4*, 4494-4498.
23. Shang, S.; Tan, Z.; Dong, S.; Danishefsky, S. J., An Advance in Proline Ligation. *J. Am. Chem. Soc.* **2011**, *133*, 10784-10786.
24. Siman, P.; Karthikeyan, S. V.; Brik, A., Native Chemical Ligation at Glutamine. *Org. Lett.* **2012**, *14*, 1520-1523.
25. Gieselman, M. D.; Xie, L.; van der Donk, W. A., Synthesis of a Selenocysteine-Containing Peptide by Native Chemical Ligation. *Org. Lett.* **2001**, *3*, 1331-1334.
26. Quaderer, R.; Sewing, A.; Hilvert, D., Selenocysteine-Mediated Native Chemical Ligation. *Helv. Chim. Acta* **2001**, *84*, 1197-1206.
27. Hondal, R. J.; Nilsson, B. L.; Raines, R. T., Selenocysteine in Native Chemical Ligation and Expressed Protein Ligation. *J. Am. Chem. Soc.* **2001**, *123*, 5140-5141.
28. Metanis, N.; Keinan, E.; Dawson Philip, E., Traceless Ligation of Cysteine Peptides Using Selective Deselenization. *Angew. Chem. Int. Ed.* **2010**, *49*, 7049-7053.
29. Townsend, S. D.; Tan, Z.; Dong, S.; Shang, S.; Brailsford, J. A.; Danishefsky, S. J., Advances in Proline Ligation. *J. Am. Chem. Soc.* **2012**, *134*, 3912-3916.
30. Malins, L. R.; Payne, R. J., Synthesis and Utility of β-Selenol-Phenylalanine for Native Chemical Ligation–Deselenization Chemistry. *Org. Lett.* **2012**, *14*, 3142-3145.
31. Mitchell, N. J.; Malins, L. R.; Liu, X.; Thompson, R. E.; Chan, B.; Radom, L.; Payne, R. J., Rapid Additive-Free Selenocystine–Selenoester Peptide Ligation. *J. Am. Chem. Soc.* **2015**, *137*, 14011-14014.
32. Kulkarni, S. S.; Watson, E. E.; Premdjee, B.; Conde-Frieboes, K. W.; Payne, R. J., Diselenide–Selenoester Ligation for Chemical Protein Synthesis. *Nat. Protoc.* **2019**, 1.
33. Hackeng, T. M.; Griffin, J. H.; Dawson, P. E., Protein Synthesis by Native Chemical Ligation: Expanded Scope by Using Straightforward Methodology. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10068-10073.
34. Mitchell, N. J.; Kulkarni, S. S.; Malins, L. R.; Wang, S.; Payne, R. J., One-Pot Ligation–Oxidative Deselenization at Selenocysteine and Selenocystine. *Chem. Eur. J.* **2017**, *23*, 946-952.
35. Malins, L. R.; Mitchell, N. J.; McGowan, S.; Payne, R. J., Oxidative Deselenization of Selenocysteine: Applications for Programmed Ligation at Serine. *Angew. Chem. Int. Ed.* **2015**, *54*, 12716-12721.
36. Sayers, J.; Karpati, P. M. T.; Mitchell, N. J.; Goldys, A. M.; Kwong, S. M.; Firth, N.; Chan, B.; Payne, R. J., Construction of Challenging Proline–Proline Junctions Via Diselenide–Selenoester Ligation Chemistry. *J. Am. Chem. Soc.* **2018**, *140*, 13327-13334.
37. Mitchell, N. J.; Sayers, J.; Kulkarni, S. S.; Clayton, D.; Goldys, A. M.; Ripoll-Rozada, J.; Barbosa Pereira, P. J.; Chan, B.; Radom, L.; Payne, R. J., Accelerated Protein Synthesis Via One-Pot Ligation-Deselenization Chemistry. *Chem* **2017**, *2*, 703-715.
38. Watson, E.; Ripoll-Rozada, J.; Lee, A.; Wu, M.; Franck, C.; Pasch, T.; Premdjee, B.; Sayers, J.; Pinto, M.; Martins, P.; Jackson, S.; Pereira, P.; Payne, R., Rapid Assembly and Profiling of an Anticoagulant Sulfoprotein Library. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 201905177.
39. Premdjee, B.; Adams, A. L.; Macmillan, D., Native N-Glycopeptide Thioester Synthesis through N-->S Acyl Transfer. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4973-5.
40. Wang, D.; Bresnahan, W.; Shenk, T., Human Cytomegalovirus Encodes a Highly Specific Rantes Decoy Receptor. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 16642-7.
41. Wang, X.; Sanchez, J.; Stone, M. J.; Payne, R. J., Sulfation of the Human Cytomegalovirus Protein UL22A Enhances Binding to the Chemokine Rantes. *Angew. Chem. Int. Ed.* **2017**, *56*, 8490-8494.
42. Thompson, R. E.; Liu, X.; Alonso-García, N.; Pereira, P. J. B.; Jolliffe, K. A.; Payne, R. J., Trifluoroethanethiol: An Additive for Efficient One-Pot Peptide Ligation– Desulfurization Chemistry. *J. Am. Chem. Soc.* **2014**, *136*, 8161-8164.
43. Lin, X.-Z.; Li, Y.-M.; Tang, Y., Syntheses of carboxylic selenides, diselenides and their seleno-polysaccharides. *Huaxue Shijie* **2009**, 50, 40-43.