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Accelerating chemoselective peptide bond formation using bis(2-selenylethyl)amido peptide selenoester surrogates†

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Given the potential of peptide selenoesters for protein total synthesis and the paucity of methods for the synthesis of these sensitive peptide derivatives, we sought to explore the usefulness of the bis(2-selenylethyl)amido (SeEA) group, *i.e.* the selenium analog of the bis(2-sulfanylethyl)amido (SEA) group, for accelerating peptide bond formation. A chemoselective exchange process operating in water was devised for converting SEA peptides into the SeEA ones. Kinetic studies show that SeEA ligation, which relies on an initial *N*,*Se*-acyl shift process, proceeds significantly faster than SEA ligation. This property enabled the design of a kinetically controlled three peptide segment assembly process based on the sequential use of SeEA and SEA ligation reactions. The method was validated by the total synthesis of hepatocyte growth factor K1 (85 AA) and biotinylated NK1 (180 AA) domains.

Introduction

The field of protein chemical synthesis has flourished substantially during the last decades to a point where the use of chemical methods is considered as a useful alternative to recombinant techniques for accessing small functional protein domains. Chemical synthesis is especially useful when proteins having unusual architectures[1–6] or well-defined modifications at specific sites are desirable. An application of protein chemical synthesis which is of utmost importance in biological sciences is the study of some complex post-translational modifications such as glycosylation,[7] ubiquitination[8–10] or sumoylation.[11,12]

Today, synthetic proteins are usually produced by assembling unprotected peptide segments in water. The native chemical ligation (NCL) is a popular chemoselective amide bond forming reaction in the field,[13,14] although other ligations show great promise as well.[15–19] The NCL reaction between a C-terminal peptide thioester and an N-terminal cysteinyl (Cys) peptide yields a native peptide bond to cysteine. Recently, C-terminal peptide selenoesters were also shown to be useful reactants for amide bond formation with the advantage of being significantly more reactive than peptide thioesters.[20,21] Unfortunately, the application of peptide selenoesters for protein synthesis is difficult due to the limited access to these sensitive peptide derivatives, which were produced up to now using Boc solid phase peptide synthesis (SPPS) methods.[20,22]

The powerfulness of the NCL reaction has stimulated the development of several methods for accessing peptide thioesters using the Fmoc SPPS.[23] In particular, several *N*,*S*-acyl shift systems relying on the *N*-(2-sulfanylethyl)amide structure were shown to rearrange into thioesters in acidic media.[24–29] The fact that *N*-(2-sulfanylethyl)amides rearrange spontaneously into thioesters in strong aqueous acids has been noticed in the 1950s by Martin and coworkers[30] using *N*-acetyl-β-mercaptoethylamine as a model system. This property is also shared by the peptide bond to cysteine.[31] In contrast, the capacity of some *N*-(2-sulfanylethyl)amides[32–35] such as the bis(2-sulfanylethyl) amido (SEA) group[32,36] to rearrange into thioesters in water at neutral[32] or mildly acidic[37–39] pH is a recent observation (Scheme 1). The reaction of a SEA peptide with an N-terminal cysteinyl peptide yields a native peptide bond to cysteine and proceeds efficiently in the presence of 4-mercaptophenylacetic acid (MPAA[40]). The usefulness of the SEA ligation has been demonstrated by the total synthesis of several functional proteins.[11,12,41–45]

Given the promise of peptide selenoester chemistry for protein total synthesis, we sought to explore the potential of the bis(2-selenylethyl)amido (SeEA) amido group,[46] *i.e.* the selenium analog of the SEA group (Scheme 1), for accelerating the chemoselective formation of the peptide bond to Cys. We first developed a mild and chemoselective exchange process for converting SEA peptides into SeEA derivatives in water.

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† Electronic supplementary information (ESI) available: Calculations, synthetic protocols and characterization for all compounds. See DOI: 10.1039/c5sc03459k

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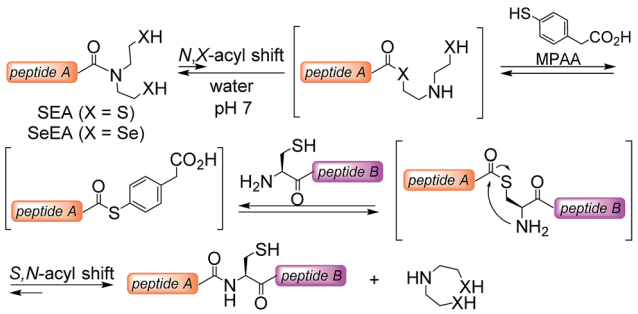


Scheme 1 Principle of SEA (X = S) and SeEA (X = Se) chemoselective ligations.

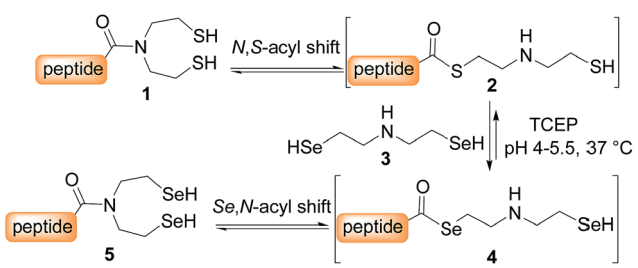
Importantly, SeEA peptides proved to be more reactive than classical peptide alkylthioesters and significantly more reactive than SEA peptides in model ligation experiments conducted at neutral pH. This property enabled designing a one-pot kinetically controlled[47] three peptide segment assembly process involving the sequential use of SeEA and SEA ligations.

Synthesis of SeEA peptides

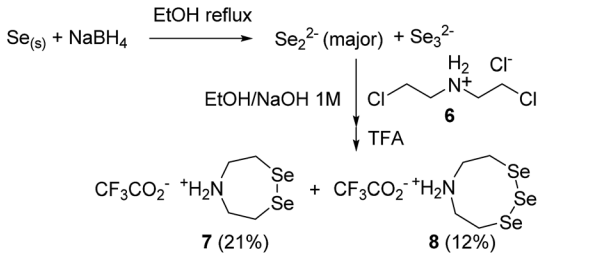
SEA peptides are easily accessible by the Fmoc SPPS using SEA polystyrene[32,48] or PEG-based resins.[11,12] We therefore sought to convert SEA peptides **1** into SeEA derivatives **5** by using the exchange reaction shown in Scheme 2, which takes advantage of the capacity of SEA amide form **1** to equilibrate in water with the SEA transient thioester form **2**.[29,32,49] We reasoned that the latter might participate to a selenol–thioester exchange reaction with an excess of the bis(2-selenylethyl)amine **3** to produce, after an *Se*,*N*-acyl shift, the target SeEA peptide **5**. Overall, the exchange process shown in Scheme 2 corresponds to a chemoselective transamidation reaction[36] which is applied to unprotected SEA peptide segments.

The trifluoroacetate salt of cyclic diselenide **7**, *i.e.* 1,2,5-diselenazepane, was envisioned as a good precursor for diselenol **3**. Its synthesis has been described elsewhere[46] by reducing metallic selenium with sodium borohydride and reacting the resulting diselenide dianion Se_2^- with bis(2-chloroethyl)amine hydrochloride **6** (Scheme 3). The reaction yielded also the trifluoroacetate salt of triselenide **8**, *i.e.* 1,2,3,6-triselenazocane, which was isolated and characterized in this work.

The first attempts to transamidate model peptide **9a** with diselenide **7** in the presence of TCEP yielded only trace amounts



Scheme 2 Conversion of SEA peptides into SeEA derivatives by transamidation.



Scheme 3 Preparation of cyclic di or triselenides **7** and **8**.

of the target SeEA peptide **10a** (Fig. 1A and B). Instead, we observed the formation of a compound whose molecular mass corresponded to peptide **10a** minus one selenium atom and to whom the structure of peptide **11a** was assigned on the basis of the following arguments.

The deselenization of selenocysteine into alanine by TCEP, which is reminiscent of the desulfurization of thiols by phosphites,[50,51] is a well-known process which has been noticed as a serious side-reaction or utilized as a useful synthetic transformation.[52–54] However, a similar deselenization process when

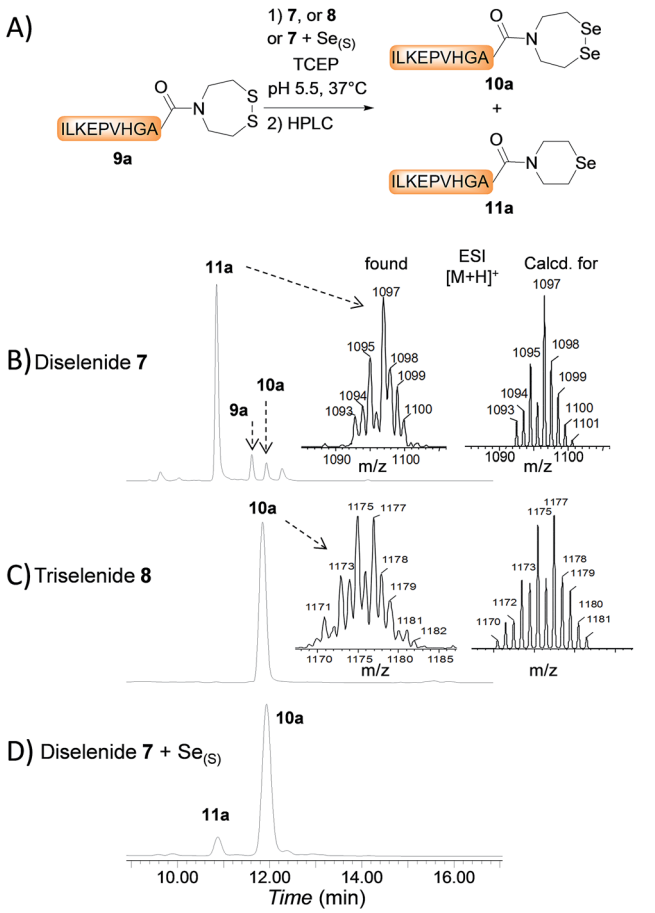


Fig. 1 Conversion of SEA peptides into SeEA derivatives by transamidation (A). HPLC of the crude exchange reaction with diselenide **7** (10 equiv) (B), triselenide **8** (10 equiv) (C) or diselenide **7** (10 equiv) plus metallic selenium (2 equiv) (D). Peptide **9a** 10 mM, TCEP 100 mM, pH 5.5, 37 °C, 24 h.

applied to SeEA peptide **10a** would yield a peptide featuring a C-terminal *N*-(2-selenylethyl)-*N*-ethyl amide moiety, whose molecular weight is 2 units greater to those measured for **11a**. This mode of deselenization can thus be ruled out.

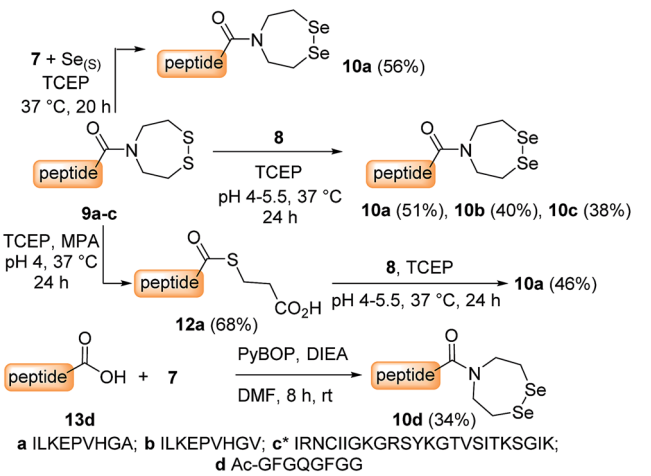
In contrast, the extrusion of selenium from diselenides to produce selenoethers has received much attention in the past and is a simple explanation to the formation of peptide **11a** from diselenide **10a**.[55] Since this free-radical process is catalysed by tertiary phosphines,[56] a potential solution to this problem was to replace TCEP by dithiothreitol (DTT) in the exchange reaction. However, while DTT is capable of reducing acyclic diselenides such as selenocystine bonds,[57] it is unable to reduce cyclic diselenide **7**.[46] This fact imposed the use of TCEP in the exchange reaction and thus to find solution for avoiding the extrusion of selenium. Gratefully, the use of triselenide **8** as a precursor for diselenol **3** yielded exclusively the target SeEA peptide **10a** (51%, Fig. 1C and Scheme 4). The exchange procedure using triselenide **8** and TCEP enabled also the successful preparation of several SeEA peptides in good yield such as SeEA peptide **10b** which features a sterically demanding valine residue at the C-terminus or the 24 amino acids SeEA peptide **10c** which includes an internal cysteine residue.

The triselenide **8** is reduced *in situ* by TCEP into diselenol **3** with the concomitant production of the selenophosphine derived from TCEP, *i.e.* Se = TCEP, which is indeed easily detected by LC-MS (see ESI†). We have recently shown that Se = TCEP can inhibit the free-radical process leading to the deselenization of Sec by TCEP.[58] Therefore, the presence of Se = TCEP in the exchange mixture might inhibit the formation of peptide **11a** as well. In support of this hypothesis, the combined use of diselenide **7** and of metallic selenium as an alternative for the *in situ* formation of Se = TCEP led to a significant decrease in the proportion of peptide **11a** in the exchange mixture and allowed the isolation of **10a** in good yield (**10a** 56%, Fig. 1D and Scheme 4).§

Interestingly, SeEA peptides could also be prepared from C-terminal peptide thioesters by using triselenide **8** (**12a** → **10a** Scheme 4) or diselenide **7** and metallic selenium (**29** → **30**, Scheme 6). This exchange reaction is an interesting alternative for accessing SeEA peptides, since a large variety of synthetic methods are now available for producing peptide thioesters.[23] Note that although SeEA peptides are probably produced in the exchange mixture in their reduced diselenol form due to the excess of TCEP, they eluted during analytical or preparative HPLC only as cyclic diselenides such as **10**. This is probably due to the rapid air oxidation of the diselenols into the corresponding cyclic diselenides during workup.

The last synthetic access to SeEA peptides which is depicted in Scheme 4 is based on the coupling of diselenide **7** to a peptide acid using PyBOP/DIEA activation (**13d** → **10d**, Scheme 4). This method which has been described elsewhere[46] is not compatible with peptides featuring functional residues. It is thus of limited scope in comparison with the exchange processes discussed above.

To summarize at this stage, the chemoselective exchange of SEA peptides or peptide thioesters by bis(2-selenylethyl)amine **3** generated *in situ* by reduction of diselenide **7** or triselenide **8** constitutes a mild access to SeEA peptides. The high stability of SeEA peptides during the HPLC purification step and upon storage thanks to their tertiary amide structure is also worth mentioning.



Scheme 4 Synthesis of SeEA peptides. *The Cys residue is temporarily protected by a *tert*-butylsulfenyl (*t*BuS) group in **9c**. It is removed during the exchange process.

Kinetic studies

We next determined the kinetic rates of the reaction of SEA peptides **9a,b**, SeEA peptides **10a,b** or MPA peptide thioesters **12a,b** with Cys peptide **14** (Fig. 2A). The ligations with SeEA peptides **10a,b** were performed in the presence of Se = TCEP to avoid the deselenization of the SeEA group by TCEP.[58] The fraction ligated was quantified by HPLC (Fig. 2B). The data were nicely fitted to a first order kinetic law from which the $t_{1/2}$ were determined (see the insert in Fig. 2B). Interestingly, the SeEA peptides proved to be significantly more reactive than SEA peptides. For example, the $t_{1/2}$ for SEA peptide **9a** with a C-terminal alanine residue was 3.3 h, while the $t_{1/2}$ for the SeEA analog **10a** was 0.24 h only, that is ~14 fold lower. Peptide **15a** produced by reacting SeEA peptide **10a** with Cys peptide **14** was isolated by HPLC with a 56% yield. No hydrolysis of the SeEA peptide nor side-reactions with nucleophilic residues such as lysine could be detected by LC-MS. Moreover, analysis of peptide **15a** by chiral GC-MS after total acid hydrolysis showed a D-Ala content <0.43%.[59] Similarly, an ~8 fold decrease of the $t_{1/2}$ was observed for valine as C-terminal residue (SEA peptide **9b** $t_{1/2}$ 23 h, SeEA peptide **10b** $t_{1/2}$ 2.9 h). Interestingly, SeEA peptides **10a,b** were also more reactive than the corresponding MPA peptide thioesters **12a,b** (Fig. 2). Therefore, the observed order of reactivity at pH 7 was SeEA peptides > MPA peptide thioester ≫ SEA peptide.

We further performed the experiment depicted in Fig. 3 with the aim to identify the rate limiting step of the SEA ligation process, and propose an explanation for the significant impact of the S → Se substitution on the reactivity of the bis(2-chalcogenoethyl)amido group. The first experiment corresponds to a typical SEA ligation process (Fig. 3A, condition A). In these

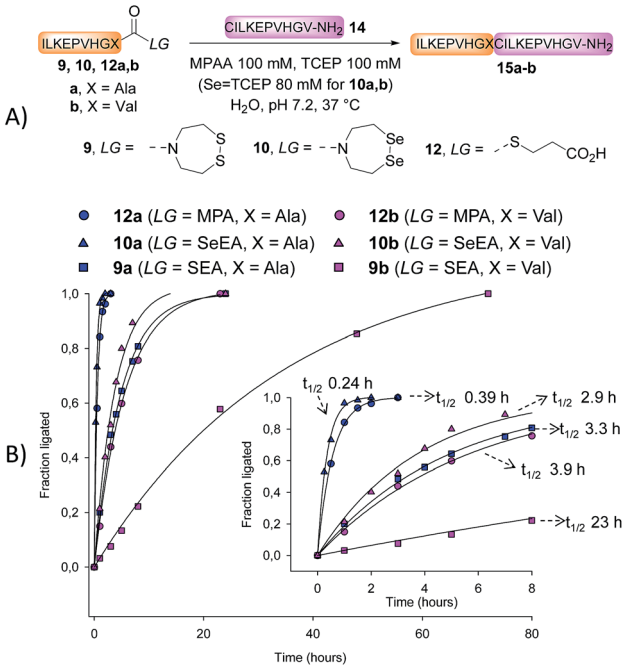


Fig. 2 Kinetic rates for the reaction of SEA peptides **9a**,**b**, SeEA peptides **10a**,**b** or MPA peptide thioesters **12a**,**b** with Cys peptide **14**. The data were fitted to a first order kinetic law (continuous curves). The half-times ($t_{1/2}$) of the reactions are indicated in the insert. Peptide concentration was 3.5 mM.

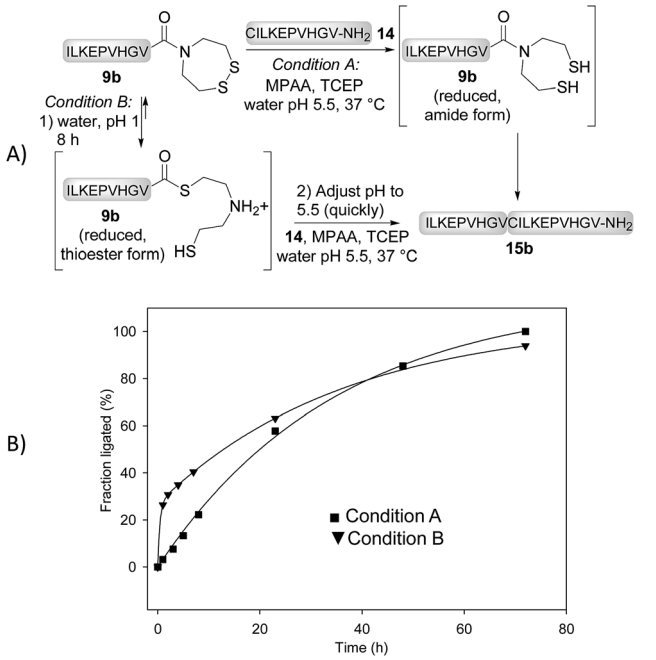


Fig. 3 SEA ligation without (condition A) or with pre-equilibration of the SEA amide form into the SEA thioester form (condition B).

conditions, the SEA amide dithiol form is the only species observed for the reduced SEA peptide **9b** during ligation. In the second experiment (Fig. 3A, condition B), the SEA peptide **9b** was first reduced and incubated at pH 1 and 37 °C for 8 h to convert the SEA amide form into the SEA thioester form which represents ~90% at equilibrium.[29] Then, peptide **14** was added together with MPAA and the pH was quickly adjusted to 5.5. Fig. 3B shows the kinetic rate for these two ligation reactions as determined by HPLC. The data obtained using condition A obeys to a first-order kinetic law with a reaction half-life ($t_{1/2}$) of 27.8 h. In contrast, the kinetic data obtained using condition B clearly show an initial burst of ligation product **15b** formation followed by a slower phase. The first phase is due to the rapid consumption of the SEA thioester form produced during the pre-equilibration step. However, it is also competitively converted back to the SEA amide form, which is significantly less reactive and at the origin of the slower phase. The significant increase of the ligation rate which is observed when the SEA amide form is pre-equilibrated into the SEA thioester form supports the *N*,*S*-acyl shift of the SEA group to be the rate limiting step of the SEA ligation process.

Another interesting observation is that the rate of SEA ligation is accelerated while the pH is decreased from pH 7.2 to 5.5 (Fig. 4). The significant increase of the SEA ligation rate upon acidification, which has also been observed with peptidyl prolyl SEA peptides[38] or with peptides featuring a SEA group on an aspartic or glutamic side-chain,[37] shows that the transfer of a proton occurs in the transition state of the rate limiting step, that is the *N*,*S*-acyl shift.

Preliminary DFT computations using *N*-formyl Gly-SEA **16a** as a model for SEA peptides revealed that amongst the different plausible neutral or anionic transitions states which were identified up to now for the *N*,*S*-acyl shift, the anionic and concerted transition state TS_{SEA} shown in Fig. 5A is by far the one displaying the lowest barrier of activation. In TS_{SEA}, the neutral 2-sulfenylethyl limb is involved in an intramolecular S–H–N interaction. The intramolecular transfer of the thiol proton to the amide nitrogen in TS_{SEA} is fully consistent with the experimentally observed pH-dependency of the SEA ligation

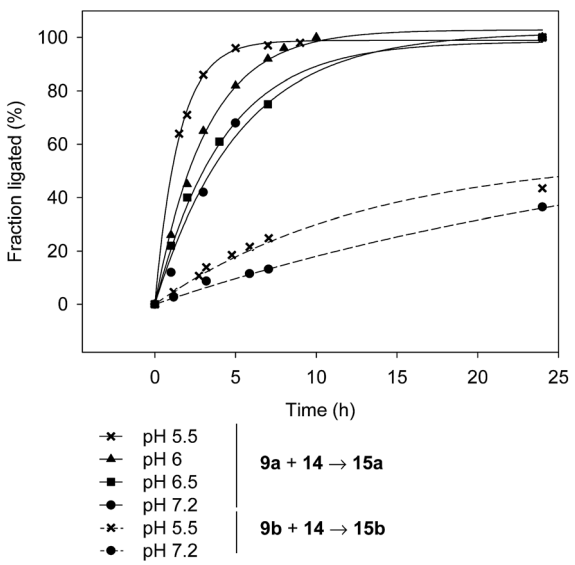


Fig. 4 The effect of the pH on the rate of SEA ligation (peptide **9a** or **9b** 7 mM, peptide **14** 10.5 mM, MPAA 200 mM, TCEP 200 mM, 37 °C).

reaction. The N-protonation of amides is known to weaken the $n_N \rightarrow \pi_{C=O^*}$ conjugation, to disrupt the amide bond planarity and thus to increase the electrophilicity of the carbonyl toward nucleophiles.[60] N-protonation is often used by biocatalysts for catalyzing biochemical processes such as amide bond isomerization,[61] hydrolysis or cleavage.[62] The N-protonation of amides ($pK_a^N \sim -7$ (ref. 63)), although thermodynamically unfavourable over O-protonation ($pK_a^O \sim 0$), can be facilitated within constrained systems which favour a hydrogen bond donation to the amide nitrogen.[61]

Importantly, the intramolecular N-protonation/nucleophilic attack mechanism permitted by the presence of two *N*-mercaptoethyl groups in close proximity to the amide group might explain the capacity of the SEA group to act as a good thioester surrogate at neutral pH,[32] in comparison with related *N*,*S*-acyl shift systems which feature only one mercaptoethyl limb such as *N*-methyl cysteine[25] or *N*-butyl-*N*-(2-sulfanylethyl)amido[27] thioester surrogates. It also allows proposing an explanation for the greater reactivity of the SeEA group compared to the SEA one. Indeed, the pK_a of selenols such as selenocysteamine or selenocysteine (p$K_a \sim 5.5$ (ref. 64)) are about 3 pK_a units lower than the pK_a of the sulfur analogs, cysteamine or cysteine (p$K_a \sim$ 8.2 (ref. 65 and 66)). This difference in pK_a, which is reminiscent of the higher acidity of hydrogen selenide (pK_a 3.8) compared to hydrogen sulfide (pK_a 7.0), is also found in S(Se)EA models **16a**,**b** those pK_a was estimated using ACD/Labs software (Fig. 5B).[67] Therefore, the intramolecular protonation of the amide nitrogen and thus the activation of the amide carbonyl might be more pronounced for SeEA peptides. Moreover, the SEA group is mostly present at neutral pH as the dithiol form, *i.e.* the productive monoanionic species of type **16a** (Fig. 5A) represents less than 1% of the total. In contrast, the monoanionic species for the SeEA analog is significantly more populated (~7%). Thus, the higher acidity of selenols relative to thiols might contribute to the higher reactivity of the SeEA group by both increasing the concentration of the productive monoanionic species and lowering the barrier of activation for the *N*,*Se*-acyl shift. DFT computations aiming at identifying the transition states for the *N*,*Se*-acyl shift are in progress and will be reported in due course.

Kinetically controlled ligation using SEA and SeEA latent thio(seleno)ester peptides

The availability of fast and slow reacting thioester functionalities or thioester surrogates can be useful for designing kinetically controlled ligations (KCLs), which involve an NCL reaction between a Cys peptide and a fast reacting thioester in the presence of a slow reacting one. The field has been pioneered by Kent and coworkers who reacted a peptide arylthioester in the presence of a peptide alkylthioester.[47] Later on, other KCLs have been developed using *O*,*S*-[68] or *N*,*S*-acyl shift systems.[33,38,44,69,70] Here we show that SeEA ligation can be performed in the presence of a SEA moiety thanks to the large difference of reactivity between SeEA and SEA groups towards Cys peptides. In particular, the sequential use of SeEA and SEA ligations enabled to develop the three peptide segment assembly method depicted in Scheme 5.

The reduction of the SeEA peptide segment **10c** (or **10d**) required the presence of TCEP at the first stage of the process. Here again, Se = TCEP (80 mM) proved to be a useful additive

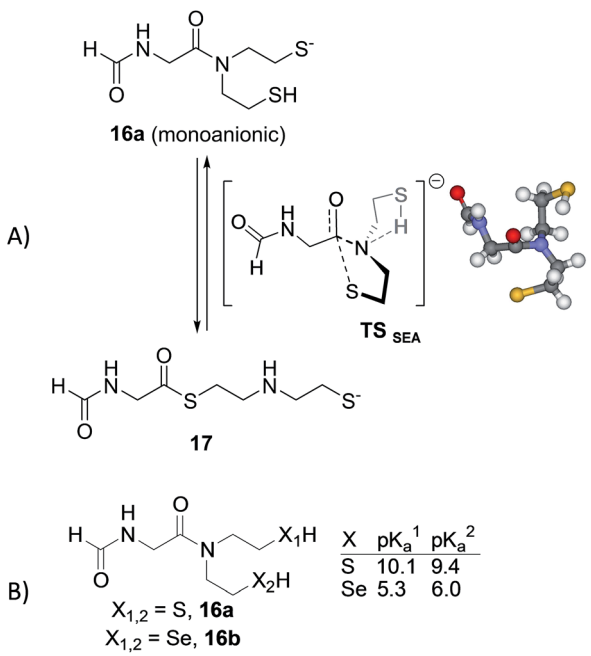
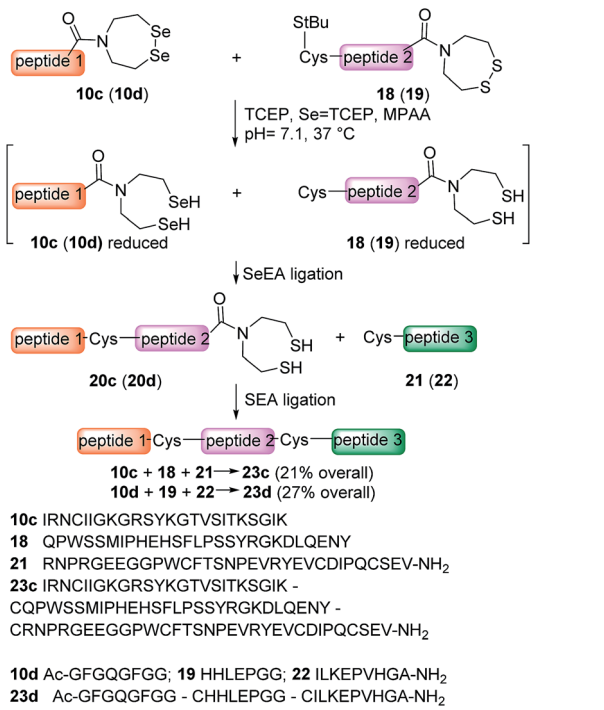


Fig. 5 (A) Hypothetical anionic transition state structure for the intramolecular *N*-to-*S* acyl transfer of the SEA group. (B) pK_as for the SEA thiol or SeEA selenol groups were estimated using ACS/Labs software.



Scheme 5 Kinetically controlled ligation using sequential SeEA and SEA ligations.

for minimizing the deselenization of the SeEA group by TCEP. Once the SeEA ligation was complete, the addition of the Cys peptide **21** (or **22**) trigged the SEA ligation step and the formation of the target polypeptide. The assembly of the model peptide segments **10d**, **19** and **22** involved the formation of two Gly–Cys junctions and yielded the 26 amino acids peptide **23d** (27% overall after HPLC purification). The method was also applied to the synthesis of the 85 amino acids Kringle 1 domain (K1) of hepatocyte growth factor/scatter factor (HGF/SF).[42,71] This example involved the assembly of peptide segments **10c** (HGF/SF 125–148), **18** (HGF/SF 149–176) and **21** (HGF/SF 177–209) through the sequential formation of Lys–Cys and Tyr–Cys junctions. Fig. 6 highlights the efficiency of the one-pot process which yielded the K1 polypeptide **23c** with 21% overall yield after HPLC purification. The K1 polypeptide produced in this work using the SeEA/SEA KCL method was found to be identical by LC-MS to a sample obtained by another route (see ESI†).[42]

Synthesis of large SeEA peptides and total synthesis of biotinylated NK1 domain of hepatocyte growth factor

So far, KCL methods were used for accessing proteins composed of less than 100 amino acids. One reason for this is the difficulty in accessing large peptide segments (>50 AA) equipped with a reactive C-terminal thio or selenoester group. Usually, large peptides are prepared by coupling chemoselectively several unprotected peptide segments together. However, such processes are hardly compatible with the presence of highly reactive functional groups such as selenoesters. A potential solution to this problem is to introduce the selenoester moiety after the chain assembly.

An appealing alternative is to use a latent selenoester during the assembly of the peptide. With such a strategy, the formation of side-products during the assembly of the latent selenoester is minimized, while the KCL process can be triggered in a subsequent step when needed.

In a previous communication we showed that the SeEA group is compatible with the NCL and SEA ligations in the presence of reducing thiols such as MPAA or DTT.[46] We show here that the sequential use of NCL and SEA ligations in a one-pot process permitted the synthesis of the 118 amino acids SeEA peptide **31** as illustrated in Scheme 6 (21% based on **26**, one-pot process III). Peptide **31** corresponds to HGF 31–148 and was assembled using MPA thioester peptide **26**, SEA peptide **27** and SeEA peptide **30**.

Peptide **26** was produced using one-pot process I by ligating MPA thioester **24** with SEA peptide **25** at pH 7 in the presence of MPAA, followed by the *in situ* exchange of the SEA group by MPA (46% for the two steps). SEA peptide **27** was obtained by standard Fmoc SPPS starting from SEA polystyrene resin.[32,48] The third segment, *i.e.* SeEA peptide **30**, was produced starting from

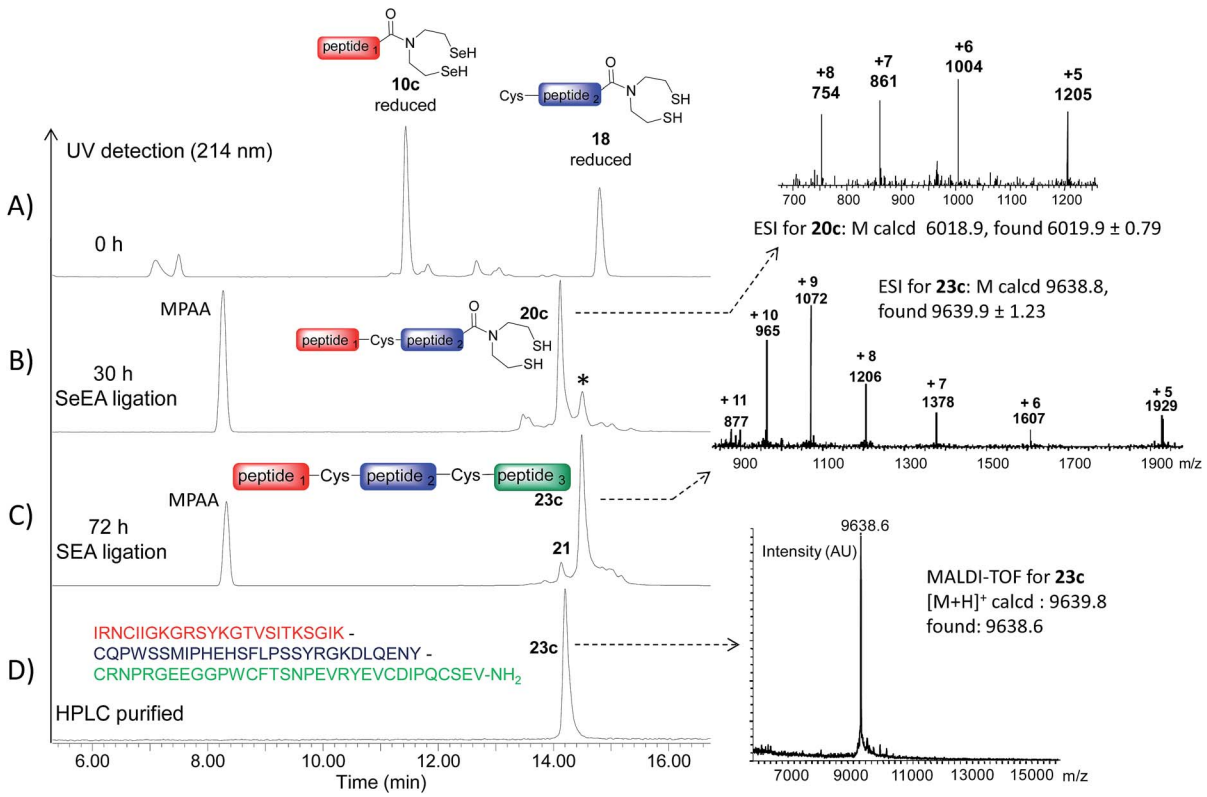
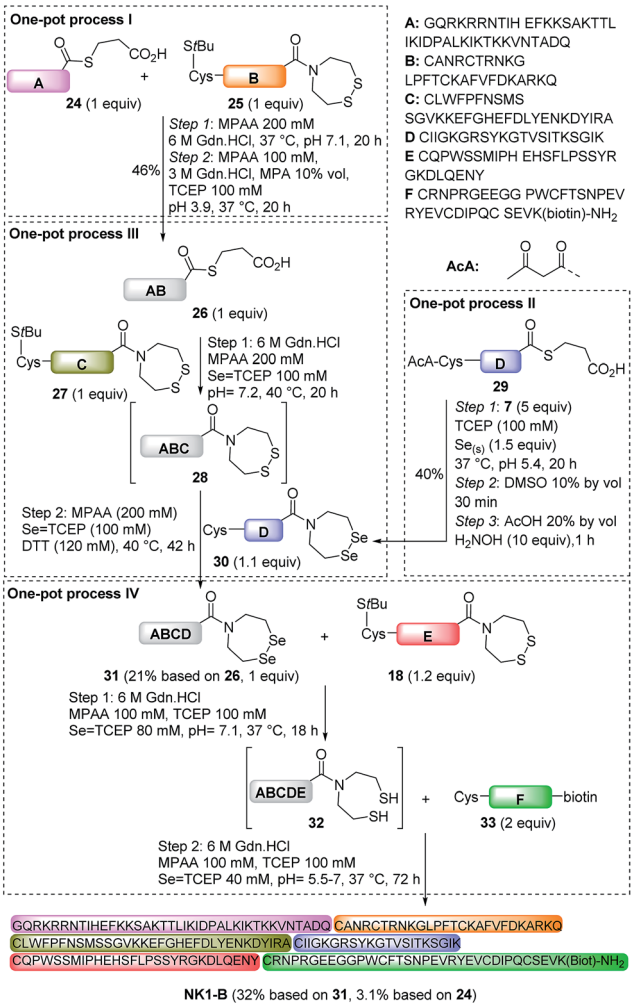


Fig. 6 Monitoring of the one-pot assembly of K1 polypeptide using HPLC. (A) Few minutes after dissolving peptide segments **10c** and **18** (4 mM each). (B) SeEA ligation step after 30 h. The peak marked by an asterisk corresponds to the partial cyclization of peptide **18**. (C) SEA ligation step 72 h after the addition of Cys peptide **21**. For (A–C), the analysis were run on a C3 Zorbax column with a linear gradient of 0% eluent B (ACN/H_2O (4/1 by vol) containing 0.1% TFA) in eluent A (water containing 0.1% TFA) to 100% eluent B in eluent A over 30 min, UV detection at 214 nm. (D) Purified K1 polypeptide. The analysis was run on a C18 Xbridge column using the above conditions.



Scheme 6 Total synthesis of SeEA peptide **31** by a sequential NCL/SEA ligation process (118 amino acids, one-pot process III) and of **NK1-B** protein by a SeEA/SEA KCL process (180 amino acids, one-pot process IV).

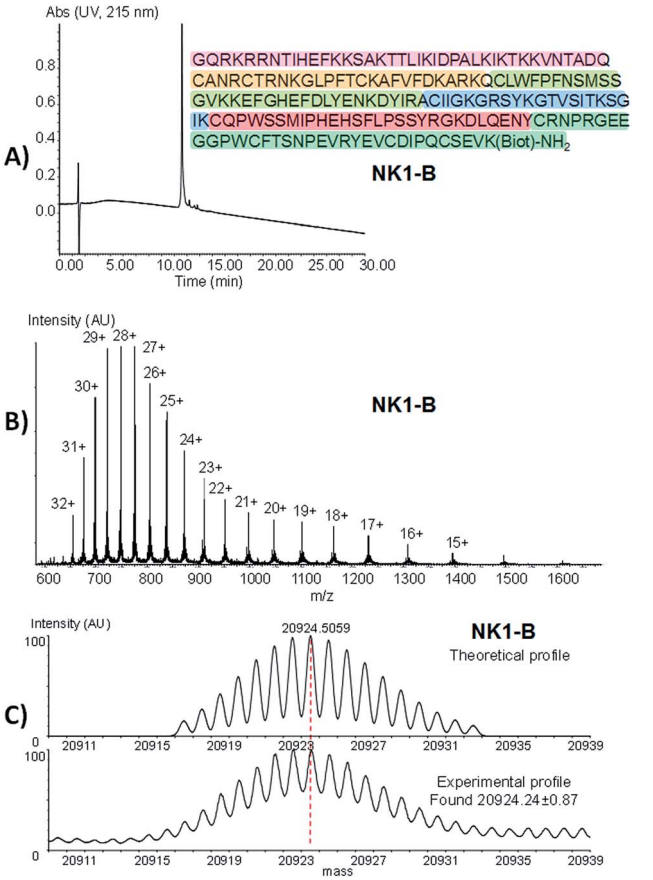


Fig. 7 Characterization of **NK1-B** protein. (A) LC analysis of **NK1-B**. (A) LC trace, eluent A 0.10% formic acid (FA) in water, eluent B 0.10% FA in CH_3CN/water: 4/1 by vol. C3 Zorbax 300SB 3.5 μm (4.6 × 150 mm) column, gradient 0–100% B in 30 min (1 mL min^{-1}, detection 215 nm). (B) ESI MS high resolution spectrum. (C) Deconvoluted spectrum and comparison with the theoretical profile.

MPA thioester **29** using one-pot process II. One-pot process II involved first the introduction of the SeEA group by treatment with diselenide **7**, metallic selenium and TCEP. The second step allowed the formation of the cyclic diselenide by oxidation with DMSO, while the N-terminal acetoacetyl group was removed in the final step by addition of hydroxylamine.[72] The successful preparation of SeEA peptide **31** set the stage for the assembly of the biotinylated NK1 domain of HGF (**NK1-B**), a protein composed of 180 amino acids (Scheme 6, one-pot process IV). NK1 protein was modified by a C-terminal biotinylated lysine residue to allow the development of binding assays in the future. **NK1-B** was assembled using the SeEA/SEA KCL method depicted in Scheme 6 (one-pot process IV), which is almost identical to the SeEA/SEA KCL discussed above (see Scheme 5). Gratifyingly, the sequential assembly of SeEA peptide **31**, SEA peptide **18** and Cys peptide **33** furnished successfully **NK1-B** polypeptide (32% based on **31**, 3.1% based on **24**, Fig. 7), showing the usefulness of S(Se)EA chemistry for accessing challenging proteins.

Conclusions

In conclusion, SeEA peptides are highly stable peptide derivatives which behave as latent selenoesters upon reduction of the cyclic diselenide. They are easily accessible through the chemoselective transamidation of SEA peptides or the selenol–thioester exchange of thioester peptides in water by an excess of bis(2-selenylethyl)amine. This reagent is formed *in situ* by reduction of 1,2,5-diselenazepane or 1,2,3,6-triselenazocane by TCEP. The presence of Se = TCEP in the exchange mixture minimizes the extrusion of one selenium atom from SeEA diselenide. SeEA peptides react faster than peptide alkylthioesters or SEA peptides with cysteinyl peptides. The large difference of reactivity between SeEA and SEA functionalities enabled designing a one-pot kinetically controlled three peptide segment assembly process working in the N-to-C direction. The method was validated by the total synthesis of hepatocyte growth factor K1 (85 AA) and biotinylated NK1 (**NK1-B**, 180 AA) domains. **NK1-B** is by far the largest polypeptide produced by using a KCL process. The synthesis of a latent SeEA selenoester with a size >100 amino acids and the assembly of a 180 amino

acids protein by a series of one-pot processes show the great potential of S(Se)EA chemistries for accessing challenging proteins.

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Notes and references

§ Note that triselenide **8** was found to decompose partially into diselenide **7** plus selenium upon storage (see ESI†). Therefore, the combined use of diselenide **7** and metallic selenium is a useful alternative to triselenide **8** for accessing SeEA peptides.

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