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Ring-Closing Alkyne Metathesis Approach toward the Synthesis of Alkyne Mimics of Thioether A-, B-, C-, and DE-Ring Systems of the Lantibiotic Nisin Z

Nourdin Ghalit,[†] Alex J. Poot,[†] Alois Fürstner,[‡] Dirk T. S. Rijkers,[†] and Rob M. J. Liskamp*,[†]

Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands, and Max-Planck-Institut für Kohlenforschung, D-45470 Mülheim/Ruhr, Germany

r.m.j.liskamp@pharm.uu.nl

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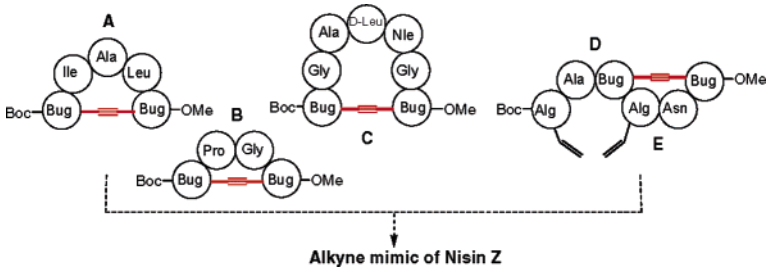
ABSTRACT



Ring-closing alkyne metathesis toward the synthesis of the alkyne-brigded A-, B-, C-, and (D)E-ring mimics of the peptide antibiotic nisin Z is described. We have successfully synthesized alkyne-bridged cyclic peptides containing 4–7 amino acid residues in yields ranging from 18 to 82%.

Replacement of natural conformational constraints such as thioether (sulfide) or disulfide bridges by unnatural constraints is an attractive approach to obtain mimics with increased metabolic stability.[1] Moreover, these constraints might also lead to a better control of the three-dimensional shape that is essential for biological activity. The antimicrobial peptide nisin Z poses a particular challenge as a target for this aim since it contains five consecutive thioether bridges (Figure 1) that play an important role in the dual mode of action of nisin as a bactericide.[2] Nisin binds via its N-terminus, comprising the ABC-ring system, to lipid II, which is an essential precursor for cell wall biosynthesis.

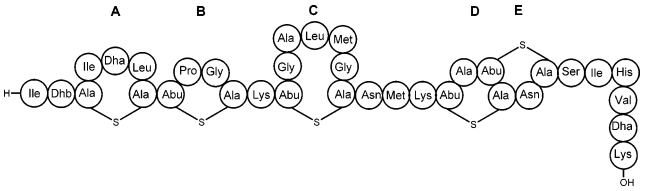


Figure 1. Structure of the lantibiotic nisin Z.

[†] Utrecht University.
[‡] Max-Plank-Institut.

(1) (a) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606. (b) Williams, R. M.; Lui, *J. Org. Chem.* **1998**, *63*, 2130. (c) Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* **1998**, *63*, 2133. (d) Stymiest, J. L.; Mitchell, B. F.; Wong, S.; Vederas, J. C. *Org. Lett.* **2003**, *5*, 47.

(2) (a) Jung, G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1051. (b) Chatterjee, C.; Paul, M.; Xie, L.; van der Donk, W. A. *Chem. Rev.* **2005**, *105*, 633.

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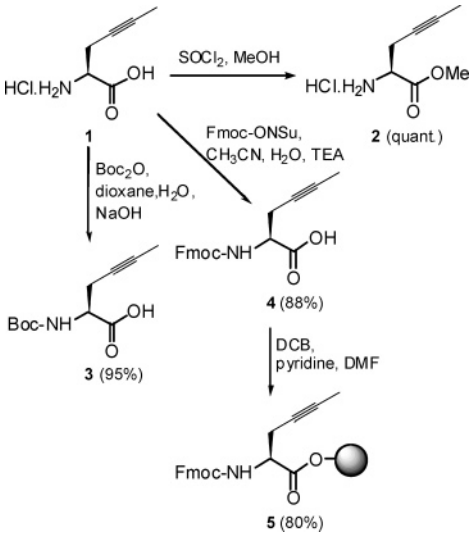
As a result, the C-terminus, comprising the knotted DE-ring, can form pores in the phospholipid membrane. This ultimately leads to cell leakage and causes a collapse of the vital ion gradients across the membrane.[3,4]

Recently, we communicated the preorganization-induced synthesis of a crossed alkene-bridged mimic of the knotted DE-ring system by ring-closing metathesis (RCM).[5] We now wish to report our results on obtaining the even more rigid triple bond, containing alkyne mimics of the A-, B-, C-, and (D)E-thioether rings of nisin Z, by ring-closing alkyne metathesis (RCAM).[6]

In the macrocyclic series, ring-closing metathesis (RCM) leads in most cases to a mixture of cis and trans double-bond-containing alkene derivatives. However, after application of ring-closing alkyne metathesis (RCAM),[7] the triple bond can be selectively reduced to either the cis isomer via Lindlar's catalyst[8] or to the trans isomer by trans-selective hydrosilylation followed by protodesilylation.[9]

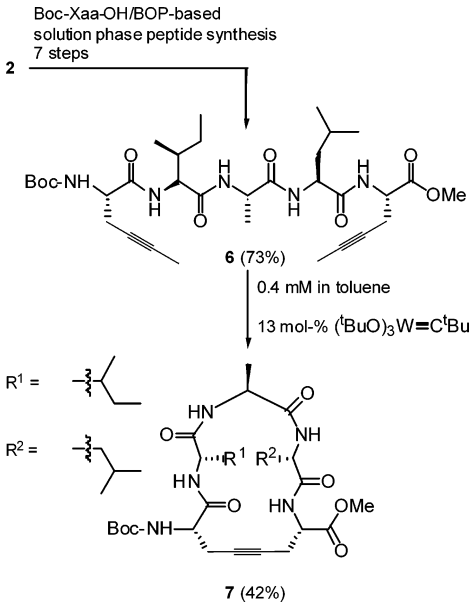
The synthesis of the required (*S*)-2-amino-4-hexynoic acid (2-butyne-glycine: "Bug", **1**)[10] for incorporation in the RCAM precursors was carried out according to the method of Belokon,[11,12] subjecting the Ni(II) complex of the Schiff base derived from glycine and (*S*)-2-(*N*′-(*N*-benzylprolyl)-amino)benzophenone to alkylation with 1-bromo-2-butyne in the presence of base. After column chromatography separation of the addition products (95% yield, diastereometric ratio 96:4) and treatment with acid, (*S*)-2-amino-4-hexynoic acid **1** was obtained as its hydrochloride salt in 80% yield with an enantiomeric excess of >98%. (determined by Chiral HPLC of **4**). Acid **1** was converted to the required derivatives for solution- and solid-phase peptide synthesis (Scheme 1). Thus, methyl ester **2** (quantitative), the *N*-α-Boc derivative **3** (95%), and the *N*-α-Fmoc derivative **4** (88%) were prepared. The latter compound was coupled to plain ArgoGel resin using Sieber conditions[13] to give **5** (Scheme 1).

Scheme 1. Derivatization of (*S*)-2-Amino-4-hexynoic Acid **1**



Linear RCAM-precursor peptide **6**, corresponding to the sequence of the A-ring in nisin, was synthesized in solution starting from ester **2** in seven steps with an overall yield of 73%. RCAM of **6** (0.04 mM) was performed in the presence of the tungsten−alkylidyne complex (^{t}BuO)$_3$W≡C^{t}Bu as a catalyst[14] in toluene at 80 °C to give alkyne bridged cyclic peptide **7** in a yield of 42% (Scheme 2).[15]

Scheme 2. Synthesis of Alkyne Mimic A (**7**)



When this reaction was carried out at a higher concentration, lower yields were obtained and oligomerization was a dominant side reaction. The synthesis of **7** is the first example

(3) Hasper, H. E.; de Kruijff, B.; Breukink, E. *Biochemistry* **2004**, *43*, 11567.

(4) Hsu, S.-T.; Breukink, E.; Tischenko, E.; Lutters, M. A. G.; de Kruijff, B.; Kaptein, R.; Bonvin, A. M. J. J.; van Nuland, N. A. J. *Nat. Struct. Mol. Biol.* **2004**, *11*, 963.

(5) Ghalit, N.; Rijkers, D. T. S.; Kemmink, J.; Versluis, C.; Liskamp, R. M. J. *Chem. Commun.* **2005**, 192.

(6) RCAM on peptides, see: (a) Aguilera, B.; Wolf, L. B.; Nieczypor, P.; Rutjes, F. P. J. T.; Overkleeft, H. S.; van Hest, J. C. M.; Schoemaker, H. E.; Wang, B.; Mol, J. C.; Fürstner, A.; Overhand, M.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem.* **2001**, *66*, 3584. (b) IJsselstijn, M.; Aguilera, B.; van der Marel, G. A.; van Boom, J. H.; van Delft, F. L.; Schoemaker, H. E.; Overkleeft, H. S.; Rutjes, F. P. J. T.; Overhand, M. *Tetrahedron Lett.* **2004**, *45*, 4379. (c) IJsselstijn, M.; Kaiser, J.; van Delft, F. L.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Amino Acids* **2003**, *24*, 263.

(7) See for example: (a) Fürstner, A.; Seidel, G. *Angew. Chem., Int. Ed.* **1998**, *37*, 1734. (b) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012. (c) Fürstner, A.; Davies, P. *Chem. Commun*. **2005**, 2307.

(8) Campos, K. R.; Cai, D.; Journet, M.; Kowal, J. J.; Larsen, R. D.; Reider, P. J. *J. Org. Chem.* **2001**, *66*, 3634.

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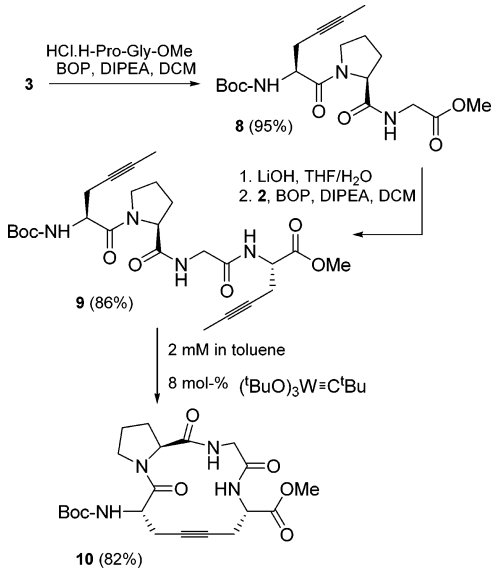
of RCAM of a peptide without any preorganization of the backbone, as was the case for proline or β-turn motif-containing sequences.[6b,c]

RCAM precursor (**9**) of the alkyne mimic of ring B was synthesized in solution starting from Boc-protected (*S*)-2-amino-4-hexynoic acid **3** in an overall yield of 86% (Scheme 3). Treatment of **9** with the tungsten−alkylidyne catalyst

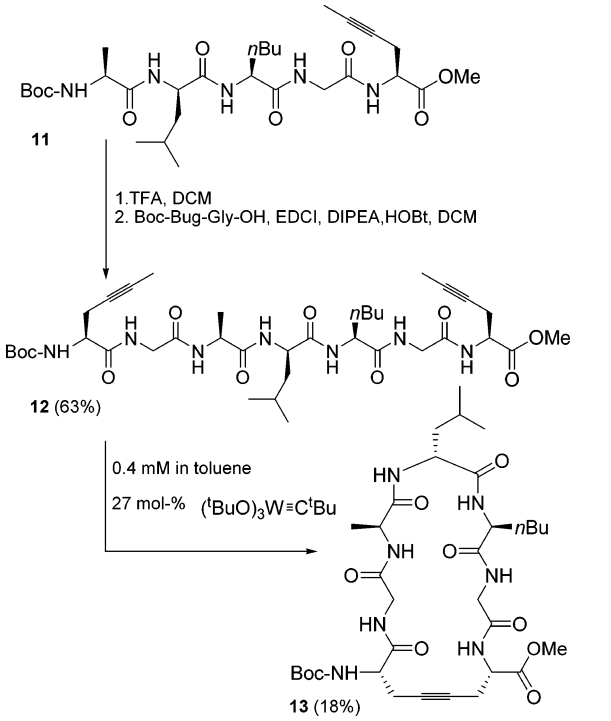
Scheme 3. Synthesis of Alkyne Mimic B (**10**)



resulted in cyclic tetrapeptide **10** in a yield of 82%. This increased yield, as compared to that of **7**, can be explained by a certain degree of preorganization induced by the proline residue, in agreement with literature data.[6b,c] Moreover, the alkyne ring-closure leading to **10** was also faster (45 min) than that affording **7** (2 h).

Starting from pentapeptide methylester **11**, we synthesized the RCAM-precursor **12** for the alkyne mimic of fragment C by fragment coupling of Boc−Bug−Gly−OH, in an overall yield of 63% (Scheme 4). The isolated yield was somewhat lower than that of **9** and **6**, mainly due to the low solubility of **12** and its precursors. On the basis of previous experience, we decided to replace the central leucine with a D-leucine residue, to favor a turn-like conformation, since it was known that the sequence with all L-amino acid residues did not cyclize under ring-closing metathesis conditions in

Scheme 4. Synthesis of Alkyne Mimic C (**13**)



the presence of second-generation Grubbs Ru catalyst.[16] Nevertheless, treatment of **12** with the tungsten−alkylidyne catalyst resulted in cyclic heptapeptide **13** in only 18% yield. The main product that was isolated consisted of insoluble oligomers.

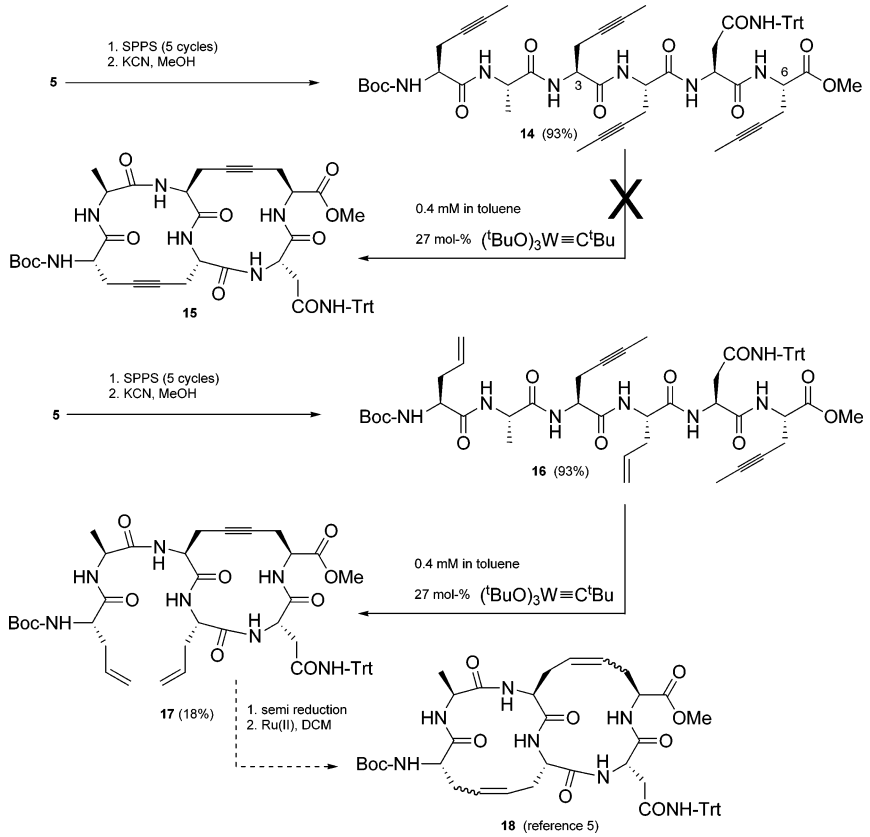
Our first approach to synthesize the crossed DE-ring system (Scheme 5) was based on the preorganization-induced synthesis of both ring systems in a single RCM reaction step as was recently reported for the synthesis of bisalkene DE-ring mimic **18**.[5] Therefore, the RCAM precursor with the amino acid sequence comprising the DE-ring with four alkyne moieties was synthesized on the solid-phase starting from solid-phase resin derivative **5** and using 2-butyne glycine derivatives **3** and **4**. After treatment with KCN in MeOH, protected hexapeptide methyl ester **14** was obtained. Unfortunately, upon RCAM, no monocyclic intermediates or bicyclic **15** were formed. After 24 h, the formation of polymeric material was observed; the conversion was still incomplete, and starting material was recovered.

Therefore, we changed our strategy by synthesizing peptide **16** containing two allylglycine residues and two 2-butyne-glycine residues. Since the ruthenium alkene metathesis catalyst and the tungsten alkyne metathesis catalyst can operate orthogonally to each other, it was envisioned to first treat **16** with the tungsten−alkylidyne catalyst in order to synthesize the monocyclic alkyne-bridged peptide **17**. This might be immediately followed by a RCM reaction in the presence of second-generation Grubbs catalyst[16] or a RCM reaction after first reducing the triple bond to the corre-

(15) **General Procedure for Ring-Closing Alkyne Metathesis.** All RCAM reactions were carried out under Ar in flame-dried glassware using Schlenk techniques. Precursor peptide **6** (46 mg, 70 μmol) was dissolved in dry toluene (200 mL), and the tungsten−alkylidyne catalyst (^{t}BuO)$_3$W≡C^{t}Bu (4 mg, 9 μmol) was added. The obtained reaction mixture was heated to 80 °C and stirred for 2 h. The reaction was monitored by TLC until no changes in product distribution could be observed. Then, H_2O (1 mL) was added to quench the catalyst, and the solvent was removed by evaporation. The residue was purified by column chromatography with DCM/MeOH 97.5:2.5 v/v as the eluent. Cyclic pentapeptide **7** was obtained as an off-white powder in 42% yield (18 mg). R_f = 0.56 (DCM/MeOH 9:1 v/v). ESMS calcd for $C_{29}H_{47}N_5O_8$ 594.4, found 594.7 $[M + H]^+$, 538.6 $[(M - C_4H_8) + H]^+$, 494.5 $[(M - C_2H_5O_2) + H]^+$. HRMS calcd for $C_{29}H_{47}N_5O_8$-Na 616.33218, found 616.33223. NMR assignments: see Supporting Information.

(16) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.

Scheme 5. Approach to the Synthesis of the Crossed Alkyne DE-Mimic



sponding double bond. These procedures would then lead to a DE mimic with the double bond originating from the triple bond with a defined (cis or trans) geometry.

After RCAM of precursor peptide **16** under high dilution (250 μM) conditions for 90 min, the reaction mixture was quenched by the addition of H_2O. HPLC analysis showed that two products were formed in a ratio of 3:2, which could be separated by preparative HPLC. According to NMR and LC-MS/MS, **17** was the major product and was obtained in 18% isolated yield (5 mg). As a side product, a dimer (connected through Bug3 and Bug6 residues) was identified by LC-MS/MS. Unfortunately, due to the extremely low solubility of **17**, the reduction of the triple bond and the subsequent alkene ring-closing metathesis reaction were unsuccessful.

In conclusion, we have successfully applied ring-closing alkyne metathesis for the synthesis of alkyne-bridged peptides of up to 4−7 amino acid residues in yields ranging from 18 to 82%. These cyclic peptides are rigid mimics of the thioether rings of nisin. The resulting mimics will be used to assemble AB- and ABC-alkyne mimics of nisin Z. These fragments will be tested for their potency to bind to lipid II as possible antibiotics. Furthermore, stereoselective reduction of the triple bonds may lead to derivatives of which the influence of the geometry of the resulting double bonds on the affinity toward lipid II can be evaluated.

Acknowledgment. We thank Cees Versluis for measuring the LC-MS/MS spectra.

Supporting Information Available: Experimental procedures, $^1H/^{13}C$ NMR data, mass analyses, and HPLC data for compounds **1**−**4**, **6**−**14**, **16**, and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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