

CYCLIC PEPTIDES

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Variants of custom peptide cyclization available from Bachem

Our customers can choose from various approaches for obtaining cyclic peptides.

A wealth of cyclic peptides has been isolated from natural sources. The compounds show an amazing structural diversity. Cyclization of peptides is as well an important tool for structure-activity studies and drug development. As ring formation limits the flexibility of the peptide chain, it allows inducing or stabilizing active conformations. Moreover, cyclic peptides are less sensitive to enzymatic degradation.

The broad choice of cyclization variants leaves much room for optimizing the properties of a cyclopeptide. Numerous chemistries together with the required amino acid derivatives are at hand for ring closure.

Types of cycles

Covalent bonds can be formed between various positions of a peptide. Most types of linkages require incorporation

of non-standard amino acid derivatives. The linear precursors usually are assembled by solid-phase peptide synthesis. Partially protected linear peptides can be obtained by Fmoc-SPPS on chlorotrityl resin or Sasrin resin.

Variants of cyclization (see Fig. 1)

- 1) End-to-end (head-to-tail) cyclization
- 2) End-to-side chain cyclization (requires a trifunctional amino acid)
- 3) Side chain-to-side chain cyclization (requires 2 trifunctional amino acids. Reactive side-chains can also be generated by alkylating the amide nitrogen with a substituent bearing a second functionality)

Chemistries of cyclization

Stability, length, and rigidity of the linkage are important parameters to be evaluated, when optimizing the structure of a cyclic peptide.

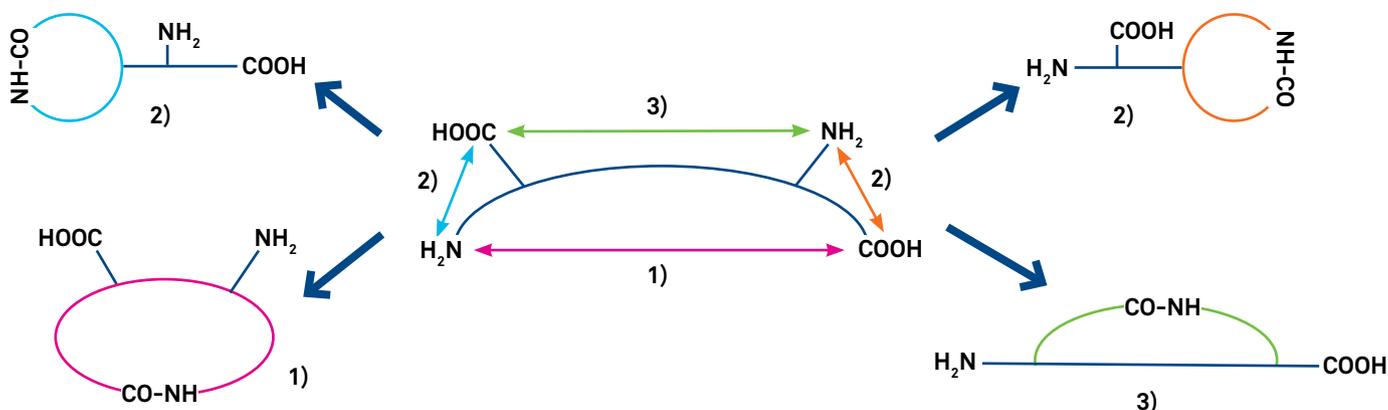


Figure 1: Variants of peptide cyclization. Most peptides can be cyclized by amide bond formation (lactamization, as shown). Modification of the termini or incorporation of suitable amino acid derivatives allows cyclization by other covalent bonds, e.g. lactone, thioether, 1,2,3-triazole

Irrespective of the chemistry used, ring formation is conducted under high dilution conditions as to minimize oligomerization. Another strategy circumvents the tedious work-up of high-dilution reactions: selectively cleavable protecting groups split off on-resin (often used in combination with side-chain anchoring) allow cyclizations on the carrier.

Disulfide bridges and mimics

Disulfide bridge formation is the most common variant of side chain-to-side chain cyclization and Nature's approach for stabilizing active conformations. The bridging is reversible, as S-S bonds can be cleaved enzymatically or by reductants as DTT. Multiple disulfide bridging is often observed in bioactive peptides. When synthesizing such molecules by chemical means, the cysteine pairs can be linked simultaneously or consecutively, the latter being more laborious and considerably costlier for the customer. An array of selectively cleavable sulfhydryl protecting groups has been developed for consecutive bridging, for further details please see our e-brochure "Cysteine Derivatives". Short peptides containing multiple disulfide bridges are extraordinarily stable, especially in combination with head-to-tail cyclization. Trisulfide bridges can also be generated from Cys-containing peptides. Cysteine can be replaced by sulfhydryl-containing amino acids as Pen or Hcy. Replacing terminal cysteines by cysteamine and/or mercaptoalkylcarboxylic acids markedly improves resistance to enzymatic degradation. Atosiban (H-6722), desmopressin (H-7675), and eptifibatide (H-6654, Fig. 2) may serve as examples for such an exchange. Moreover, end-to-end cyclized cystine peptides can be accessed by this approach.

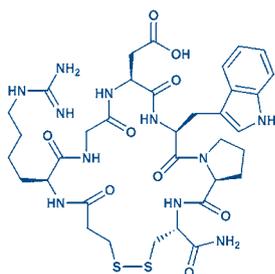


Figure 2: The RGD mimic eptifibatide.

Disulfide bonds are rather flexible, they are sensitive to strong oxidants and bases and, of course, to reductants. They may be replaced by sulfide (thioether) bonds, which can be generated by reacting the cysteine thiol with an N-terminal chloro- or bromoacetyl moiety. As the reactivity of the halogen in ω -haloalkylcarboxylic acids decreases with increasing chain length, use of the preformed thioether derivative is an attractive alternative, e.g. when synthesizing the oxytocin analog carbetocin (H-5832, Fig 3). Replacing both sulfur atoms by aliphatic carbon yields a

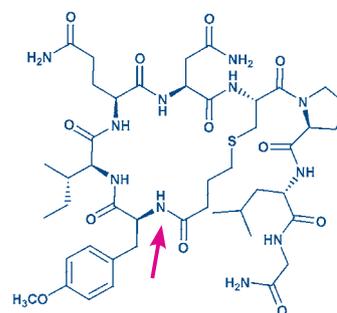


Figure 3: Structure of carbetocin. The arrow points at the site of ring formation.

completely stable cystine mimic. For this end, two cysteines have to be replaced by an α -amino- α,ω -dicarboxylic acid (e.g. by incorporating Fmoc-Asu(OtBu)) or an appropriately protected α,ω -diamino- α,ω -dicarboxylic acid. In the eel calcitonin analog elcatonin (H-2214), α -aminosuberic acid (Asu) replaces the disulfide bridge of the native peptide. Insertion of an α,ω -diamino- α,ω -dicarboxylic acid can be circumvented by replacing the cysteines by ω -alkenyl- α -amino acids. Cyclization is achieved by ring-closing metathesis (RCM) followed by hydrogenation. RCM leaves a double bond, a mixture of the cis and trans isomer is formed. Application of RCM is by no means restricted to the synthesis of stapled peptides, and less sophisticated olefin derivatives such as ω -alkenoic acids can be incorporated in the linear precursor peptide.

Amide linkages

Cyclic carboxamides are also known as lactams. For head-to-tail cyclization in solution, the linear precursor has to be protected except for the terminal amino and carboxyl moieties. Ring formation involving side chains require selectively cleavable protecting groups. OAl/Aloc or highly acid-labile groups are chosen for obtaining such compounds by Fmoc-SPPS.

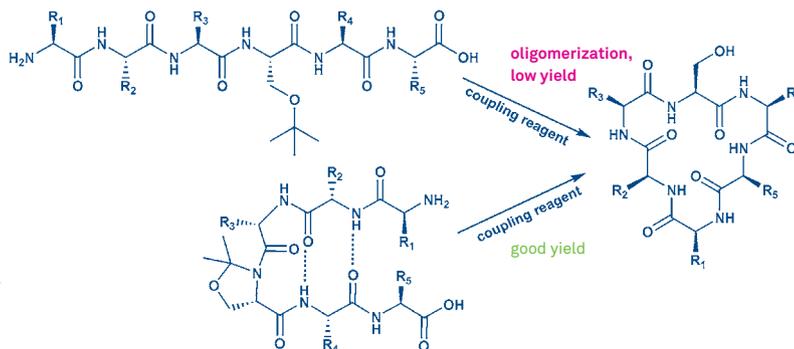


Figure 4: Cyclization of a hexapeptide employing a pseudoproline generated from serine.

The ease of cyclization depends on the conformation of the peptide. A cis amide bond is required for proper folding, for generating a bend. Hence ring closing is facilitated or even only made possible by incorporation of Pro residues, N-methylamino acids, pseudoproline, or Dmb backbone protection (such modifications will promote all types of ring closures). The beneficial effect of the incorporation of a pseudoproline moiety is shown in Fig. 4. For further information please see our e-brochure "Pseudoproline Dipeptides".

The outcome of such a cyclization can be strongly influenced by the choice of coupling reagent and linkage site. So, when synthesizing a cyclic hexapeptide lacking Gly or Pro, one of the 6 amide bonds has to be chosen for ring closure. The corresponding protected linear precursor is obtained by SPPS. Besides modest cyclization yields or failure, concomitant racemization could pose a problem.

Lactam Variants

end-to-end - fully protected peptide with free termini

end-side chain - trifunctional amino acid:

C-terminus-Lys(Aloc), Asp/Glu(OAll)-N-terminus

side chain-side chain - 2 trifunctional amino acids:

Lys(Aloc) and Asp or Glu allyl ester

Orn, Dab, and Dab are alternatives for lysine, Asp/Glu can be replaced by the homologous Aad or Asu. Our e-brochure "Orthogonality of Protecting Groups" presents a compilation of suitable amino acid derivatives and combinations. Native chemical ligation offers an alternative to "standard" cyclization methods.

Other Types of Cycles

Cyclic depsipeptides contain at least one ester bond, which is formed during the penultimate synthetic step followed by deblocking. Suitably protected hydroxycarboxylic acids are required for end-to-end cyclization, whereas selective deprotection of Ser/Thr and Asp/Glu allow side chain-side chain esterification. Cyclic peptide thioesters can be obtained in the same manner.

Cycles, even from completely deprotected peptides, can be generated by 1,3-dipolar cycloaddition (click chemistry) when incorporating azido and alkyne derivatives into the linear peptide, e.g. Pra and Lys(N₂) for side chain ring formation, N-terminal ω-azidocarboxylic acids and C-terminal propargylamides allow end-to-end cyclization. The resulting triazole is a rigid peptide bond surrogate. Fig. 5 shows two examples. Further information can be obtained from our e-brochure "Click Chemistry".

RCM as well allows cyclization of unprotected peptides.

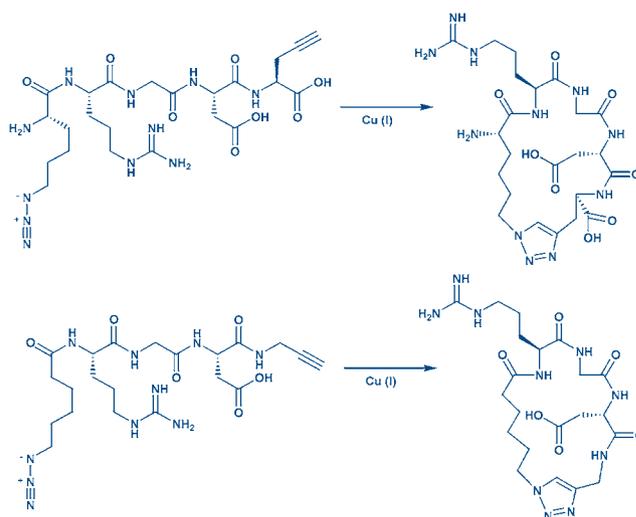


Figure 5: Side chain-side chain and end-to-end cyclization by click chemistry.

References

- Moroder, L., Besse, D., Musiol, H. J., Rudolph-Bohner, S. and Siedler, F. Oxidative folding of cystine-rich peptides vs regioselective cysteine pairing strategies, *Biopolymers* 40, 207-234 (1996)
- Davies, J. S. The cyclization of peptides and depsipeptides, *J. Pept. Sci.* 9, 471 (2003)
- White, C. J. and Yudin, A. K. Contemporary strategies for peptide macrocyclization, *Nat. Chem.* 3, 509-524 (2011)
- Reinwarth, M., Nasu, D., Kolmar, H. and Avrutina, O. Chemical synthesis, backbone cyclization and oxidative folding of cystine-knot peptides: promising scaffolds for applications in drug design, *Molecules* 17, 12533-12552 (2012)
- Roxin, A. and Zheng, G. Flexible or fixed: a comparative review of linear and cyclic cancer-targeting peptides, *Future Med. Chem.* 4, 1601-1618 (2012)
- Bockus, A. T., McEwen, C. M. and Lokey, R. S. Form and function in cyclic peptide natural products: a pharmacokinetic perspective, *Curr Top Med Chem* 13, 821-836 (2013)
- Thakkar, A., Trinh, T. B. and Pei, D. Global analysis of peptide cyclization efficiency, *ACS Combinatorial Science* 15, 120-129 (2013)
- Lau, Y. H., de Andrade, P., Wu, Y. and Spring, D. R. Peptide stapling techniques based on different macrocyclisation chemistries, *Chem. Soc. Rev.* 44, 91-102 (2015)
- Marti-Centelles, V., Pandey, M. D., Burguete, M. I. and Luis, S. V. Macrocyclization Reactions: The importance of conformational, configurational, and template-induced preorganization, *Chem. Rev.* 115, 8736-8834 (2015)
- Rohrbacher, F., Deniau, G., Luther, A. and Bode, J. W. Spontaneous head-to-tail cyclization of unprotected linear peptides with the KAHA ligation, *Chem. Sci.* 6, 4889-4896 (2015)

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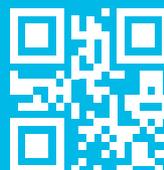
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