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Access to Cyclic or Branched Peptides Using Bis(2-sulfanylethyl)amido Side-Chain Derivatives of Asp and Glu

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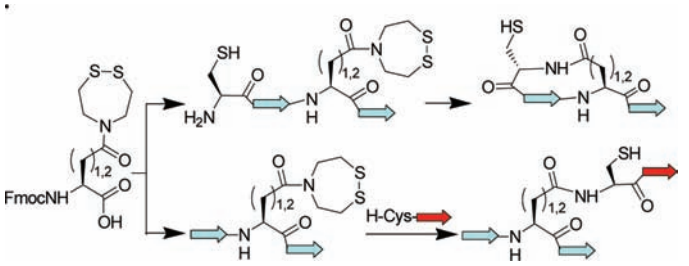
ABSTRACT



Bis(2-sulfanylethyl)amido (SEA) side-chain derivatives of aspartic and glutamic acids enable the synthesis of tail-to-side chain cyclic or branched peptides using standard Fmoc-SPPS followed by SEA native peptide ligation.

The importance of peptide cyclization for studying peptide conformation, creating new structures, or for developing peptide therapeutics is well established.[1] In particular, side-chain lactam bridges linking two amino acid residues that are several residues apart in the linear sequence or head-to-tail backbone peptide cyclization enable rigidification of the structure and improvement of in vivo stability.

Native chemical ligation (NCL),[2] that is the reaction of a C-terminal peptide thioester with an N-terminal cysteine peptide, is now an established method for producing backbone-cyclized peptides[3] or proteins.[4] Often, the rate of cyclization is enhanced by proximity effects induced by the folded state of the peptide or protein.[5] The application of NCL to the synthesis of side-chain cyclized peptides is less frequent. Head-to-side-chain cyclization by ligating a C-terminal thioester with a Cys residue located on a lysine side chain was recently used by Kent's group for creating a novel protein scaffold.[6] The alternative tail-to-side-chain cyclization mode is rare, probably due to the difficulty of installing a thioester group on amino acid side chains such as aspartic or glutamic acids. Interestingly, this mode of cyclization is found in the lasso peptides.[7] Note that access to peptides featuring a thioester group on the side chain of Asp or Glu derivatives would also facilitate the synthesis of branched peptides using NCL chemistry.[8]

Obviously, the design of masked side-chain thioester derivatives of Asp and Glu compatible with Fmoc-SPPS is an important goal which should facilitate access to various

(1) For a recent review on peptide macrocyclization, see: White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509–24.

(2) (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776–9. (b) Kent, S. B. *Chem. Soc. Rev.* **2009**, *38*, 338–51.

(3) (a) Shao, Y.; Lu, W.; Kent, S. B. H. *Tetrahedron Lett.* **1998**, *39*, 3911–3914. (b) Camarero, J. A.; Muir, T. W. *Chem. Commun.* **1997**, 1369–1370. For recent applications, see: (c) Clark, R. J.; Craik, D. J. *Biopolymers* **2010**, *94*, 414–22. (d) Clark, R. J.; Craik, D. J. *Methods Enzymol.* **2012**, *503*, 57–74. For a recent application using peptide hydrazides as thioester precursors, see: (e) Zheng, J.-S.; Tang, S.; Guo, Y.; Chang, H.-N.; Liu, L. *ChemBioChem* **2012**, *13*, 542–546.

(4) (a) Camarero, J. A.; Muir, T. W. *J. Am. Chem. Soc.* **1999**, *121*, 5597–5598. (b) Iwai, H.; Pluckthun, A. *FEBS Lett.* **1999**, *459*, 166–72. (c) Camarero, J. A.; Fushman, D.; Sato, S.; Giriat, I.; Cowburn, D.; Raleigh, D. P.; Muir, T. W. *J. Mol. Biol.* **2001**, *308*, 1045–62.

(5) Camarero, J. A.; Pavel, J.; Muir, T. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 347–349.

(6) Mandal, K.; Pentelute, B. L.; Bang, D.; Gates, Z. P.; Torbeev, V. Y.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2012**, *51*, 1283–1488.

(7) Wilson, K.-A.; Kalkum, M.; Ottesen, J.; Yuzenkova, J.; Chait, B. T.; Landick, R.; Muir, T.; Severinov, K.; Darst, S. A. *J. Am. Chem. Soc.* **2003**, *125*, 12475–12483.

(8) One report describes the synthesis of a branched peptide by NCL of a Glu(SBz) containing peptide with a Cys peptide. See: Dolphin, G. T. *J. Am. Chem. Soc.* **2006**, *128*, 7287–7290.

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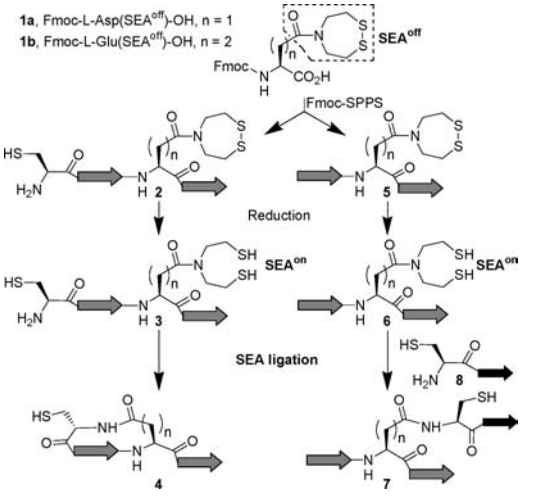
cyclic or branched peptide scaffolds. We describe herein a potential solution to this important problem.

The reaction of a bis(2-sulfanylethyl)amido (SEA^{on}) group with an N-terminal cysteine residue in water and at neutral pH results in the formation of a native peptide bond (Scheme 1).[9,10] This reaction, called SEA ligation, is triggered by an intramolecular *N,S*-acyl shift[11] which leads to the in situ formation of a transient thioester.[12] The transient thioester reacts subsequently with the N-terminal Cys peptide as in NCL to give an X-Cys junction. Oxidation of SEA^{on} results in a cyclic disulfide called SEA^{off} having a 1,2,5-dithiazepan-5-carbonyl structure. SEA^{off} is a self-protected form of SEA^{on}. SEA^{off} and SEA^{on} can be easily interconverted by reduction/oxidation.[13]

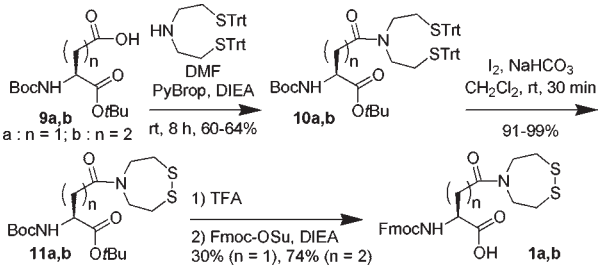
We anticipate that the SEA^{off} group should survive the repetitive piperidine treatments used for removing the Fmoc group during SPPS, given the known stability of dialkyldisulfides in the presence of nitrogen nucleophiles.[14] Consequently, we examined the utility of side-chain SEA^{off} derivatives of Asp and Glu for accessing branched or tail-to-side-chain cyclic peptides using Fmoc-SPPS and intermolecular or intramolecular SEA native peptide ligation (Scheme 1).

Scheme 1. Side-Chain SEA^{off} Derivatives of Asp and Glu Enable the Synthesis of Branched or Tail-to-Side Chain Cyclic Peptides



The synthesis of Fmoc-Asp(SEA^{off})-OH **1a** and Fmoc-Glu(SEA^{off})-OH **1b** was carried out by first coupling bis[[2-[triphenylmethyl]sulfanyl]ethyl]]amine[9] to the side chain of Boc-Asp-O*t*Bu or Boc-Glu-O*t*Bu using bromotripyrrolidinophosphonium hexafluorophosphate (PyBrop)/*N,N*-diisopropylethylamine (DIEA) activation (Scheme 2). Iodine oxidation yielded the corresponding SEA^{off} derivatives **11a**,**b**. Finally, the removal of the *tert*-butyloxycarbonyl (Boc) and *tert*-butyl (*t*Bu) ester groups in tri-

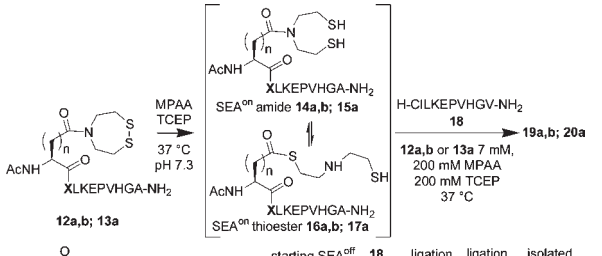
Scheme 2. Synthesis of Fmoc-Asp(SEA^{off})-OH **1a** and Fmoc-Glu(SEA^{off})-OH **1b**



fluoroacetic acid (TFA) followed by reaction with 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) furnished the target Fmoc-Asp(SEA^{off})-OH and Fmoc-Glu(SEA^{off})-OH derivatives **1a**,**b**.

With amino acids **1a**,**b** in hand, we undertook in a first approach the synthesis of model Asp(SEA^{off}) peptides **12a** and **13a** and the synthesis of model Glu(SEA^{off}) peptide **12b** (Scheme 3). These peptides were prepared under standard Fmoc-strategy SPPS conditions and TFA cleavage steps. HPLC isolation yielded peptides **12a** (42%), **12b** (32%), and **13a** (56%) in excellent purity.

Scheme 3. Synthesis of Branched Peptides **19a,b** and **20a**



X	n	starting SEA^{off} peptide	18 (equiv)	ligation pH	ligation product	isolated yield (%)
Ile	1	**12a**	1.5	7.3	**19a**	22
Ile	2	**12b**	1.5	7.3	**19b**	39
Ala	1	**13a**	2	5.5	**20a**	84

We next examined the utility of peptides **12a**,**b** and **13a** for the synthesis of branched peptides using SEA native peptide ligation in the presence of 4-(mercaptophenyl)-acetic acid (MPAA)[15] as shown in Scheme 3. For this, Glu(SEA^{off}) peptide **12b** was reduced in situ at pH 7.3 by

(9) Ollivier, N.; Dheur, J.; Mhidia, R.; Blanpain, A.; Melnyk, O. *Org. Lett.* **2010**, *12*, 5238–41.

(10) SEA ligation can proceed also at mildly acidic pH; see: Hou, W.; Zhang, X.; Li, F.; Liu, C. F. *Org. Lett.* **2011**, *13*, 386–389.

(11) Ollivier, N.; Behr, J. B.; El-Mahdi, O.; Blanpain, A.; Melnyk, O. *Org. Lett.* **2005**, *7*, 2647–50.

(12) (a) Dheur, J.; Ollivier, N.; Vallin, A.; Melnyk, O. *J. Org. Chem.* **2011**, *76*, 3194–3202. (b) Dheur, J.; Ollivier, N.; Melnyk, O. *Org. Lett.* **2011**, *13*, 1560–3.

(13) Ollivier, N.; Vicogne, J.; Vallin, A.; Drobecq, H.; Desmet, R.; El Mahdi, O.; Leclercq, B.; Goormachtigh, G.; Fafeur, V.; Melnyk, O. *Angew. Chem., Int. Ed.* **2012**, *51*, 209–213.

(14) Parker, A. J.; Kharasch, N. *Chem. Rev.* **1959**, *59*, 583–628.

(15) Johnson, E. C.; Kent, S. B. *J. Am. Chem. Soc.* **2006**, *128*, 6640–6.

(16) (a) Ligations of **12a**,**b** with **18** at pH 7.3 proceeded at a significantly lower rate than ligation between C-terminal Ala-SEA^{off} peptide H-ILKEPVHGA-SEA^{off} and **18** (see ref 9), for which $t_{1/2}$ was ~2 h.

tris(2-carboxyethyl)phosphine (TCEP) into Glu(SEA^{on}) amide and thioester peptides **14b** and **16b** in the presence of Cys peptide **18**. HPLC analysis of the reaction mixture showed that the ligation proceeded successfully without significant side-reactions (Figure 1). The half-time ($t_{1/2}$) of the reaction, that is, the time required to reach 50% conversion, was ~19 h (93% conversion after 100 h, Figure 2). The ligation product **19b** was isolated in 39% yield after HPLC purification. The reaction with Asp(SEA^{off}) peptide **12a** proceeded similarly, albeit with a significantly and unexpected lower rate ($t_{1/2}$ ~48 h) than for the Glu(SEA^{off}) counterpart **12b**.[16a]

Ligation of Asp(SEA^{off}) peptide **13a**, featuring an Ala residue in position 2, with Cys peptide **18** at pH 7.3 was also examined to probe the sensitivity of the reaction to aspartimide formation. Interestingly, Asp(SEA^{off})-Ala peptide **13a** reacted as efficiently as Asp(SEA^{off})-Ile analogue **12a** ($t_{1/2}$ ~52 h, Figure 2), with only few percents of aspartimide formation (see Supporting Information). Lowering the pH to 5.5 led to a significant decrease of the time ($t_{1/2}$ ~12 h, Figure 2) and permitted the isolation of the branched product **20a** in 84% yield after HPLC purification.

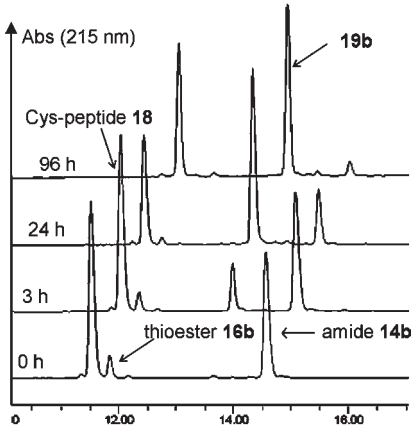


Figure 1. HPLC analysis of the ligation of Glu(SEA^{off}) peptide **12b** with Cys-peptide **18** (see Scheme 3 for experimental details).

Dithiol peptides **14** (Scheme 3), obtained by in situ reduction of SEA^{off} derivatives **12**, equilibrate with thioester peptides **16**. The lower reactivity of Asp(SEA^{off}) peptide **12a** might be due to a destabilization of the thioester form **16a** or to a reduction of the *N*,*S*-acyl shift kinetic rate compared to Glu(SEA^{off}). To clarify this point we determined the fraction of dithiol amide **14** and thioester **16** at equilibrium as a function of pH and the time course for the equilibration. For this, peptides **12** were reduced with TCEP at pH 4−5, that is at a pH which favors the amide form **14**.[12a] For studying the equilibrium between **14** and **16**, the pH was adjusted to the appropriate value between pH 1 and 6, and the mixture was left for 24 h at 37 °C. The yields of amide and thioester forms were determined by HPLC (Figure 3A). The time course for the

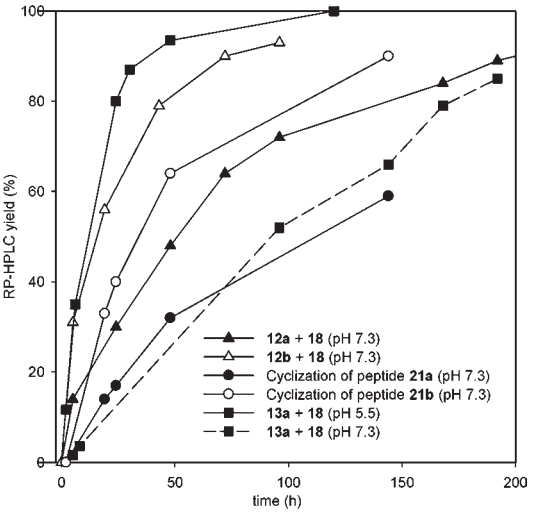


Figure 2. Time course of intermolecular (**12a**,**b** + **18** or **13a** + **18**) or intramolecular (**21a**,**b**) ligations (see Scheme 3 or Figure 4 for experimental conditions). Yields were determined by HPLC (215 nm).

equilibration between **14** and **16** was determined similarly at pH 1 (Figure 3B). Figure 3A shows that the proportion of amide and thioester forms **14** and **16** was very similar for Asp and Glu peptides, whereas Glu peptide amide **14b** equilibrated faster than Asp analog **14a**. These data suggest that the *N*,*S*-acyl shift is the rate limiting step for the ligation of Asp or Glu(SEA^{off}) derivatives with Cys peptides.[17] In line with these results, previous studies have

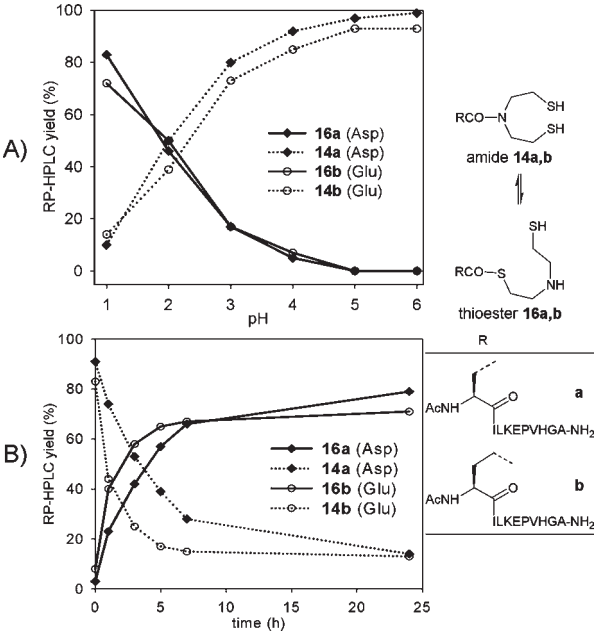
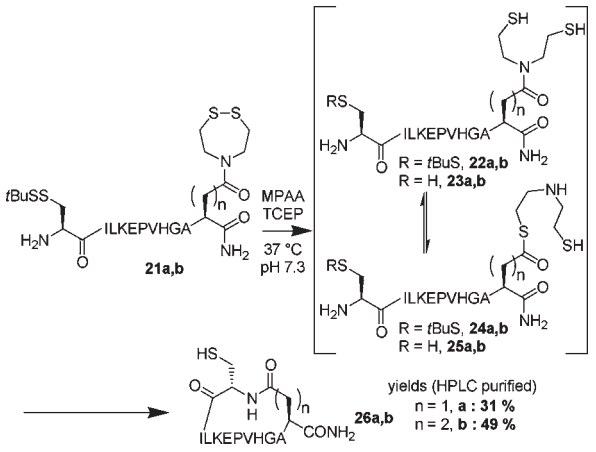


Figure 3. Study of the position of equilibrium (A) and rate of equilibration (B) for peptides **14**/**16**.

Scheme 4. Tail to Side-Chain Cyclization Using SEA Ligation



shown that the rate of transthioesterification of C-terminal SEA^{off} peptides by 3-mercaptopropionic acid was correlated with the rate of the *N,S*-acyl shift.[12a]

We next examined the cyclization of peptides **21a**,**b**, featuring an N-terminal Cys(S*t*Bu) residue and an Asp or Glu(SEA^{off}) group at the C-terminus (Scheme 4). In situ reduction of both acyclic and cyclic disulfides by TCEP triggered the SEA intramolecular ligation. Gratifyingly, both reactions showed the formation of the cyclic peptides **26a**,**b**. As for the intermolecular side chain ligations, cyclization of Glu(SEA^{off}) peptide **21b** proceeded faster than cyclization of Asp analogue **21a** (Figure 2). The HPLC analysis of the reaction mixture for Glu(SEA^{off}) peptide **21b** shows the clean conversion of reduced peptide **23b** into cyclic peptide **26b** and the absence of significant side reactions such as oligomerization (Figure 4). Cyclization of Asp derivative **21a** proceeded similarly but was complicated by the partial deamidation of the primary amide group within cyclic peptide **26a**. Consequently, the isolated yield for **26a** was lower. Besides careful mass spectrometry and NMR analysis, the structure of cyclic peptides **26a**,**b** was confirmed by MALDI-TOF post source decay fragmentation analysis after alkylation of Cys thiol with iodoacetamide and trypsin digestion (see the Supporting Information).

(17) The small amounts of thioester **16b** observed in Figure 1 are formed during the acidic workup and extraction allowing removal of MPAA.

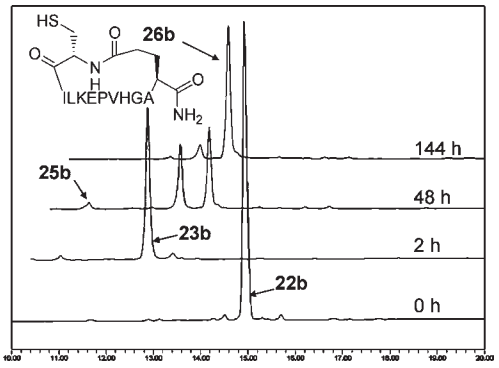


Figure 4. HPLC analysis of the cylization of Glu(SEA^{off}) peptide **21b** (**21b** 1 mM, 200 mM MPAA, 200 mM TCEP, 37 °C, pH 7.3). UV detection at 215 nm.

In conclusion, bis(2-sulfanylethyl)amido side-chain derivatives of Fmoc-Asp-OH and Fmoc-Glu-OH are compatible with standard Fmoc-SPPS. The side-chain SEA^{off} group enables the synthesis of branched or tail-to-side chain cyclic peptides by intermolecular or intramolecular SEA native peptide ligation with an N-terminal Cys residue. Aspartimide formation was not significant when Asp(SEA^{off}) residue was followed by Ala residue. The *N,S*-acyl shift of the bis(2-sulfanylethyl)amido group and SEA ligations proceeded faster for Glu than for Asp derivatives. Overall, the data presented here show that Fmoc-Asp(SEA^{off})-OH and Fmoc-Glu(SEA^{off})-OH are useful amino acid derivatives for the synthesis of complex peptide scaffolds.

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Supporting Information Available. Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.