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Selectively Activatable Latent Thiol and Selenolesters Simplify the Access to Cyclic or Branched Peptide Scaffolds

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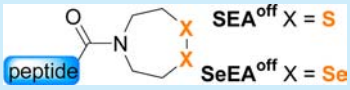
Supporting Information

ABSTRACT: The cyclic dichalcogenides based on the bis(2-chalcogenoethyl)amide structure are latent *N,S* (SEA, chalcogen = S) or *N,Se* (SeEA, chalcogen = Se) acyl shift systems. The large difference in the reducing potential between SEA and SeEA dichalcogenides allows their sequential and selective activation by reduction. Based on these concepts, one-pot three or four peptide segment assembly processes were designed, facilitating access to branched or cyclic peptide scaffolds.



The native chemical ligation reaction (NCL),[1] which consists of the chemoselective reaction of a peptide thiol or selenolester[2] with a cysteinyl (Cys) peptide, is a major tool for protein total synthesis. In recent years, several efficient sequential[3−5] or convergent[6,7] peptide segment assembly strategies have been developed based on the NCL reaction. In particular, efforts have focused on the design of one-pot three[3−6,8] and four[9−11] peptide segment assembly methods with the aim of facilitating access to small proteins or novel peptidic scaffolds.[12] The majority of these synthetic strategies rely on the temporary protection of some Cys residues to avoid side reactions and to direct the ligation at a specific site. Several Cys protecting group (PG) strategies have been developed in the field.[12] The masking of Cys residue in the form of a thiazolidine[3] has found widespread use, although recent studies show that the development of easily removable PGs for Cys is still an important goal in the field of protein synthesis.[8,11,13] In this work, we explore the inverse strategy by developing *protected and selectively activatable* thiol(selenol)ester surrogates (−COR_2 and −COR_3 in Figure 1), which, in combination with the peptide alkylthiolester functionality (−COR_1 in Figure 1), should facilitate considerably the stepwise and chemoselective formation of peptide bonds and access to sophisticated cyclic[14] and/or branched peptide scaffolds.[15]

In synthetic organic chemistry, one common strategy for designing a set of selectively removable PGs is to use structurally similar PGs, which display a graduated reactivity toward a type of reagent. We sought to extend this concept to the design of activatable thiol(selenol)ester surrogates. We were particularly interested in using disulfide bond reducing agents for the activation steps, with the objective to trigger the ligations sequentially and selectively by using a step increase of the reducing potential of the mixture (Figure 1). With these concepts, we also hoped to facilitate the development of one-pot processes to save time and yield.

The development of latent and easily activatable thiolester surrogates is challenging, thus explaining why only a few of such systems have been described to date.[16,17] The *bis*(2-sulfanylethyl)amido cyclic disulfide (SEA^{off}),[4,18] which belongs to the family of *N,S*-acyl shift systems,[19,20] is one of these. Indeed,

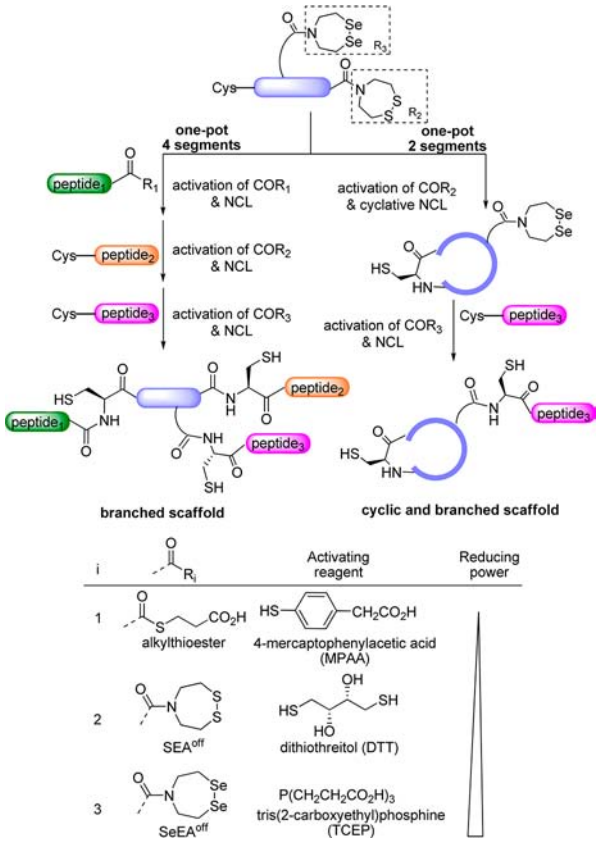


Figure 1. Selectively activatable S(Se)EA^{off} latent thiol(selenol)esters facilitate the synthesis of complex peptide scaffolds.

a peptide alkylthiolester can be ligated selectively in the presence of the SEA^{off} group (−COR_2 in Figure 1) if the reaction mixture contains only MPAA,[21] which is a weakly reducing thiol used to catalyze the NCL reaction. While an excess of MPAA reduces

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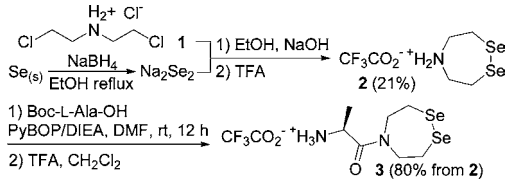
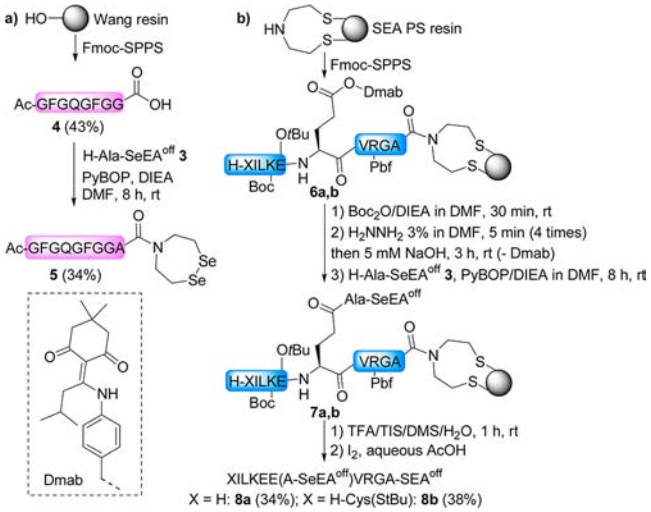
Scheme 1. Synthesis of 1,2,5-Diselenazepane 2 and of SeEA^{off} Alanine Derivative 3 (Trifluoroacetate Salts)



Scheme 2. Synthesis of the SeEA^{off} Peptides in Solution (a) or on the Solid Phase (b)



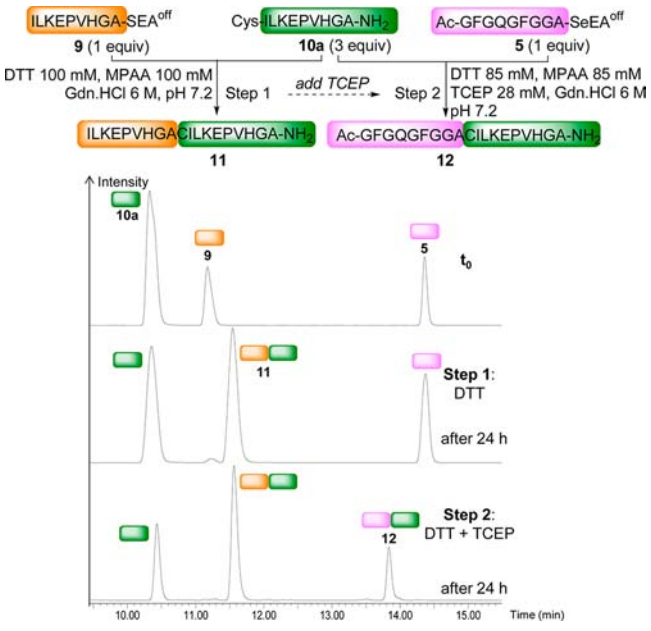
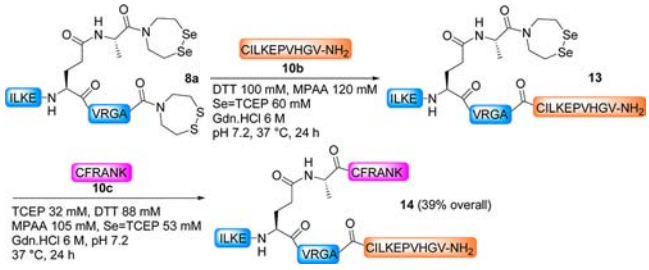


Figure 2. Sequential and selective activation of SEA^{off} and SeEA^{off} groups with DTT and TCEP, respectively.

acyclic disulfides such as cystine bonds, thereby allowing the NCL reaction to proceed in the absence of additional reducing agents, the activation/reduction of the SEA^{off} group requires a stronger reducing agent such as DTT. In this work we show that the selenium analogue of SEA^{off} group, i.e., the SeEA^{off} cyclic diselenide (Figure 1), is inert toward DTT but can be activated by reduction with TCEP. The SeEA^{off} group, which plays the role

Scheme 3. One-Pot Synthesis of Branched Peptide 14 by Sequential SEA/SeEA Ligations



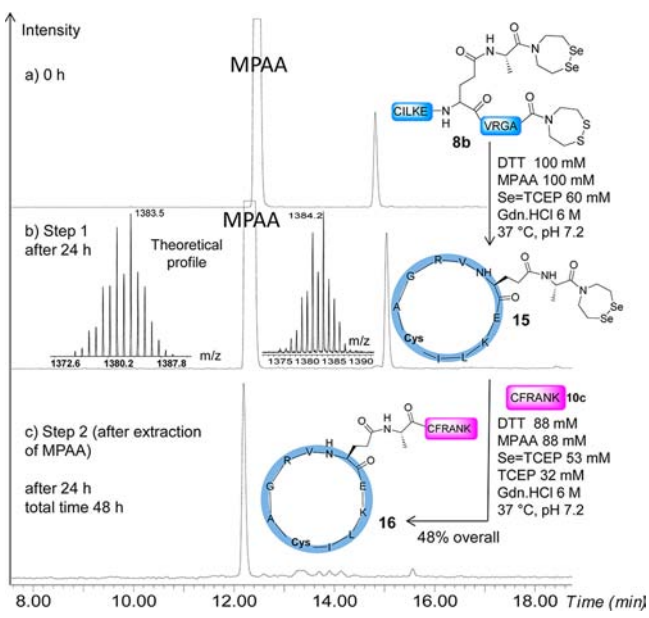


Figure 3. One-pot synthesis of cyclic and branched peptide **16** by sequential SEA/SeEA ligations. HPLC of crude reaction mixtures. (a) Few seconds after the addition of DTT; (b) Cyclative SEA ligation after 24 h; (c) SeEA ligation with peptide **10c** after 24 h.

of $-COR_3$ in Figure 1, is the first latent selenolester described to date. The selective and sequential activation of alkylthiolester and SEA and SeEA functionalities allowed the processes described in Figure 1 to be performed in a single vessel without any intermediate isolation steps.

The synthesis of the SeEA^{off} peptides used in this study required first the preparation of the trifluoroacetate salt of 1,2,5-diselenazepane **2** (Scheme 1), which was obtained by reacting *bis*(2-chloroethyl)amine hydrochloride **1** with diselenide dianion Se_2^{2-}. It was coupled subsequently to Boc-L-Ala-OH using benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)/*N*,*N*-diisopropylethylamine (DIEA) activation. Finally, the Boc group was removed with trifluoroacetic acid (TFA) to produce the SeEA^{off} alanine derivative **3** in good yield.

The 1,2,5-diselenazepane moiety is highly stable toward the classical reagents used for peptide synthesis in solution or on the solid phase. Therefore, SeEA^{off} peptides **5** and **8a,b** used in this study could be prepared by straightforward methodology (Scheme 2). C-terminal SeEA^{off} peptide **5** was obtained by coupling peptide acid **4** to SeEA^{off} alanine derivative **3** using PyBOP/DIEA activation, while the branched SEA^{off}/SeEA^{off} peptides **8a,b** were prepared by solid phase peptide synthesis (SPPS) using 9-fluorenylmethyloxycarbonyl (Fmoc) protocols

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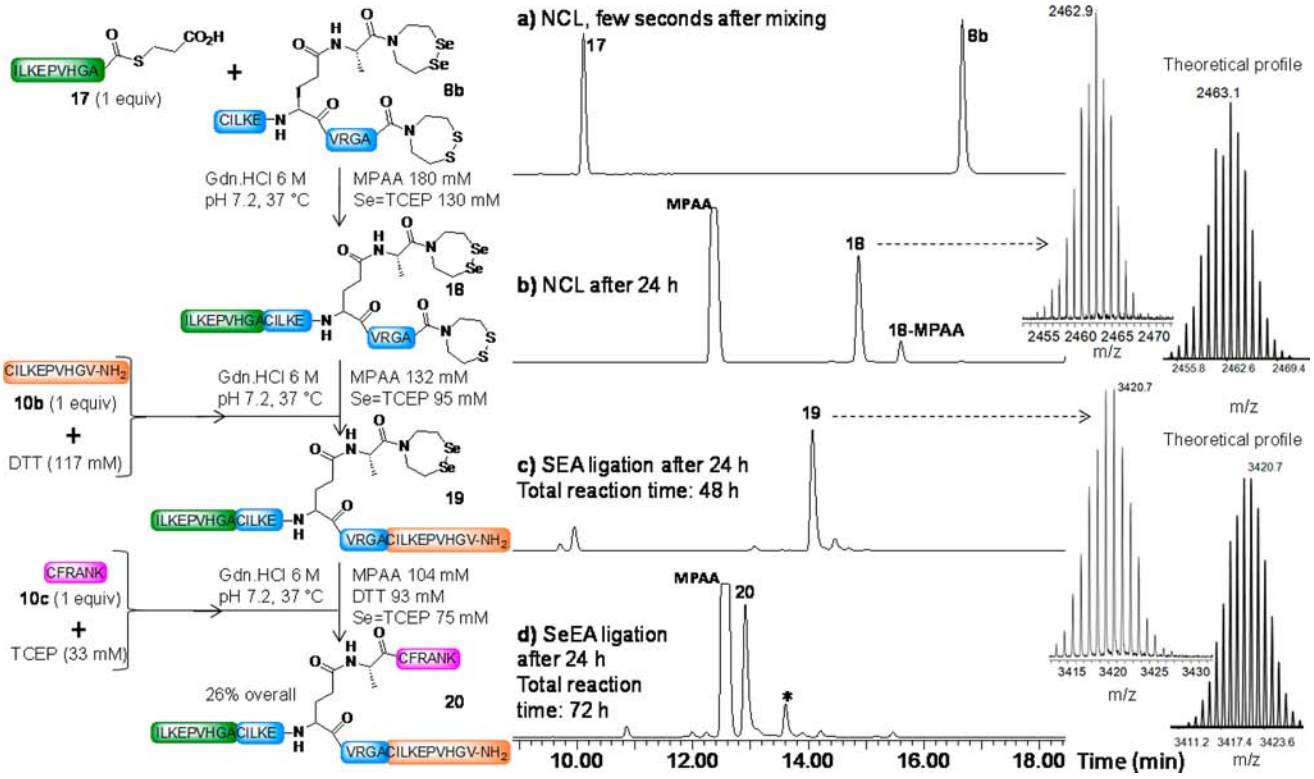

Figure 4. One-pot sequential NCL/SEA/SeEA ligations. LC−MS of crude reaction mixtures (MPAA was extracted after acidification in panels a and c). (a) NCL at $t = 0$; (b) NCL at $t = 24$ h. **18-MPAA** is the mixed disulfide between **18** and MPAA; (c) SEA ligation at $t = 24$ h; (d) SeEA ligation at $t = 24$ h. *The excess of peptide **10b** from the previous step reacts with SeEA group only upon addition of TCEP.

on a SEA polystyrene resin (SEA PS).[18] In brief, one of the glutamic acid residues was introduced as a Dmab protected derivative to allow a selective on-resin deprotection of the γ-carboxyl group using hydrazine hydrate and sodium hydroxide. Then, the SeEA^{off} alanine derivative **3** was coupled to the free γ-carboxyl group using PyBOP/DIEA activation, and the peptides were cleaved from the solid support using TFA and appropriate scavengers. Finally, the SEA^{off} group was formed by iodine oxidation of the SEA dithiol moiety prior to the HPLC purification step.[18,22]

The proof of concept for the selective activation of SEA^{off} latent thiolester in the presence of SeEA^{off} latent selenolester is presented in Figure 2. In this experiment, 1 equiv of each SeEA^{off} peptide **5** and SEA^{off} peptide **9** were solubilized with an excess of Cys peptide **10a** in the presence of DTT and MPAA (Step 1). In these conditions we observed the exclusive formation of peptide **11** resulting from the activation and ligation of SEA^{off} peptide **9**, while peptide **12**, which may have formed by the concomitant activation and ligation of the SeEA^{off} group, was not detected *even after prolonged reaction times*. Then, the addition of TCEP triggered the activation of the SeEA^{off} group and the successful formation of ligation product **12** (Step 2). It is known that the reducing potential of DTT is strong enough to reduce at least partially some acyclic diselenides such as selenocystine bonds.[23] The greater resistance of the SeEA^{off} group to the reduction by DTT is ascribed to the 7-membered ring structure of the SeEA^{off} diselenide, by analogy with the greater reduction potential of dithiols that can form cyclic disulfides in comparison with monothiols.[24] In line with our observations, previous work showed that the 6-membered ring diselenide derived from diselenothreitol (DST) could not be reduced by a large excess of DTT.[25]

The high selectivity of the sequential SEA/SeEA activation process described above found a first application in the one-pot synthesis of branched peptide **14** (Scheme 3) or of cyclic and branched peptide scaffold **16** (Figure 3). The assembly of peptide **14** involved as a first step an intermolecular SEA ligation between bifunctional scaffold **8a** and Cys peptide **10b**, while in the second example with trifunctional peptide **8b**, the SEA ligation step was intramolecular and permitted the cyclization of the peptide scaffold. In both cases, the second SeEA ligation step was performed with Cys peptide **10c** in the presence of Se═TCEP, which is a useful inhibitor of the deselenization of the SeEA group by the added TCEP.[26] The cyclic structure of peptide **16** was confirmed by alkylating the Cys thiols with iodoacetamide and characterizing the trypsin digest of the alkylated peptide by mass spectrometry (see Supporting Information).

In the last example shown in Figure 4, we took advantage of the possibility to selectively activate the alkylthiolester, SEA^{off} latent thiolester, and SeEA^{off} latent selenolester functionalities in this order to design a simple and efficient one-pot four peptide segment assembly process.

The works describing the conception of such one-pot methods are rare due to the difficulty in performing sequentially two deprotection (or activation) steps and three NCL reactions without intermediate isolation steps. Two elegant studies exploited a specific cysteine protecting group strategy.[10,11] Another study relied on the differential reactivity of thiolester surrogates for setting up a kinetically controlled ligation scheme.[9] Fundamentally, the process described in Figure 4 differs from the previous studies in several aspects. First, only one carbonyl functionality can react at a time, i.e., the process is not kinetically controlled. An important consequence is that there is no

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dependency of the selectivity of peptide bond formation on the nature of the reactive groups or on particular proximity effects. For example, the partial activation of the SEA^{off} group during the first step of the one-pot process (**8b** → **18**, Figure 4) would yield inevitably the cyclic peptide **15** (see Figure 3) as a side-product, which was not observed. More generally, LC−MS analysis of the reaction mixtures shows that the SEA^{off} and SeEA^{off} groups are unaffected until the proper reducing agent is introduced in the mixture. Second, the activation of SEA and SeEA thiol(selenol)-ester surrogates by DTT and TCEP, respectively, occurs within minutes. Finally, these popular reducing agents are harmless to polypeptides, while the pH remains constant and neutral throughout the process.

Today, several selectively removable Cys protecting groups are at the disposal of the peptide or protein chemist. In contrast, selectively activatable latent thiol(selenol)ester surrogates are much less developed, although they could facilitate access to sophisticated scaffolds using NCL related reactions. The SeEA^{off} cyclic diselenide is the first latent *N*,*Se*-acyl shift system reported to date. This latent selenolester is highly stable toward reducing thiols, thereby allowing the reduction of acyclic disulfides and the activation of the SEA^{off} cyclic disulfide or of the alkylthioester group in its presence. Simple, mild and highly selective one-pot three or four peptide segment assembly processes were designed by triggering the reactivity of alkylthioester and SEA^{off} and SeEA^{off} functionalities by the addition of MPAA, DTT, and TCEP. We believe that selectively activatable SEA^{off}/SeEA^{off} latent thiol(selenol)esters will facilitate access to complex peptide scaffolds of biological interest. We have also found that SeEA^{off} peptides react faster than classical peptide thiolesters or SEA peptides with Cys peptides. These results and a detailed mechanistic study will be published in due course.

■ ASSOCIATED CONTENT

Supporting Information

Procedures and characterization for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01817.

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Notes

The authors declare no competing financial interest.

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