Synthesis of Triazole Bridged Vancomycin Mimics

Synthese van Triazool Gebrugde Vancomycine Mimetica

(met een samenvatting in het Nederlands)

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door

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Synthesis of Triazole Bridged Vancomycin Mimics

Jinqiang Zhang

2012

for my dear parents

献给我亲爱的父母

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Chapter 1

General Introduction

1.1 Cyclic peptides and synthetic approaches toward cyclic peptides

Cyclic peptides are amino acid derived macrocyclic compounds that occupy a unique and important segment in medicinal chemistry because of their broad biological activities as diverse as antimicrobial agents,[1-4] protease inhibitors,[5-8] agonists and antagonists of G protein-coupled receptors,[9] and protein-protein interaction inhibitors.[10,11] Along with the naturally occurring cyclic peptides, such as the ACE inhibitor K-13 (**1**),[12] the immunosuppressant drug cyclosporine (**2**),[13] the protease inhibitors cyclotheonamide A and B (CtA and CtB) (**3**),[14] and the antibiotic daptomycin (**4**)[15] (Figure 1), synthetic cyclic peptides have also been recognized as a great resource and inspiration for drug discovery.[16]

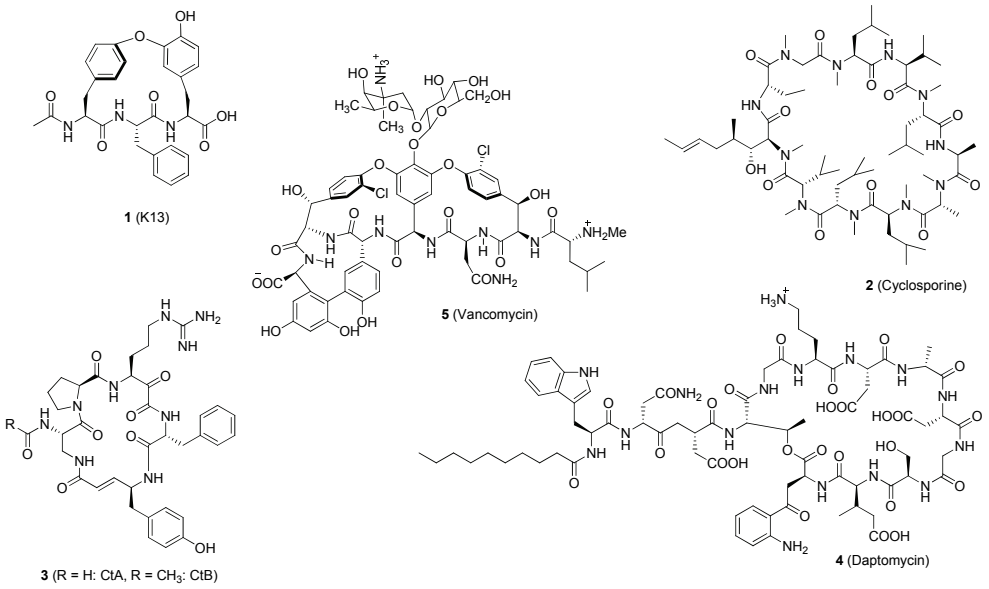

Figure 1. Examples of naturally occurring bioactive cyclic peptides.

In peptide engineering, macrocyclization is recognized as an efficient way to restrict the conformational freedom of a peptide when it binds to the target enzyme or receptor which often leads to increased affinity[9, 17] and selectivity.[18] Cyclic peptides also possess other beneficial properties such as reduced polarity, increased proteolytic stability and consequently improved druggability.[19] Cyclic peptides could be obtained by cyclization of the linear peptide precursor via different covalent bond formation reactions, that lead to disulfide-,[1-3] lactone-,[20] lactam-,[21] biaryl- and biaryl ether[12, 22] bridges. With respect to the latter constraint, one of the

outstanding examples is the glycopeptide antibiotic vancomycin[34, 35] (Figure 1), which is also the inspiration source and starting point of the research described in this thesis.

The development of synthetic methods toward cyclic peptides is a challenging task for the medicinal chemist, since only a limited number of approaches for efficient peptide macrocyclization are presently available. The macrocyclization step is always suffered with low yield or uncontrolled oligomerization and requires a highly diluted substrate concentration and thus long reaction time. However, a number of synthetic approaches have been successfully applied for the construction of versatile macrocyclic structures. The traditional methodologies such as lactonization and lactamization are the most commonly used approaches to achieve (peptide)macrocycles.[23] S_NAr-based macrocyclization is widely utilized to synthesize macrocycles containing the biaryl ether linkage.[22, 24] With the great contribution from Grubbs,[25] the ruthenium-based ring-closing metathesis (RCM) approach has been the most widely employed method for the synthesis of macrocycles including cyclic peptidomimetics.[26] Palladium-catalyzed cross-coupling reactions, such as the Stille coupling, the Heck reaction, the Suzuki reaction and the Sonogashira reaction, is another extensively explored category for creating versatile constrained macrocycles.[27]

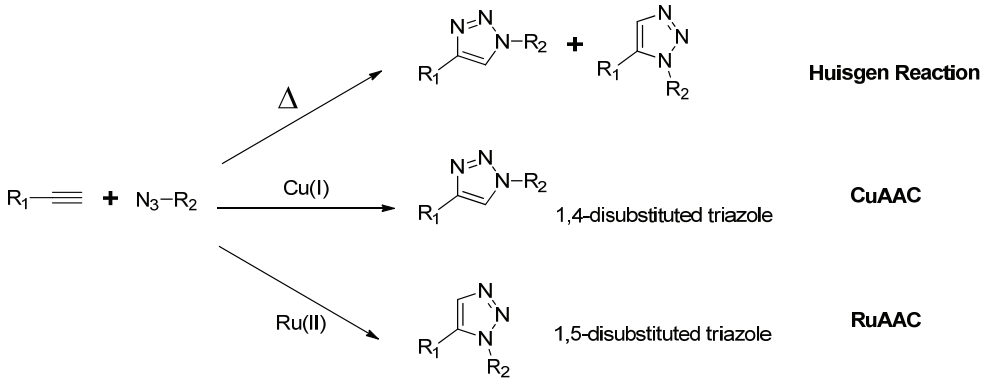

Figure 2. The 1,3-dipolar cycloaddition reaction between an alkyne and an azide.

The groups of Meldal[28] and Sharpless[29] independently found that the copper(I)-catalyzed 1,3-dipolar alkyne-azide cycloaddition (CuAAC) reaction yielded, under very mild conditions, regioselectively the 1,4-disubstituted triazole moiety, which was an important improvement of the traditional Huisgen

cycloaddition[30] (Figure 2). CuAAC- which is the most prominent example of 'click chemistry'- had become the most intensively utilized reaction in many areas, ranging from material science to chemical biology.[31] This powerful chemoselective reaction has provided the medicinal chemist a great platform for exploring novel macrocyclic structures.[32]

In this thesis, 'click chemistry' is the main theme and our main goal is to explore the applicability of CuAAC (as well as RuAAC) chemistry for the synthesis of simplified vancomycin DE- and CDE-ring mimics.

1.2 Vancomycin and its peptidomimetics

Vancomycin (**5**) (Figure 3) is the most representative member of the family of glycopeptide antibiotics which is the most important class of drugs for the treatment of resistant bacterial infections.[33] After its isolation by Eli Lilly in 1955, vancomycin was introduced into clinical practice in 1958, but its structure was not disclosed until 1983.[34] The main clinical use of vancomycin is the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, for which it is regarded as the antibiotic of last resort.[35] However, after more than 50 years of clinical use, the emergence of resistant Gram-positive pathogens including vancomycin-resistant *Enterococci* (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) would be a fatal disaster for the public health care since new antibiotics have not been developed so far, which has stimulated many efforts to develop new vancomycin analogues and peptidomimetics.[36]

Vancomycin inhibits bacterial cell wall synthesis by binding to the peptidoglycan peptide terminus D-Ala-D-Ala found in cell wall precursors.[37] The constrained structure of vancomycin forms a hydrophobic binding pocket and binds the D-Ala-D-Ala sequence through van der Waals contacts, and the complex is further stabilized by forming five hydrogen bonds lining in the binding pocket (Figure 3).[38] In the most prevalent resistant phenotypes (VanA and VanB), the bacteria remodeled their precursor peptidoglycan terminus from D-Ala-D-Ala into D-Ala-D-Lac.[39, 40] The binding affinity of vancomycin for the altered ligand is reduced (1000-fold), resulting in a corresponding loss in antibaterial activity (1000-fold)[40] since the loss of a hydrogen bond and a repulsive lone pair interaction between the vancomycin residue 4 carbonyl moiety and the D-Ala-D-Lac ester oxygen.

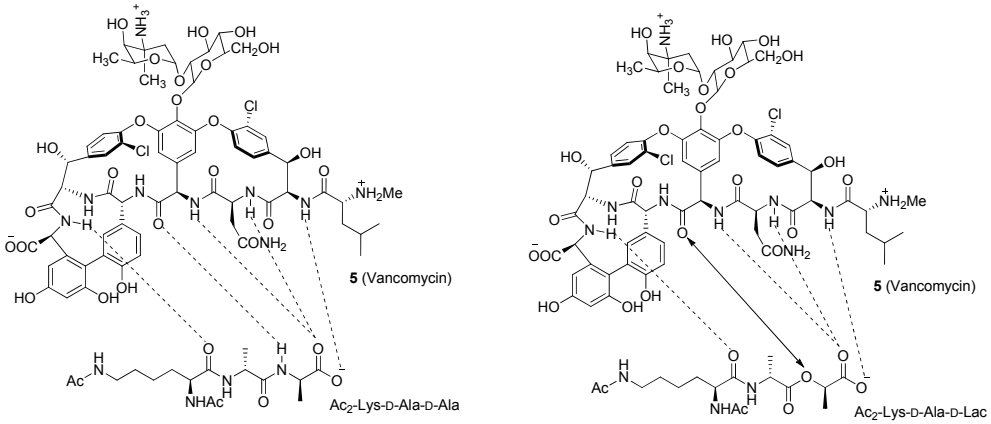

Figure 3. Vancomycin and its binding interactions with the peptidoglycan peptide terminus Ac_2-Lys-D-Ala-D-Ala and Ac_2-Lys-D-Ala-D-Lac.

Based on vancomycin's mechanism of action against bacteria, newly designed vancomycin analogues should have an altered binding pocket which is able to bind not only D-Ala-D-Lac but also maintain binding to D-Ala-D-Ala. The significant contributions from Dale Boger's group have shown that modification of residue 4 within vancomycin's peptide backbone could achieve dual binding to D-Ala-D-Ala and D-Ala-D-Lac (Figure 4). In their first successful case, the reengineered [Ψ[CH_2NH]Tpg^4]-vancomycin aglycon (**6**) with a methylene moiety at residue 4 instead of a carbonyl, eliminated the repulsive lone pair interaction, and increased the binding affinity with D-Ala-D-Lac, and exhibited MICs of 31 μg/mL against VanA *Enterococcus faecalis* (BM4166), being 40-fold more potent than vancomycin or its aglycon (MICs of 2000 and 640 μg/mL).[41] Another modification of residue 4 led to the redesigned [Ψ[C(=NH)NH]Tpg^4]vancomycin aglycon (**7**), which could act as either a hydrogen bond donor or acceptor. Indeed, **7** showed improved dual binding affinities and antibacterial activities (MIC = 0.31 μg/mL) against VanA *E. faecalis* (VanA VRE, BM4166) and an equivalent activity against vancomycin-sensitive bacteria as vancomycin.[42] These elegant results reconfirmed the mechanism of vancomycin binding interactions and proved that it is possible to overcome the resistance by an appropriately redesigned vancomycin backbone.

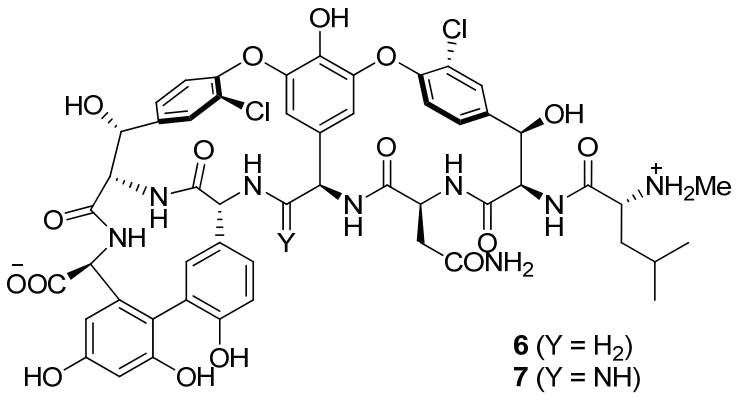

Figure 4. Reengineered vancomycin aglycon derivatives **6** and **7**.

Unfortunately, the complicated structure of vancomycin deterred detailed structural modifications and a large scale synthesis of these analogues seemed to be very challenging. Therefore, it is the mission of a medicinal chemist to develop simplified mimics of vancomycin, which could exhibit antibacterial activity comparable to vancomycin although which are much more easily accessible by organic synthesis.

With respect to this, some simplified vancomycin mimics have been developed featuring part of the vancomycin's cavity structure. A library of vancomycin DE-ring mimics was synthesized by a combinatorial approach as described by Ellman and coworkers.[43] The synthesized compounds incorporated a simplified vancomycin DE-ring cavity as an invariant core and a linear tripeptide side chain which could freely rotate to eliminate the repulsive interaction with the D-Ala-D-Lac ester oxygen. The library was employed for the identification of synthetic receptors targeting vancomycin resistant bacteria and one of these receptors (**8**) (Figure 5) was found to bind Ac_2-Lys-D-Ala-D-Lac 5-fold more tightly than vancomycin in aqueous solution.

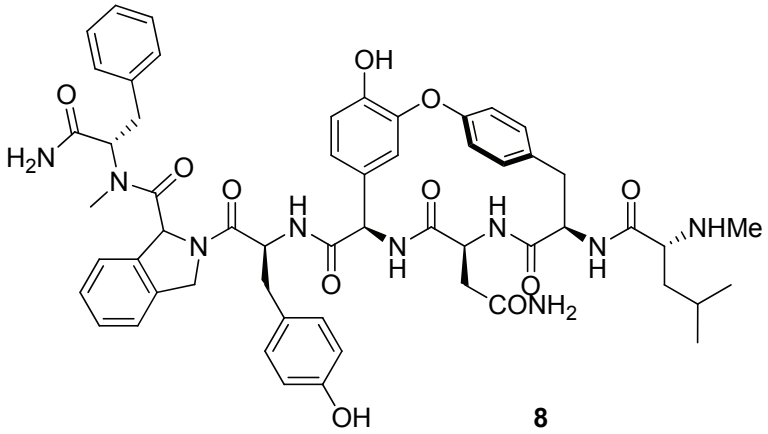

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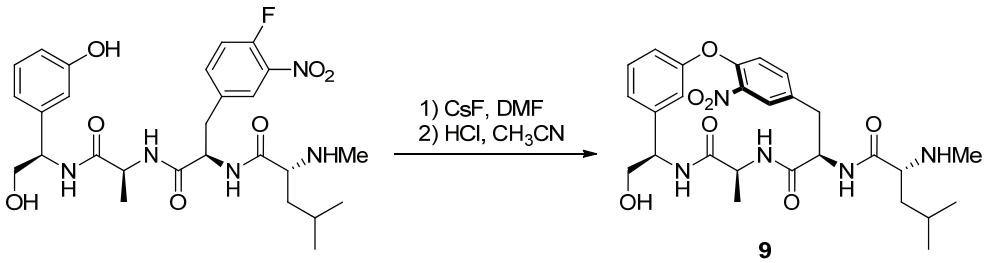
Figure 5. Simplified vancomycin mimics as developed by Ellman and coworkers.

Zhu and coworkers reported the synthesis of a 16-membered macrocycle to mimic the carboxyl binding pocket of the vancomycin DE-ring fragment.[44] The synthesis featured an intramolecular cycloetherification based on an S_NAr reaction to form efficiently the biaryl ether bond (Scheme 1). The resulting cyclic tripeptide (**9**) showed interesting binding interactions with Ac-D-Ala-OH with a dissociation constant (*K*d) of 5×10^{-4} M.



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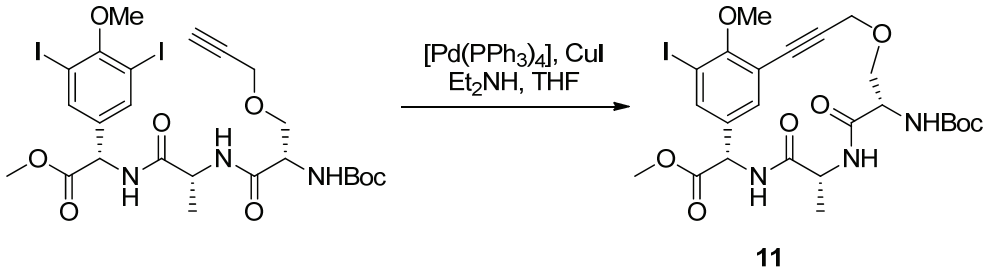
Scheme 1. Synthesis of a simplified vancomycin mimics by an intramolecular S_NAr reaction.

Our group has also been working on the development of simplified mimics inspired by vancomycin by employing different synthetic approaches. A solid phase synthesis of simplified vancomycin mimics was developed by Arnusch and Pieters.[45] An on-bead S_NAr reaction was successfully applied to enable the required coupling of two peptide side chains (Scheme 2). The synthesized mimics (exemplified by **10**) showed binding with the tetra-*n*-butyl ammonium salt of Ac-D-Ala-OH in CH_3CN with an association constant of 7.3×10^3 M^{-1}.

Scheme 2. Synthesis of vancomycin mimics **10** on solid phase.

A macrocyclization strategy based on a Pd-catalyzed Sonogashira reaction was employed to synthesize alkyne-bridged cyclic tripeptides as highly constrained mimics of the vancomycin DE-ring fragment, as communicated by ten Brink *et al*.[46] However, the intramolecular Sonogashira reaction suffered from low yield because of the rigid alkyne-bridge (Scheme 3). Modeling data of the cyclic peptides (**11**) showed a distorted triple bond and a cavity-like structure that might serve as a binding pocket for the guest ligand.



Scheme 3. Synthesis of vancomycin mimics **11** by an intramolecular Sonogashira reaction.

Moreover, a bicyclic mimics of the vancomycin CDE-ring was described by ten Brink as well.[47] The bicyclic pentapeptide (**12**) was prepared by ring-closing metathesis (RCM) (Figure 6). Unfortunately, the resulting products were obtained as a mixture of stereoisomers since the poor *E*/*Z* selectivity of the RCM reaction and due to racemization of the central hydroxyphenylglycine derivative. Via molecular modeling, the isomer with lowest energy conformation showed a cavity-like structure that may be suitable for ligand binding.

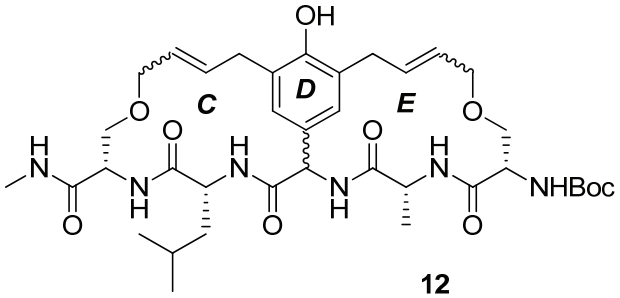


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Figure 6. Bicyclic mimics **12** representing the vancomycin CDE-ring fragment as synthesized by RCM.

Our ongoing efforts toward the development of novel synthetic methodology to access conformationally restricted cyclic peptides, especially the simplified vancomycin mimics, stimulated the research as described in this thesis, in which 'click chemistry' was employed as a chemical tool for the synthesis of constrained cyclic peptides as vancomycin peptidomimetics.

1.3 Application of CuAAC reaction in the synthesis of cyclic peptidomimetics

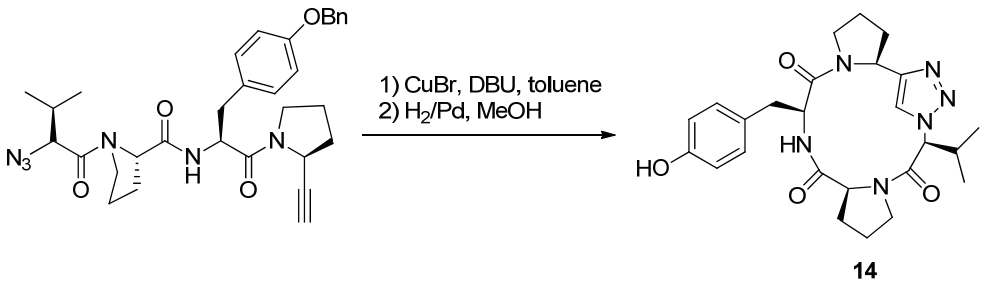
As mentioned above, 'click chemistry' is a powerful strategy for the synthesis of novel macrocycles. Because of its orthogonal nature to other coupling reactions and high functional group tolerance, the Cu(I)-catalyzed alkyne-azide cycloaddition reaction (CuAAC, Figure 2) has been utilized for preparing various cyclic peptidomimetics. The CuAAC cyclization reaction could be achieved in the manner of either head-to-tail or side chain-to-side chain, which was also found in a variety of natural cyclic peptides. In many cases, the cyclic peptidomimetics obtained by CuAAC were biologically active comparable to their parent natural products, which in turn stimulated the wide application of CuAAC reaction in the synthesis of other peptidomimetics.

Ghadiri and coworkers reported the synthesis of 1,4-disubstituted triazole containing cyclic peptidomimetics as ligands for the somatostatin receptor (SSTR).[48] In this study the triazole moiety was used to mimic a locked *trans*-amide bond in the cyclic tetrapeptide. A small library of stereoisomeric cyclic peptidomimetics (**13**) was synthesized employing the CuAAC reaction with TBTA as Cu(I) ligand to suppress competing homodimer formation (Scheme 4).

The biological activity of the new compounds was evaluated against five human SSTR subtypes 1-5, and exhibited diverse selectivity profiles.

Scheme 4. Synthesis of cyclic peptidomimetics as SSTR ligands using CuAAC cyclization.

One of the advantages of the CuAAC cyclization is that it can be applied to synthesize some otherwise inaccessible cyclic peptidomimetics. For instance, the total synthesis of the highly constrained cyclotetrapeptide Pro-Tyr-Pro-Val, which was isolated in 1993 from the bacteria *Lactobacillus helveticus* and reported as a potent tyrosinase inhibitor,[49,50] was precluded since the difficult cyclization of the linear peptide precursor by a general lactamization approach. However, van Maarseveen and coworkers reported the synthesis of a cyclic tetrapeptide analogue **14** (cyclo-[Pro-Val-Ψ(triazole)-Pro-Tyr]) by CuAAC cyclization in an impressive 70% yield (Scheme 5).[51] Gratifyingly, the peptidomimetic **14** retained its biological activity compared to the parent natural product against tyrosinase.



Scheme 5. Synthesis of a triazole-containing cyclic tetrapeptide by CuAAC cyclization.

These two research examples illustrated the head-to-tail cyclization by CuAAC reaction, while the side chain-to-side chain cyclization was also employed to prepare cyclic peptidomimetics and in some cases, this approach resulted into even more complicated cyclic structures. The Meldal group recently reported the

synthesis of bicyclic mimics of Tachyplesin I (TP-I), which is a 17-residue bicyclic peptide containing two disulfide bonds (Figure 7).[52] The Cys-3 and Cys-7 in TP-I were replaced by propargylglycine (Pgl) and Cys-12 and Cys-16 were replaced by (*S*)-2-amino-4-azido-butyric acid (2-Abu(γ-N_3)) or 5-azido-norvaline (Nva(δ-N_3)), respectively, to give two linear TP-I analogues: [^{3,7}Pgl,^{12,16}2-Abu(γ-N_3)]-TP-I and [^{3,7}Pgl,^{12,16}Nva(δ-N_3)]-TP-I. By employing CuAAC cyclization on the solid support, two bicyclic TP-I analogues (**15**) were synthesized and the two disulfide bonds in TP-I were replaced by triazole moieties. The antimicrobial activity of the triazole-bridged analogues were comparable or even better than those for the wild-type TP-I, which confirmed the structural similarity of the novel TP-I analogues with the natural TP-I.

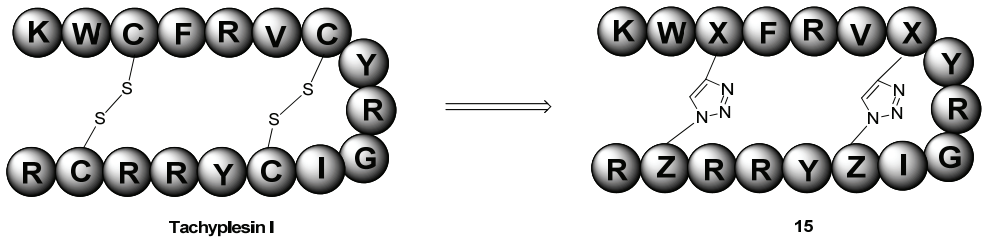


Figure 7. TP I and its triazole-bridged mimetics (X = Pgl, Z = 2-Abu(γ-N_3), or Nva(δ-N_3)).

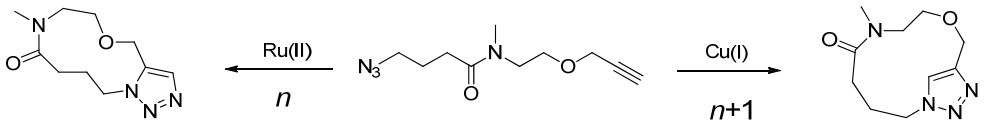
The orthogonal nature of CuAAC reaction could also be employed to create novel cyclic peptidomimetics by combination with other cyclization methods. Suga and coworkers reported the ribosomal synthesis of bicyclic peptides via an orthogonal CuAAC reaction followed by a thioether formation reaction.[53] With a series of biosynthesized linear peptide precursors (e.g., **16**) containing two pairs of orthogonal reacting groups, Cys-Cab (4-(2-chloroacetyl)aminobutyric acid) and Aha (azidohomoalanine)-Pgl (propargylglycine), the bicyclic peptide scaffold (e.g., **17**) was formed in two independent cyclization steps (Scheme 6). The advantage of using such biosynthesized peptides is that it can be readily expanded to the preparation of libraries of bicyclic peptides with a uniform skeleton and accelerate the discovery of novel peptide-based drugs against various biologically relevant targets by combination with an appropriate in vitro peptide-display technique.

Scheme 6. Synthesis of bicyclic peptidomimetics using CuAAC and an orthogonal thioether cyclization reaction.

With the successful application of the CuAAC reaction in the preparation of a variety of cyclic peptidomimetics, it is our expectation that it could also be applied in our newly designed vancomycin mimics and that the triazole moiety could be used as an alternative for the biaryl ether bridges in vancomycin.

1.4 RuAAC reaction and its application in the synthesis of cyclic peptides

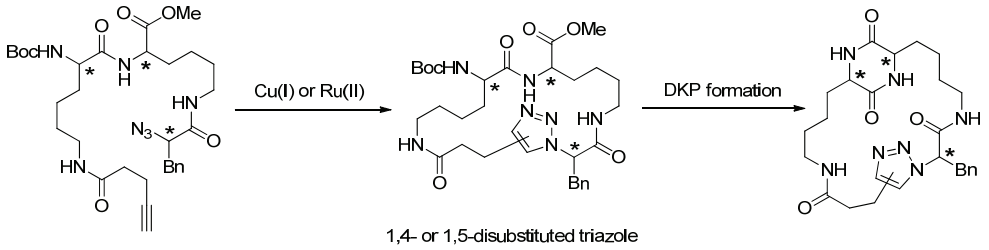
The great success of the CuAAC reaction promoted the development of synthetic methods toward the selective access of the complementary regioisomer, the 1,5-disubstituted triazole moiety. With the important contribution from Fokin and coworkers, the ruthenium-catalyzed alkyne-azide cycloaddition reaction (RuAAC) (Figure 2) was successfully established in 2005.[54] Since the emergence of this novel RuAAC reaction, it has been employed to achieve diverse structures in combination with the CuAAC reaction. Although the application of this reaction in the synthesis of cyclic peptidomimetics is still growing, there are already a few research reports to mention its great potential for the application in drug discovery.
Marcaurelle and coworkers reported in 2009 for the first time the application of RuAAC for the intramolecular cyclization of triazole-containing *N*-methyl lactams.[55] By employing both CuAAC as well as RuAAC cyclization, the macrocyclic structural diversity was achieved by the choice of the catalyst. Using a common substrate, the RuAAC and CuAAC reaction led to the formation of two structurally unique macrocycles with either a 1,5- or a 1,4-triazole moiety resulting in a ring with *n* or *n* + 1 membered macrocycle (Scheme 7).



Scheme 7. Intramolecular cyclization via CuAAC and RuAAC cyclization.

Inspired by this study, it was decided to expand the synthetic strategy and explore the application of RuAAC cyclization for the synthesis of 1,5-disubstituted triazole-bridged vancomycin mimics. Unfortunately, the application of RuAAC cyclization was proved unsuccessful in the synthesis of cyclic peptidomimetics in a limited number of publications.[56] However, we believed that the intramolecular RuAAC cyclization could be achieved if appropriate reaction conditions were implemented. Indeed, after careful optimization of the cyclization conditions, the RuAAC cyclization was successfully applied in the synthesis of our desired vancomycin DE-ring mimics (Chapter 3).

Shortly thereafter, Spring and coworkers also successfully applied the RuAAC reaction for the synthesis of a library of triazole-containing cyclic peptidomimetics.[57] In their diversity-oriented synthesis, the CuAAC or RuAAC cyclization was followed by a DKP formation step to achieve additional structural complexity and rigidity, and a small library of 14 cyclic peptidomimetics consisting of either a 1,4- or a 1,5-disubstituted triazole and a DKP ring were synthesized (Scheme 8).



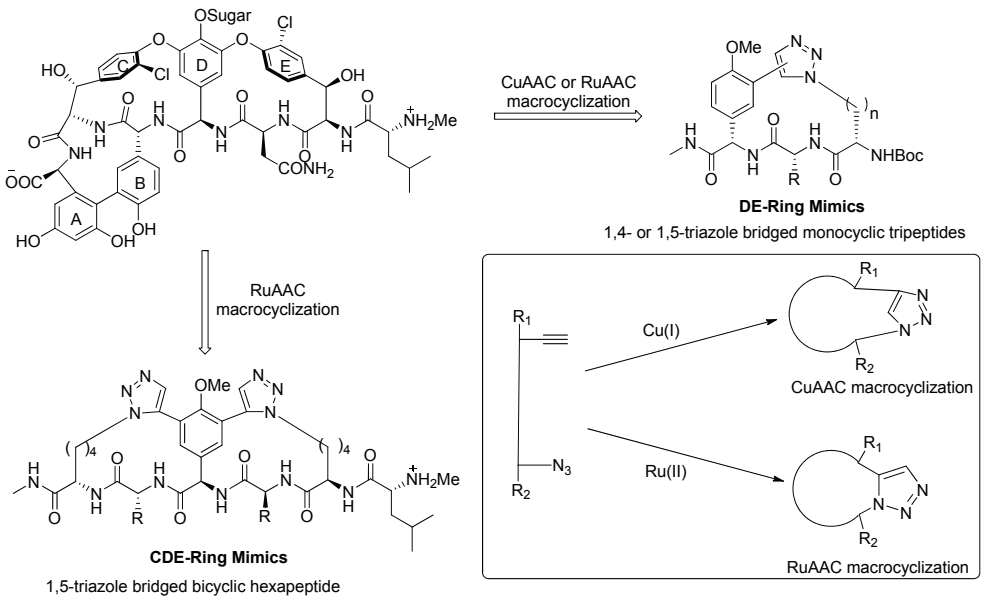
Scheme 8. Synthesis of 1,4- or 1,5-disubstituted triazole-containing cyclic peptidomimetics by CuAAC or RuAAC followed by DKP formation.

The RuAAC and CuAAC cyclization reactions are important complementary tools for the synthesis of cyclic peptidomimetics. As they share the common substrate, the diverse macrocyclic structures can easily be obtained through catalyst control. On the other hand, since the conformational difference between the generated 1,4- and 1,5-disubstituted triazole, it is possible that one specific

conformation is favored in the macrocyclic structure of the newly synthesized peptidomimetic. In general, the combinatorial application of CuAAC and RuAAC is a very promising synthetic protocol in the synthesis of cyclic peptidomimetics.

1.5 Outline of this thesis

The ultimate goal of the research described in this thesis is to develop biologically active vancomycin-based peptidomimetics. Based on previous research reported by our group on the synthesis of vancomycin mimics and inspired by the wide application of CuAAC reaction in the synthesis of cyclic peptidomimetics, it was decided to synthesize 1,4-disubstituted triazole-bridged vancomycin mimics. During the progress of this research, the RuAAC reaction was included as an alternative and complementary chemical tool for the CuAAC cyclization reaction to expand the structural diversity of the vancomycin mimics. Eventually, it was shown that the intramolecular RuAAC cyclization was a more efficient approach to obtain monocyclic vancomycin DE-ring mimics. Finally, the RuAAC cyclization was successfully applied in the synthesis of the desired bicyclic vancomycin CDE-ring mimics (Scheme 9).



Scheme 9. Rationale and general strategy of the research described in this thesis.

In **chapter 2**, a CuAAC based macrocyclization approach was successfully applied for the synthesis of relatively small and rather constrained cyclic tripeptide systems to mimic the DE-ring of the vancomycin. After the successful synthesis of a series of linear tripeptide precursors, a CuAAC macrocyclization approach was developed. Although this strategy preferentially led to the formation of cyclic dimers, two cyclic monomeric tripeptides, derived from ornithine and lysine, were successfully synthesized with a TBTA-promoted CuAAC macrocyclization protocol.

Chapter 3, describes a successfully developed Ru(II)-catalyzed click-type macrocyclization protocol, and a series of cyclic tripeptides as vancomycin DE-ring mimics was synthesized in good yield. The most constrained cyclic tripeptide obtained had only 13 atoms in the macrocyclic ring, while the lysine-derived tripeptide was of the same size as the vancomycin DE-ring containing 16 atoms. The RuAAC macrocyclization proved to be effective for the synthesis of 1,5-disubstituted triazole-containing cyclic tripeptides, and was superior compared to the CuAAC-based click cyclization with respect to the prevalent synthesis of monomeric cyclic peptides.

In **Chapter 4**, the synthesis of a 1,5-triazole-bridged bicyclic vancomycin CDE-ring peptidomimetic using RuAAC macrocyclization was successfully achieved. Based on the optimized synthetic route, the linear hexapeptide could be synthesized in 10 steps from the commercially available hydroxyphenylglycine with good to excellent yield. The RuAAC macrocyclization of the linear hexapeptide successfully provided the desired bicyclic peptidomimetic, and the method could be in principal applied to synthesize a small library of bicyclic peptidomimetics for screening the biological activity as vancomycin mimetics. The methodology developed in this study proved that the RuAAC macrocyclization had very good intramolecular selectivity and could be applied in the synthesis of more complicated bicyclic compounds with a biological relevance.

Chapter 5 described in more detail the NMR characterization, conformational studies by molecular modeling, the binding affinity and the *in vitro* biological evaluation of the triazole-bridged vancomycin mimics. The successful synthesis of the triazole bridged monocyclic and bicyclic peptidomimetics of vancomycin was confirmed by NMR spectroscopy in combination with HPLC and MALDI-TOF analysis. Molecular modeling was performed to get the structural information of the synthesized cyclic peptidomimetics and the structural similarity with vancomycin was investigated by superimposing the energy minimized structure

onto a vancomycin-related antibiotic in complex with the natural substrate. Next, ITC experiments were carried out to determine the binding affinity of these cyclic peptidomimetics with the natural ligand of vancomycin. Binding toward D-Ala-D-Ala was observed however with lower affinity compared to vancomycin. Finally, the antibacterial activity of the bicyclic peptidomimetic was tested and unfortunately no obvious inhibition of the bacterial growth was observed.

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Chapter 2

Synthesis of 1,4-Triazole-Bridged Vancomycin DE-Ring Peptidomimetics via CuAAC Macrocyclization

Parts of this chapter have been published: Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2011**, *13*, 3438-3441.

2.1 Introduction

Cyclic peptides are currently an attractive research topic since they have important biological activity as diverse as antimicrobial agents,[1] protease inhibitors,[2] agonists and antagonists of G Protein-coupled receptors,[3] and protein-protein interaction inhibitors.[4] By introduction of a cyclic covalent constraint, the activity and selectivity of biologically active peptides can be increased since the unfavourable entropy loss during receptor binding is reduced.[5,6] Such a covalent constraint is, however, not limited to head-to-tail cyclization, and there are several examples in nature of bioactive peptides that contain other covalent constraints like, disulfide bridges,[1a-c] lactone- and lactam bridges,[7] and biaryl ether bridges.[8] With respect to the latter, one of the outstanding examples is the glycopeptide antibiotic vancomycin (Figure 1). Vancomycin is currently reserved for the treatment of life-threatening bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA).[9] Because of the emerging resistance to vancomycin[10], it is of utmost importance to design and synthesize novel vancomycin mimics as potential peptide-based antibiotics.

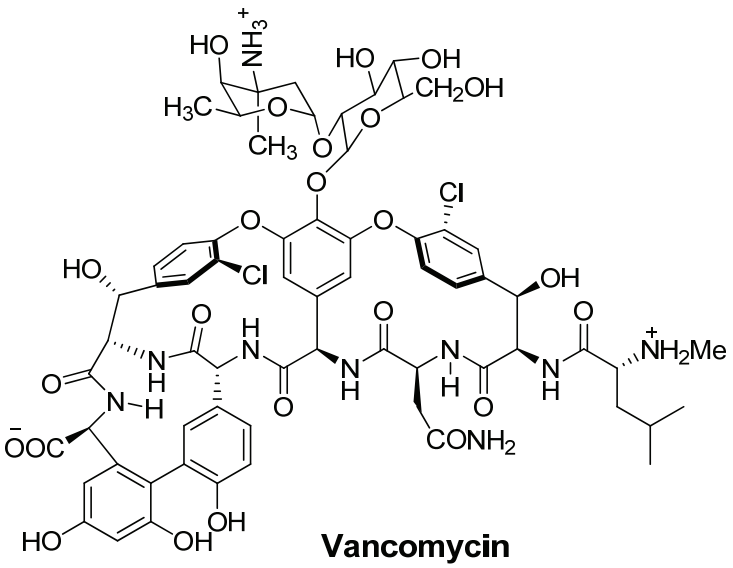


Figure 1. Structure of vancomycin.

There is a great challenge in the development of approaches to control the conformation and shape of peptides, which are usually very flexible. The ongoing

development of novel synthetic methodology to access conformationally restricted cyclic peptides encouraged us to develop effective approaches to mimic the bioactive conformation of vancomycin as closely as possible. Previously, our group had employed ring-closing metathesis (RCM) to synthesize a bicyclic pentapeptide **1** to mimic the central CDE-ring system of vancomycin,[11] and also a constrained DE-ring derived cyclic tripeptide **2**[12] as a vancomycin mimics was synthesized by using the Sonogashira cross-coupling to introduce the alkyne functionality (Figure 2).

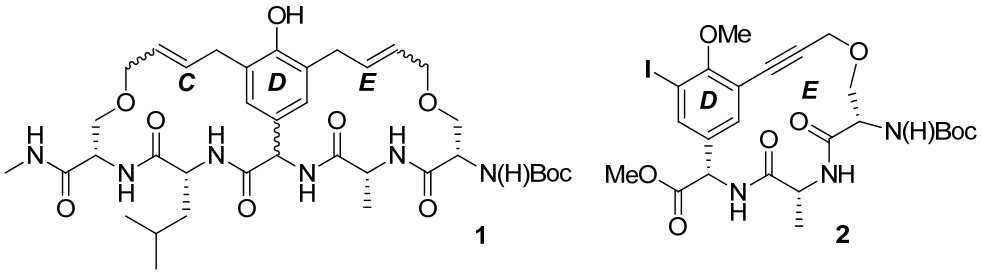


Figure 2. Constrained vancomycin mimics synthesized in our group.

The discovery of the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction,[13] which gives regioselectively the 1,4-disubstituted triazole, has stimulated the widespread application of this 1,3-dipolar cycloaddition for, among others, the synthesis of cyclic peptides.[14] Next to our goal towards the synthetic accessibility of small cyclic peptides, especially to uncover promising alternatives for the biaryl ether bridge in the vancomycin-related class of glycopeptide antibiotics, the introduction of a 1,4-triazole moiety employing the CuAAC reaction is a new attractive alternative that possibly lead to potentially bioactive peptidomimetics.

2.2 Results and Discussion

2.2.1 Design and synthesis of the vancomycin DE-ring mimics by CuAAC macrocyclization

Vancomycin has a tricyclic structure containing two biaryl ether bridges.[9] As an initial study, the DE-ring section of vancomycin was selected as target for the

synthetic mimics. The introduction of a 1,4-triazole moiety to replace the biaryl ether bridge leads to the desired DE-ring mimics of vancomycin (Figure 3).

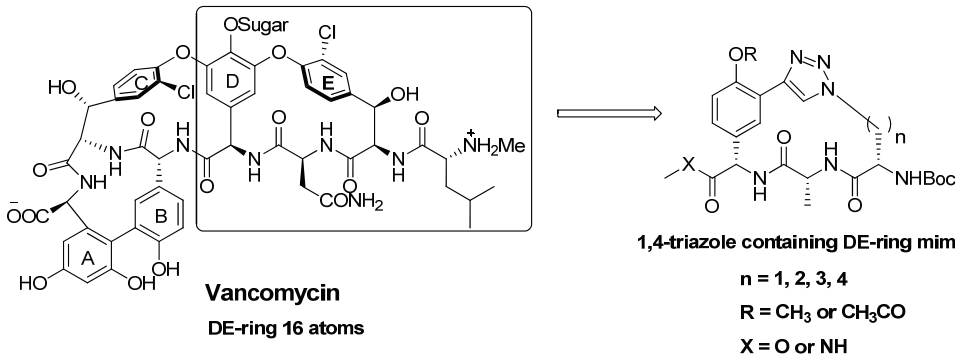
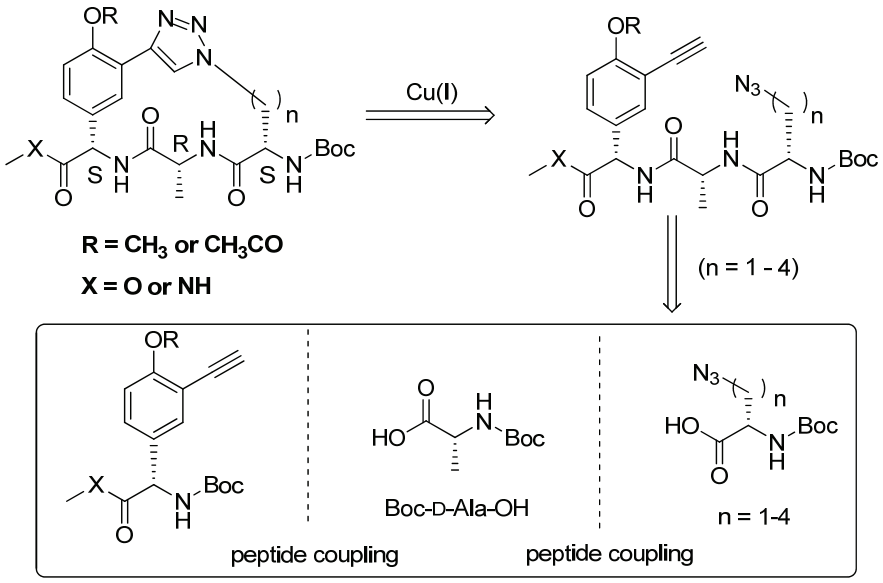


Figure 3. Design of the vancomycin DE-ring mimics containing 1,4-triazole bridge.

These DE-ring mimics consist of a cyclic tripeptide and the side chains of the C- and N-terminal amino acids were linked together by a 1,4-triazole bridge. In the original design, the hydroxyl group of the hydroxyphenylglycine could be protected either as acetyl ester or methyl ether and the C-terminal amino acid could be converted to a methyl ester or a methyl amide. However, during the synthesis, it was found that appropriate protection of the hydroxyl group was important for a successful synthetic route. The final choice was a methyl ether as hydroxyl group protecting group while the C-terminal carboxylic acid was converted into a methyl amide. The ring size of the designed DE-ring mimics ranged from 14 to 17 atoms by varying the side chain of the N-terminal azidoamino acid (n = 1, 2, 3, and 4 respectively).

In principle, the 1,4-triazole moiety can be conveniently incorporated by the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The triazole ring can be introduced intramolecularly employing the CuAAC reaction as the final macrocyclization step, or intermolecularly at an early stage of the synthesis, followed by a macrolactamization step by means of peptide coupling reagents. To highlight the CuAAC reaction as a powerful tool for synthesizing small cyclic peptides, the focus was set on a reliable CuAAC macrocyclization method (‘click cyclization’) for the synthesis of the designed series of DE-ring mimics, which can be further expanded to a small library of cyclic peptides. The macrolactamization was also carried out as an alternative route for comparison.



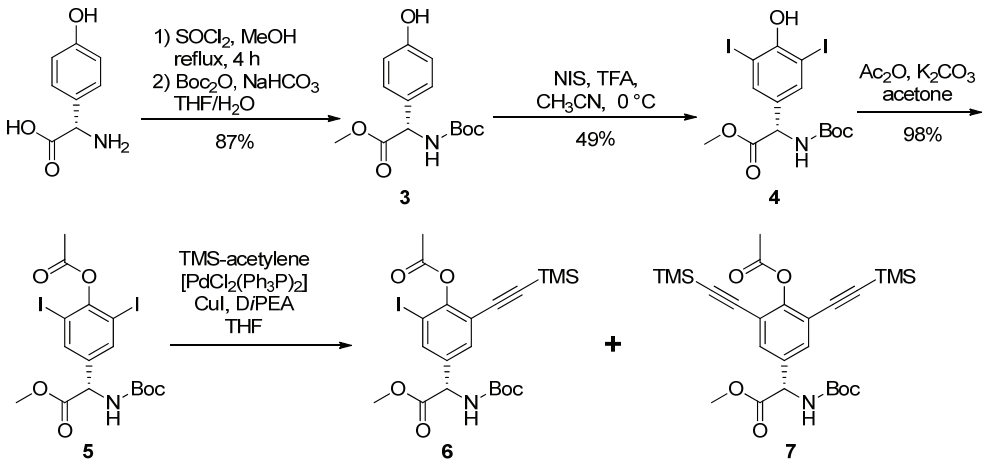
Scheme 1. Retrosynthetic analysis of the 1,4-triazole containing cyclic tripeptides as vancomycin DE-ring mimics.

The retrosynthetic route of the DE-ring mimics using CuAAC macrocyclization is shown in Scheme 1. The CuAAC reaction of the linear tripeptide precursor functionalized by an alkyne as well as an azide moiety would yield the desired cyclic peptide via side chain cyclization. The assembly of the linear tripeptide could be achieved by peptide coupling of the three amino acid building blocks, which include the C-terminal alkyne-functionalized L-4-hydroxyphenylglycine and the N-terminal side-chain modified azidoamino acid.

2.2.2 Synthesis of the alkyne-functionalized 4-hydroxyphenylglycine building block

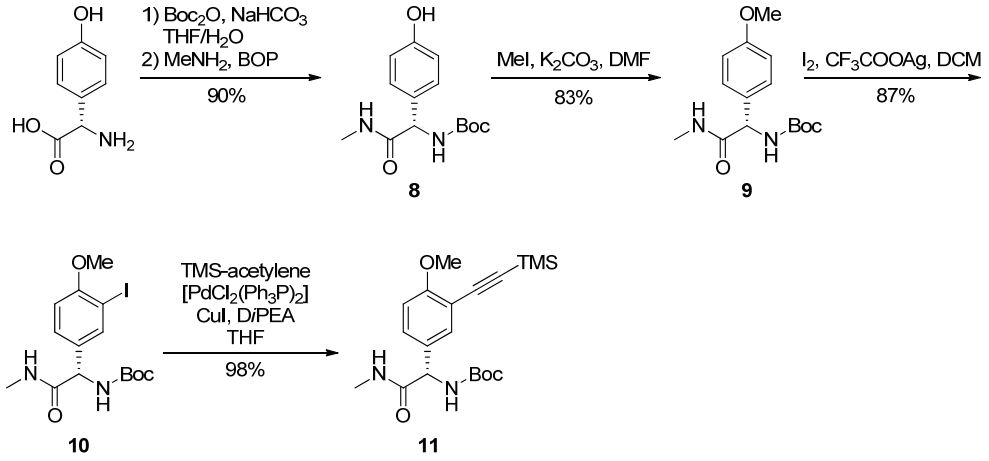
To synthesize the linear tripeptide precursors, the first step was the preparation of the alkyne-functionalized hydroxyphenylglycine derivative. The first attempt started with commercially available L-4-hydroxyphenylglycine (Scheme 2) and followed the strategy as described for the synthesis of the alkyne-bridged vancomycin DE-ring mimic **2** as shown in Figure 2, in which 3,5-diiodo-4-hydroxyphenylglycine (**4**) was synthesized as an important intermediate,[12] which

could be further functionalized with an alkyne by a Pd-catalyzed Sonogashira cross coupling reaction as shown in scheme 2.[15]



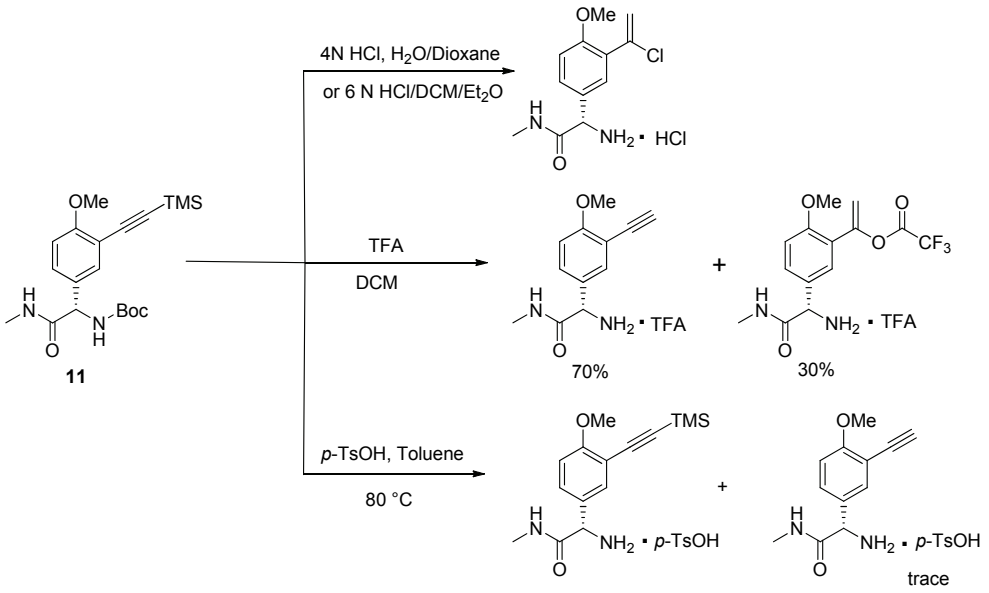
Scheme 2. First attempted synthesis route towards the alkyne-functionalized L-4-hydroxy-phenylglycine building block.

Firstly, L-4-hydroxyphenylglycine was converted into a methyl ester and the α-amine functionality was protected by a Boc-group to give compound **3**. The diiodide compound **4** was obtained using a modified procedure by treatment compound **3** with NIS and a catalytic amount of TFA in 49% overall yield.[16] The reported procedure without adding TFA was difficult to reproduce and gave a yield of only lower than 30%. Next, the phenolic hydroxyl group was protected as an acetyl ester in the presence of Ac_2O and K_2CO_3 as base. With the fully protected diiodide compound **5** in hand, the alkyne was introduced via a Pd-catalyzed Sonogashira cross coupling reaction with TMS-acetylene. Unfortunately, the mono- as well as the di-alkyne functionalized compounds were obtained. Reducing the amount of the TMS-acetylene present in the reaction mixture preferentially resulted in the formation of the mono-alkyne **6**. Nevertheless, purification of **6** remained to be difficult, while the scale was also rather limited. Since the preparation of compound **6** was not trivial, it was not an ideal building block at an early stage for continuing the synthesis. Therefore, an alternative synthetic approach was developed, which is shown in Scheme 3.



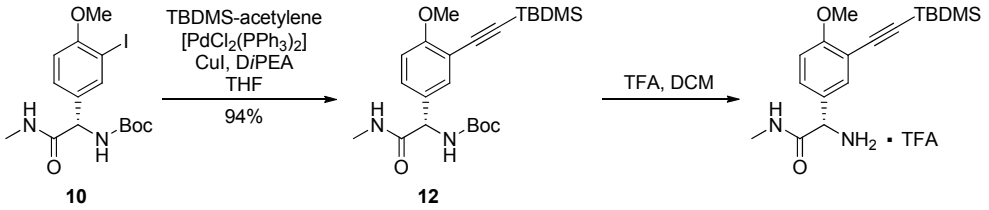
Scheme 3. Second synthesis route towards the alkyne-functionalized L-4-hydroxyphenylglycine building block.

Based on the experience of the first synthetic strategy, the second approach was initiated by synthesizing the 3-iodo-4-hydroxyphenylglycine derivative according to a literature procedure that described a CF_3COOAg catalyzed iodonation of 4-methoxyphenylglycine to give selectively 3-iodo-4-methoxy-phenylglycine.[17] Therefore, the α-amino group of L-4-hydroxyphenylglycine was first protected by a Boc-group. Then, the carboxylic acid was converted into a methyl amide to suppress possible racemization[18] of the sensitive hydroxyphenylglycine[19] core under basic reaction conditions in the later stage of the synthesis. Methylation of the phenolic hydroxyl group of **8** with methyl iodide in DMF and K_2CO_3 as base resulted in methyl ether **9** which was subjected to iodination by treatment of iodine in the presence of silver trifluoroacetate to give mono-iodo compound **10**.[17] Next, the required alkyne was introduced via a Pd-catalyzed Sonogashira cross-coupling using TMS-acetylene to afford **11** in an excellent yield (98%).



Scheme 4. Unprecedented reactivity of the TMS-acetylene moiety during Boc-group removal under acidic conditions.

After the successful synthesis of **11**, the next step was to remove the Boc-group to elongate the peptide sequence. Unfortunately, it turned out that the TMS-group was not completely impervious towards acid treatment needed for removal of the Boc-group (Scheme 4). When being treated with HCl (either 4 N HCl in H_2O/dioxane or 6 N HCl in DCM/diethyl ether), the TMS-group was also removed while HCl was added to the triple bond. As an alternative, treatment with 10% TFA in DCM also led to removal of the TMS-group, while 30% of the deprotected acetylene underwent an addition reaction with TFA. Boc-removal was successful only in the presence of *p*-toluenesulfonic acid (*p*-TsOH)[20] since only traces of the TMS-deprotected compound were found. However, this reaction required heating at 80 °C in toluene and the excess of *p*-TsOH need to be removed by washing with *aq*. Na_2CO_3, which is rather impractical for repetitive peptide synthesis. Therefore, a more acid-stable alkyne protecting group was required.



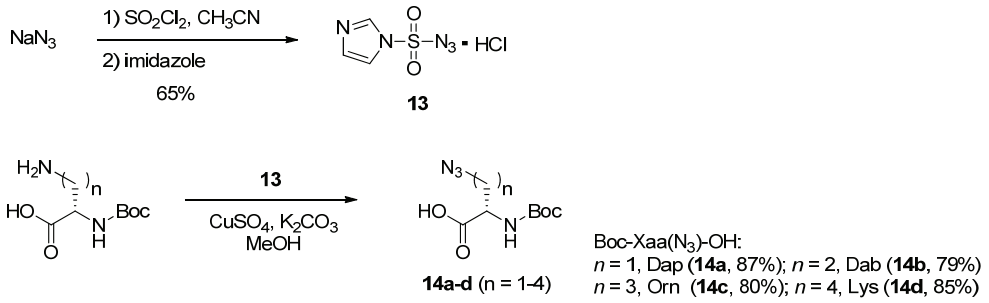
Scheme 5. Application of the TBDMS-functionality as more acid-stable acetylene protecting group.

For this purpose, TBDMS-protected acetylene was chosen to introduce the alkyne moiety. Due to the bulkiness of the TBDMS-group, it was expected to resist Boc-cleavage conditions. Iodo compound **10** was reacted with TBDMS-acetylene using the same reaction conditions to introduce the TMS-acetylene and the desired TBDMS-protected alkyne was successfully incorporated to give compound **12** in 94% yield. Gratifyingly, after treatment of **12** with 10% TFA, the TBDMS-group was found to be stable and the desired free amine as TFA salt was obtained as the only product as shown in Scheme 5. Therefore, the TBDMS-protected alkyne-functionalized compound **12** was an ideal building block as the alkyne-functionalized amino acid for further peptide synthesis.

2.2.3 Synthesis of the azidoamino acids

The synthesis of the azidoamino acids was relatively easy to perform. Suitably protected and commercially available diamino acids with the ω-amine functionality left unprotected were used and underwent a Cu(II)-catalyzed diazotransfer reaction to obtain the corresponding azido derivatives in high yield. Diazotransfer reagent **13**, imidazole-1-sulfonyl azide hydrochloride, was reported as a very efficient, inexpensive, and shelf-stable diazotransfer reagent, which could be easily obtained by reaction of NaN_3 with SO_2Cl_2, followed by treatment with imidazole.[21]

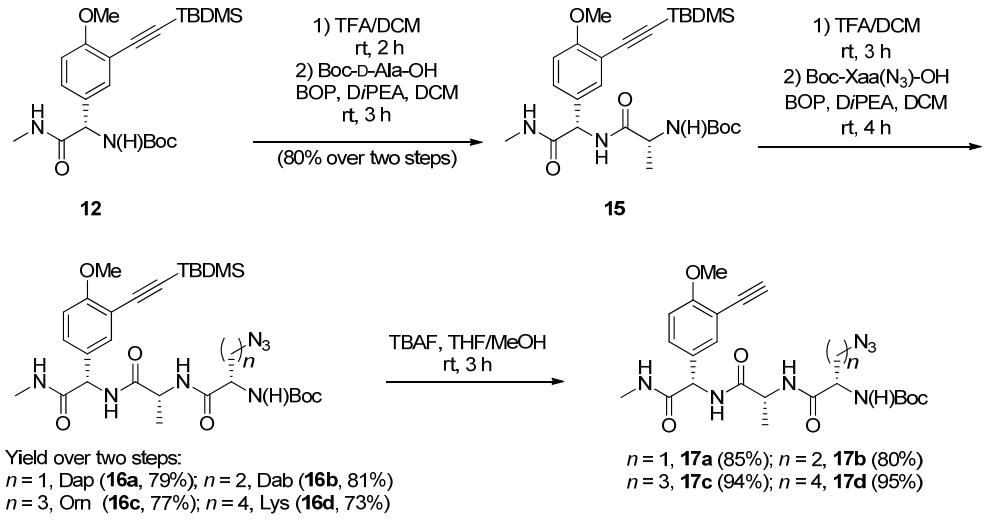
Since at this stage in the synthesis of the vancomycin mimetics it was not known what the optimal length of the side chain of the azidoamino acid should be required for cyclization by CuAAC. Therefore four diamino acids: (*S*)-2,3-diaminopropionic acid (Dap), (*S*)-2,4-diaminobutanoic acid (Dab), ornithine (Orn), and lysine (Lys), respectively, were converted into their corresponding azidoamino acids (**14a-d**) as shown in Scheme 6.



Scheme 6. Synthesis of the azidoamino acids by a Cu(II)-catalyzed diazotransfer reaction.

2.2.4 Final assembly of the linear tripeptide precursors

The alkyne-functionalized hydroxyphenylglycine building block was treated with TFA to remove the Boc-group and the obtained free amine was coupled with Boc-D-Ala-OH in the presence of BOP/D*i*PEA as coupling reagents to give dipeptide **15** in good yield (80%). Subsequently, after removal of the Boc-group, the four azidoamino acids (**14a-d**) were incorporated via a BOP/D*i*PEA-mediated coupling to give four tripeptides (**16a-d**) in good yield (73% to 81%). Finally, the TBDMS-group was removed by treatment with TBAF and the 'click' precursors **17a-d** were obtained in high yield (80 to 95%) as shown in Scheme 7.



Scheme 7. Synthesis of the linear tripeptide precursors for 'click' macrocyclization

2.2.5 Development and optimization of the CuAAC macrocyclization

With the linear tripeptide precursors in hand, the next step was to develop the reaction protocol for the CuAAC macrocyclization. The synthesis of such peptide-based macrocycles by using the CuAAC reaction has been thoroughly studied and various Cu(I) sources and solvent combinations, such as CuI/DMF, $CuSO_4$/sodium ascorbate/H_2O-DMF (or H_2O-^{t}BuOH), CuI (or CuBr)/toluene and $Cu(CH_3CN)_4PF_6$ (or $Cu(CH_3CN)_4BF_4$)/toluene have been described in the literature.[14, 22-26] The reaction conditions play a pivotal role in the outcome of the macrocyclization, however, currently there is no universal applicable reaction protocol which means that each 'click' macrocyclization needs to be individually optimized.

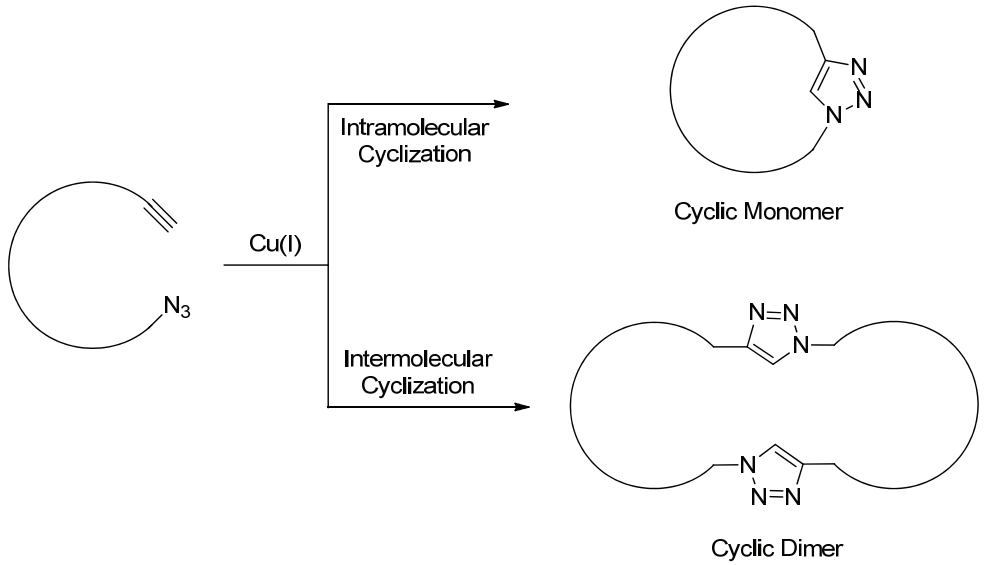
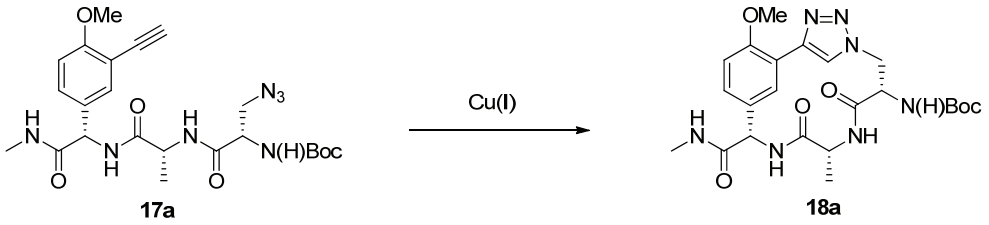


Figure 4. Cu(I)-catalyzed macrocyclization may lead to intramolecular as well as to intermolecular cyclization products.

The most challenging problem in the CuAAC macrocyclization is to overcome the mysterious dimerization effect (cyclodimerization),[23] which may lead to cyclic dimers as the prevalent reaction products (Figure 4), especially in small-sized and constrained ring systems. In the designed vancomycin DE-ring mimics, the ring size ranges from 14 to 17 atoms, and the ring is rather constrained since it contains a *meta*-substituted phenyl moiety and the resulting 1,4-triazole moiety forces the whole ring into a stretched rather than a bend conformation.

Therefore, it was expected that 'click' macrocyclization of precursors **17a-d** may gave the cyclic monomer as well as cyclic oligomers.



17a **18a**

Scheme 8. Cu(I)-catalyzed macrocyclizations of linear precursor **17a**.

In a first approach, the linear tripeptide **17a** with the shortest side chain was chosen as model compound to screen and optimize the reaction conditions, as shown in Scheme 8 and summarized in Table 1, respectively.

Table 1. Optimization of the Cu(I)-catalyzed macrocyclization[a] of precursor **17a**.

Entry	Catalyst (mol-%)	Solvent	Substrate Conc. (mM)	Temperature (°C)	Conversion (%)[b]
1	$CuSO_4$, sodium ascorbate (10%)	^{t}BuOH/H_2O (95:5 v/v)	5	r.t.	trace
2	$CuSO_4$, sodium ascorbate (10%)	^{t}BuOH/H_2O (95:5 v/v)	20	40	trace
3	$Cu(CH_3CN)_4PF_6$ (20%)	toluene/MeOH (4:1 v/v)	5	60	~30
4	$Cu(CH_3CN)_4PF_6$ (100%)	toluene/MeOH (4:1 v/v)	5	r.t.	100[c]
5	$Cu(CH_3CN)_4PF_6$ (100%)	toluene/MeOH (4:1 v/v)	1	r.t.	~70
6	$Cu(CH_3CN)_4PF_6$ (150%)	toluene/MeOH (4:1 v/v)	1	r.t.	100[c]

[a] All the reactions were run for 24h. [b] Conversion was determined by recovery of the starting material. [c] No starting material was recovered.

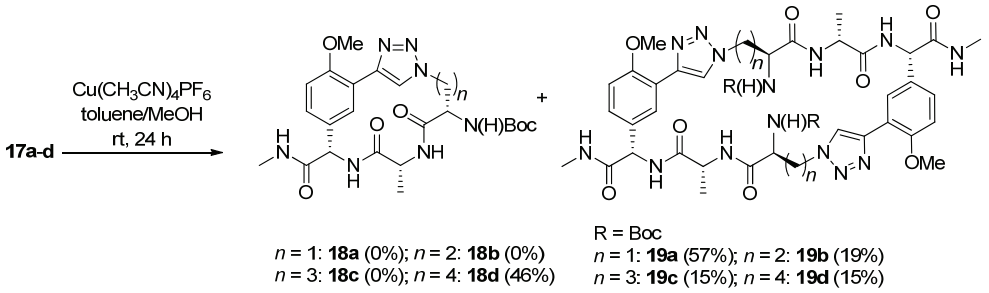
Using ^{t}BuOH/H_2O as solvent combination and $CuSO_4$/sodium ascorbate as the Cu(I) source, the reaction did not go to completion neither at different substrate concentration nor at an elevated temperature (Entries 1 and 2, Table 1). Next,

$Cu(CH_3CN)_4PF_6$ was chosen as the catalyst and toluene as solvent according to a report of Rowley-Kelly *et al.*,[24] in which the efficient synthesis of small lactam derivatives containing a triazole ring was described. Because of the poor solubility of the tripeptide in neat toluene, methanol was added as co-solvent. In the presence of 20 mol-% of catalyst at 60 °C, the cyclization reaction proceeded with only 30% conversion (Entry 3, Table 1). Inspired by a protocol from James *et al.*,[25] who reported the use of an excess of Cu(I) catalyst to promote the macrocyclization under highly diluted reaction conditions, the catalyst loading was increased to 100 mol-% and, to our delight, the 'click' cyclization reaction proceeded to complete conversion (Entry 4, Table 1). However, by lowering the substrate concentration to 1 mM to favor intramolecular cyclization, the conversion was also decreased to ~70% (Entry 5, Table 1). Finally, in the presence of 150 mol-% (1.5 equiv) of the Cu(I) catalyst in a highly diluted reaction mixture, the macrocyclization went to completion (Entry 6, Table 1). These optimized reaction conditions were applied in the macrocylizations of precursor peptides **17a-d**.

2.2.6 Synthesis of the 1,4-triazole containing mimics of the vancomycin DE-ring by CuAAC cyclization

The optimized reaction conditions for CuAAC macrocyclization, namely 1.5 equiv of the soluble Cu(I) catalyst $Cu(CH_3CN)_4PF_6$ at highly diluted substrate concentration (1 mM), were used for the linear precursors **17a-d** and all reactions went to complete conversion after 24 h at room temperature as shown in Scheme 9 and Table 2. Not unexpectedly, cyclization of precursor **17a** containing the shortest azidoamino acid side chain led to the formation of dimer **18a** as the major product, and even its trimer (not shown), as was judged by HPLC and MALDI-TOF, was obtained in an appreciable yield (19%, Table 2), while the cyclic monomer could not be identified. The cyclization of linear tripeptide **17b** with a side chain one methylene longer, gave an improved dimer/trimer ratio ~2:1 compared to **17a** (~3:1), but the isolated yield was quite low partly because of the poor solubility of the cyclic products. Apparently, the length of the ornithine azide **17c** was still not sufficient for formation of the desired click macrocycle **18c**, but now the corresponding cyclic trimer was not formed and dimer **19c** was the only obtained reaction product in a low yield (15%), partly due to its poor solubility. Gratifyingly, the precursor containing the lysine derived azide side chain **17d** led to the formation of the desired macrocycle **18d** as the major product (46%) together with

dimer **19d** as a minor side product (15%), which indicated that a minimal ring size of 17 atoms was required in this series of cyclic tripeptides obtained by CuAAC macrocyclization.



Scheme 9. Cu(I)-catalyzed macrocyclizations of the linear tripeptides **17a-d**.

Table 2. Results of the Cu(I)-catalyzed macrocyclizations of the linear tripeptides **17a-d**.

Compound	Catalyst (mol%)	Solvent	Substrate Conc. (mM)	Yield (%)[a]	Monomer (%)[b]	Dimer (%)[b]	Trimer (%)[b]
17a	$Cu(CH_3CN)_4PF_6$ (150%)	toluene/MeOH (4:1 v/v)	1	76	0	57	19
17b	$Cu(CH_3CN)_4PF_6$ (150%)	toluene/MeOH (4:1 v/v)	1	27	0	19	8
17c	$Cu(CH_3CN)_4PF_6$ (150%)	toluene/MeOH (4:1 v/v)	1	15	0	15	0
17d	$Cu(CH_3CN)_4PF_6$ (150%)	toluene/MeOH (4:1 v/v)	1	61	46	15	0

[a] Isolated yield. [b] Yield as determined by LCMS after pre-purification by column chromatography.

2.2.7 TBTA-promoted intramolecular selectivity of CuAAC macrocyclization

During the progress of the CuAAC macrocyclizations as described above, a paper by Gagan *et al.* described the Cu(I)-catalyzed macrocyclizations of rather small ring systems.[26] They found that addition of the Cu(I)-ligand TBTA (Figure 5) promoted the intramolecular cyclization and favored the synthesis of monocyclic compounds. Inspired by their protocol, it was decided to investigate the role of TBTA on the CuAAC macrocyclizations of the linear precursors **17a-d**.

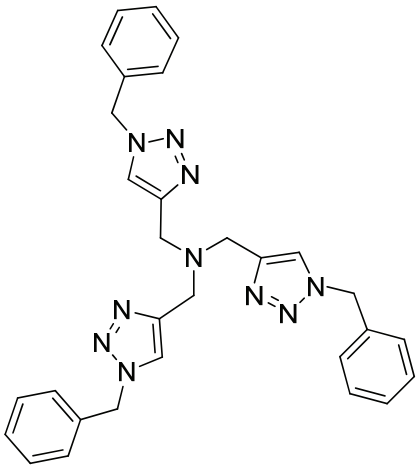
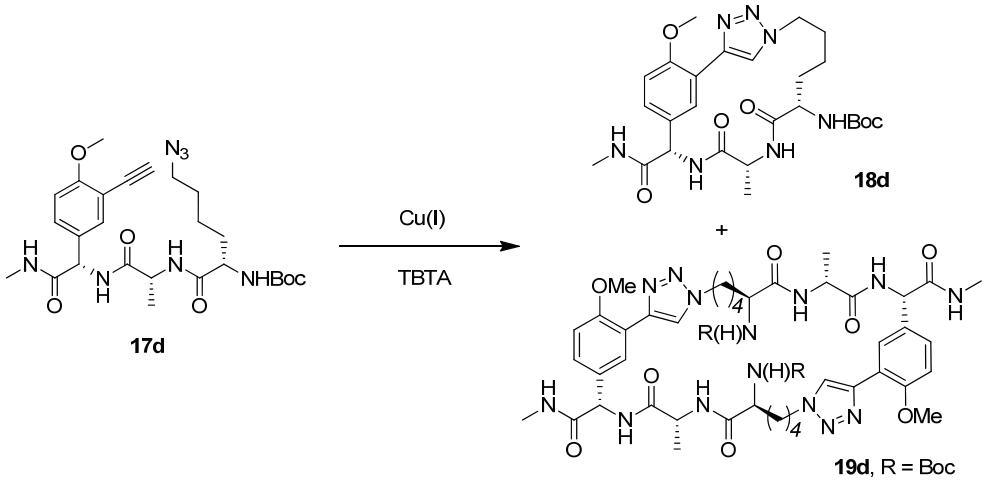


Figure 5. Structure of TBTA as a Cu(I)-ligand to promote intramolecular cyclization.



Scheme 10. CuAAC macrocyclization of linear tripeptide **17d** in the presence of TBTA.

Since only the lysine-derived linear tripeptide **17d** was successfully converted into a cyclic monomer, this precursor was chosen to optimize the CuAAC macrocyclizations in the presence of TBTA as shown in Scheme 10 and summarized in Table 3.

Table 3. CuAAC macrocyclizations in the presence of TBTA of linear tripeptide **17d**.[a]

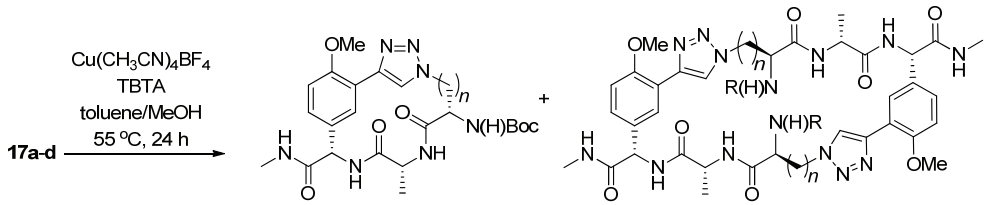
entry	Catalyst (mol%)	TBTA (mol%)	Substrate Conc. (mM)	Solvent	Temperature (°C)	Monomer/Dimer ratio	Conversion (%)[b]
1	$Cu(CH_3CN)_4PF_6$ (150)	0	1	toluene/MeOH (4:1 v/v)	r.t	2.7:1	95
2	$Cu(CH_3CN)_4PF_6$ (150)	5	1	toluene/MeOH (4:1 v/v)	r.t	3.6:1	97
3	$Cu(CH_3CN)_4PF_6$ (150)	10	1	toluene/MeOH (4:1 v/v)	r.t	3.5:1	95
4	$Cu(CH_3CN)_4BF_4$ (5)	5	20	DCM	55	1.6:1	74
5	$Cu(CH_3CN)_4BF_4$ (15)	15	5	DCM/MeOH (46:1 v/v)	r.t	2.2:1	97
6	$Cu(CH_3CN)_4BF_4$ (5)	5	1	toluene/MeOH (4:1 v/v)	r.t	3.3:1	100
7	$Cu(CH_3CN)_4BF_4$ (5)	0	1	toluene/MeOH (4:1 v/v)	r.t	0.9:1	37
8	$Cu(CH_3CN)_4BF_4$ (5)	5	1	toluene/MeOH (4:1 v/v)	55	3.5:1	100
9	$Cu(CH_3CN)_4BF_4$ (0)	0	1	toluene/MeOH (4:1 v/v)	55	0[c]	46
10	$Cu(CH_3CN)_4BF_4$ (1)	1	1	toluene/MeOH (4:1 v/v)	55	2.1:1	37
11	$Cu(CH_3CN)_4BF_4$ (10)	10	1	toluene/MeOH (4:1 v/v)	55	3.2:1	100

[a] All the reactions were run for 24h. [b]Conversion was determined by HPLC analysis of the residual starting material in the reaction mixture. [c] No monomer was detected.

For careful comparison, the cyclization of **17d** was repeated using the previously optimized CuAAC protocol without TBTA (Entry 1, Table 3). It was consistent with the previous results and a monomer/dimer ratio of 2.7:1 was obtained. In first instance, 5 and 10 mol-% TBTA was added to investigate its beneficial effect on the cyclization reaction (Entries 2 and 3, Table 3). Indeed, the

monomer/dimer ratio was increased to 3.6:1 and 3.5:1, respectively. As the next step in the optimization process, the conditions reported by Gagan *et al.* were used. Thus, dichloromethane was used as solvent with a substrate concentration of 20 mM at a reaction temperature of 55 °C with $Cu(CH_3CN)_4BF_4$ as catalyst (Entry 4, Table 3). This was not an improvement, since both the monomer/dimer ratio (1.6:1) and the conversion (74%) were lower. To remedy this, the substrate concentration was reduced to 5, respectively 1 mM while MeOH was added as co-solvent in the presence of 5 mol-% catalyst as well as TBTA, the conversion was restored to 100% and the monomer/dimer ratio was found to be 3.3:1 (Entries 5 and 6, Table 3). Under these conditions, the presence of TBTA is required (Entry 7, Table 3) while at an elevated temperature, the monomer/dimer ratio was further improved (Entry 8, Table 3) As a negative control experiment, the cyclization was carried out only by heating in the absence of both catalyst and TBTA (Entry 9, Table 3), and gave only a small amount of cyclic dimer. Further exploration by changing the catalyst loading (Entries 10 and 11, Table 3) did not result in a significant improvement with respect to cyclization efficiency and selectivity.

Based on this optimization, the best cyclization conditions were represented by entry 8 (Table 3). With this newly optimized protocol, the intramolecular cyclization selectivity was improved (monomer/dimer ratio was increased from 2.7:1 to 3.5:1) and the catalyst loading was decreased from 150 mol-% to only 5 mol-%.



Scheme 11. CuAAC macrocyclization of the linear peptide precursors **17a-d** in the presence of TBTA.

Since the TBTA-assisted CuAAC was optimized for precursor **17d**, the other three precursor peptides **17a-c**, which were not obtained as their monocyclic derivatives so far, were subjected toward the newly optimized macrocyclization protocol (Scheme 11). These data are summarized in Table 4. For careful

comparison of the results, the macrocyclization using the old protocol without TBTA was also repeated.

Table 4. CuAAC macrocyclization of the linear peptide precursors **17a-d**.[a]

Compound	Catalyst (mol%)	TBTA (mol%)	Solvent	Temperature (°C)	Yield (%)[b]	Monomer (%)[c]	Dimer (%)[c]	Trimer (%)[c]
17a	$Cu(CH_3CN)_4BF_4$ (5)	5	toluene/MeOH (4:1 v/v)	55	90	0	66	24
17a	$Cu(CH_3CN)_4PF_6$ (150)	0	toluene/MeOH (4:1 v/v)	r.t.	59[d]	0	43[d]	16[d]
17b	$Cu(CH_3CN)_4BF_4$ (5)	5	toluene/MeOH (4:1 v/v)	55	75	0	53	22
17b	$Cu(CH_3CN)_4PF_6$ (150)	0	toluene/MeOH (4:1 v/v)	r.t.	46[d]	0	34[d]	12[d]
17c	$Cu(CH_3CN)_4BF_4$ (5)	5	toluene/MeOH (4:1 v/v)	55	95	45	39	11
17c	$Cu(CH_3CN)_4PF_6$ (150)	0	toluene/MeOH (4:1 v/v)	r.t.	45[d]	0	19[d]	26[d]
17d	$Cu(CH_3CN)_4BF_4$ (5)	5	toluene/MeOH (4:1 v/v)	55	87	62	18	7
17d	$Cu(CH_3CN)_4PF_6$ (150)	0	toluene/MeOH (4:1 v/v)	r.t.	69[d]	41[d]	17[d]	11[d]

[a] All the reactions were run for 24h. [b] Yields were the total ratio of the cyclic monomer, dimer and trimer determined by HPLC. [c] Ratio of the cyclic monomer, dimer or trimer determined by HPLC. [d] The results are not completely comparable with Table 2 since the yield and ratio were determined after pre-purification by column chromatography.

In case of the linear tripeptides **17a-b**, with the shorter azide-functionalized side chain, it was still not possible to synthesize their corresponding cyclic monomer. However, the cyclic dimer/trimer ratio was increased and the apparent yield (not the isolated yield) was also increased since intermolecular oligomerization was disfavored in the presence of TBTA. However, an interesting aspect of this new protocol is the fact that it can be used for the convenient synthesis of relatively small cyclic dimeric peptides with a symmetrical peptide

backbone, like gramicidin,[27] as a potent antibiotic peptide. Gratifyingly, the macrocyclization of the ornithine-derived linear tripeptide **17c** resulted in the formation the desired cyclic monomer **18c** as the main product (45%) along with cyclic dimer (39%) (Figure 6). This was an important result since cyclic monomer **18c** was of the same ring size (16 atoms) as the vancomycin DE-ring and it could act as an improved structural mimic compared to **18d** which contains a 17-membered ring system. As already indicated in Table 3, the lysine-derived linear tripeptide **17d** could be converted into the cyclic monomer by CuAAC macrocyclization, however, the presence of TBTA gave superior results with respect to the cyclic monomer/dimer ratio and overall yield as well as a reduced catalyst loading.

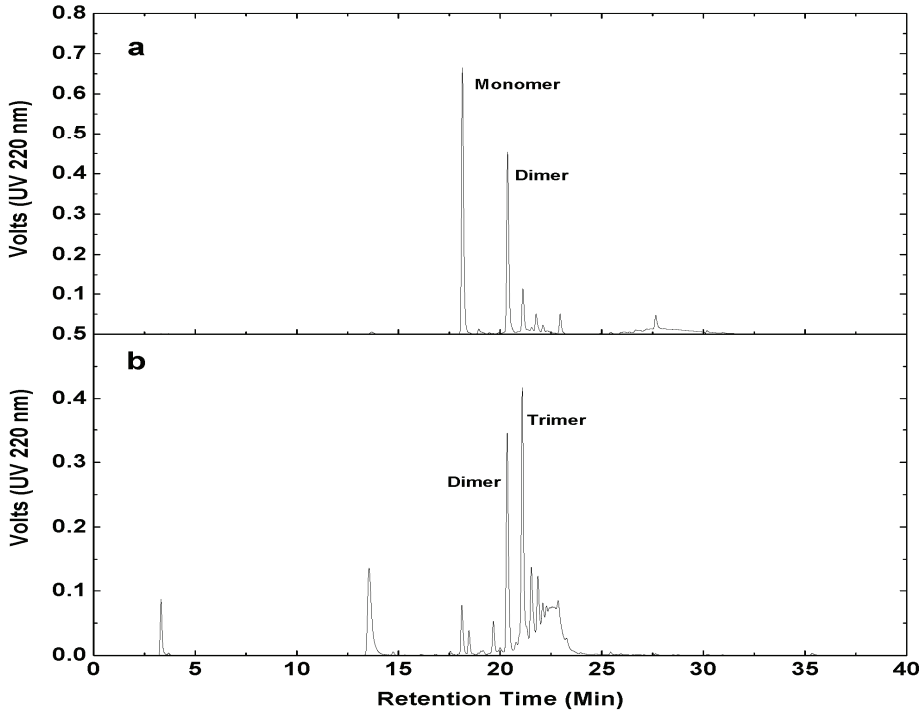
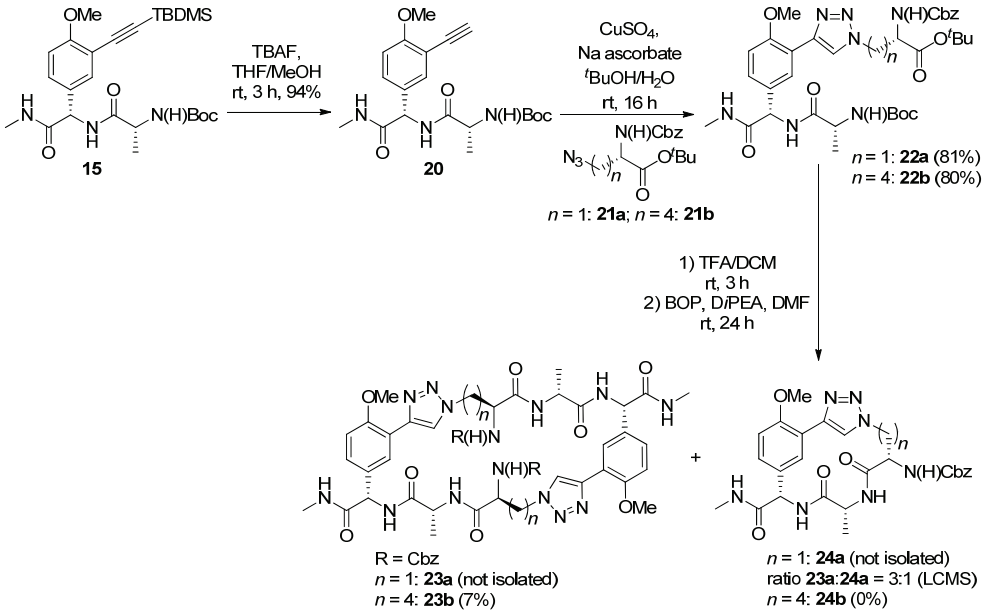


Figure 6. CuAAC macrocyclization of linear tripeptide **17c**: (a) with the addition of TBTA and (b) without the addition of TBTA.

2.2.8 Synthesis of the 1,4-triazole containing mimics of the vancomycin DE-ring by macrolactamization

The research described above investigated the application of CuAAC macrocyclization strategy as the final step to introduce the 1,4-triazole bridge. An attractive alternative approach for the synthesis of the 1,4-triazole containing DE-ring mimetics is a macrolactamization as cyclization step, in which the triazole moiety has been assembled via an intermolecular reaction. Since a large set of peptide coupling reagents is known, it is expected that macrolactamization will be easier to optimize and probably give higher yields than the previously described 'click' macrocyclization.



Scheme 12. Synthesis of the 1,4-triazole containing cyclic peptides via macrolactamization.

To evaluate this approach, dipeptide **15** was treated with TBAF to yield the free alkyne-dipeptide **20**. Then, the 1,4-triazole moiety was introduced by a Cu(I)-mediated 'click' reaction between dipeptide **20** and azidoamino acids **21a** or **21b**, respectively (Scheme 12). The precursors **22a** with the shortest 1,4-triazole containing side chain and **22b** with the longest 1,4-triazole containing side chain were obtained in high yield. After removal of both acid labile protecting groups (^{t}Bu/Boc) by treatment with TFA in DCM, a BOP/D*i*PEA-mediated

macrolactamization was performed at highly diluted (0.5 mM) reaction conditions in DMF as solvent, to suppress dimerization and/or oligomerization. The work-up procedure was quite problematic because of the poor solubility of the crude cyclization products. After aqueous washing steps to remove the coupling reagents, the residue was analyzed by HPLC. Different from the 'click' macrocyclization, macrolactamization of the precursor with the shortest chain led to the formation of the dimer **23a** but also the desired monocycle **24a** could be identified albeit present in a complex reaction mixture, while the ratio between **23a**:**24a** was found to be 3:1 based on LCMS analysis. However, macrolactamization of **22b** gave only the cyclic dimer **23b** in an isolated yield of 7% with preparative RP-HPLC as judged by HPLC, ^{1}H-NMR and MALDI-TOF analysis.

Surprisingly, the macrolactamization strategy showed no advantage compared to the CuAAC macrocyclization with respect to the prevalent formation of the cyclic monomer. These results also indicated that the introduction of 1,4-triazole moiety prior to macrolactamization disfavored cyclization probably due to its preorganized (e.g. stretched) conformation that may prevent formation of the cyclic monomer.

2.3 Conclusion

This chapter described the development of a CuAAC based approach towards the synthesis of relatively small and rather constrained cyclic peptide systems to mimic the DE-ring of the peptide antibiotic vancomycin.[28] After the successful linear synthesis of a series of tripeptide precursors, a CuAAC macrocyclization approach was developed. Although this strategy preferentially led to the formation of cyclic dimers, cyclic monomers could also be obtained with a ring containing at least 16 atoms employing the TBTA-promoted CuAAC macrocyclization protocol. Two cyclic monomeric tripeptides, derived from ornithine and lysine, were successfully synthesized and could act as structural mimics of the vancomycin DE-ring. The preferentially formed cyclic dimers were also of considerable interest, since symmetrical relatively small dimeric peptides form the backbone of potent antibiotic peptides like gramicidin.

2.4 Experimental section

2.4.1 General experimental procedures

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. The solvents were obtained as peptide synthesis grade and stored on molecular sieves (4 Å).
Column chromatography was performed using *Silicycle SiliFlash P60* silica gel (particle size 40-63 μm).
Thin Layer Chromatography (TLC) was performed on *Merck* precoated silica gel 60F254 glass plates. Compound spots were visualized by UV-quenching, ninhydrin, or Cl_2/TDM.
Melting point was measured on a Büchi Schmelzpunktbestimmungsapparat according to Dr. Tottoli and was uncorrected.
Optical rotation was measured on a *JASCO* P-1010 Polarimeter using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 mL) are indicated.
^{1}H NMR was acquired on a *Varian* Mercury 300 MHz or a *Varian* Innova 500 MHz spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to TMS (0.00 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br). ^{13}C NMR was acquired on a *Varian* Mercury 75 MHz or a *Varian* Innova 125 MHz spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the solvent residual signal, $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or DMSO-d_6 (39.0 ppm).
Analytical HPLC was performed on an automated HPLC system (*Shimadzu*) equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using an *Alltech* Alltima C8 column (pore size: 100 Å, particle size: 5 μm; 250 × 4.6 mm) at a flow rate of 0.5 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 40 min (method A), or 60 min (method B)].
Preparative RP-HPLC was performed on an automated preparative HPLC system (*Applied Biosystems*) equipped with a UV/Vis detector operating at 214 nm using an *Alltech* Alltima C8 column (pore size: 100 Å, particle size: 10 μm; 250 × 22 mm)

at a flow rate of 2.0 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 90 min].

ESI-MS was performed on a *Shimadzu* LCMS-QP8000 electrospray ionization mass spectrometer.

MALDI-TOF MS was performed on a *Shimadzu* Kratos AXIMA-CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and P14R as reference.

2.4.2 Synthesis and compound analyses

***N*$^{\alpha}$-L-Boc-4-hydroxyphenylglycine methyl ester 3**:[11] $SOCl_2$ (6 mL) was added dropwise to methanol (60 mL) at 0 °C. Afterwards, L-4-hydroxyphenylglycine (5.0 g, 29.9 mmol) was added to the solution in portions. The mixture was brought to reflux for 4 h. After cooling down, the solvent was removed to give 4-hydroxyphenylglycine methyl ester as a white solid. In the next step, it was dissolved in 100 mL of H_2O/THF (1:1, v/v) and to this solution Boc_2O (6.52 g, 29.9 mmol) and $NaHCO_3$ (7.54 g, 89.7 mmol) were added. After stirring for 4 h, the mixture was diluted with 100 mL of EtOAc. The aqueous layer was separated and the organic layer was washed successively with 1N $KHSO_4$ (100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL). After drying over Na_2SO_4, the solvent was removed and the crude product was recrystallized in hexane/EtOAc (60 mL, 1:1, v/v). *N*$^{\alpha}$-L-Boc-4-hydroxyphenylglycine methyl ester **3** was obtained as a white solid (7.32 g, 87%); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.20 (d, *J* = 8.5 Hz, 2H), 6.76 (d, *J* = 8.6 Hz, 2H), 5.57 – 5.47 (m, 1H), 5.29 (s, 2H), 3.71 (s, 3H), 1.43 (s, 9H).

3,5-Diiodo-*N*$^{\alpha}$-L-Boc-4-hydroxyphenylglycine methyl ester 4:[11, 16] Compound **3** (563 mg, 2 mmol) was dissolved in CH_3CN (8 mL) and TFA (44 μl, 0.6 mmol) was added to this solution. NIS (989 mg, 4.4 mmol) was added in small portions at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was stirred for 24 h at room temperature. The solvent was removed and the residue was redissolved in EtOAc (50 mL). The resulting solution was washed with saturated Na_2SO_3 (50 mL, twice) and brine (50 mL, once). After drying over Na_2SO_4, the solvent was removed and the residue was purified by column chromatography (EtOAc/hexane, 1:7, v/v). 3,5-Diiodo-*N*$^{\alpha}$-L-Boc-4-hydroxyphenylglycine methyl ester **4** was obtained as a

light yellow solid (522 mg, 49%); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.66 (s, 2H), 5.58 (s, 1H), 5.16 (d, *J* = 6.5 Hz, 1H), 3.74 (s, 3H), 1.43 (s, 9H).

Compound 5:[11] Compound **4** (500 mg, 0.94 mmol) was dissolved in acetone (10 mL). Acetic anhydride (114 μL, 1.20 mmol) and K_2CO_3 (195 mg, 1.40 mmol) were added to the solution. After stirring for 2 h at room temperature, the solvent was removed and the residue was redissolved in EtOAc (50 mL). The resulting solution was washed successively with 1N $KHSO_4$ (50 mL), saturated $NaHCO_3$ (50 mL) and brine (50 mL). After drying over Na_2SO_4, the solvent was removed and compound **5** was obtained as light yellow solid (530 mg, 98%); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.77 (s, 2H), 5.62 (s, 1H), 5.23 (d, *J* = 6.5 Hz, 1H), 3.76 (s, 3H), 2.40 (s, 3H), 1.43 (s, 9H).

Compound 6 and 7: Compound **5** (533 mg, 1.0 mmol), $[PdCl_2(PPh_3)_2]$ (22.5 mg, 0.1 mmol), and CuI (28.6 mg, 0.15 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated three times). Then, THF (5.0 mL) (purged with dry N_2 for 1 h prior to use) was added by a syringe, followed by the addition of D*i*PEA (554 μL, 4.0 mmol) and TMS-acetylene (304 μL, 2.2 mmol). After stirring for 15 h at room temperature under N_2, the solvent was subsequently removed under reduced pressure and the residue was redissolved in EtOAc (50 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once), saturated $NaHCO_3$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:5 to 1:4, v/v). A small amount of compound **6** was isolated as a yellowish solid (112 mg, 21%) and compound **7** was difficult to be isolated as a pure compound; For compound **6**, ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.74 (dd, *J* = 2.1, 0.4 Hz, 1H), 7.57 – 7.37 (m, 1H), 5.59 (s, 1H), 5.23 (d, *J* = 6.5 Hz, 1H), 3.73 (s, 4H), 2.35 (s, 4H), 1.42 (s, 14H), 0.22 (s, 10H).

***N*$^{\alpha}$-L-Boc-4-hydroxyphenylglycine methylamide 8**:[18] L-4-Hydroxyphenyl-glycine (6.68 g, 40 mmol) was dissolved in THF/H_2O (240 mL, 1:1, v/v). To this solution $NaHCO_3$ (10.08 g, 120 mmol) and Boc_2O (10.48 g, 48 mmol) were added at 0 ^{o}C. After stirring for 1 h at 0 ^{o}C, the mixture was stirred overnight. The turbid solution was extracted with Et_2O (150 mL, twice). The aqueous solution was acidified with $KHSO_4$ to pH 2-3 and was extracted with EtOAc (100 mL, twice).

The combined organic phase was washed with H_2O (100 mL, once) and brine (100 mL, once) and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, N^{α}-L-Boc-4-hydroxyphenylglycine was obtained and used in the next step without further purification. N^{α}-L-Boc-4-hydroxyphenylglycine (5.35 g, 20 mmol) was dissolved in DCM (150 mL) to give a turbid solution. To this solution BOP (13.29 g, 30 mmol) was added, and followed by the addition of $MeNH_2$ (2M in THF, 40 mL, 30 mmol) dropwise during 10 min at 0 ^{o}C. The mixture was allowed to warm up to room temperature and stirred for 20 h. The solvents were removed in vacuum and the residue was redissolved in EtOAc (100 mL). The resulting solution was washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:1, v/v). Compound **8** was obtained as a white solid (5.04 g, 90%). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.42 (s, 9H), 2.71 (d, *J* = 4.8 Hz, 3H), 4.98 (s, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 7.16 (d, *J* = 8.5 Hz, 1H).

***N*$^{\alpha}$-L-Boc-4-methoxyphenylglycine methylamide 9**: Compound **8** (5.00 g, 17.8 mmol) was dissolved in DMF (40 mL). To this solution K_2CO_3 (9.84 g, 71.2 mmol) and MeI (5.54 g, 39.2 mmol) were added. The mixture was stirred at room temperature for 4 h. Then, the reaction mixture was diluted with EtOAc (250 mL) and the resulting solution was successively washed with H_2O (250 mL, once), 1 N $KHSO_4$ (250 mL, once), and brine (250 mL, once). The organic phase was dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:1, v/v). Compound **9** was obtained as a white solid (4.32 g, 83%); R_f = 0.32 (hexane/EtOAc, 1:1, v/v); mp = 159-161 °C; $[\alpha]_D^{20}$ = +142.5 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (s, 9H), 2.72 (d, *J* = 4.8 Hz, 3H), 3.75 (s, 3H), 5.15 (br s, 1H), 5.89 (br s, 1H), 6.40 (br s, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 6.27 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6, 28.5, 55.5, 57.9, 80.2, 144.4, 128.6, 130.9, 155.5, 159.6, 171.4; MS (ESI) *m/z* calcd for $C_{12}H_{22}N_2NaO_4$ $[M+Na]^+$ 317.15, found 316.50.

Compound 10: Compound **9** (4.60 g, 15.6 mmol) was dissolved in $CHCl_3$ (200 mL). To this solution CF_3COOAg (7.59 g, 34.4 mmol) and I_2 (4.77 g, 18.8 mmol) were added.[2] The obtained solution mixture was stirred at room temperature for 15 h. The reaction was quenched by the addition of saturated $Na_2S_2O_3$ (150 mL)

and the aqueous phase was extracted with $CHCl_3$ (100 mL, twice). The combined organic layer was dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:4 to 1:1, v/v). Compound **10** was obtained as a white solid (5.70 g, 87%). R_f = 0.38 (hexane/EtOAc, 1:1, v/v); mp = 155-157 °C; $[\alpha]_D^{20}$ = +119.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9H), 2.75 (d, *J* = 4.8 Hz, 3H), 3.83 (s, 9H), 5.13 (br s, 1H), 5.91 (br s, 1H), 6.31 (br s, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 7.27-7.30 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.73 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.7, 28.6, 56.6, 57.2, 80.4, 86.6, 111.2, 128.6, 132.9, 138.2, 155.5, 158.2, 170.8; MS (ESI) *m/z* calcd for $C_{12}H_{21}IN_2NaO_4$ $[M+Na]^+$ 443.04, found 442.60.

Compound 11: Compound **10** (1.80 g, 4.28 mmol), $[PdCl_2(PPh_3)_2]$ (150 mg, 0.214 mmol), and CuI (81.5 mg, 0.428 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated three times). Then, THF (30 mL) (purged with dry N_2 for 1 h prior to use) was added by a syringe, followed by the addition of D*i*PEA (1.17 mL, 8.56 mmol) and TMS-acetylene (1.79 mL, 12.9 mmol). After stirring the reaction mixture at room temperature for 15 h under N_2, the solvent was subsequently removed under reduced pressure and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, once), saturated $NaHCO_3$ (100 mL, once) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:2, v/v). Compound **11** was obtained as a yellowish solid (1.65 g, 98%). R_f = 0.46 (hexane/EtOAc, 1:1, v/v); $[\alpha]_D^{20}$ = +122.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.42 (d, *J*=2.1, 1H), 7.32 – 7.20 (m, 2H), 6.82 (d, *J*=8.6, 1H), 5.80 (s, 1H), 5.68 (s, 1H), 5.00 (s, 1H), 3.86 (s, 3H), 2.79 (d, *J*=4.8, 3H), 1.40 (s, 9H), 0.26 (s, 7H).

Compound 12: Compound **10** (2.10 g, 5.00 mmol), $[PdCl_2(PPh_3)_2]$ (175 mg, 0.25 mmol), and CuI (95.2 mg, 0.50 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated three times). Then, THF (25 mL) (purged with dry N_2 for 1 h prior to use) was added by a syringe, followed by the addition of D*i*PEA (1.37 mL, 10.0 mmol) and TBDMS-acetylene (1.79 g, 12.5 mmol). After stirring the reaction mixture at room temperature for 15 h under N_2, the solvent was subsequently removed under reduced pressure and the residue was redissolved in EtOAc (100 mL). The

resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, once), saturated $NaHCO_3$ (100 mL, once) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:4 to 1:3, v/v). Compound **12** was obtained as a yellowish solid (2.04 g, 94%). R_f = 0.46 (hexane/EtOAc, 1:1, v/v); mp = 43-45 °C; $[\alpha]_D^{20}$ = +120.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.18 (s, 6H), 1.00 (s, 9H), 1.40 (s, 9H), 2. 79 (d, *J* = 4.8 Hz, 3H), 3.85 (s, 3H), 4.97 (br s, 1H) 5.61 (br s, 1H), 5.78 (br s, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 7.24-7.28 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = -4.4, 17.0, 26.4, 26.8, 28.5, 56.2, 57.9, 80.3, 97.8, 101.5, 111.4, 113.3, 129.0, 130.8, 132.9, 155.4, 160.7, 170.8; MS (ESI) *m/z* calcd for $C_{23}H_{36}N_2NaO_4Si$ $[M+Na]^+$ 455.23, found 454.85.

Diazotransfer reagent 13:[21] To a suspension of NaN_3 (3.26 g, 50 mmol) in CH_3CN (50 mL), SO_2Cl_2 (4.1 mL, 50 mmol) was added dropwise at 0 °C. After addition, the resulting mixture was stirred vigorously for 20 h at room temperature. Then, the obtained mixture was cooled down to 0 °C and imidazole (6.51 g, 95 mmol) was added in portions. This solution mixture was stirred for 3 h at room temperature. Finally, the mixture was diluted with 100 mL of EtOAc and washed with H_2O (100 mL, twice), saturated $NaHCO_3$ (100 mL, once) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was cooled to 0 °C and a HCl/ethanol solution (obtained by addition of AcCl (4.3 mL) to an icc-cooled ethanol (20 mL)) was added dropwise while stirring. The resulting precipitate was collected and washed with cold EtOAc (25 mL, twice) to give the diazotransfer reagent **13** (6.82 g, 65%).

Azidoamino acids 14a-d: The azidoamino acids **14a-d** were synthesized using imidazole-1-sulfonyl azide **13** as the diazotransfer reagent starting from the corresponding Boc-protected diamino acids according to a literature procedure.[21] For a general procedure: Boc-Lys-OH (493 mg, 2 mmol) was dissolved in MeOH (10 mL). To this solution K_2CO_3 (677 mg, 4.9 mmol), $CuSO_4$ (4.99 mg, 0.02 mmol) and diazotransfer reagent **13** (503 mg, 2.4 mmol) were successively added. The reaction mixture was stirred for 20 h at room temperature. Then the solvent was removed and the residue was redissolved in 30 mL of H_2O. The resulting solution was acidified with $KHSO_4$ to pH 2-3 and then extracted with EtOAc (50 mL, twice). The combined organic phase was dried over Na_2SO_4. After filtration and

removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:6, v/v). Azidoamino acid **14d** was obtained as viscous colorless oil (463 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.07 (br s, 1H), 5.04 (d, *J* = 7.4 Hz, 1H), 4.32 (s, 1H), 3.29 (t, *J* = 6.6 Hz, 2H), 1.89 (s, 1H), 1.78 – 1.55 (m, 3H), 1.58 – 1.33 (m, 11H).

The azidoamino acids **14a-c** were synthesized using the same procedure as described above for **14d**.

Azidoamino acid **14a** was obtained as viscous colorless oil (400 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.27 (s, 1H), 5.44 (d, *J* = 6.7 Hz, 1H), 4.49 (s, 1H), 3.90 – 3.60 (m, 2H), 1.46 (s, 9H).

Azidoamino acid **14b** was obtained as viscous colorless oil (388 mg, 79%). ^{1}H NMR (300 MHz, DMSO) δ = 7.15 (d, *J* = 8.1 Hz, 1H), 3.96 (td, *J* = 9.4, 4.7 Hz, 1H), 3.37 (m, 1H), 2.03 – 1.67 (m, 2H), 1.38 (s, 9H).

Azidoamino acid **14c** was obtained as viscous colorless oil (414 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) 5.04 (br s, 1H), 4.34 (s, 1H), 3.44 (t, *J* = 6.4 Hz, 2H), 2.03 – 1.89 (m, 1H), 1.78 – 1.64 (m, 3H), 1.46 (s, 9H).

Dipeptide 15: Compound **12** (1.30 g, 3.00 mmol) was dissolved in DCM (80 mL). To this solution TFA (8 mL) was added. After stirring for 2 h, the volatiles were removed under reduced pressure. The residue was coevaporated with Et_2O (20 mL, four times) to remove the residual TFA. The solid residue was dried under vacuum for 1 h and subsequently redisssolved in DCM (80 mL). To the obtained solution, Boc-D-Ala-OH (624 mg, 3.3 mmol) and BOP (1.99 g, 4.5 mmol) were added, followed by the addition of D*i*PEA (1.20 mL, 9.0 mmol). The reaction mixture was stirred for 3 h at room temperature. The solvent was removed under reduced pressure and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, once) and brine (100 mL, once), and subsequently dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:4 to 1:3, v/v). Dipeptide **15** was obtained as a white solid (1.20 g, 80% over two steps). R_f = 0.14 (hexane/EtOAc, 1:1, v/v); mp = 90-92 °C; $[\alpha]_D^{20}$ = +98.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.18 (s, 6H), 1.00 (s, 9H), 1.32 (d, *J* = 6.9 Hz), 1.44 (s, 9H), 2.79 (d, *J* = 4.8 Hz, 3H), 3.84 (s, 3H), 4.08-4.17 (br m, 1H), 5.02 (br s, 1H), 5.28 (d, *J* = 6.9 Hz, 1H), 6.16 (br s, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 7.16 (br s, 1H), 7.24-7.27 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.39 (d, *J* = 2.4 Hz); ^{13}C NMR (75 MHz,

$CDCl_3$): δ = -4.4, 17.0, 18.6, 26.4, 26.6, 28.6, 50.5, 56.0, 56.3, 80.4, 97.8, 101.5, 111.3, 111.5, 113.3, 129.1, 129.9, 132.5, 155.8, 160.7, 170.5, 172.6; MS (ESI) *m/z* calcd for $C_{26}H_{42}N_3O_5Si$ $[M+H]^+$ 504.29, found 504.00; calcd for $C_{26}H_{41}N_3NaO_5Si$ $[M+Na]^+$ 526.27, found 525.85.

General procedure for the synthesis of tripeptides 16a-d: Dipeptide **15** (400 mg, 0.795 mmol) was dissolved in DCM (30 mL) and to this solution TFA (3 mL) was added. The obtained reaction mixture was stirred for 3 h after which TLC analysis indicated that the deprotection of the Boc-group was complete. The solvent and TFA were removed under reduced pressure. The residual TFA was removed by coevaporation with Et_2O (10 mL, four times). The solid residue was dried under vacuum for 1 h and subsequently redisssolved in DCM (30 mL). To this solution, azidoamino acid **14a** (0.875 mmol, 1.1 eq), BOP (387 mg, 0.875 mmol) and D*i*PEA (327 μL, 2.63 mmol) were added successively. The reaction mixture was stirred at room temperature for 4 h. Then, the solvent was removed under reduced pressure and the residue was redissolved in EtOAc (50 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (30 mL, twice), saturated $NaHCO_3$ (30 mL, once) and brine (30 mL, once), and subsequently dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:4 to 2:3, v/v). Tripeptide **16a** was obtained as a white solid (385 mg, 79%). R_f = 0.51 (DCM/CH_3OH, 9:1, v/v); mp = 180-182 °C; $[\alpha]_D^{20}$ = +120.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.16 (s, 6H), 0.97 (s, 9H), 1.42 (d, *J* = 6.0 Hz, 3H), 1.49 (s, 9H), 2.82 (d, *J* = 3.0 Hz, 3H), 3,39 (br s, 1H), 3,50 (br s, 1H), 3.83 (s, 3H), 5.04 (br s, 1H), 5.18 (br s, 1H), 5.98 (d, *J* = 7.5 Hz), 6.72-6.75 (br s, 1H and d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.50 (s, 1H), 7.59 (br s, 1H), 8.54 (br s, 1H), 8.67 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = -4.8, 17.0, 19.8, 26.3, 26.5, 28.7, 49.1, 53.7, 55.1, 56.1, 80.1, 97.4, 101.8, 111.3, 112.9, 128.0, 130.3, 132.3, 156.1, 160.4, 169.6, 170.7, 172.1; MS (ESI) *m/z* calcd for $C_{29}H_{46}N_7O_6Si$ $[M+H]^+$ 616.33, found 616.05; calcd for $C_{29}H_{45}N_7NaO_6Si$ $[M+Na]^+$ 638.33, found 638.40.

The tripeptides **16b-d** were synthesized using the same procedure as described for tripeptide **16a**.

Tripeptide 16b was obtained as a white solid (406 mg, 81%). R_f = 0.51 (DCM/CH_3OH, 9:1, v/v); mp = 179-180 °C; $[\alpha]_D^{20}$ = +100.9 (c = 1.0, $CHCl_3$); ^{1}H

NMR (500 MHz, $CDCl_3$): δ = 0.15 (s, 6H), 0.96 (s, 9H), 1.43 (d, *J* = 5.0 Hz, 3H), 1.49 (s, 9H), 1.86 (br s, 1H), 1.95 (br s, 1H), 2.82 (s, 3H), 3.05 – 3.09 (br m, 2H), 3.82 (s, 3H), 4.86 (br s, 1H), 5.22 (br s, 1H), 6.04 (d, *J* = 6.0 Hz, 1H), 6.75-6.78 (d, *J* = 9.0 Hz, 1H and br s, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.49 (s, 1H), 7.71 (br s, 1H), 8.53 (br s, 1H), 8,85 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = -4.8, 17.0, 19.7, 26.4, 28.8, 33.6, 48.0, 49.2, 52.2, 55.0, 56.1, 79.7, 97.3, 101.8, 111.3, 112.9, 127.6, 130.4, 132.1, 156.2, 160.4, 170.7, 171.3, 172.2; MS (ESI) *m/z* calcd for $C_{30}H_{48}N_7O_6Si$ $[M+H]^+$ 630.34, found 630.45; calcd for $C_{30}H_{47}N_7NaO_6Si$ $[M+Na]^+$ 652.33, found 652.75.

Tripeptide 16c was obtained as a white solid (396 mg, 77%). R_f = 0.51 (DCM/CH_3OH, 9:1, v/v); mp = 215-217°C; $[\alpha]_D^{20}$ = +71.8 (c = 1.0, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$/CD_3OD, 1:1, v/v): δ = 0.16 (s, 6H), 0.99 (s, 9H), 1.33 (d, *J* = 7.0 Hz, 3H), 1.38 (s, 9H), 1.63-1.65 (m, 3H), 1.81 (br s, 1H), 2.75 (s, 3H), 3.85 (s, 3H), 4.01 (d, *J* = 7.0 Hz, 1H), 4.35-4.39 (m, 1H), 5.26 (s, 1H), 6.89 (d, *J* = 3.5 Hz, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.39 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD 1:1, v/v): δ = -4.7, 16.6, 16.9, 25.2, 25.8, 25.9, 27.9, 29.4, 49.2, 50.9, 54.7, 55.7, 57.1, 79.9, 97.0, 101.6, 111.3, 113.0, 129.1, 129.5, 133.0, 156.5, 160.8, 171.6, 173.1, 173.6; MS (ESI) *m/z* calcd for $C_{31}H_{50}N_7O_6Si$ $[M+H]^+$ 644.36, found 644.25; calcd for $C_{31}H_{49}N_7NaO_6Si$ $[M+Na]^+$ 666.34, found 666.45.

Tripeptide 16d was obtained as a white solid (382 mg, 73%). R_f = 0.51 (DCM/CH_3OH, 9:1, v/v); mp = 224-226 °C; $[\alpha]_D^{20}$ = +83.7 (c = 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ = 0.15 (s, 6H), 0.96 (s, 9H), 1.26 (br s, 2H), 1.42 (d, *J* = 5.0 Hz, 3H), 1.49 (br s, 12H), 1.67(br s, 1H), 2.82 (s, 3H), 2.87-2.96 (m, 2H), 3.82 (s, 1H), 4.78 (br s, 1H), 5.23, (br s, 1H), 6.11 (br s, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.50 (s, 1H), 7.84 (br s, 1H), 8.60 (br s, 1H), 8.86 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = -4.0, 17.0, 20.0, 22.9, 26.4, 28.8, 34.5, 49.1, 51.4, 53.8, 54.8, 56.1, 79.4, 97.4, 101.8, 111.1, 112.8, 127.5, 130.8, 132,3, 156.2, 160.3, 170.7, 172.0, 172.1; MS (ESI) *m/z* calcd for $C_{32}H_{52}N_7O_6Si$ $[M+H]^+$ 658.37, found 658.80; calcd for $C_{32}H_{51}N_7NaO_6Si$ $[M+Na]^+$ 680.36, found 680.90.

General procedure for the synthesis of tripeptides 17a-d:
Tripeptide **16a** (185 mg, 0.300 mmol) was dissolved in THF/MeOH (20 mL, 19:1, v/v) and to this solution TBAF (142 mg, 0.450 mmol) was added at 0 °C. After stirring for 3 h, TBDMS-group was removed completely as indicated by TLC.

Subsequently, the solvents were removed under reduced pressure, and the residue was redissolved in EtOAc (30 mL) and the resulting solution was washed with saturated NH_4Cl (30 mL, once) and brine (30 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:2 to 1:1, v/v). Tripeptide **17a** was obtained as a white solid (128 mg, 85%). R_f = 0.47 (DCM/CH_3OH, 9:1, v/v); ^1H NMR (500 MHz, CD_3OD): δ = 1.34 (d, *J* = 7.0 Hz, 3H), 1.44 (s, 9H), 2.76 (s, 3H), 3.59-3.61 (m, 2H), 3.86 (s, 3H), 4.24-4.26 (m, 1H), 4.37-4.41 (m, 1H), 5.29 (s, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 9.0 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.4, 25.4, 27.5, 49.5, 52.1, 54.6, 55.2, 57.0, 79.3, 80.0, 81.8, 111.1, 112.0, 129.3, 129.6, 133.1, 156.4, 160.8, 170.9, 171.6, 173.1; MS (ESI) *m/z* calcd for $C_{23}H_{32}N_7O_6$ $[M+H]^+$ 502.24, found 501.90; calcd for $C_{23}H_{31}N_7NaO_6$ $[M+Na]^+$ 524.22, found 523.85.

The tripeptides **17b-d** were synthesized using the same procedure as described for tripeptide **17a**.

Tripeptide 17b was obtained as a white solid (124 mg, 80%). R_f = 0.47 (DCM/CH_3OH, 9:1, v/v); ^1H NMR (500 MHz, CD_3OD): δ = 1.35 (d, *J* = 7.0 Hz, 3H), 1.39 (s, 9H), 1.82-1.88 (m, 1H), 1.98-2.01 (m, 1H), 2.76 (s, 3H), 3.42 (d, *J* = 5.5 Hz, 2H), 3.86 (s, 3H), 4.11-4.14 (m, 1H), 4.37-4.40 (m, 1H), 5.27 (s, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.5, 25.4, 27.5, 31.1, 48.7, 49.3, 52.8, 55.2, 57.1, 79.3, 79.7, 81.8, 111.1, 112.0, 129.3, 129.7, 133.1, 156.6, 160.8, 171.7, 173.2, 173.4; MS (ESI) *m/z* calcd for $C_{24}H_{34}N_7O_6$ $[M+H]^+$ 516.26, found 515.90; calcd for $C_{24}H_{33}N_7NaO_6$ $[M+Na]^+$ 538.24, found 537.95.

Tripeptide 17c was obtained as a white solid (149 mg, 94%). R_f = 0.47 (DCM/CH_3OH, 9:1, v/v); ^1H NMR (500 MHz, CD_3OD): δ = 1.35 (d, *J* = 7.0 Hz, 3H), 1.39 (s, 1H), 1.62-1.69 (br m, 3H), 1.81 (br s, 1H), 2.76 (s, 3H), 3.34 (d, *J* = 6.0 Hz, 2H), 3.86 (s, 3H), 4.02 (br s, 1H), 4.36-4.40 (m, 1H), 5.28 (s, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.5, 25.2, 25.4, 27.5, 29.2, 49.2, 50.8, 54.7, 55.2, 57.1, 79.3, 79.6, 81.8, 111.1, 112.0, 129.3, 129.7, 133.1 156.6, 160.8, 171.7, 173.2, 173.8; MS (ESI) *m/z* calcd for $C_{25}H_{36}N_7O_6$ $[M+H]^+$ 530.27, found 529.95; calcd for $C_{25}H_{35}N_7NaO_6$ $[M+Na]^+$ 552.25, found 551.95.

Tripeptide 17d was obtained as a white solid (155 mg, 95%). R_f = 0.47 (DCM/CH_3OH, 9:1, v/v); ^1H NMR (500 MHz, CD_3OD): δ = 1.35 (d, *J* = 7.0 Hz, 3H), 1.38 (s, 9H), 1.40-1.45 (br m, 2H), 1.57-1.67 (br m, 3H), 1.73-1.75 (br m, 1H), 2.76 (s, 3H), 3.29-3.32 (m, 2H), 3.86 (s, 3H), 3.97-4.00 (m, 1H), 4.35-4.40 (m, 1H), 5.27 (s, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.5, 23.0, 25.4, 27.5, 28.4, 31.5, 49.2, 51.1, 55.1, 55.2, 57.1, 79.3, 79.5, 81.8, 111.1, 112.0, 129.3, 129.7, 133.1, 156.7, 160.8, 171.7, 173.3, 174.1; MS (ESI) *m/z* calcd for $C_{26}H_{38}N_7O_6$ $[M+H]^+$ 544.29, found 544.05; calcd for $C_{26}H_{37}N_7NaO_6$ $[M+Na]^+$ 566.27, found 566.15.

General procedure for the Cu(I)-catalyzed click cyclization:
The catalyst $Cu(CH_3CN)_4PF_6$ (0.15 mmol) was placed in a capped flask. The flask was evacuated and refilled with dry N_2 (three times). Then, toluene (80 mL) and MeOH (15 mL) were added and subsequently followed by a solution of compound **17a-d** (0.1 mmol in 5 mL of MeOH). The solvents were purged with dry N_2 for 1 h prior to use. The obtained reaction mixture was stirred under N_2 at room temperature for 24 h. Then, the solvents were removed under reduced pressure and the residue was absorbed on silica gel and purified by column chromatography.

Cyclization of 17a: After column chromatography, two fractions were obtained (total amount that was recovered: 38 mg, 76%). The first fraction (25 mg) was a single spot on TLC and ^1H NMR analysis showed the presence of the 1,4-triazole proton (δ = 8.18 ppm) an indication that a cyclic product was formed. HPLC analysis of this fraction revealed a main component (t_R = 20.0 min (method A)). After purification by preparative RP-HPLC, the identity of this component was verified by MALDI-TOF analysis and it was found to be the cyclic dimer **19a**. ^1H NMR (300 MHz, CD_3OD): δ = 1.18 (d, *J* = 6.9 Hz, 6H), 1.41 (s, 18H), 2.74 (s, 6H), 3.89 (s, 6H), 4.29-4.36 (m, 2H), 4.56-4.63 (m, 6H), 5.35 (s, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 7.29-7.32 (dd, *J* = 8.7, 2.1 Hz, 2H), 8.04 (d, *J* = 2.1 Hz, 2H), 8.18 (s, 2H); MALDI-TOF MS *m/z* calcd for $C_{46}H_{62}N_{14}NaO_{12}$ $[M + Na]^+$ 1025.46, found 1025.76; calcd for $C_{46}H_{62}KN_{14}O_{12}$ $[M + K]^+$ 1041.43, found 1041.77.
The second fraction (13 mg) was analyzed by HPLC and showed to contain a main component with a t_R = 20.8 min (method A). After purification by preparative RP-HPLC, MALDI-TOF analysis confirmed that this main component was the cyclic trimer. MALDI-TOF MS *m/z* calcd for $C_{69}H_{93}N_{21}NaO_{18}$ $[M + Na]^+$ 1526.69, found 1526.75; calcd for $C_{69}H_{93}KN_{21}O_{18}$ $[M + K]^+$ 1542.66, found 1542.76.

Cyclization of 17b: After column chromatography, one product fraction was obtained (14 mg 27%) which was a single spot on TLC. HPLC analysis of this fraction indicated the presence of two peaks, t_R = 19.9 and t_R = 20.9 min, respectively (method A). Purification by preparative RP-HPLC revealed two isolated compounds and both were analyzed by MALDI-TOF. The compounds were identified to be the cyclic dimer **19b** and the cyclic trimer, respectively. Since an acidic buffer (0.1% TFA) was used for the preparative HPLC purification, the Boc-group of the isolated cyclic peptides was partially cleaved-off. The obtained cyclic dimer **19b** was characterized by ^{1}H NMR and showed the presence of the 1,4-triazole proton (δ = 8.18 ppm). MALDI-TOF MS *m/z* calcd for $C_{48}H_{66}N_{14}NaO_{12}$ $[M + Na]^+$ 1053.49, found 1053.93; calcd for $C_{48}H_{66}KN_{14}O_{12}$ $[M + K]^+$ 1069.46, found 1069.90.
For the corresponding cyclic trimer: MALDI-TOF MS *m/z* calcd for $C_{72}H_{99}N_{21}NaO_{18}$ $[M + Na]^+$ 1568.74, found 1569.05; calcd for $C_{72}H_{99}KN_{21}O_{18}$ $[M + K]^+$ 1584.71, found 1585.05.

Cyclization of 17c: After column chromatography, one product fraction was obtained (8 mg, 15%) which was a single spot on TLC. ^{1}H NMR analysis revealed the presence of the 1,4-triazole proton (δ = 8.17 ppm). HPLC analysis indicated the presence of one main compound, t_R = 20.4 min (method A). The compound was identified by MALDI-TOF to be the cyclic dimer **19c**. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.32 (d, *J* = 7.2 Hz, 6H), 1.44 (s, 18H), 1.61-1.76 (m, 4H), 1.95-2.05 (m, 4H), 2.69 (s, 6H), 3.82 (s, 6H), 4.09-4.15 (m, 2H), 4.31-4.38 (m, 2H), 4.42-4.47 (m, 4H), 5.31 (s, 2H), 6.97 (d, *J* = 8.7 Hz, 2H), 7.27-7.31 (dd, *J* = 8.7, 1.8 Hz, 2H), 8.05 (d, *J* = 1.8 Hz, 2H), 8.17 (s, 2H); MALDI-TOF MS *m/z* calcd for $C_{50}H_{70}N_{14}NaO_{12}$ $[M + Na]^+$ 1081.52, found 1081.67; calcd for $C_{50}H_{70}KN_{14}O_{12}$ $[M + K]^+$ 1097.49, found 1097.66.

Cyclization of 17d: After column chromatography, two fractions were obtained (total amount that was recovered: 33 mg, 61%). The first fraction (20 mg) contained a main compound as judged by analytical HPLC, t_R = 18.8 min (method A). After purification by preparative RP-HPLC, the identity of this compound was verified by MALDI-TOF analysis and it was found to be the cyclic monomer **18d**. MALDI-TOF MS *m/z* calcd for $C_{26}H_{37}N_7NaO_6$ $[M + Na]^+$ 566.27, found 566.80; calcd for $C_{26}H_{37}KN_7O_6$ $[M + K]^+$ 582.24, found 582.78.

The second fraction (13 mg) was analyzed by HPLC and showed to contain a main component, t_R = 20.6 min (method A). After purification by preparative RP-HPLC, MALDI-TOF analysis identified this compound as the cyclic dimer **19d**. MALDI-TOF MS *m/z* calcd for $C_{52}H_{74}N_{14}NaO_{12}$ $[M + Na]^+$ 1109.55, found 1109.87; calcd for $C_{52}H_{74}KN_{14}O_{12}$ $[M + K]^+$ 1125.52, found 1125.80.
Since an acidic buffer (0.1% TFA) was used for the preparative RP-HPLC purification, the Boc-group of the isolated cyclic peptides was partially cleaved-off. The obtained cyclic dimer **19d** and cyclic monomer **18d** were characterized by ^{1}H-NMR and showed the presence of the 1,4-triazole proton (δ = 8.17 ppm).

General procedure for the TBTA-promoted Cu(I)-catalyzed click cyclization:
The catalyst $Cu(CH_3CN)_4PF_6$ (0.005 mmol) and TBTA (0.005 mmol) was placed in a capped flask. The flask was evacuated and refilled with dry N_2 (three times). Then toluene (80 mL) and MeOH (15 mL) were added and subsequently followed by a solution of compound **17a-d** (0.1 mmol in 5 mL of MeOH). The solvents were purged with dry N_2 for 1 h prior to use. The obtained reaction mixture was stirred under N_2 at room temperature for 24 h. Then, the solvents were removed under reduced pressure and the residue was analyzed by HPLC and LC-MS.

Compound 20: Dipeptide **15** (1.01 g, 2.00 mmol) was dissolved in THF (50 mL) and to this solution TBAF (1.27 g, 4.00 mmol) was added at 0 °C and the obtained reaction mixture was stirred for 3 h. Then, the solvent was removed under reduced pressure and the residue was redissolved in EtOAc (50 mL) and the obtained solution was washed with saturated NH_4Cl (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/DCM, 1:3 to 2:3, v/v). Dipeptide **20** was obtained as a white solid (735 mg, 94%). R_f = 0.50 (DCM/CH_3OH, 9:1, v/v); mp = 73-74 °C; $[\alpha]_D^{20}$ = +107.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD, 1:1, v/v): δ = 1.32 (d, *J* = 7.2 Hz, 3H), 1.45 (s, 9H), 2.78 (d, *J* = 4.5 Hz, 3H), 3.89 (s, 3H), 4.08-4.12 (m, 1H), 5.32 (d, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 7.31-7.35 (dd, *J* = 2.4, 8.7 Hz, 1H), 7.46 (d, *J* = 2.4 Hz, 1H), 7.74 (br s, 1H), 7.95 (d, *J* = 6.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD, 1:1, v/v): δ = 17.7, 26.3, 28.2, 50.4, 55.9, 56.3, 79.5, 80.3, 81.8, 111.0, 111.7, 129.5, 132.9, 156.2, 160.5, 171.0, 173.5; MS (ESI) *m/z* calcd for $C_{20}H_{28}N_3O_5$ $[M+H]^+$ 390.20, found 390.35; calcd for $C_{20}H_{27}N_3NaO_5$ $[M+Na]^+$ 412.18, found 412.40.

Compounds 21a-b:

These compounds were synthesized starting from corresponding Cbz-protected diamino acids using the same methods as described for the synthesis of azidoamino acids **14a-d**, respectively. The *tert*-butyl ester moiety was introduced using 2,2,2-trichloro-acetamidate/BF_3 etherate, and the corresponding *tert*-butyl esters were obtained in good yield and purity.[12] For compound **21a**, ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 9H), 3.72 (t, J = 3.6 Hz, 2H), 4.40 (t, J = 3.6 Hz, 1H), 5.13 (s, 2H), 5.60 (br 1H), 7.31-7.37 (m, 5H). For compound **21b**, ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.25-1.43 (m, 2H), 1.46 (s, 9H), 1.57-1.71 (m, 3H), 1.77-1.89 (m, 1H), 3.25 (t, J = 6.6 Hz, 2H), 4.27 (m, 1H), 5.10 (s, 2H), 5.36 (br, 1H), 7.30-7.36 (m, 5H).

Compounds 22a-b: Alkyne **20** (39 mg, 0.10 mmol) was dissolved in *tert*-BuOH/H_2O (5 mL, 1:1, v/v). To this solution, azide **21a** (0.11 mmol) was added, followed by the addition of $CuSO_4$ (1.6 mg in 20 μL H_2O) and sodium ascorbate (2.6 mg in 20 μL H_2O). The reaction mixture was stirred at room temperature for 10 h. Next, the reaction mixture was diluted with H_2O (5 mL) and the aqueous phase was extracted with EtOAc (25 mL, twice). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:100 to 2:100, v/v). Triazole containing peptide **22a** was obtained as a white solid (58 mg, 81%). R_f = 0.48, (DCM/CH_3OH, 9:1, v/v); mp = 122-124 °C; $[\alpha]_D^{20}$ = +108.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (d, J = 6.9 Hz, 3H), 1.42 (s, 9H), 1.43 (s, 9H), 2.75 (d, J = 4.8 Hz, 3H), 3.84 (s, 3H), 4.15 (t, J = 6.9 Hz, 1H), 4.67 (d , J = 6.6 Hz, 1H), 4.77-4.91 (m, 2H), 5.11-5.17 (s, 2H, and br s, 1H), 5.16 (br s, 1H), 5.40 (d, J = 6.9 Hz, 1H), 5.74 (d, J = 6.9 Hz, 1H), 6.57 (br s, 1H), 6.93 (d, J = 8.7 Hz, 1H), 7.31-7.35 (m, 6H), 7.97 (s, 1H), 8.26 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 26.8, 28.1, 28.5, 50.7, 51.0, 54.8, 55.7, 57.2, 67.4, 80.4, 84.1, 111.7, 119.7, 124.6, 126.9, 128.2, 128.4, 128.7, 130.7, 136.2, 143.8, 155.8, 156.0, 167.8, 170.6, 172.4; MS (ESI) *m/z* calcd for $C_{35}H_{48}N_7O_9$ $[M+H]^+$ 710.35, found 710.55.

Compound **22b** was synthesized using the same procedure as described for **22a** by coupling of alkyne **20** and azide **21b**. Triazole containing peptide **22b** was obtained as a white solid (60 mg, 80%). R_f = 0.48, (DCM/CH_3OH, 9:1, v/v); mp = 90-92 °C; $[\alpha]_D^{20}$ = +71.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (d, J = 7.2

Hz, 3H), 1.39 (s, 9H), 1.41 (s, 9H), 1.62-1.72 (m, 2H), 1.80-1.98 (m, 3H), 2.16 (br s, 1H), 2.72 (d, *J* = 4.5 Hz, 3H), 3.88 (s, 3H), 4.15-4.23 (m, 2H), 4.32-4.36 (m, 2H), 5.06 (s, 2H), 5.30 (d, *J* = 6.9 Hz, 1H), 5.42-5.47 (m, 2H), 6.85 (br s, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 7.29-7.33 (m, 6H), 7.47 (d, *J* = 6.9 Hz, 1H), 7.96 (s, 1H), 8.27 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.5, 22.4, 26.7, 28.2, 28.5, 30.1, 32.4, 50.1, 50.6, 54.2, 55.8, 57.1, 67.1, 80.3, 82.6, 111.6, 119.9, 123,4, 126.8, 128.0, 128.3, 128.7, 130.7, 136.5, 142.9, 155.7, 156.1, 170.8, 171.5, 172.5; MS (ESI) *m/z* calcd for $C_{38}H_{54}N_7O_9$ $[M+H]^+$ 752.40, found 752.65; calcd for $C_{38}H_{53}N_7NaO_9$ $[M+Na]^+$ 774.38, found 774.50.

General procedure for the macrolactamization:
Compound **22a-b** (0.05 mmol) was dissolved in DCM (15 mL). To this solution TFA (3 mL) was added, and the obtained reaction mixture was stirred for 3 h at room temperature. Then, the volatiles were removed under reduced pressure and the residue was coevaporated with DCM (10 mL, three times) to remove the residual TFA. The resulting solid residue was dried under vacuum for 1 h. Next, the residue was dissolved in DMF (100 mL) and BOP (33 mg, 0.075 mmol) and D*i*PEA (21 μL, 0.15 mmol) were added to this solution. The obtained reaction mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure the residue was purified. The products were analyzed by HPLC and characterized by MALDI-TOF MS and ^{1}H NMR.

Marcolactamization of compound 22a: Since the poor solubility of the obtained crude products, the residue was washed with CH_3CN/H_2O (1:4, v/v, 10 mL, four times) to remove the soluble coupling reagent. Then the crude product was analyzed by HPLC. It showed complicated product mixture and at least two compounds were present and could be assigned as the cyclic monomer **24a**, t_R = 20.0 min (method A) and the cyclic dimmer, t_R = 21.1 min (method A) with a monomer/dimer ratio of 1:3. MALDI-TOF gave a peak corresponding to the cyclic dimer **23a** while the cyclic monomer could not be identified. For cyclic dimer **23a**, MALDI-TOF MS *m/z* calcd for $C_{52}H_{59}N_{14}O_{12}$ $[M+H]^+$ 1071.44, found 1071.82; calcd for $C_{52}H_{58}N_{14}NaO_{12}$ $[M+Na]^+$ 1093.43, found 1093.81; calcd for $C_{52}H_{58}KN_{14}O_{12}$ $[M+K]^+$ 1109.40, found 1109.82.

Macrolactamization of compound 22b: Since the poor solubility of the obtained crude products, the residue was washed with CH_3CN/H_2O (1:4, v/v, 10 mL, four

times) to remove the soluble coupling reagent. Then the crude product was analyzed by HPLC. Based on analytical HPLC, a single compound was identified, t_R = 21.5 min (method A), which was characterized by MALDI-TOF as the cyclic dimer **23b**. A small amount cyclic dimer **23b** (1.6 mg) could be isolated by preparative RP-HPLC and characterized by ^{1}H NMR. For cyclic dimer **23b**, ^{1}H NMR (300 MHz, CD_3OD/DMSO, 1:1, v/v): δ = 1.03 (d, *J* = 7.2 Hz, 6H), 1.13 – 1.29 (m, 6H), 1.47-1.54 (m, 4H), 1.70-1,81 (m, 4H), 2.55 (d, *J* = 4.5 Hz, 6H), 3.82 (s, 6H), 3.91-3.98 (m, 2H), 4.27-4.36 (m, 6H), 4.96 (s, 4H), 5.32 (d, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 7.23-7.33 (m, 14H), 8.09 (d, *J* = 7.8 Hz, 2H), 8.13 (d, *J* = 2.4 Hz, 2H), 8.18 (d, *J* = 4.8 Hz, 2H), 8.25 (s, 2H), 8.38 (d, *J* = 7.8 Hz, 2H); MALDI-TOF MS *m/z* calcd for $C_{58}H_{71}N_{14}O_{12}$ $[M+H]^+$ 1155.54, found 1155.58; calcd for $C_{58}H_{70}N_{14}NaO_{12}$ $[M+Na]^+$ 1177.52, found 1177.54; calcd for $C_{58}H_{70}N_{14}KO_{12}$ $[M+K]^+$ 1193.49, found 1193.55.

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Chapter 3

Synthesis of 1,5-Triazole-Bridged Vancomycin DE-Ring Peptidomimetics via RuAAC Macrocyclization

Parts of this chapter have been published: Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2011**, *13*, 3438-3441.

3.1 Introduction

To explore suitable alternatives for the biaryl bridges in vancomycin, the introduction of a 1,5-triazole moiety to constrain the DE-ring in vancomycin is a relevant follow-up study after the successful application of CuAAC[1] (which gives 1,4-disubstituted triazole derivatives) for the synthesis of the vancomycin DE-ring mimics. As a complementary reaction of CuAAC, the Ru(II)-catalyzed azide-alkyne cycloaddition (RuAAC) reaction leads to the 1,5-disubstituted triazole in a regioselective manner,[2] and the 1,5-disubstituted triazole-containing cyclic peptides may have improved biological activity compared to the corresponding 1,4-disubstituted derivatives.[3] However, the RuAAC reactions are rather restricted with respect to their reaction conditions and RuAAC macrocyclization proved to be difficult for the synthesis of cyclic peptides.[3, 4] Therefore, it is of high importance to develop an optimized RuAAC-based cyclization for the synthesis of cyclic peptides to obtain diversity in side-chain to side-chain connectivity patterns. At the time this research project was started to design the 1,5-triazole containing vancomycin DE-ring mimics, there was no report in the literature that described a successful application of RuAAC reaction as the cyclization step for the synthesis of cyclic peptides.[5] However, a report by Rowley-Kelly *et al.* in 2009 was the first in a series that described the Ru(II)-catalyzed intramolecular 1,5-disubstituted triazole ring formation to obtain small molecules,[6] and proved to be a very attractive procedure for application in the synthesis of cyclic peptides.

3.2 Design and synthesis of the 1,5-disubstituted triazole-bridged vancomycin DE-ring mimics by RuAAC macrocyclization

As a suitable alternative for the 1,4-disubstituted triazole-bridged vancomycin DE-ring mimics, the rationale for design of the 1,5-disubstituted triazole-bridged mimics was generally the same as described in the previous chapter (Figure 1), in which the biaryl bridge of the DE-ring was replaced by a 1,4-disubstituted triazole moiety.

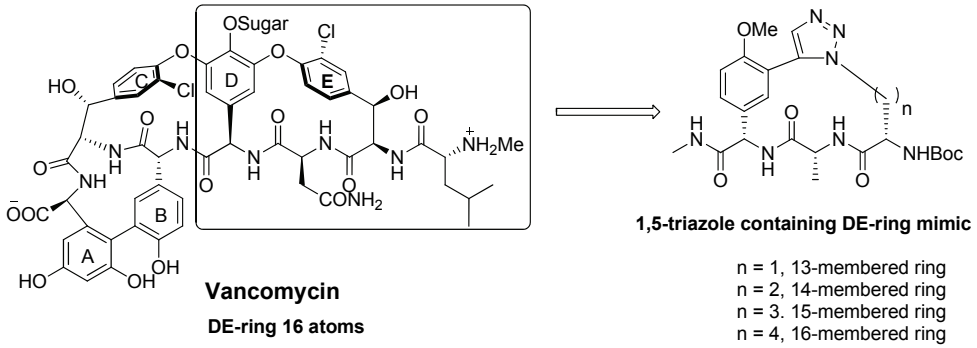
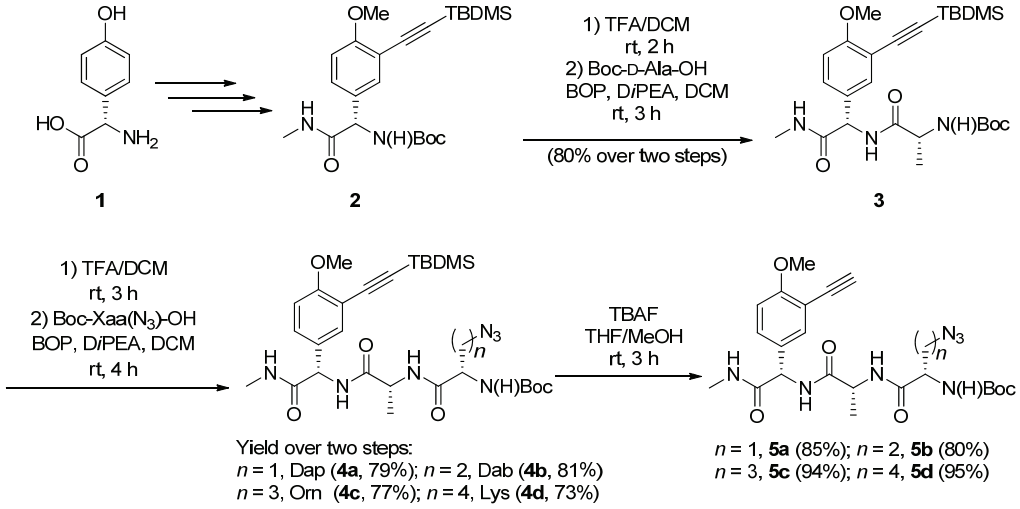


Figure 1. Design of the 1,5-disubstituted triazole-bridged vancomycin DE-ring mimics.

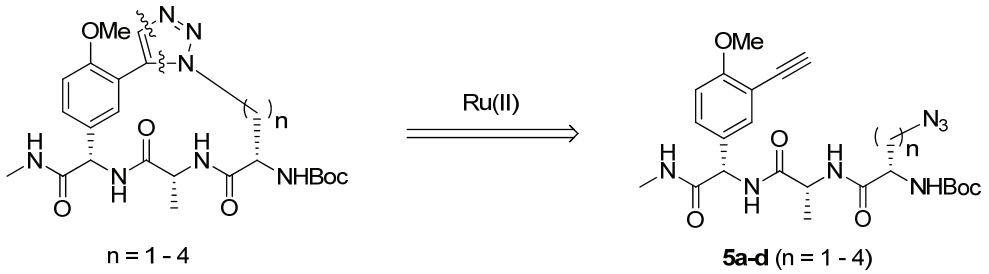
Apparently, the desired 1,5-disubstituted triazole-bridged mimics can be conveniently synthesized by employing RuAAC chemistry starting from the same linear tripeptide precursors **5a-d** as used in the synthesis of 1,4-disubstituted triazole-bridged mimics which have been described in detail in chapter 2, and summarized in Scheme 1.



Scheme 1. Synthesis of the linear tripeptide precursors **5a-d** for RuAAC macrocyclization.

The retrosynthetic route of the DE-ring mimics using RuAAC macrocyclization is shown in Scheme 2. Since the results of the CuAAC macrocyclization indicated the scope and limitations to access these small cyclic peptides containing the stretched 1,4-disubstituted triazole ring system, which

showed predominantly dimer formation in most of the cases, it would be foreseeable that the RuAAC reaction may also yield cyclic dimers as byproducts. However, in the 1,5-disubstituted triazole system, the two substituents are positioned under a smaller angle, which might be beneficial for a more easily obtainable bent site in the small ring system and leads to the desired cyclic monomer.

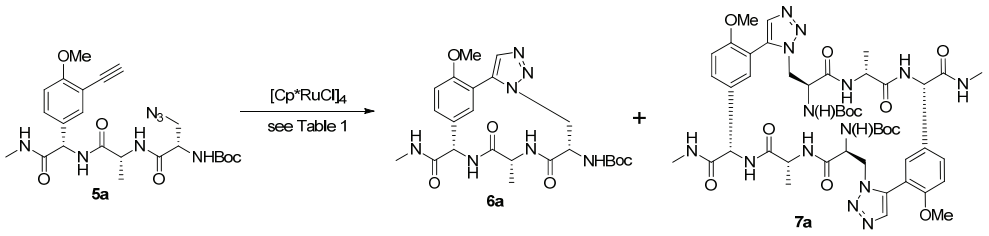


Scheme 2. Retrosynthetic route of the 1,5-triazole containing vancomycin DE-ring mimics.

3.2.1 Optimization of the RuAAC cyclization conditions

As mentioned above, the Ru(II)-catalyzed macrocyclization has not been well studied previously. To carry out the Ru(II)-catalyzed macrocyclization of the precursor peptides **5a-d**, the development of workable RuAAC macrocyclization conditions is required. In line with the development of CuAAC macrocyclization, tripeptide **5a** was chosen as the model compound to investigate the optimal reaction conditions. Based on a literature search[2] and the report from Rowley-Kelly *et al.*,[6] $[Cp^*RuCl]_4$ was selected as catalyst since it has a good regioselectivity for the formation of 1,5-disubstituted triazole. It was decided to choose in first instance toluene as solvent, according to the procedure described by Rowley-Kelly. However, due to the poor solubility of the tripeptides in toluene, an additional amount of MeOH (20%) was required. With 5 mol-% $[Cp^*RuCl]_4$ at a substrate concentration of 1 mM, the reaction gave no or only trace amounts of the cyclic tripeptide at three different temperatures (Entry 1-3, Table 1). Considering the protic solvent like methanol has an adverse effect on the RuAAC reaction,[2a] therefore, toluene was replaced by THF containing only 5 vol-% MeOH. The substrate concentration was also increased to 5 mM, although it was realized that an increased concentration may frustrate the desired intramolecular cyclization. Gratifyingly, under these reaction conditions, a 68% conversion of the starting

material was achieved (Entry 4, Table 1). As the reaction was still carried out in a relatively diluted solution compared to the previously reported Ru-catalyzed reactions (~100 mM),[2b] it was decided to increase the catalyst loading to 15 mol-% in order to increase the cyclization efficiency. Under these optimized reaction conditions (Entry 5, Table 1), a complete consumption of the linear tripeptide **5a** was obtained.



Scheme 3. Optimization of the Ru(II)-catalyzed macrocyclization with linear tripeptide **5a**

Table 1. Optimization of the Ru(II)-catalyzed macrocyclizations.[a]

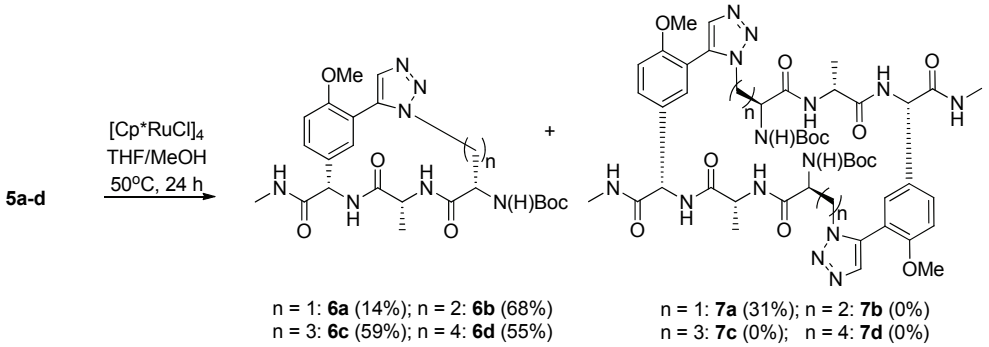
Entry	Catalyst (%)	Solvent	Substrate Conc. (mM)	Temperature (°C)	Conversion[b] (%)
1	5	toluene/MeOH (4:1 v/v)	1	r.t.	no reaction
2	5	toluene/MeOH (4:1 v/v)	1	50	no reaction
3	5	toluene/MeOH (4:1 v/v)	1	80	trace
4	5	THF/MeOH (19:1 v/v)	5	50	68
5	15	THF/MeOH (19:1 v/v)	5	50	100[c]

[a] All the reactions were run for 24h. [b] Conversion was determined by recovery of the starting material. [c] No starting material was recovered.

Under the optimized reaction conditions, the Ru-catalyzed macrocyclization proceeded smoothly and reached quantitative conversion after 24 h at 50 °C. The crude reaction mixture was purified by a two-step approach, and three compounds were isolated and characterized by MALDI-TOF analysis as the cyclic monomer **6a**, the cyclic dimer **7a**, and the cyclic trimer (not shown), respectively (Scheme 3).

The ratio of the monomer/dimer/trimer was 2:4:1 determined LC-MS. This was a very encouraging result, since it indicated that a 1,5-disubstituted triazole allowed the formation of a monomeric cyclic tripeptide with the shortest side chain, to obtain the highly constrained ring system of 13 atoms.

3.2.2 Synthesis of the 1,5-disubstituted triazole-bridged vancomycin DE-ring mimics by RuAAC macrocyclization



Scheme 3. Ru(II)-catalyzed macrocyclizations of the linear tripeptides **5a-d**.

Table 2. Results of the Ru(II)-catalyzed macrocyclizations of the linear tripeptides **5a-d.**

Comp.	Catalyst (mol-%)	Conc. (mM)	Yield (%)[a]	Monomer (%)[b]	Dimer (%)[b]	Trimer (%)[b]
5a	15	5	53	14	31	8
5b	15	5	68	100	0	0
5c	15	5	59	100	0	0
5d	15	5	55	100	0	0

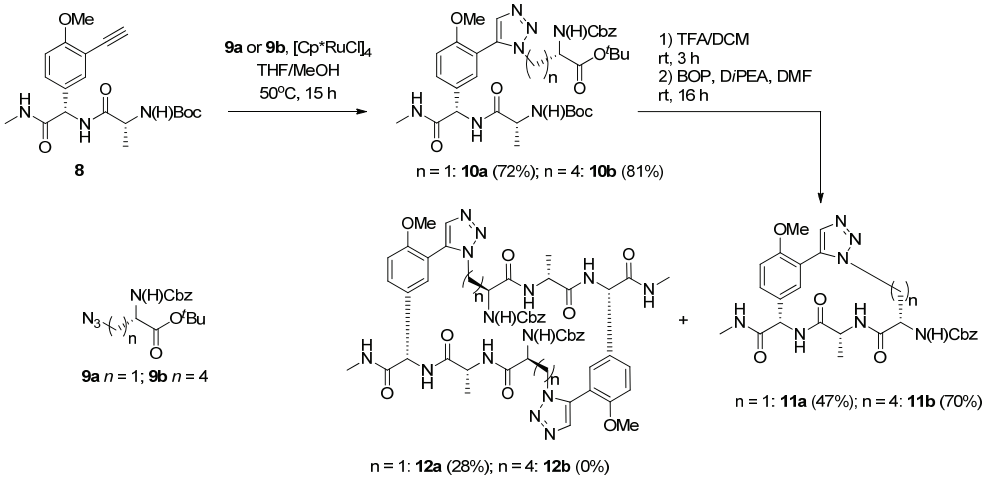
[a] Isolated yield. [b] Yield as determined by LCMS.

Based on the optimized reaction protocol, the Ru(II)-catalyzed cyclization of precursors **5b-d** was studied. Under these reaction conditions, the macrocyclization of these precursor tripeptides resulted in the exclusive formation of the monomeric cyclic tripeptides **7b-d** while dimer and/or trimer formation was completely absent. The cyclic peptides could be isolated by column chromatography with high purity and good yield (55% to 68%). These observations were consistent with the expectation mentioned before that the 1,5-disubstituted triazole moiety can

facilitate the intramolecular cyclization due to its bended geometry. The most constrained cyclic tripeptide obtained had only 13 atoms in the macrocyclic ring while the lysine-derived tripeptide was of the same size as the vancomycin DE-ring and contained 16 atoms. The affinity evaluation with Ac-Ala-Ala-OH and structure modeling results of these cyclic tripeptides will be discussed in Chapter 5.

3.3 Synthesis of the 1,5-triazole containing mimics of the vancomycin DE-ring by macrolactamization

Since it was observed that the products of the Ru(II)-catalyzed macrocyclization were predominantly the monomeric cyclic peptides, it was assumed that the introduction of a 1,5-disubstituted triazole moiety prior to cyclization would orient the linear tripeptides in such a conformation that macrolactamization would be favored and lead to a more efficient intramolecular cyclization. To prove this hypothesis it was decided to carry out the macrolactamization as an alternative strategy (Scheme 4).



Scheme 4. Synthesis of the 1,5-triazole containing cyclic peptides via macrolactamization.

For this purpose, dipeptide **8** was first reacted with azidoamino acids **9a** or **9b** via a Ru(II)-catalyzed cycloaddition reaction (Scheme 4). The fully protected tripeptides **10a-b** were obtained in good yield. After removal of both acid labile protecting groups (^{t}Bu/Boc) by treatment with TFA in DCM, a BOP-mediated macrolactamization of the linear peptides was carried out in a diluted reaction

mixture to avoid dimerization and/or oligomerization. It was found that precursor **10a** with the shortest side chain gave 28% dimer **12a** besides the desired monocycle **11a** (47%). In sharp contrast to precursor **10b** which even led to a slightly higher yield of the cyclic monomer **11b** (70%) compared to 'click' cyclization to give **6d** (55%, Scheme 3), while formation of cyclic dimer **12b** was completely absent. These results clearly demonstrated that assembly of the 1,5-triazole prior to macrolactamization is beneficial for the efficient formation of the cyclic monomer.

3.4 Conclusion

A Ru(II)-catalyzed click-type macrocyclization protocol was successfully developed and a series of cyclic tripeptides as vancomycin DE-ring mimics was synthesized in good yield.[7] Due to its β-turn-like conformation, the 1,5-disubstituted triazole moiety favored the formation of cyclic monomers and allowed the formation of highly constrained ring structures. The Ru(II)-catalyzed macrocyclization proved to be effective for the synthesis of 1,5-disubstituted triazole-containing cyclic tripeptides, and showed superiority over the CuAAC-based click cyclization with respect to the prevalent synthesis of monomeric cyclic peptides. Along with the results of the synthesis of 1,4-triazole containing cyclic peptides using either Cu(I)-catalyzed macrocyclization or macrolactamization, it was concluded that the formation of cyclic monomer or cyclic dimer of the series of tripeptide not only depends on the ring size, but also strongly influenced by the geometric conformation of the triazole moiety in the cyclic peptides. As a new alternative, the Ru(II)-catalyzed macrocyclization is a very promising chemical tool for the synthesis of small cyclic peptides and can be utilized as a diverse supplemental chemical tool besides the CuAAC macrocyclization.

3.5 Experimental section

3.5.1 General experimental procedures

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. The solvents were obtained as peptide synthesis grade and stored on molecular sieves (4 Å).

Column chromatography was performed on *Silicycle SiliFlash P60* silica gel (particle size 40-63 μm).

Thin Layer Chromatography (TLC) was performed on *Merck* precoated silica gel 60F254 glass plates. Compound spots were visualized by UV-quenching, ninhydrin, or Cl_2/TDM.

Melting point was measured on a Büchi Schmelzpunktbestimmungsapparat according to Dr. Tottoli and was uncorrected.

Optical rotation was measured on a *JASCO* P-1010 Polarimeter using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 mL) are indicated.

^{1}H NMR was acquired on a *Varian* Mercury 300 MHz or a *Varian* Innova 500 MHz spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to TMS (0.00 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br). ^{13}C NMR was acquired on a *Varian* Mercury 75 MHz or a *Varian* Innova 125 MHz spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the solvent residual signal, $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or DMSO-d_6 (39.0 ppm).

Analytical HPLC was performed on an automated HPLC system (*Shimadzu*) equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using an *Alltech* Alltima C8 column (pore size: 100 Å, particle size: 5 μm; 250 × 4.6 mm) at a flow rate of 0.5 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 40 min (method A), or 60 min (method B)].

Preparative RP-HPLC was performed on an automated preparative HPLC system (*Applied Biosystems*) equipped with a UV/Vis detector operating at 214 nm using an *Alltech* Alltima C8 column (pore size: 100 Å, particle size: 10 μm; 250 × 22 mm) at a flow rate of 2.0 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 90 min].

ESI-MS was performed on a *Shimadzu* LCMS-QP8000 electrospray ionization mass spectrometer.

MALDI-TOF MS was performed on a *Shimadzu* Kratos AXIMA-CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and P14R as reference.

3.5.2 The Ru(II)-catalyzed click cyclizations of 5a-d

General procedure for the Ru(II)-catalyzed click cyclization:
The catalyst $[Cp^*RuCl]_4$ was placed in a capped flask. The flask was evacuated and refilled with dry N_2 (three times). Then, the flask was filled with THF (19 mL) and a solution of compound **5a-d** (0.1 mmol in 1 mL of MeOH) was added. The solvents were purged with dry N_2 for 1 h prior to use. The obtained reaction mixture was stirred at 50 °C under N_2 for 24 h. Then, the solvents were removed under reduced pressure and the residue was absorbed on silica gel and purified by column chromatography.

Cyclization of 5a: After column chromatography, two fractions were obtained (total amount that was recovered: 27 mg, 53%). The first fraction (15 mg) was analyzed by HPLC and turned out to contain one main compound, t_R = 31.4 min (method B). The second fraction (12 mg) was analyzed by HPLC and showed two main components. The two fractions were separately purified by preparative RP-HPLC and three compounds were isolated. The isolated three compounds were analyzed by MALDI-TOF and could be characterized as the cyclic monomer **6a**, the cyclic dimer **7a** and the cyclic trimer, respectively. Since an acidic buffer (0.1% TFA) was used for the preparative RP-HPLC purification, the Boc-group of the isolated cyclic peptides was partially cleaved-off. The obtained cyclic monomer **6a** and cyclic dimer **7a** were further characterized by ^{1}H NMR and showed the presence of the 1,5-triazole proton (δ = 7.77 ppm). For cyclic monomer **6a**, MALDI-TOF MS *m/z* calcd for $C_{23}H_{32}N_7O_6$ $[M + H]^+$ 502.24, found 502.50; calcd for $C_{23}H_{31}N_7NaO_6$ $[M + Na]^+$ 524.22, found 524.53; calcd for $C_{23}H_{31}KN_7O_6$ $[M + K]^+$ 540.20, found 540.49.
For the cyclic dimer **7a**, MALDI-TOF MS *m/z* calcd for $C_{46}H_{62}N_{14}NaO_{12}$ $[M + Na]^+$ 1025.46, found 1025.48; calcd for $C_{46}H_{62}KN_{14}O_{12}$ $[M + K]^+$ 1041.43, found 1041.42.
For the cyclic trimer, MALDI-TOF MS *m/z* calcd for $C_{69}H_{93}N_{21}NaO_{18}$ $[M + Na]^+$ 1526.69, found 1526.10; calcd for $C_{69}H_{93}KN_{21}O_{18}$ $[M + K]^+$ 1542.66, found 1542.10.

Cyclization of 5b: After column chromatography, a single product fraction was obtained (35 mg, 68%). Based on HPLC analysis the isolated product was pure, t_R = 31.6 min (method B). ^{1}H NMR, ^{13}C NMR, and MALDI-TOF analysis confirmed

that the isolated product was the desired cyclic monomer **6b**. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.43 (d, *J* = 7.5 Hz, 3H), 1.47 (s, 9H), 2.21-2.35 (m, 1H), 2.68 (s, 3H), 3.80 (s, 3H), 4.03-4.09 (m, 1H), 4.22-4.49 (m, 3H), 5.51 (s, 1H), 7.05 (d, *J* = 8.7 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.47-7.51 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 15.8, 25.3, 27.5, 33.2, 44.9, 51.3, 54.0, 55.1, 55.3, 80.1, 111.5, 115.6, 127.3, 130.9, 131.3, 133.6, 156.1, 157.2, 171.0, 173.4; MALDI-TOF MS *m/z* calcd for $C_{24}H_{34}N_7O_6$ $[M + H]^+$ 516.26, found 516.58; calcd for $C_{24}H_{33}N_7NaO_6$ $[M + Na]^+$ 538.24, found 538.55; calcd for $C_{24}H_{33}KN_7O_6$ $[M + K]^+$ 554.21, found 554.54.

Cyclization of 5c: After column chromatography, a single product fraction was obtained (31 mg, 59%), which was pure according to analytical HPLC, t_R = 31.3 min (method B). ^{1}H NMR, ^{13}C NMR, and MALDI-TOF analysis confirmed the product was the desired cyclic monomer **6c**. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.40 (s, 3H), 1.43 (s, 9H), 1.66-1.73 (m, 2H), 2.79 (s, 3H), 3.81 (s, 3H), 3.88 (br s, 1H), 4.11-4.18 (m, 1H), 4.26 (t, *J* = 6.3 Hz, 2H), 5.45 (s, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 7.27 (s, 1H), 7.45-7.48 (d, *J* = 8.7 Hz, 1H), 7.70 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 14.2, 23.8, 25.5, 25.9, 28.1, 49.9, 53.7, 54.1, 54.3, 78.5, 109.8, 114.7, 128.8, 129.7, 129.8, 131.8, 133.6, 154.7, 155.8, 169.7, 171.7, 172.3; MALDI-TOF MS *m/z* calcd for $C_{25}H_{36}N_7O_6$ $[M + H]^+$ 530.27, found 530.58; calcd for $C_{25}H_{35}N_7NaO_6$ $[M + Na]^+$ 552.25, found 552.58; calcd for $C_{25}H_{35}KN_7O_6$ $[M + K]^+$ 568.23, found 568.58.

Cyclization of 5d: After column chromatography, a single product fraction was obtained (20 mg, 55%), which was pure according to analytical HPLC, t_R = 32.2 min (method B). ^{1}H NMR, ^{13}C NMR, and MALDI-TOF analysis confirmed the product was the desired cyclic monomer **6d**. ^{1}H NMR (500 MHz, CD_3OD): δ = 0.91-0.98 (br m, 2H), 1.39 (d, *J* = 7.0 Hz, 9H), 1.44 (s, 9H), 1.75 (br s, 2H), 1.96 (br s, 1H), 2.77 (s, 3H), 3.82 (s, 3H), 3.91 (br s, 1H), 4.00 (br s, 1H), 4.29 (t, *J* = 8.5 Hz, 2H), 5.49 (s, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.64 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.1, 21.2, 25.3, 27.5, 29.9, 31.2, 50.5, 54.7, 55.2, 56.0, 79.9, 111.2, 116.5, 130.2, 130.7, 131.8, 132.6, 135.6, 156.6, 157.4, 171.6, 172.9, 173.2; MALDI-TOF MS *m/z* calcd for $C_{26}H_{38}N_7O_6$ $[M + H]^+$ 544.29, found 544.59; calcd for $C_{26}H_{37}N_7NaO_6$ $[M + Na]^+$ 566.27, found 566.60; calcd for $C_{26}H_{37}KN_7O_6$ $[M + K]^+$ 582.24, found 582.66.

3.5.3 Macrolactamization of linear tripeptide 10a-b

Compound 10a: Alkyne **8** (39 mg, 0.10 mmol) and $[Cp^*RuCl]_4$ (5.4 mg, 0.005 mmol) were placed in a capped flask. The flask was evacuated and refilled with dry N_2 (three times). In a separate flask, azide **9a** was dissolved in THF (5 mL) which was purged with dry N_2 for 1 h prior to use, and this solution was transferred to the alkyne/catalyst mixture. The resulting reaction mixture was thoroughly flushed with dry N_2 (twice). The obtained reaction mixture was stirred at 50 °C under N_2 for 15 h. Then, the solvent was removed under reduced pressure and the residue was purified by column chromatography (MeOH/DCM, 1:100 to 2:100, v/v). Compound **10a** was obtained as a white solid (51 mg, 72%). R_f = 0.48, (DCM/MeOH, 9:1, v/v); mp = 120-122 °C; $[\alpha]_D^{20}$ = +67.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31, (d, *J* = 6.9 Hz, 3H), 1.37 (s, 9H), 1.42 (s, 9H), 2.76 (d, *J* = 4.8 Hz, 3H), 3.72 (s, 3H), 4.16 (t, *J* = 6.6 Hz, 1H), 4.41-4.68 (m, 2H), 4.75-4.98 (m, 2H), 5.13 (br, 1H), 5.37 (br, 1H), 5.84 (d, *J* = 7.5 Hz), 6.90 (d, *J* = 8.7 Hz, 1H), 7.18-7.34 (m, 6H), 7.40-7.44 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.51-7.55 (m, 1H), 7.62 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3, 26.8, 28.0, 28.5, 49.2, 50.6, 54.7, 55.9, 56.7, 67.2, 67.8, 80.4, 83.8, 112.1, 115.9, 128.2, 128.5, 128.8, 130.4, 130.8, 131.0, 134.4, 134.9, 136.0,155.9, 156.9, 168.1, 170.4, 172.6; MS (ESI) *m/z* calcd for $C_{35}H_{48}N_7O_9$ $[M+H]^+$ 710.35, found 710.50; calcd for $C_{35}H_{47}N_7NaO_9$ $[M+Na]^+$ 732.33, found 732.85.

Compound 10b was synthesized using the same procedure as described for **10a** by coupling of alkyne **8** and azide **9b**. Compound **10b** was obtained as a white solid (61 mg, 81%). R_f = 0.48, (DCM/MeOH, 9:1, v/v); mp = 85-87 °C; $[\alpha]_D^{20}$ = +57.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.12-1.20 (m, 2H), 1.28 (d, *J* = 6.0 Hz, 3H), 1.38 (s, 9H), 1.42 (s, 9H), 1.51-1.67 (m, 2H), 1.72-1.88 (m, 2H), 2.76 (d, *J* = 4.2 Hz, 3H), 3.74 (s, 3H), 4.03-4.23 (m, 4H), 5.04 (s, 2H), 5.09 (d, *J* = 6.6 Hz, 1H), 5.53 (d, *J* = 6.9 Hz, 1H), 5.85 (br s, 1H), 6.90-6.93 (d, *J* = 8.7 Hz, 1H), 6.98 (d, *J* = 3.9 Hz, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 7.29-7.34 (m, 5H), 7.43-7.46 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.56 (s, 1H), 7.64 (d, *J* = 6.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3, 22.3, 26.7, 28.1, 28.5, 29.6, 32.1, 48.2, 50.7, 54.7, 55.9, 56.3, 67.0, 80.5, 82.6, 111.9, 116.2, 128.4, 128.7, 130.3, 130.9, 133.7, 134.6, 136.5, 155.8, 156.3, 157.0, 170.5, 172.1, 172.7; MS (ESI) *m/z* calcd for $C_{38}H_{54}N_7O_9$ $[M+H]^+$ 752.40, found 752.75; calcd for $C_{38}H_{53}N_7NaO_9$ $[M+Na]^+$ 774.38, found 774.50.

General procedure for macrolactamization:
The linear compound **10a-b** (0.05 mmol) was dissolved in DCM (15 mL). To this solution, TFA (3 mL) was added, and the obtained reaction mixture was stirred for 3 h at room temperature. Then, the volatiles were removed under reduced pressure and the residue was coevaporated with DCM (10 mL, three times) to remove the residual TFA. The resulting solid residue was dried under vacuum for 1 h. Next, the residue was dissolved in DMF (100 mL) and BOP (33 mg, 0.075 mmol) and D*i*PEA (21 μL, 0.15 mmol) were added to this solution. The resulting reaction mixture was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure the residue was purified. The product was analyzed by HPLC and characterized by MALDI-TOF MS and ^{1}H NMR.

Macrolactamization of compound 10a: The residue was redissolved in EtOAc (30 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (30 mL, once), saturated $NaHCO_3$ (30 mL, once) and brine (30 mL, once), and then dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the obtained cyclic peptide was analyzed with HPLC. Based on analytical HPLC, two main peaks, which could be assigned as the cyclic monomer **11a**, t_R = 19.3 min (method A) and the cyclic dimer **12a**, t_R = 20.8 min (method A), respectively, with a monomer/dimer ratio of 5:3. Both compounds were isolated by preparative RP-HPLC to give the cyclic monomer **11a** (10.6 mg) and the cyclic dimer **12a** (4.4 mg), and their identity was confirmed by MALDI-TOF analysis and ^{1}H NMR. For cyclic monomer **11a**: ^{1}H NMR (300 MHz, DMSO): δ = 1.31 (d, *J* = 7.2 Hz, 3H), 2.73 (d, *J* = 4.5 Hz, 3H), 3.74 (s, 3H), 3.98-4.08 (m, 1H), 4.24-4.39 (m, 2H), 4.75-4.81 (m 1H), 5.12 (s, 2H), 5.58 (d, *J* = 9.6 Hz, 1H), 6.78 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.19-7.23 (m, 1H), 7.31-7.38 (m, 5H), 7.63 (d, *J* = 6.9 Hz, 1H), 7.75 (s, 1H), 8.08 (d, *J* = 9.6 Hz, 1H), 8.28 (d, *J* = 6.9 Hz, 1H), 8.63 (d, *J* = 4.5 Hz, 1H); MALDI-TOF MS *m/z* calcd for $C_{26}H_{30}N_7O_6$ $[M+H]^+$ 536.23, found 536.82; calcd for $C_{26}H_{29}N_7NaO_6$ $[M+Na]^+$ 558.21, found 558.86; calcd for $C_{26}H_{29}N_7KO_6$ $[M+K]^+$ 574.18, found 574.81. For the cyclic dimer **12a**: MALDI-TOF MS *m/z* calcd for $C_{52}H_{59}N_{14}O_{12}$ $[M+H]^+$ 1071.44, found 1072.04; calcd for $C_{52}H_{58}N_{14}NaO_{12}$ $[M+Na]^+$ 1093.43, found 1094.06; calcd for $C_{52}H_{58}N_{14}KO_{12}$ $[M+K]^+$ 1109.40, found 1110.01.

Marcolactamization of compound 10b: The crude macrocyclization product was redissolved in EtOAc (30 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (30 mL, once), saturated $NaHCO_3$ (30 mL, once) and brine (30

mL, once), and then dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the obtained cyclic peptide was purified by column chromatography. The obtained product was analyzed by HPLC and showed to be a single product. It was characterized by MALDI-TOF MS and ^{1}H NMR and ^{13}C NMR, and the identity was confirmed as the cyclic monomer **11b**. Cyclic monomer **11b** was isolated in a good yield of 69% (19.9 mg). HPLC analysis, t_R = 19.6 min (method A). ^{1}H NMR (300 MHz, CD_3OD): δ = 0.90-0.97 (m, 1H), 1.19-1.46 (m, 5H), 1.74-1.78 (m, 3H), 2.76 (s, 3H), 3.79 (s, 3H), 3.98-4.04 (m, 2H), 4.22-4.30 (m, 2H), 5.02-5.12 (m, 2H), 5.46 (s, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 7.27-7.38 (m, 6H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.62 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ = 15.9, 21.2, 25.3, 30.0, 31.2, 50.5, 54.9, 55.2, 55.9, 66.8, 111.2, 116.5, 128.0, 128.3, 130.2, 130.6, 131.7, 132.6, 135.6, 136.8, 157.4, 171.6, 172.7, 173.1; MALDI-TOF MS *m/z* calcd for $C_{29}H_{36}N_7O_6$ $(M+H)^+$ 578.27, found 578.55; calcd for $C_{29}H_{35}N_7NaO_6$ $[M+Na]^+$ 600.25, found 600.51; calcd for $C_{29}H_{35}N_7KO_6$ $[M+K]^+$ 616.23, found 616.47.

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Chapter 4

Synthesis of 1,5-Triazole-Bridged Vancomycin CDE-Ring Peptidomimetics via RuAAC Macrocyclization

4.1 Introduction

Vancomycin is an outstanding example in which peptide side chain cyclization leads to cavity or shell-like structures resulting in a high binding affinity to its natural ligand. However, due to the intriguing and complex structure of vancomycin, its total synthesis[1] and structural modification[2] is still highly challenging. Since vancomycin is currently reserved for the treatment of life-threatening bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA),[3] the emerging resistance against vancomycin is a serious problem[4] and the demand for newly designed antibiotics, like simplified vancomycin mimics which retain antibacterial activity, is of utmost urgency.[5]

In the previous chapters, it has been shown that the biaryl ether bridge of vancomycin DE-ring can be effectively mimicked by either the 1,4- or 1,5-disubstituted triazole functionality, using Cu(I)- or Ru(II)-catalyzed azide-alkyne cycloaddition chemistry (CuAAC or RuAAC), respectively. Particularly, the lysine-containing 1,5-triazole bridged DE-ring mimic, which is of the same ring size of the natural DE-ring, showed an excellent resemblance with the corresponding part of vancomycin, as judged by modeling and overlay data based on the X-ray structure of vancomycin in complex with Ac_2-Lys-D-Ala-D-Ala (see **Chapter 5** for details). Encouraged by these results, the stage was set to synthesize a bicyclic peptidomimetic of the vancomycin CDE-ring, in which the two biaryl ether bridges were replaced by 1,5-triazole moieties.

4.2 Design and retrosynthetic analysis of the vancomycin CDE-ring mimics by RuAAC macrocyclization

The design of the vancomycin CDE-ring mimics is inspired by the successful synthesis of the DE-ring mimics, in which the biaryl ether bridge has been replaced by a triazole moiety. For the CDE-ring mimics, both biaryl bridges in the vancomycin CDE-ring system will be replaced by two 1,5-triazole moieties which will be introduced via a side chain linkage (Figure 1). The AB-ring has been omitted from the mimic and the heptapeptide backbone was simplified into a hexapeptide by deletion of the C-terminal aromatic amino acid residue. The P1 and P5 positions have been substituted by a lysine-derived ε-azido amino acid and are linked together via a triazole bridge with the central alkyne-functionalized hydroxyphenylglycine residue at position P3. To complete the peptide sequence,

D-leucine and L-alanine, respectively, have been incorporated at position P2 and P4 instead of the original amino acid residues in vancomycin for simplifying the synthesis. The *N*-Me-D-leucine residue was retained at P6.

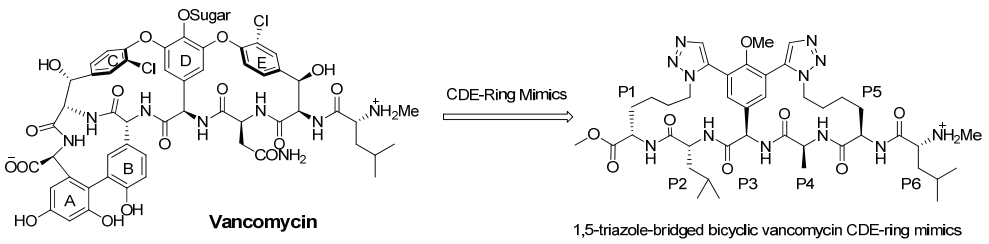
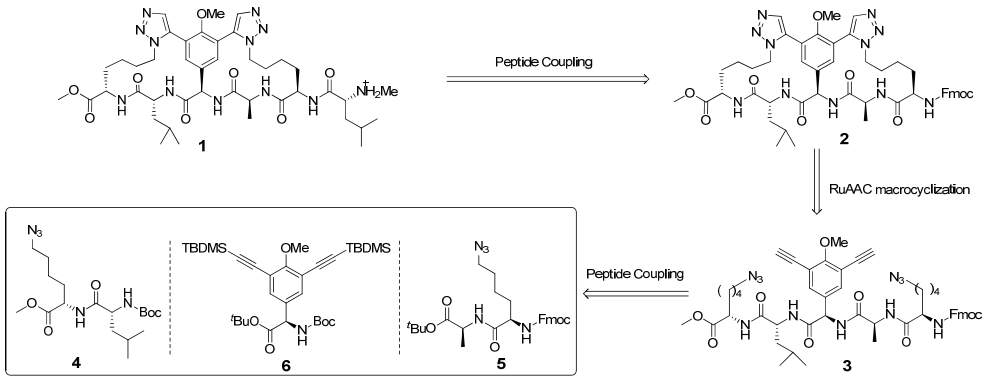


Figure 1. Design of the 1,5-triazole-bridged bicyclic vancomycin CDE-ring mimics.



Scheme 1. Retrosynthetic route of the bicyclic vancomycin CDE-ring mimics.

The retrosynthetic analysis of the bicyclic vancomycin CDE-ring mimic involves four key stages (Scheme 1). The first stage is the synthesis of building blocks **4**-**6** to assemble (as the second stage) the linear pentapeptide **3** which is functionalized with the desired alkyne and azide functionalities. The third stage is the macrocyclization of the linear peptide via RuAAC reaction to afford the triazole-bridged bicyclic pentapeptide **2**. Finally, the fourth stage involves the incorporation of *N*-Me-D-leucine to obtain the desired bicyclic vancomycin CDE-ring mimic **1**.

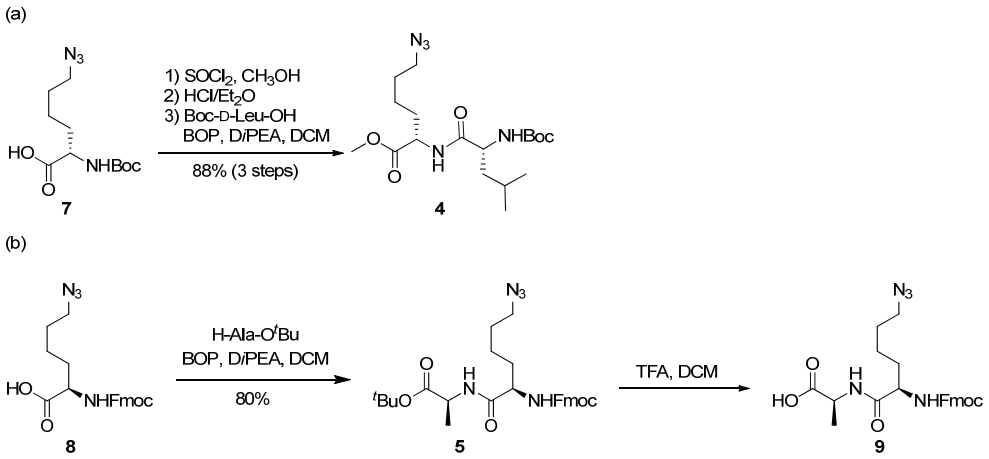
The linear pentapeptide **3** was convergently synthesized by coupling two dipeptide segments (**4** and **5**) to the central alkyne-functionalized hydroxyphenylglycine residue **6**. The coupling of these fragments could be started either from the C-terminus or from the N-terminus of the central amino acid residue **6**. Then, the removal of the alkyne protecting groups will generate the linear

pentapeptide **3** which can be subsequently converted into the desired bicyclic intermediate pentapeptide **2** via a RuAAC macrocyclization step. As shown in the previous chapter, the RuAAC macrocyclization has an excellent intramolecular selectivity, therefore, it is reasonable to expect that this key step will only lead to the desired bicyclic pentapeptide **2**.

4.3 Results and Discussion

4.3.1 Synthesis of the C- and N-terminal dipeptide segments 4 and 5

For the synthesis of the linear pentapeptide **3**, the first step was to prepare the required dipeptide segments **4** and **5**.



Scheme 2. Synthesis of the (a) C-terminal and (b) N-terminal dipeptide segments.

The C-terminal dipeptide **4** was easily obtained from lysine-derived ε-azidoamino acid **7**, which was synthesized from Boc-Lys-OH by a diazotransfer reaction.[6] Then, azidoamino acid **7** was subsequently converted into its methyl ester using $SOCl_2$ in CH_3OH, while the Boc-group was partially cleaved by the in situ generated HCl. To ensure that the Boc-group was completely removed an additional treatment with HCl/Et_2O was performed. Next, $HCl{\cdot}H_2N$-Lys(N_3)-OMe was coupled with Boc-D-Leu-OH using BOP/D*i*PEA as coupling reagents to obtain dipeptide **4** in an excellent overall yield (88%). To avoid DKP-formation[7]

dipeptide **4** should not be stored as its corresponding amine and the Boc-group should be removed just before the next coupling reaction.

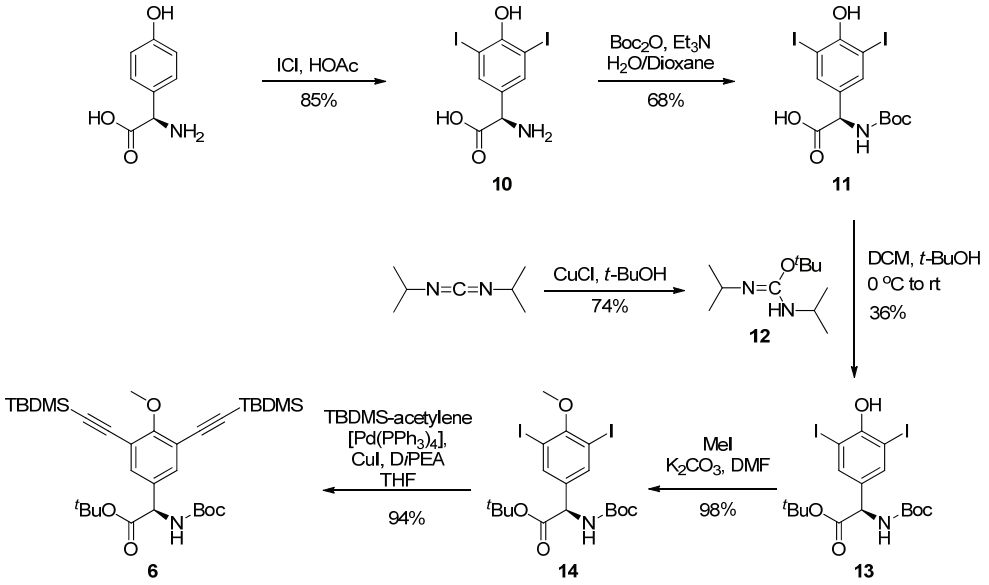
The N-terminal dipeptide **5** was synthesized starting from Fmoc-D-Lys(N_3)-OH, which was synthesized from Fmoc-D-Lys-OH by a diazotransfer reaction.[6] BOP/D*i*PEA was used to couple acid **8** with H-Ala-O^{t}Bu to give dipeptide **5**, which was converted into dipeptide acid **9** after removal of *tert*-butyl group by TFA.

4.3.2 Synthesis of the alkyne-functionalized hydroxyphenylglycine derivative

The alkyne-functionalized hydroxyphenylglycine derivative **6** is a key intermediate in the assembly of the linear peptide. Both alkyne-functionalities could be introduced by a Pd-catalyzed Sonogashira cross coupling reaction (Scheme 3) analogously as described in **Chapter 3** for the monocyclic DE-ring mimics.

The synthesis started with the iodination of D-hydroxyphenylglycine by using ICl in acetic acid, and diiodide **10** was obtained in 85% yield without purification in line with the literature procedure.[8] In the next step, amine **10** was protected with a Boc-group to give **11** in 68% yield. Then, the carboxyl functionality was converted into a *tert*-butyl ester by using reagent **12**[9] in a rather disappointing yield of only 36% because of the poor selectivity between the phenolic hydroxyl group and the carboxylic acid moiety. Subsequent methylation of the phenolic hydroxyl functionality with MeI in DMF gave the fully protected diiodide **14**. Finally, both alkyne moieties were introduced using TBDMS-protected acetylene via a Pd-catalyzed Sonogashira cross coupling reaction to give the desired phenylglycine residue **6**.

This synthetic route afforded *tert*-butyl ester **13** in a quite low yield, which limited the scale up of the following two steps. It was expected that further optimization could be realized to improve the yield of this step. However, the desired key intermediate **6** was successfully obtained and the focus was now on the assembly of the linear pentapeptide **3**



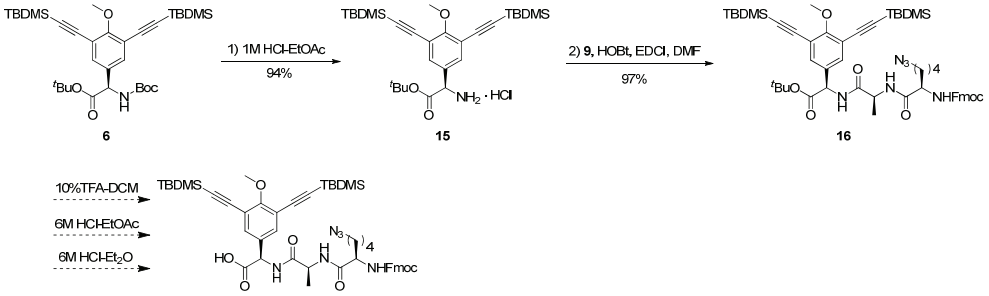
Scheme 3. Synthesis of the alkyne-functionalized hydroxyphenylglycine derivative.

4.3.3 Synthesis of the linear pentapeptide starting from the N-terminus of hydroxyphenylglycine

The assembly of the linear pentapeptide **3** could be started either from the C-terminus or from the N-terminus of the central amino acid **6** by removal the *tert*-butyl ester or the Boc-group, respectively. Since the Boc-group can be selectively removed in the presence of a ^{t}Bu-ester,[10] it was decided to elongate the peptide sequence starting from the N-terminus of the hydroxyphenylglycine derivative **6** (Scheme 4).

Hydroxyphenylglycine derivative **6** was treated with 1M HCl in EtOAc to remove selectively the Boc-group. The resulting amine **15** was coupled with the dipeptide **9** mediated by EDCI/HOBt in DMF to afford tripeptide **16** in 97% yield. Then, tripeptide **16** was treated with acid to cleave the *tert*-butyl ester. Unfortunately, it turned out that this deprotection was rather problematic. Treatment of **16** with 10% TFA in DCM, gave a complex mixture, while treatment with 6M HCl in EtOAc or 6M HCl in Et_2O did not result in a clean cleavage. Although the TBDMS-group was found to be relatively stable in an acidic environment (see **Chapter 2**), it was incompatible with reaction conditions to

remove the ^{t}Bu-ester functionality. It was assumed that electronic properties of the phenylglycine moiety play a role in the acid stability of the TBDMS-group.

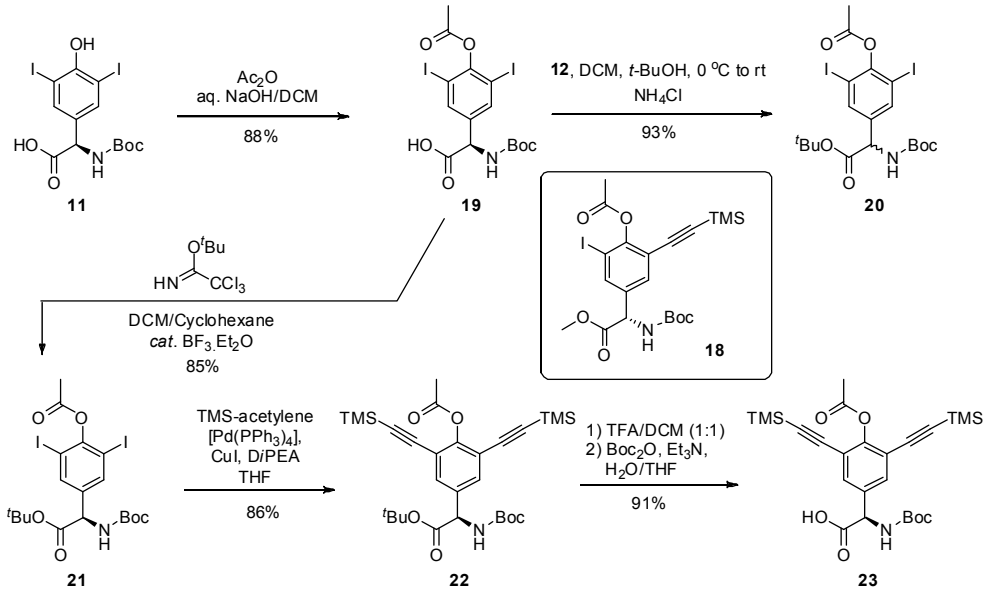


Scheme 4. Synthesis of the linear pentapeptide starting from the N-terminus of hydroxyphenylglycine.

The failure to remove of the *tert*-butyl ester interrupted further peptide synthesis. Since it was realized that compound **6** might be not the most suitable building block, a more robust alkyne-functionalized hydroxyphenylglycine derivative was required.

4.3.4 Synthesis of a more robust bisalkyne-functionalized hydroxyphenylglycine derivative

The electronic properties of the moiety as suitable protecting group of the phenolic hydroxyl functionality turned out to have an important role in the acid stability of the silyl derivatives that protect the alkyne. In case of an electron donating group (methoxy), the acid stability of silicium-based protecting groups is less than in case of an electron withdrawing group (acetoxy). These differences have already been mentioned in **Chapter 2**, during the synthesis of compound **18** (Scheme 5). Therefore, it was decided to synthesize phenylglycine derivative **22** in which the hydroxyl group was converted in an electron withdrawing moiety by virtue of an acetyl ester while the alkyne was protected by a TMS-group (Scheme 5).

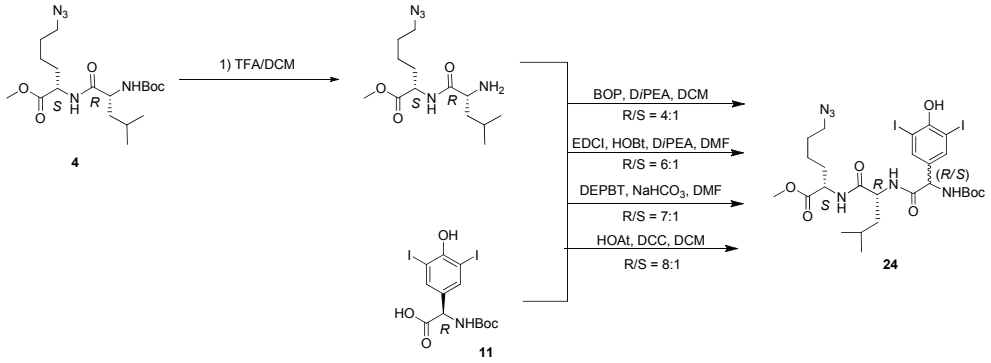


Scheme 5. Synthesis of a more robust bisalkyne-functionalized phenylglycine derivative.

Compound **11** was first converted into acetyl ester using acetic anhydride in *aq*. 1N NaOH/DCM to give ester **19** in high yield.[11] Subsequently, the carboxylic acid was converted into the corresponding *tert*-butyl ester by using reagent **12** in an excellent yield (93%) as previously described. However, it was found that this method induced racemization of the hydroxyphenylglycine residue, when basic conditions were applied.[12] As an alternative reagent, *tert*-butyl 2,2,2-trichloroacetimidate, was used in the presence of a catalytic amount of $BF_3{\cdot}Et_2O$,[13] which gave *tert*-butyl ester **21** in good yield (85%) without racemization under these mild reaction conditions. In the next step, both alkyne functionalities were introduced by a Pd-catalyzed Sonogashira cross coupling reaction in the presence of TMS-acetylene, and dialkyne **22** was obtained in an excellent yield of 86%. Gratifyingly, treatment of **22** with TFA in DCM resulted in the simultaneous removal of the Boc and ^{t}Bu protecting groups while the TMS-functionalities remained intact. After reprotection of the α-amine, acid **23** was obtained in an overall yield of 91%. In the next section the synthesis of the pentapeptide precursor is described starting with the coupling of acid **23** to the corresponding amine of dipeptide **4**.

4.3.5 Synthesis of the linear pentapeptide starting from the C-terminus of the phenylglycine derivative.

With the hydroxyphenylglycine derivative **23** in hand, it was decided to start the assembly of the linear pentapeptide from the C-terminus. It was also rational because of the high sensitivity toward racemization of the hydroxyphenylglycine during the peptide coupling. The C^{α} position of the hydroxyphenylglycine could be epimerized which would result in the formation of diastereoisomeric products during peptide coupling as a result of activation of the carboxylic acid.[14] It was expected that racemization of the phenylglycine building block could not be avoided during the peptide coupling. At this stage of the synthesis it was anticipated that the diastereomeric tripeptides could be more easily separated by column chromatography than in an advanced stage of peptide assembly.



Scheme 6. Model study to explore the most optimal coupling conditions to suppress racemization.

To find out the coupling conditions with a minimal degree of racemization, a model reaction with phenylglycine derivative **11** was carried out, as shown in Scheme 6. It became clear that racemization was apparent, it was, however, strongly dependent on the coupling reagents used. The diastereomeric ratio was determined by ^{1}H-NMR based on the proton signal of the methyl ester. A BOP/D*i*PEA-mediated coupling resulted in a diastereomeric ratio of 4:1 (*R,R,S*:*S,R,S*) (Figure 2a). As the second attempt, EDCI/HOBt was used and it was found that the diastereomeric ratio was slightly improved, *R*,*R*,*S*:*S*,*R*,*S* = 6:1 (Figure 2b). The coupling reagent DEPBT was reported to be one of the most effective reagents to suppress racemization during peptide fragment coupling.[15] In our case, it was found that coupling of **11** still led to the formation of

diastereoisomers with a ratio of 7:1 (*R*,*R*,*S*:*S*,*R*,*S*) (Figure 2c). The best conditions were found when DCC/HOAt were used as coupling reagents, since only 11% of epimerization at the C^{α} position of the phenylglycine derivative was found (Figure 2d).

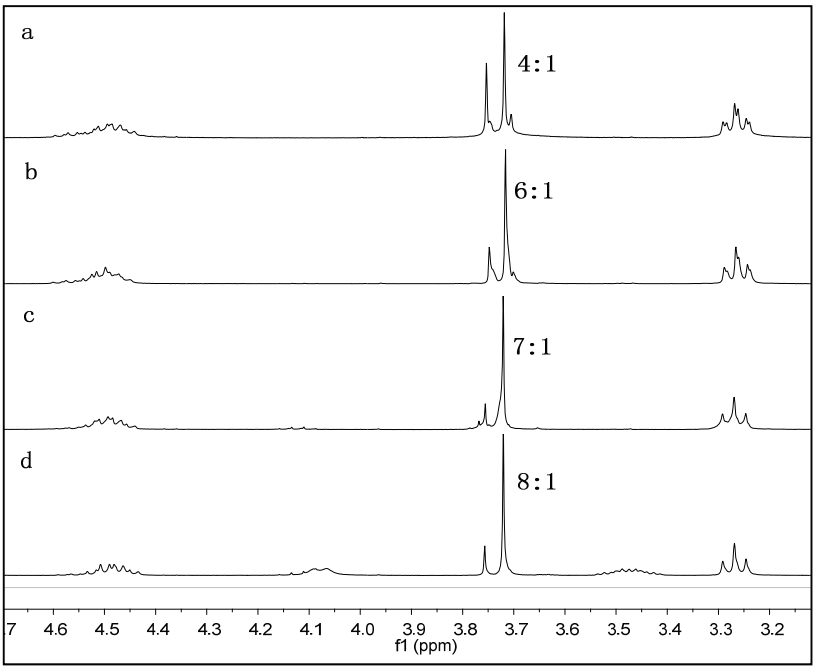
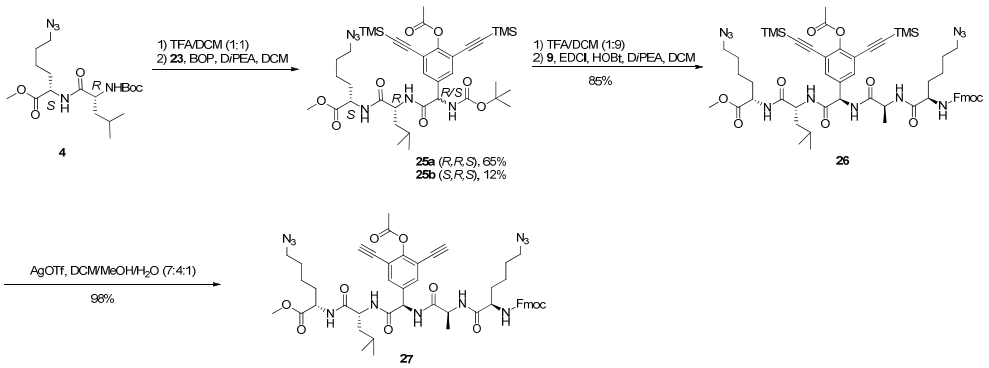


Figure 2. Partial ^{1}H NMR spectrum of the diastereoisomer **24** prepared using different coupling reagents: (a) BOP/D*i*PEA, (b) EDCI/HOBt, (d) DEPBT and (d) DCC/HOAt.

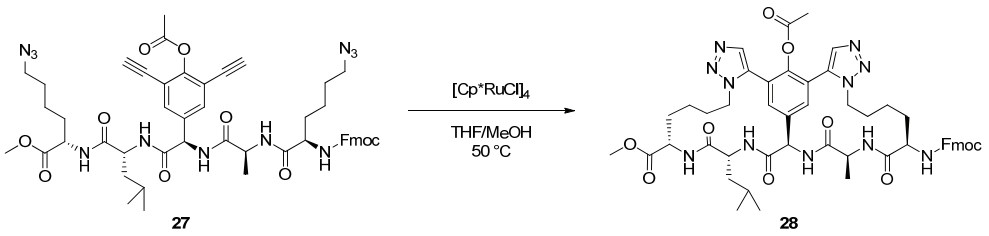
Based on this model reaction and as a first attempt, acid **23** was coupled with the amine derived from dipeptide **5** in the presence of DCC/HOAt. Unfortunately, the conversion was very low while only in the presence of BOP/D*i*PEA a reasonable conversion could be achieved (Scheme 7). Gratifyingly, the desired *R*,*R*,*S*-**25a** could be isolated in a stereochemically pure form in 65% yield, while its diastereoisomer *S*,*R*,*S*-**25b** was isolated in 12% yield. Subsequently, tripeptide **25a** was treated with TFA to remove the Boc-group, and then coupled with dipeptide acid **9** using EDCI/HOBt/D*i*PEA to afford the linear pentapeptide **26** in good yield (85%). Finally, the removal of both TMS-groups was achieved with AgOTf[16] instead of TBAF, to avoid premature cleavage of the Fmoc-group[17] and the linear pentapeptide precursor **27** was obtained in an excellent yield (98%).



Scheme 7. Synthesis of the linear pentapeptide **27** starting from the C-terminus of the hydroxyphenylglycine derivative.

4.3.6 First attempt of RuAAC macrocyclization of precursor 27

In a first attempt, the RuAAC macrocyclization conditions as described in **Chapter 3**, were tried to install the bicyclic ring starting from precursor peptide **27** (Scheme 8).



Scheme 8. First attempt of RuAAC macrocyclization of precursor **27**.

Precursor **27** was subjected to a RuAAC macrocyclization reaction in the presence of 15 mol-% $[Cp^*RuCl]_4$ at a substrate concentration of 5 mM and the cyclization was run at 50 °C. According to TLC analysis the reaction was not complete after 24 h, however, a clear spot-to-spot conversion was observed. Therefore, it was decided to increase the catalyst loading to 30 mol-%. To our delight, the complete consumption of the linear peptide **27** was achieved according to TLC. Next, the reaction product was analyzed by HPLC, which showed two peaks that did not correspond to starting material (Figure 3). The two new compounds that corresponded to these peaks were further analyzed by LC-MS and gave the same mass as the starting material, an indication that cyclization into a

monomer had occurred. After isolation by preparative HPLC, both compounds were analyzed by ^{1}H-NMR to identify the proton that correlated to the triazole moiety. Unfortunately, the structural assignment remained unclear due to overlapping signals in the aromatic part of the spectrum (Fmoc-group). Based on HPLC and mass analysis it was assumed that the isolated compounds might be atropisomers of the constrained bicyclic vancomycin mimic **28**.

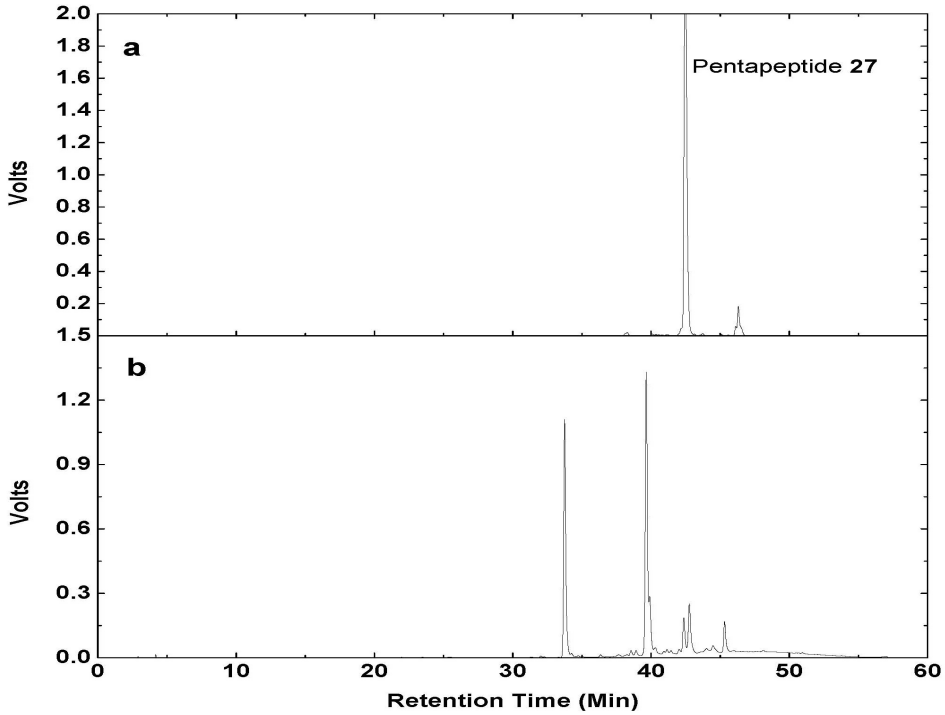
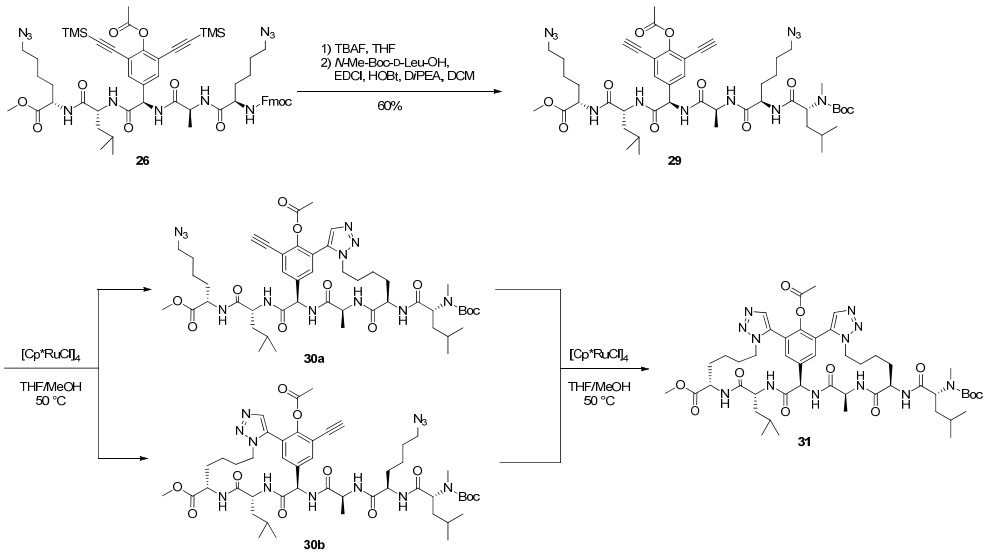


Figure 3. HPLC analysis of a) the linear pentapeptide **27** and b) the reaction mixture after RuAAC macrocyclization of the linear pentapeptide **27**.

4.3.7 Synthesis and RuAAC macrocyclization of the linear hexapeptide 29

Since the identity of the RuAAC macrocyclization product could not unambiguously verified, it was decided to perform the cyclization on a hexapeptide level in which the Fmoc-group was replaced by a *N*-Me-Boc-D-leucine residue (Scheme 9).



Scheme 9. Synthesis and RuAAC macrocyclization of the linear hexapeptide **29**.

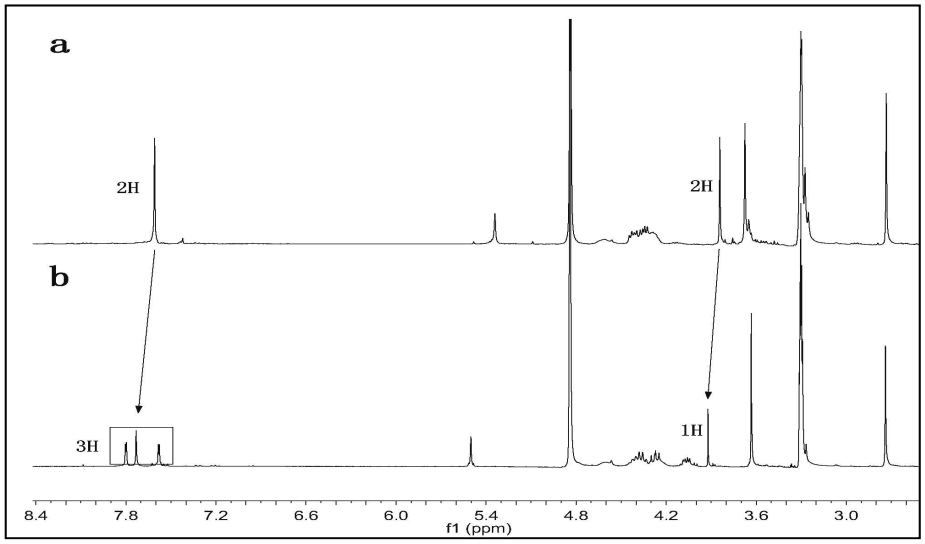


Figure 4. Partial ^{1}H NMR spectrum of (a) the linear hexapeptide **29** and (b) the monocyclic compound **30a** or **30b**.

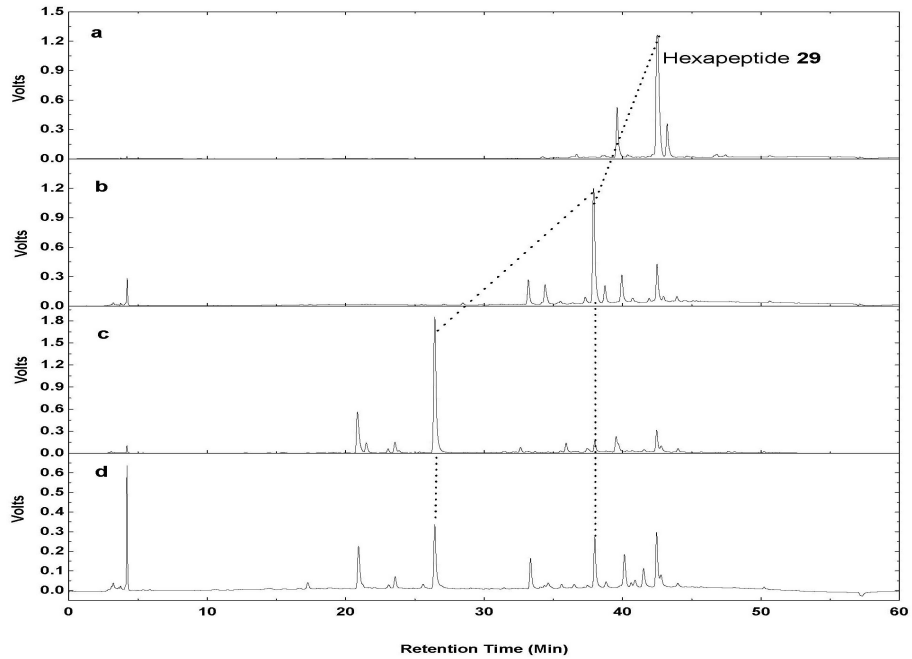


Figure 5. HPLC analysis of RuAAC macrocyclization of hexapeptide **29**: (a) hexapeptide **29**, (b) RuAAC macrocyclization of the hexapeptide **29** (30 mol-% $[Cp^*RuCl]_4$, 24 h), (c) RuAAC macrocyclization of the monocyclic compound **30**, and (d) RuAAC macrocyclization of the hexapeptide **29** (50 mol-% $[Cp^*RuCl]_4$, 24 h).

Pentapeptide **26** was treated with TBAF, to remove both TMS-groups as well as the Fmoc-group in a single reaction step,[17] and the corresponding α-amine was directly coupled to *N*-Me-Boc-D-Leu-OH using EDCI/HOBt in DCM. Hexapeptide **29** was obtained after tedious column chromatography in 60% yield, and was subjected to a RuAAC macrocyclization. In this case, HPLC analysis indicated one major component (t_R = 37.9 min) in the reaction mixture (Figure 5b). This compound was isolated and was characterized by ^{1}H NMR. The presence of only one alkyne proton (δ = 3.92) and three proton signals in the aromatic region (Figure 4) indicated that this compound was a monocyclic species as represented by **30a** or **30b** (Scheme 9). Therefore, the monocyclic compound (**30a** or **30b**) underwent a second RuAAC macrocyclization and the reaction mixture was analyzed by HPLC and indicated a new peak at t_R = 26.4 min (Figure 5c). By increasing the catalyst to 50 mol-%, the reaction mixture showed both of these two peaks (Figure 5d). This observation indicated that the compound with retention time of 26.4 min might be the desired bicyclic compound **33**. Unfortunately, the isolation and characterization

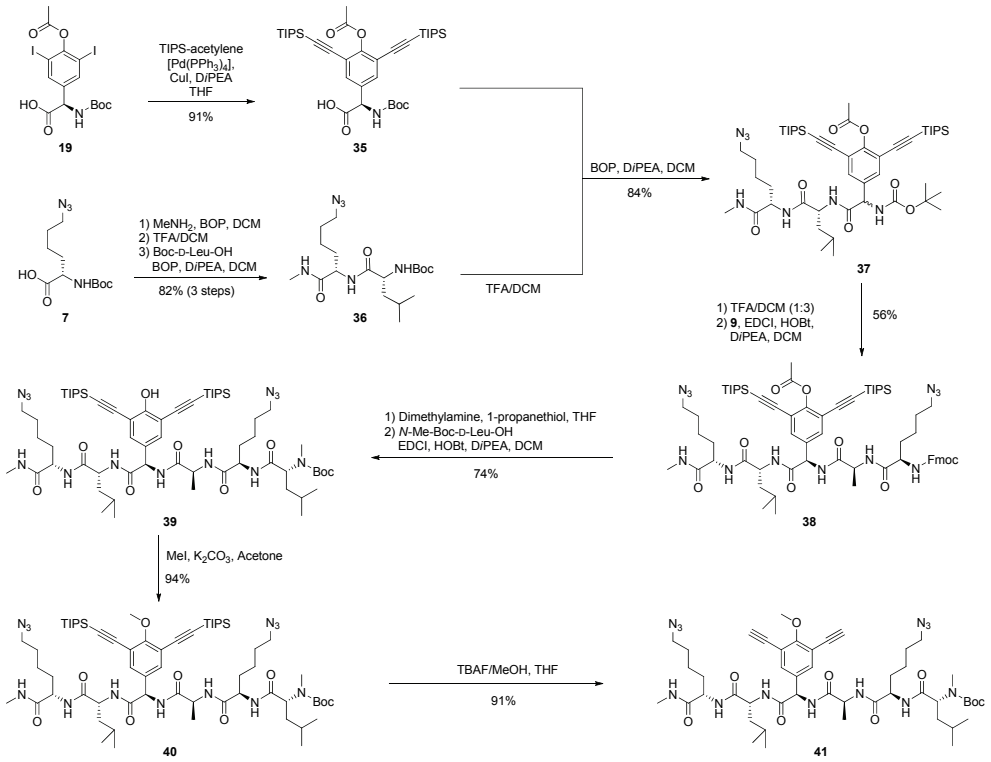
of this compound failed due to the very low amount of material. To obtain a sufficient amount of material for structural analysis, it was decided to optimize the whole synthesis of the hexapeptide **29**, as shown in Scheme 10.

4.3.8 Optimized synthesis of the linear hexapeptide

With the progress toward the completion of the synthesis of the desired bicyclic CDE-ring mimic of vancomycin, the whole blueprint of the synthesis became clear and some drawbacks of the synthesis needed to be optimized. First, the alkyne-functionalized hydroxyphenylglycine derivative should be modified with a more bulky protecting group, like triisopropylsilyl, to achieve an increased stability under acidic reaction conditions. Although the TMS-group displayed sufficient stability at a small scale, scaling up the synthesis was hampered, especially during acid treatments. Second, it was decided to replace the C-terminal methyl ester by a methyl amide to improve mimicry of the CDE-ring since the amide may participate in the hydrogen bonding interaction with the natural ligand of vancomycin.

During the optimization studies, it was found that acid **19** was a suitable substrate for a Pd-catalyzed Sonogashira cross-coupling to obtain TIPS-protected bisalkyne-functionalized phenylglycine derivative **35**. With this improved synthesis of the important building block **35**, the assembly of the hexapeptide macrocyclization precursor could be scaled up and required less synthesis steps. The next step was to couple acid **35** with the dipeptide amine derived from fragment **36**. The fully protected dipeptide **36** was obtained in two steps from azidoamino acid **7** and Boc-D-Leu-OH. Tripeptide **37** was obtained as a mixture of diastereoisomers which could not be isolated as a stereochemically pure entity. Therefore, tripeptide **37** was used as a diastereomeric mixture in the next synthesis steps. After removal of the Boc-group and coupling dipeptide acid **9** by using EDCI/HOBt as coupling reagents, pentapeptide **38** was isolated by column chromatography as a single stereoisomer in an overall yield of 56%. Next, the removal of the Fmoc-group was performed by using dimethylamine in THF, which also cleaved the acetyl ester. Subsequent coupling of *N*-Me-Boc-D-Leu-OH afforded successfully hexapeptide **39**, followed by methylation with MeI and K_2CO_3 as base gave fully protected hexapeptide **40** in an overall yield of 70%. Finally, the removal of both TIPS-groups was achieved by treatment with TBAF in THF containing 5 vol-% MeOH and the desired linear hexapeptide **41** was obtained in an excellent yield (91%). The presence of methanol was important since it

resulted in a clean deprotection, but it also avoided racemization of the phenylglycine derivative by tempering the nucleophilicity/basicity of the fluoride ion due to its Lewis acid properties. As a consequence, silyl removal was slower and additional equivalent of TBAF was required.

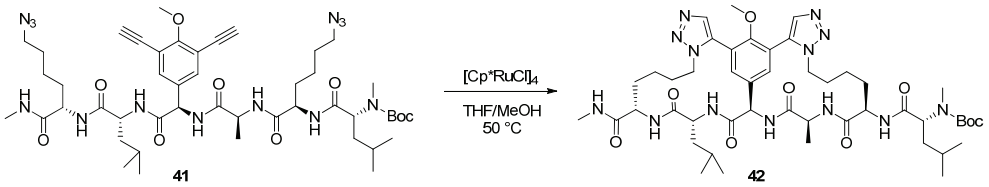


Scheme 10. Optimized synthesis towards the linear hexapeptide.

4.3.9 Optimization of the RuAAC macrocyclization of the linear hexapeptide 41

Based on the previous observations of RuAAC macrocyclization of linear pentapeptide **27** and hexapeptide **29**, it could be concluded that the RuAAC macrocyclization could be promoted by increasing the catalyst amount and the cyclization of the left- and right-part was not equally efficient. Although a two-step cyclization was possible to get the desired bicyclic compound (Scheme 9), it was not an ideal protocol for application of this RuAAC macrocyclization methodology.

Therefore, further optimization of this method was required to reach the bicyclic compound in one RuAAC macrocyclization step.



41 42

Scheme 11. RuAAC macrocyclization of the linear hexapeptide **41**.

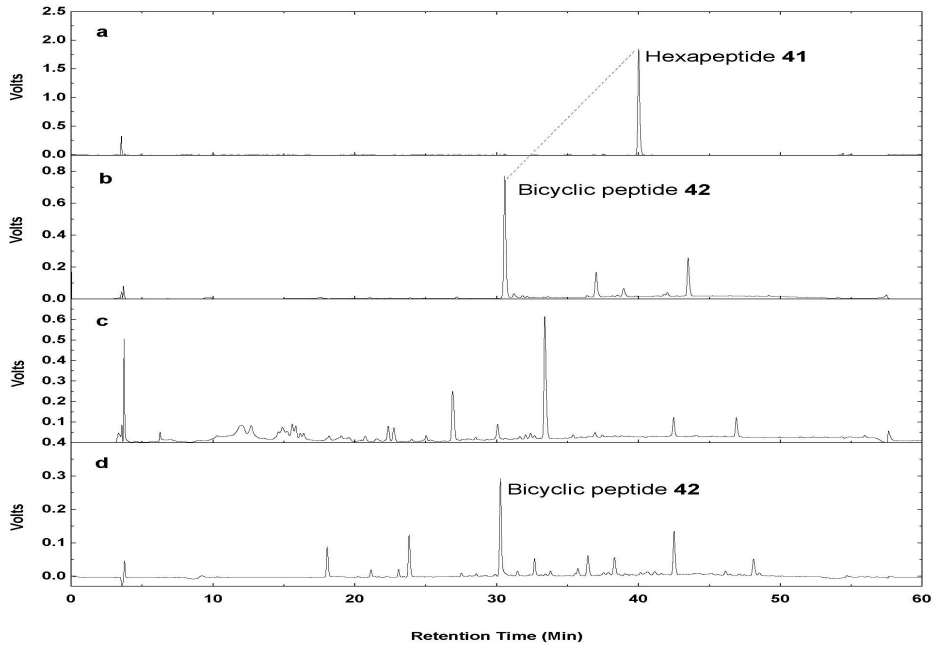


Figure 6. HPLC analysis of the RuAAC macrocyclization of hexapeptide **41**: (a) hexapeptide **41**, (b) RuAAC macrocyclization of hexapeptide **41** (100 mol-% $[Cp^*RuCl]_4$, 24 h), (c) RuAAC macrocyclization of hexapeptide **41** with new batch of catalyst (100 mol-% $[Cp^*RuCl]_4$, 24 h), and (d) RuAAC macrocyclization of hexapeptide **41** with new batch of catalyst (30 mol-% $[Cp^*RuCl]_4$, 24 h).

To optimize this RuAAC macrocyclization reaction, obviously, the first choice was to further increase the catalyst amount. Therefore, it was decided to increase the catalyst amount to 100 mol-%. To our delight, the cyclization of linear hexapeptide **41** was complete in 24 h and only one major peak was found by HPLC

analysis. The main component has a retention time of 30.6 min (Figure 6b) which was quite close with the bicyclic compound **31**. But the reaction could not be reproduced with a new batch of catalyst and gave different analytical HPLC chromatography (Figure 6c). At this point, it was realized that the old batch of catalyst might lost its catalytic activity and couldn't efficiently catalyze the RuAAC macrocyclization. While a high amount of the new batch of catalyst might lead to an 'over-action' of the reaction. Therefore, the reaction was again repeated but now with only 30 mol-% of the fresh catalyst. It was found that the reaction proceeded smoothly to completion in 24 h. Analytical HPLC showed similar results with a major component in accordance with the observation in the first attempt with 100 mol-% 'old' catalyst (Figure 6d).

The compound with retention time of 30.6 min was isolated by preparative RP-HPLC in 40% yield. It was identified by ^{1}H NMR, 2D HSQC NMR, and MALDI-TOF MS to be the desired bicyclic compound **42**. To confirm the bicyclic structure of compound **42**, a TCEP-reduction experiment was carried out on the linear peptide **41** and the obtained bicyclic compound **42**. The azide groups present in the linear peptide could be reduced to the corresponding amine by TCEP resulting in a molecular weight loss of 26 amu for each azide, while no change in molecular weight for the bicyclic compound was expected.[17] Accordingly, after treatment of the linear peptide **41** with TCEP, MALDI-TOF MS showed the corresponding free amine peak with a molecular loss of 26 and 52 amu, respectively. Treatment of the bicyclic compound **42** showed no change in molecular weight. The reduction experiments were also followed by HPLC analysis, which also showed consistent results. TCEP treatment of linear peptide **41** led to a group of new peaks, while no change was observed for the bicyclic compound **42** (Figure 7). These results confirmed the formation of the bicyclic compound **42**.

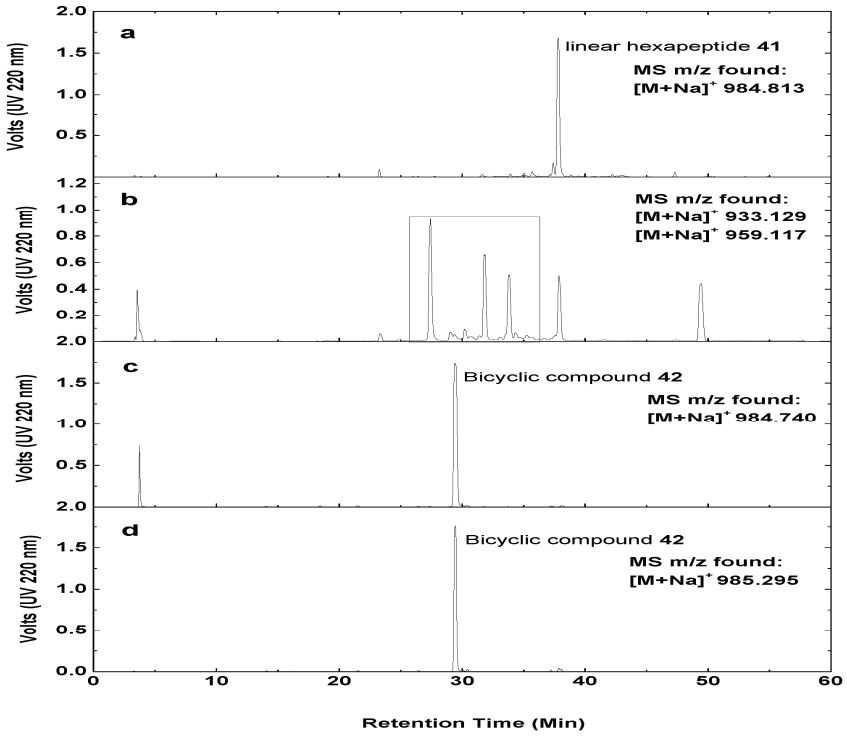
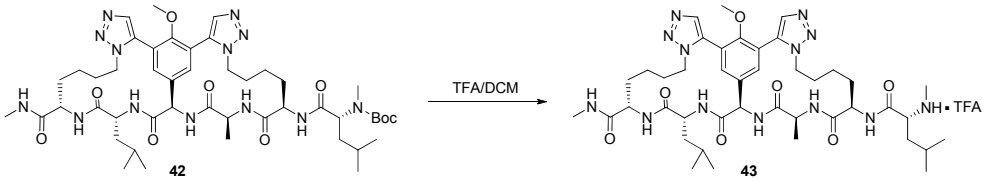


Figure 7. HPLC analysis of the TCEP reduction of azide: (a) the linear hexapeptide **41**, (b) the linear hexapeptide **41** after treatment with TCEP, (c) the bicyclic compound **42** and (d) the bicyclic compound **42** after treatment with TCEP.



Scheme 12. Deprotection of linear peptide **42**.

After final deprotection of the Boc-group, the desired bicyclic compound **43** was obtained (Scheme 12). The bicyclic compound **43** could serve as a CDE-ring mimic of vancomycin. Its structural modeling, binding affinity with the natural ligands of vancomycin, and its biological evaluation will be described in **Chapter 5**.

4.4 Conclusion

This chapter described the synthesis of 1,5-triazole-bridged vancomycin CDE-ring peptidomimetics using RuAAC macrocyclization. With the optimized synthetic route, the linear hexapeptide could be synthesized in 10 steps from the hydroxyphenylglycine with good to excellent yield. The RuAAC macrocyclization of the linear hexapeptide successfully provided the desired bicyclic compound **43**, and the method could in principal be applied to synthesize a small library of bicyclic peptidomimetics for screening the biological activity as vancomycin mimics. The methodology developed in this study proved that the RuAAC macrocyclization has very good intramolecular selectivity and could be applied in the synthesis of more complicated bicyclic compound in near future.

4.5 Experimental section

4.5.1 General experimental procedures

Chemicals were used as obtained from commercial sources without further purification unless stated otherwise. The solvents were obtained as peptide synthesis grade and stored on molecular sieves (4 Å).
Column chromatography was performed using *Silicycle SiliFlash P60* silica gel (particle size 40-63 μm).
Thin Layer Chromatography (TLC) was performed on *Merck* precoated silica gel 60F254 glass plates. Compound spots were visualized by UV-quenching, ninhydrin, or Cl_2/TDM.
Optical rotation was measured on a *JASCO* P-1010 Polarimeter using a 10 cm cell with a Na 589 nm filter. The specific concentrations (in g/100 ml) are indicated.
^{1}H NMR was acquired on a *Varian* Mercury 300 MHz or a *Varian* Innova 500 MHz spectrometer in $CDCl_3$ or CD_3OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to TMS (0.00 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br). ^{13}C NMR was acquired on a *Varian* Mercury 75 MHz or a *Varian* Innova 125 MHz spectrometer in $CDCl_3$, or CD_3OD as solvent. Chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to the solvent residual signal, $CDCl_3$ (77.0 ppm) or CD_3OD (49.0 ppm).

Analytical HPLC was performed on an automated HPLC system (*Shimadzu*) equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using an *Alltech* Alltima C8 column (pore size: 100 Å, particle size: 5 μm; 250 × 4.6 mm) or Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100Å, particle size: 5 μm; 250 × 4.6 mm) at a flow rate of 0.5 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 60 min.

Preparative RP-HPLC was performed on an automated preparative HPLC system (*Applied Biosystems*) equipped with a UV/Vis detector operating at 214 nm using an Dr. Maisch Reprosil-Pur C18-AQ column (pore size: 100 Å, particle size: 10 μm; 250 × 22 mm) at a flow rate of 2.0 mL/min [100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 90 min.

ESI-MS was performed on a *Shimadzu* LCMS-QP8000 electrospray ionization mass spectrometer.

MALDI-TOF MS was performed on a *Shimadzu* Kratos AXIMA-CFR mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix and human ACTH (18-39) as reference.

4.5.2 Synthesis and compound analyses

Dipeptide 4: Azidoamino acid **7** (1.05 g, 3.86 mmol) was dissolved in methanol (10 mL). To this solution $SOCl_2$ (1.12 mL, 15.4 mmol) was added at 0 ^oC. The reaction mixture was stirred for 3 h at room temperature. Then another 2.0 equivalent of $SOCl_2$ (0.56 mL, 7.7 mmol) was added to the solution. The mixture was stirred for 10 h, finally 6 M $HCl{\cdot}Et_2O$ (0.5 mL) was added and the mixture was stirred for another 2 h to ensure complete Boc-removal. Next, the volatiles were removed in vacuum and the resulting crude product was dried under high vacuum for 1 h. The free amine was dissolved in DCM (50 mL). To this solution Boc-D-Leu-OH (982 mg, 4.25 mmol) and BOP (1.88 g, 4.25 mmol) were added, followed by the addition of D*i*PEA (2.0 mL, 11.6 mmol). The obtained reaction mixture was stirred for 3 h. The solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc,

4:1, v/v). Dipeptide **4** was obtained as a viscous oil (1.36 g, 88%); R_f = 0.73 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +45.0 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.08 (d, *J* = 5.3 Hz, 1H), 5.25 (d, *J* = 6.0 Hz, 1H), 4.47 (td, *J* = 7.9, 5.4 Hz, 1H), 4.11 (s, 1H), 3.61 (s, 3H), 3.15 (t, *J* = 6.7 Hz, 2H), 1.76 (td, *J* = 13.7, 7.2 Hz, 1H), 1.67 – 1.37 (m, 8H), 1.33 (s, 9H), 0.82 (dd, *J* = 6.1, 3.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.9, 172.7, 155.9, 79.9, 53.1, 52.4, 51.9, 51.2, 41.3, 31.9, 28.4, 24.9, 23.1, 22.6, 22.0; MS (ESI) *m/z* calcd for $C_{18}H_{34}N_5O_5$ $[M+H]^+$ 400.26, found 400.30; calcd for $C_{18}H_{33}N_5NaO_5$ $[M+Na]^+$ 422.24, found 422.25.

Dipeptide 5 and 9: Azidoamino acid **8** (680 mg, 1.72 mmol) was dissolved in DCM (30 mL). To this solution H-Ala-O^{t}Bu·HCl (343 mg, 1.89 mmol) and BOP (836 mg, 1.89 mmol) were added, followed by the addition of D*i*PEA (0.74 mL, 4.30 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 4:1, v/v). Dipeptide **5** was obtained as a white solid (720 mg, 80%); R_f = 0.76 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +1.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (dd, *J* = 7.4, 6.9 Hz, 2H), 7.31 (td, *J* = 7.4, 1.2 Hz, 2H), 6.58 (d, *J* = 6.0 Hz, 1H), 5.39 (d, *J* = 8.0 Hz, 1H), 4.44 (dt, *J* = 9.5, 4.8 Hz, 3H), 4.22 (t, *J* = 6.8 Hz, 2H), 3.26 (t, *J* = 6.7 Hz, 2H), 1.98 – 1.78 (m, 1H), 1.76 – 1.51 (m, 3H), 1.48 – 1.40 (m, 12H), 1.37 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.1, 170.9, 156.3, 144.0, 144.0, 141.5, 128.0, 127.3, 125.3, 120.2, 82.5, 67.3, 54.8, 51.3, 49.0, 47.4, 32.6, 28.7, 28.2, 22.8, 18.8; MS (ESI) *m/z* calcd for $C_{28}H_{36}N_5O_5$ $[M+H]^+$ 522.27, found 522.00; calcd for $C_{28}H_{35}N_5NaO_5$ $[M+Na]^+$ 544.25, found 544.55.
Dipeptide 9 was obtained in quantitative yield by treatment of dipeptide **5** with TFA and was used without further purification.

Compound 10 and 11 were synthesized according to a literature procedure.[8] D-4-hydroxyphenylglycine (10.5 g, 63.0 mmol) was dissolved in AcOH (90 mL). To this solution ICl (22.5 g, 138.6 mmol) in AcOH (5.0 mL) was added dropwise during 10 min under argon. After stirring for 72 h at room temperature, the reaction mixture was poured into ice water (1000 mL). The precipitated crystals were

filtered off, washed with EtOH (100 ml twice) to provide compound **10** (22.1 g, 85%) as light brown crystals. Without further purification, it was used in the next step. Compound **10** (4.19 g, 10 mmol) was dissolved in H_2O/dioxane (60 mL, 1:1, v/v). To this solution Boc_2O (2.62 g, 12 mmol) and Et_3N (2.1 mL, 15 mmol) were added. The reaction mixture was stirred for 4 h, after which the mixture was diluted with EtOAc (50 mL) and the resulting solution was extracted with H_2O (50 mL, twice). The aqueous phase was combined and acidified with $KHSO_4$ to pH 2-3, extracted with EtOAc (100 mL, twice). The organic phase was washed with brine (150 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1, v/v, with 0.1% HOAc). Compound **11** was obtained as a light yellowish solid (3.54 mg, 68%); R_f = 0.57 (hexane/EtOAc, 1:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = -94.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (s, 1H), 7.75 (s, 2H), 4.97 (d, *J* = 4.8 Hz, 1H), 1.29 (s, 9H).

Compound 13: Compound **11** (705 mg, 1.36 mmol) was dissolved in DCM/*t*-BuOH (30 mL, 1:1, v/v) and to this solution reagent **12** (1.09 g, 5.44 mmol) was added dropwise at 0 ^{o}C during 10 min. After stirring for 15 min at 0 ^{o}C, the reaction mixture was stirred for 20 h at room temperature. The reaction mixture was diluted with DCM (50 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (30 mL, once) and brine (30 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 1:20, v/v). Compound **13** was obtained as a white solid (281 mg, 36%); R_f = 0.22 (hexane/EtOAc, 9:1, v/v); $[\alpha]_D^{20}$ = -57.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.65 (s, 2H), 5.90 (s, 1H), 5.61 (d, *J* = 6.0 Hz, 1H), 5.05 (d, *J* = 6.7 Hz, 1H), 1.42 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.5, 154.9, 153.7, 137.9, 134.0, 83.4, 82.5, 80.6, 56.2, 28.5, 28.1; MS (ESI) *m/z* calcd for $C_{17}H_{24}I_2NO_5$ $[M+H]^+$ 575.97, found 575.75.

Compound 14: Compound **13** (130 mg, 0.23 mmol) was dissolved in DMF (5 mL). To this solution MeI (21 μL, 0.33 mmol) and K_2CO_3 (45.6 mg, 0.33 mmol) were added. After stirring for 2 h, the mixture was diluted with EtOAc (30 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (30 mL, once) and brine (30 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 1:15, v/v). Compound **14** was obtained as a white solid (131 mg,

98%); R_f = 0.64 (hexane/EtOAc, 3:1, v/v); $[\alpha]_D^{20}$ = -53.4 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.74 (s, 2H), 5.60 (d, *J* = 6.1 Hz, 1H), 5.07 (d, *J* = 6.9 Hz, 1H), 3.84 (s, 3H), 1.43 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.2, 158.8, 154.9, 138.4, 137.7, 90.7, 83.6, 80.6, 60.9, 56.3, 28.5, 28.1.

Compound 6: Compound **14** (340 mg, 0.58 mmol), [$Pd(PPh_3)_4$] (67 mg, 0.058 mmol), and CuI (33.0 mg, 0.174 mmol) were placed in a flask sealed with rubber septa. The flask was evacuated and refilled with dry N_2 (repeated three times). THF (10 mL) (purged with dry N_2 for 1 h prior to use) was added into the flask by a syringe. The resulting solution was degassed again using a freeze-pump-thaw procedure (repeated three times). Then, D*i*PEA (200 μL, 1.16 mmol) and TBDMS-acetylene (440 μL, 2.32 mmol) were added to the mixture by syringe. After stirring the reaction mixture at room temperature for 20 h under N_2, the obtained suspension was filtered through a pad of celite and the filtrate was evaporated to dryness. The residue was subsequently redissolved in EtOAc (50 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:20, v/v). Compound **6** was obtained as a yellowish solid (336 mg, 94%); R_f = 0.68 (hexane/EtOAc, 5:1, v/v); $[\alpha]_D^{20}$ = -48.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35 (s, 2H), 5.54 (d, *J* = 7.0 Hz, 1H), 5.07 (d, *J* = 7.2 Hz, 1H), 3.99 (s, 3H), 1.42 (s, 9H), 1.39 (s, 9H), 0.99 (s, 18H), 0.17 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.8, 162.7, 154.9, 133.2, 132.8, 117.9, 101.0, 98.2, 83.1, 80.3, 61.2, 57.2, 28.5, 28.0, 26.3, 16.9, -4.13, -4.5, -4.9.

Compound 16: Compound **6** (122 mg, 0.199 mmol) was treated with 1 M HCl in EtOAc (6 mL) to give the free amine **15** (87 mg, 94%). The free amine **15** (23 mg, 0.045 mmol) was dissolved in DMF (2 mL) and to this solution dipeptide **9** (23 mg, 0.049 mmol), EDCI (26 mg, 0.135 mmol), and HOBt (20 mg, 0.148 mmol) were added. The obtained reaction mixture was stirred for 2 h, and subsequently diluted with EtOAc (20 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (20 mL, twice), saturated $NaHCO_3$ (20 mL, twice) and brine (20 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 2:1, v/v). Compound **16** was obtained as a white solid (42 mg, 97%); R_f = 0.61

(hexane/EtOAc, 1:1, v/v); $[\alpha]_D^{20}$ = -43.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.75 (d, *J* = 7.3 Hz, 2H), 7.56 (dd, *J* = 7.0, 3.7 Hz, 2H), 7.38 (dd, *J* = 13.4, 6.0 Hz, 4H), 7.29 (t, *J* = 7.4 Hz, 2H), 7.21 (s, 1H), 6.69 (s, 1H), 5.43 (s, 1H), 5.31 (d, *J* = 6.9 Hz, 1H), 4.59 (t, *J* = 7.2 Hz, 1H), 4.42 (d, *J* = 7.2 Hz, 1H), 4.20 (t, *J* = 6.8 Hz, 1H), 4.00 (s, 2H), 3.25 (t, *J* = 6.7 Hz, 2H), 2.00 – 1.49 (m, 2H), 1.47 – 1.17 (m, 16H), 0.99 (d, *J* = 2.5 Hz, 18H), 0.18 (s, 12H).

Compound 19: Compound **11** (2.60 g, 5.00 mmol) was dissolved in ice-cold *aq*. 1N NaOH/DCM (60 mL, 1:1, v/v) and to this mixture, Ac_2O (2.36 mL, 25.0 mmol) was added dropwise at 0 ^oC. Then, the reaction mixture was stirred at room temperature for 3 h. Subsequently, the aqueous solution was acidified with $KHSO_4$ to pH 2-3 and extracted with DCM (30 mL, twice). The organic phases were combined and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:2, v/v, with 0.1% HOAc). Compound **19** was obtained as a white solid (2.47 g, 88%); R_f = 0.61 (hexane/EtOAc, 1:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = -72.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.08 (s, 1H), 7.86 (s, 2H), 5.02 (d, *J* = 3.7 Hz, 1H), 2.39 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.5, 167.4, 157.0, 151.5, 139.5, 138.3, 90.5, 83.1, 57.3, 28.5, 28.3, 21.6.

Compound 21: Compound **19** (1.45 g, 2.58 mmol) was dissolved in DCM/cyclohexane (30 mL, 1:2, v/v). To this solution, *tert*-butyl trichloro-acetimidate (1.82 mL, 10.32 mmol) and $BF_3{\cdot}Et_2O$ (50 μL) were added. The obtained reaction mixture was stirred for 15 h. The resulting precipitated solid was filtered off and the filtrate was evaporated to dryness. The obtained residue was purified by column chromatography (EtOAc/hexane, 1:9 to 1:8, v/v). Compound **21** was obtained as a white solid (1.22 g, 85%). R_f = 0.26 (hexane/EtOAc, 9:1, v/v); $[\alpha]_D^{20}$ = -72.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.77 (s, 1H), 5.60 (d, *J* = 6.2 Hz, 1H), 5.12 (d, *J* = 7.0 Hz, 1H), 2.38 (s, 3H), 1.42 (s, 8H), 1.40 (s, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.1, 167.3, 154.8, 151.6, 139.3, 138.1, 90.6, 83.7, 80.7, 56.4, 28.5, 28.1, 21.6.

Compound 22: Compound **21** (617 mg, 1.0 mmol), $[Pd(PPh_3)_4]$ (116 mg, 0.1 mmol), and CuI (57.1 mg, 0.3 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated three times). THF (25 mL) (purged with dry N_2 for 1 h prior to use) was added to the flask via a

syringe. The resulting solution was degassed using a freeze-pump-thaw procedure (repeated three times). Then D*i*PEA (348 μL, 2.0 mmol) and TMS-acetylene (672 μL, 5.0 mmol) were added to the mixture via a syringe. After stirring the reaction mixture at room temperature for 20 h under N_2, it was subsequently filtered through a pad of celite and the filtrate was evaporated to dryness. The residue was subsequently redissolved in EtOAc (50 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:50 to 1:40, v/v). Compound **22** was obtained as a yellowish solid (480 mg, 86%); R_f = 0.26 (hexane/EtOAc, 9:1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.42 (s, 2H), 5.55 (d, *J* = 7.0 Hz, 1H), 5.10 (d, *J* = 7.4 Hz, 1H), 2.31 (s, 3H), 1.41 (s, 9H), 1.38 (s, 9H), 0.22 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 169.6, 167.7, 154.8, 153.2, 135.9, 131.9, 118.6, 100.7, 98.9, 83.3, 80.4, 57.3, 28.5, 28.0, 20.6, 0.0.

Compound 23: Compound **22** (157 mg, 0.277 mmol) was dissolved in DCM (2 mL). To this solution, TFA (2 mL) was added and the obtained mixture was stirred for 1 h. Then the solvent and TFA were removed by evaporation and the residual TFA was removed by coevaporation with DCM (5 mL, twice). After drying for 1 h at high vacuum, the crude product was dissolved in H_2O/THF (4 mL, 1:1, v/v) and to this solution Boc_2O (72.5 mg, 0.332 mmol) and Et_3N (77 μL, 0.554 mmol) were added. The reaction mixture was stirred for 4 h, after which it was diluted with EtOAc (50 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 6:1, v/v, with 0.1% HOAc). Compound **23** was obtained as a light yellowrish solid (126 mg, 91%); R_f = 0.18 (hexane/EtOAc, 1:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = -30.7 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.86 (s, 1H), 7.49 (s, 2H), 5.04 (s, 1H), 2.32 (s, 3H), 1.34 (d, *J* = 52.1 Hz, 9H), 0.23 (s, 18H); MS (ESI) *m/z* calcd for $C_{25}H_{35}NNaO_6Si_2$ $[M+Na]^+$ 524.19, found 524.20.

Tripeptides 25a-b: Dipeptide **4** (479 mg, 1.2 mmol) was dissolved in DCM (15 mL) and to this solution, TFA (15 mL) was added. The obtained reaction mixture was stirred for 1 h. The volatiles were removed and the residual TFA was removed by coevaporation with DCM (20 mL, twice). After drying for 1 h at high vacuum,

the free amine was dissolved in DCM (20 mL). To this solution, compound **23** (502 mg, 1.0 mmol) and BOP (531 mg, 1.2 mmol) were added, followed by the addition of D*i*PEA (346 μL, 2.0 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 6:1, v/v). Compound **25a** was obtained as a white solid (510 mg, 65%); R_f = 0.50 (hexane/EtOAc, 2:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = -21.0 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.45 (s, 2H), 6.60 (s, 1H), 6.32 (d, *J* = 7.9 Hz, 1H), 5.46 (d, *J* = 5.3 Hz, 1H), 5.01 (d, *J* = 5.3 Hz, 1H), 4.47 (m, 2H), 3.69 (s, 3H), 3.26 (t, *J* = 6.8 Hz, 2H), 2.32 (s, 3H), 1.91 – 1.51 (m, 9H), 1.43 (s, 9H), 1.39 (d, *J* = 7.9 Hz, 3H), 0.94 (dd, *J* = 11.9, 6.2 Hz, 6H), 0.23 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.4, 171.4, 169.5, 167.6, 155.7, 153.6, 135.0, 132.2, 119.2, 101.3, 98.5, 81.4, 58.8, 52.6, 52.2, 51.3, 40.8, 31.6, 29.9, 28.5, 25.2, 23.2, 22.8, 21.8, 20.6, -0.1; MS (ESI) *m/z* calcd for $C_{38}H_{59}N_6O_8Si_2$ $[M+H]^+$ 783.39, found 783.75; calcd for $C_{38}H_{58}N_6NaO_8Si_2$ $[M+Na]^+$ 805.38, found 805.35.

Compound **25b** was obtained as a white solid (94 mg, 12%); R_f = 0.44 (hexane/EtOAc, 2:1, v/v, with 0.1% HOAc); $[\alpha]_D^{20}$ = +40.3 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (s, 2H), 6.62 (d, *J* = 7.0 Hz, 1H), 6.14 (d, *J* = 7.8 Hz, 1H), 5.64 (d, *J* = 6.3 Hz, 1H), 5.07 (s, 1H), 4.63 – 4.38 (m, 2H), 3.74 (s, 3H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.31 (s, 3H), 1.94 – 1.79 (m, 1H), 1.77 – 1.45 (m, 7H), 1.41 (s, 9H), 1.37 – 1.32 (m, 3H), 0.99 – 0.87 (m, 1H), 0.83 (dd, *J* = 6.5, 3.5 Hz, 6H), 0.23 (s, 18H); MS (ESI) *m/z* calcd for $C_{38}H_{58}N_6NaO_8Si_2$ $[M+Na]^+$ 805.38, found 805.45.

Pentapeptide 26: Tripeptide **25a** (270 mg, 0.345 mmol) was dissolved in DCM (9 mL) and to this solution TFA (1 mL) was added. The obtained reaction mixture was stirred for 1 h,after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (10 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (10 mL). To this solution, dipeptide **9** (176 mg, 0.380 mmol), EDCI (72.6 mg, 0.380 mmol), and HOBt (51.3 mg, 0.380 mmol) were added, followed by the addition of D*i*PEA (119 μL, 0.69 mmol), and the mixture was stirred for 2 h. Subsequently the solvent was removed by evaporation and the residue was redissolved in EtOAc (50 mL). The

resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 0.5:99.5 to 1:99, v/v). Compound **26** was obtained as a white solid (330 mg, 85%); R_f = 0.39 (hexane/EtOAc, 1:1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 7.4 Hz, 2H), 7.44 (s, 2H), 7.39 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 1.2 Hz, 1H), 7.30 (d, *J* = 1.1 Hz, 1H), 7.28 (d, *J* = 1.1 Hz, 1H), 7.01 (d, *J* = 4.5 Hz, 1H), 6.93 (d, *J* = 7.5 Hz, 1H), 6.55 (d, *J* = 5.3 Hz, 1H), 6.34 (d, *J* = 8.4 Hz, 1H), 5.18 (d, *J* = 5.2 Hz, 1H), 4.66 – 4.29 (m, 3H), 4.24 – 3.96 (m, 2H), 3.58 (s, 3H), 3.23 (dd, *J* = 9.3, 4.3 Hz, 4H), 2.32 (d, *J* = 6.2 Hz, 3H), 1.91 (dt, *J* = 13.9, 7.0 Hz, 1H), 1.84 – 1.73 (m, 4H), 1.65 – 1.43 (m, 7H), 1.42 – 1.31 (m, 6H), 0.94 (dd, *J* = 8.8, 5.9 Hz, 6H), 0.23 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.6, 173.8, 173.6, 172.6, 169.0, 167.8, 157.6, 153.7, 143.9, 143.7, 141.6, 133.6, 132.7, 128.0, 127.3, 125.0, 120.2, 119.5, 101.7, 98.3, 66.9, 59.2, 54.4, 52.6, 51.3, 51.2, 50.7, 47.4, 39.3, 31.4, 30.2, 29.7, 28.6, 28.4, 25.5, 23.6, 23.1, 20.9, 20.6, 16.6, -0.0; MS (ESI) *m/z* calcd for $C_{57}H_{76}N_{11}O_{10}Si_2$ $[M+H]^+$ 1130.53, found 1131.15; calcd for $C_{57}H_{75}N_{11}NaO_{10}Si_2$ $[M+Na]^+$ 1152.51, found 1152.75.

Pentapeptide 27: Pentapeptide **26** (80 mg, 0.071 mmol) was dissolved in a mixture of MeOH/DCM/H_2O (7.2 mL, 4:7:1, v/v/v). To this solution, AgOTf (7.27 mg, 0.028 mmol) was added and the mixture was stirred for 20 h. Then, the solvents were removed in vacuum and the residue was redissolved in EtOAc (50 mL). The resulting solution was washed with H_2O (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99, v/v). Compound **27** was obtained as a white solid (69 mg, 98%); R_f = 0.19 (hexane/EtOAc, 1:1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 7.5 Hz, 2H), 7.54 (d, *J* = 9.4 Hz, 4H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.25 (m, 3H), 7.20 (s, 1H), 6.98 (s, 1H), 6.10 (d, *J* = 7.7 Hz, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 4.63 – 4.41 (m, 3H), 4.39 – 4.03 (m, 4H), 3.61 (s, 3H), 3.25 (dd, *J* = 15.9, 9.1 Hz, 4H), 2.36 (s, 3H), 1.88 – 1.51 (m, 12H), 1.50 – 1.32 (m, 8H), 1.29 – 1.22 (m, 4H), 0.86 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl3) δ = 174.5, 173.5, 172.5, 169.1, 168.2, 157.4, 154.1, 143.9, 143.8, 141.6, 133.9, 133.4, 128.0, 127.3, 125.1, 120.2, 118.5, 83.7, 83.5, 67.1, 58.8, 54.6, 52.6, 52.3, 52.2, 51.3, 50.0, 47.3, 39.6, 31.3, 30.7, 29.9,

28.7, 28.4, 25.4, 23.1, 21.1, 20.8, 16.7, 14.3; MS (ESI) *m/z* calcd for $C_{51}H_{60}N_{11}O_{10}$ $[M+H]^+$ 986.45, found 986.75.

Hexapeptide 29: Pentapeptide **27** (56.5 mg, 0.05 mmol) was dissolved in THF (2 mL) and to this solution, TBAF (63.1 mg, 0.20 mmol) was added. The reaction mixture was stirred for 5 min and subsequently quenched by addition of MeOH (0.5 mL). The resulting solution was diluted with EtOAc (25 mL) and subsequently washed with saturated $NaHCO_3$ (25 mL, once) and brine (25 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the crude product was dried under high vacuum for 1 h. The residue was dissolved in DCM (2 mL). To this solution *N*-Me-Boc-D-Leu-OH (14.7 mg, 0.06 mmol), EDCI (11.5 mg, 0.06 mmol), and HOBt (8.11 mg, 0.06 mmol) were added, followed by the addition of D*i*PEA (21 μL, 0.12 mmol). The obtained reaction mixture was stirred for 4 h, after which the solvent was removed by evaporation and the residue was redissolved in EtOAc (50 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 3:97, v/v). Compound **29** was obtained as a white solid (30 mg, 60%); R_f = 0.68 (DCM/MeOH, 9:1, v/v); ^{1}H NMR (300 MHz, CD_3OD) δ = 7.61 (s, 2H), 5.34 (s, 1H), 4.70 – 4.52 (m, 1H), 4.47 – 4.19 (m, 4H), 3.84 (s, 2H), 3.68 (s, 3H), 3.26 (d, *J* = 6.7 Hz, 4H), 2.73 (s, 3H), 2.32 (s, 3H), 1.87 – 1.51 (m, 18H), 1.47 (s, 9H), 1.37 (d, *J* = 7.1 Hz, 3H), 1.03 – 0.82 (m, 12H); MS (ESI) *m/z* calcd for $C_{48}H_{71}N_{12}O_{11}$ $[M+H]^+$ 991.54, found 991.85; calcd for $C_{48}H_{70}N_{12}NaO_{11}$ $[M+Na]^+$ 1013.52, found 1013.50.

Compound 35: Compound **19** (2.24 g, 4.0 mmol), $[Pd(PPh_3)_4]$ (462 mg, 0.4 mmol), and CuI (228 mg, 1.2 mmol) were placed in a flask sealed with a rubber septum. The flask was evacuated and refilled with dry N_2 (repeated for three times). Then, THF (40 mL) (purged with dry N_2 for 1 h prior to use) was added to the flask via a syringe. The resulting solution was degassed by a freeze-pump-thaw procedure (repeated for three times). Then, D*i*PEA (1.39 mL, 8.0 mmol) and TIPS-acetylene (3.60 mL, 16 mmol) were added to the mixture via a syringe. After stirring the reaction mixture for 20 h at room temperature under N_2, the resulting suspension was filtered through a pad of celite and the filtrate was evaporated to dryness. Subsequently the residue was redissolved in EtOAc (100 mL) and the resulting

solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:4 to 1:2, v/v, with 0.1% HOAc). Compound **35** was obtained as a yellowish solid (2.45 g, 91%). R_f = 0.26 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -51.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.99 (s, 1H), 7.51 (s, 2H), 5.04 (s, 1H), 2.28 (s, 3H), 1.26 (s, 9H), 1.11 (s, 42H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.6, 167.4, 156.9, 152.6, 135.9, 132.4, 118.6, 100.6, 96.6, 82.7, 58.2, 27.9, 20.6, 18.6, 11.2; MS (ESI) *m/z* calcd for $C_{37}H_{59}NNaO_6Si_2$ $[M+Na]^+$ 692.38, found 692.25.

Dipeptide 36: Azidoamino acid **7** (2.18 g, 8.0 mmol) was dissolved in DCM (80 mL). To this solution, BOP (3.90 g, 8.8 mmol) was added, followed by the addition of $MeNH_2$ (2M in THF, 12 mL, 24 mmol) dropwise during 20 min at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 20 h. Then, the solvents were removed by evaporation and the residue was redissolved in EtOAc (150 mL). The resulting solution was washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (EtOAc/hexane, 1:3 to 1:1, v/v) to give the methyl amide as a white solid (2.10 g, 92%). Subsequently, the obtained amide (1.43 g, 5.0 mmol) was dissolved in DCM (50 mL), and to this solution TFA (30 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed by evaporation and the residual TFA was removed by coevaporation with DCM (30 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (80 mL). To this solution Boc-D-Leu-OH (1.27 g, 5.5 mmol) and BOP (2.43 g, 5.5 mmol) were added, followed by the addition of D*i*PEA (2.59 mL, 15.0 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (100 mL, twice), saturated $NaHCO_3$ (100 mL, twice) and brine (100 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v) to afford dipeptide **36** as a viscous oil (1.64 g, 82%). R_f = 0.64 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -9.0 (c = 1.0 methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.01 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 5.25 (d, *J* = 5.7 Hz, 1H), 4.41 (q, *J* = 8.3 Hz, 1H), 4.12 – 3.92 (m, 1H), 3.24 (t, *J* =

6.6 Hz, 2H), 2.74 (d, *J* = 4.5 Hz, 3H), 1.91 (s, 1H), 1.75 – 1.52 (m, 5H), 1.52 – 1.30 (m, 12H), 0.91 (t, *J* = 5.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.2, 172.0, 156.1, 80.3, 53.8, 53.0, 51.1, 40.8, 31.4, 28.4, 28.3, 26.2, 24.7, 22.9, 22.7, 22.1; MS (ESI) *m/z* calcd for $C_{18}H_{35}N_6O_4$ $[M+H]^+$ 399.27, found 399.45; calcd for $C_{18}H_{34}N_6NaO_4$ $[M+Na]^+$ 421.25, found 421.20.

Tripeptide 37: Dipeptide **36** (877 mg, 2.2 mmol) was dissolved in DCM (15 mL), and to this solution, TFA (15 mL) was added. The reaction mixture was stirred for 1 h, after which the volatiles were removed and the residual TFA was removed by coevaporation with DCM (20 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (60 mL). To this solution compound **35** (1.34 g, 2.0 mmol) and BOP (973 mg, 2.2 mmol) were added, followed by the addition of D*i*PEA (1.04 mL, 6.0 mmol). This reaction mixture was stirred for 3 h. Then, the solvent was removed in vacuum and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (hexane/EtOAc, 3:1 to 1:1, v/v). Compound **37** was obtained as a diastereomeric mixture (1.60 g, 84%); MS (ESI) *m/z* calcd for $C_{50}H_{84}N_7O_7Si_2$ $[M+H]^+$ 950.60, found 951.00.

Pentapeptide 38: Tripeptide **37** (300 mg, 0.316 mmol) was dissolved in DCM (8 mL), and to this solution TFA (2 mL) was added. The obtained reaction mixture was stirred for 1 h. Then, the volatiles were removed in vacuum and the residual TFA was removed by coevaporation with DCM (10 mL, twice). After drying for 1 h at high vacuum, the free amine was dissolved in DCM (10 mL). To this solution, dipeptide **9** (161 mg, 0.347 mmol), EDCI (66.3 mg, 0.347 mmol), and HOBt (46.9 mg, 0.347 mmol) were added, followed by the addition of D*i*PEA (192 μL, 1.11 mmol), and the reaction mixture was stirred for 4 h. Subsequently the solvent was removed by evaporation and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent by evaporation, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Pentapeptide **38** was obtained as a white solid (230 mg, 56%); R_f = 0.68 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +19.7 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz,

$CDCl_3$) δ = 7.76 (d, *J* = 7.4 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.60 (dd, *J* = 14.2, 7.4 Hz, 2H), 7.45 (s, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.19 (s, 1H), 7.15 – 7.07 (m, 1H), 6.91 (s, 1H), 6.51 (d, *J* = 16.5 Hz, 1H), 5.20 (d, *J* = 4.4 Hz, 1H), 4.59 – 4.31 (m, 3H), 4.23 (dd, *J* = 13.5, 6.7 Hz, 3H), 4.06 – 3.92 (m, 1H), 3.27 – 3.18 (m, 4H), 2.75 (d, *J* = 3.7 Hz, 3H), 2.31 (s, 3H), 1.95 – 1.65 (m, 6H), 1.60 – 1.52 (m, 4H), 1.47 – 1.34 (m, 8H), 1.11 (s, 42H), 0.92 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.0, 173.5, 173.0, 172.7, 169.0, 167.6, 157.8, 153.5, 143.9, 143.6, 141.3, 133.3, 127.7, 127.1, 127.0, 125.2, 125.1, 119.9, 119.6, 100.0, 98.0, 67.1, 59.1, 54.0, 53.6, 52.0, 51.1, 50.6, 47.2, 39.2, 30.4, 29.7, 28.5, 28.4, 26.2, 25.2, 23.3, 23.1, 23.0, 20.7, 20.6, 18.6, 16.3, 11.2, -0.0; MS (ESI) *m/z* calcd for $C_{69}H_{101}N_{12}O_9Si_2$ $[M+H]^+$ 1297.74, found 1297.75.

Hexapeptide 39: Pentapeptide **38** (430 mg, 0.331 mmol) was dissolved in THF (10 mL) and to this solution $(CH_3)_2NH$ (8.28 mL, 2M in THF, 16.6 mmol) was added, followed by the addition of 1-propanethiol (292 μL, 3.31 mmol).[19] The reaction mixture was stirred for 1 h. After removal of the solvent under reduced pressure, the free amine was obtained. After drying for 1 h at high vacuum, it was dissolved in DCM (20 mL), and to this solution *N*-Me-Boc-D-Leu-OH (81 mg, 0.331 mmol), EDCI (69.5 mg, 0.364 mmol), and HOBt (49.2 mg, 0.364 mmol) were added, followed by the addition of D*i*PEA (126 μL, 0.728 mmol). The reaction mixture was stirred for 3 h. Then, the solvent was removed in vacuum and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, twice), saturated $NaHCO_3$ (50 mL, twice) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 1:99 to 2:98, v/v). Compound **39** was obtained as a white solid (310 mg, 74%); R_f = 0.55 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +25.5 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) δ = 7.43 (s, 2H), 5.14 (s, 1H), 4.65 – 4.48 (m, 1H), 4.40 – 4.11 (m, 3H), 3.29 (d, *J* = 6.4 Hz, 4H), 2.73 (s, 6H), 1.58 – 1.85 (m, 12H), 1.47 (s, 12H), 1.37 (d, *J* = 6.8 Hz, 4H), 1.15 (s, 44H), 1.01 – 0.82 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ = 177.8, 177.1, 177.0, 176.4, 175.4, 162.8, 136.6, 131.3, 115.4, 104.9, 101.0, 84.2, 62.2, 59.6, 58.0, 57.2, 56.5, 54.9, 54.8, 53.0, 43.1, 40.3, 34.8, 33.8, 32.2, 31.8, 31.5, 29.4, 28.6, 28.5, 26.9, 26.7, 26.6, 26.4, 25.0, 24.4, 21.9, 20.3, 15.0; MS (ESI) *m/z* calcd for $C_{64}H_{110}N_{13}O_9Si_2$ $[M+H]^+$ 1260.81, found 1261.20.

Hexapeptide 40: Hexapeptide **39** (310 mg, 0.246 mmol) was dissolved in acetone (20 mL). To this solution, MeI (46 μL, 0.738 mmol) and K_2CO_3 (102 mg, 0.738 mmol) were added. The obtained reaction mixture was stirred for 24 h. After the reaction was completed as judged by ESI-MS, the solvent was removed and the residue was redissolved in EtOAc (100 mL). The resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once), saturated $NaHCO_3$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98, v/v). Compound **40** was obtained as a white solid (295 mg, 94%); R_f = 0.55 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = +26.9 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.63 (s, 1H), 7.50 (s, 1H), 7.34 (s, 2H), 6.89 (s, 1H), 6.68 (d, *J* = 17.3 Hz, 1H), 5.19 (s, 1H), 4.53 (s, 1H), 4.42 (s, 1H), 4.26 (s, 1H), 4.01 (s, 1H), 3.99 (s, 3H), 3.26 (d, *J* = 3.1 Hz, 4H), 2.86 – 2.71 (m, 6H), 1.77 (d, *J* = 11.7 Hz, 8H), 1.57 – 1.64 (m, 4H), 1.43 (s, 12H), 1.39 (d, *J* = 6.4 Hz, 4H), 1.12 (s, 44H), 0.93 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.5, 173.0, 172.4, 169.3, 163.2, 133.4, 131.0, 119.0, 101.5, 96.8, 80.3, 77.2, 61.1, 58.8, 57.5, 53.5, 51.9, 51.1, 51.1, 50.3, 39.8, 37.0, 30.7, 28.4, 28.3, 26.1, 25.2, 25.0, 23.4, 23.2, 23.0, 21.8, 20.8, 18.6, 16.0, 11.6, 11.3, 11.2, 10.9, -0.0; MS (ESI) *m/z* calcd for $C_{65}H_{112}N_{13}O_9Si_2$ $[M+H]^+$ 1274.82, found 1275.05.

Hexapeptide 41: Hexapeptide **40** (275 mg, 0.216 mmol) was dissolved in THF/MeOH (20 mL, 19:1, v/v), and to this solution TBAF (0.65 mL, 1M in THF, 0.65 mmol) was added. After stirring for 1 h, another portion of TBAF (0.65 mL, 1M in THF) was added. The obtained reaction mixture was stirred for another 2h. Based on TLC analysis, the reaction was complete. Subsequently the solution was diluted with EtOAc (100 mL) and the resulting solution was successively washed with 1 N $KHSO_4$ (50 mL, once), saturated $NaHCO_3$ (50 mL, once) and brine (50 mL, once), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography (MeOH/DCM, 2:98 to 4:96, v/v). Compound **41** was obtained as a white solid (190 mg, 91%); R_f = 0.49 (DCM/MeOH, 9:1, v/v); $[\alpha]_D^{20}$ = -15.0 (c = 1.0 MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ = 7.52 (s, 2H), 5.28 (s, 1H), 4.73 – 4.48 (m, 1H), 4.27 (t, *J* = 9.2 Hz, 4H), 3.99 (s, 3H), 3.79 (s, 2H), 3.42 – 3.17 (m, 4H), 2.72 (d, *J* = 4.0 Hz, 6H), 1.96 – 1.51 (m, 10H), 1.51 – 1.22 (m, 20H), 0.93 (dd, *J* = 15.2, 6.1 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 177.6, 177.1, 177.0, 176.5, 174.9, 166.8, 137.7, 135.7, 120.8, 86.9, 84.1, 82.2, 81.8, 64.3, 61.2, 59.9, 57.6, 57.3, 57.2, 56.7, 54.8, 54.8, 53.0, 43.2,

40.6, 34.7, 34.6, 33.6, 32.1, 31.9, 31.2, 29.1, 28.5, 26.9, 26.7, 26.2, 25.9, 24.4, 21.7, 20.2; MS (ESI) *m/z* calcd for $C_{47}H_{72}N_{13}O_9$ $[M+H]^+$ 962.56, found 963.05; calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.54, found 985.30; MALDI-TOF MS *m/z* calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.540, found 984.813; calcd for $C_{47}H_{71}KN_{13}NO_9$ $[M+K]^+$ 1000.513, found 1000.788.

Bicyclic compound 42: Hexapeptide **41** (75 mg, 0.078 mmol) and $[Cp^*RuCl]_4$ (25.2 mg, 0.0234 mmol) were placed in a capped flask. The flask was evacuated and refilled with dry N_2 (repeated for three times). Then, THF/MeOH (15.6 mL, 19:1, v/v) was added to the flask via a syringe. The solvents were purged with dry N_2 for 1 h prior to use. The resulting solution was degassed using a free-pump-thaw procedure (repeated for three times). Then, the reaction mixture was stirred for 24 h at 50 °C under N_2, after which the solvents were removed under reduced pressure and the residue was absorbed on silica gel and purified by column chromatography (MeOH/DCM, 5:95 to 10:90, v/v). A product fraction was obtained which was further purified by preparative RP-HPLC. Pure compound **42** was obtained as a white solid after lyophilization (30 mg, 40%); R_f = 0.25 (DCM/MeOH, 9:1, v/v); HPLC analysis, t_R = 30.6 min; ^{1}H NMR (500 MHz, CD_3OD) δ = 8.02 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.55 (s, 1H), 5.73 (s, 1H), 4.59 (s, 1H), 4.45 – 4.28 (m, 3H), 4.28 – 4.05 (m, 5H), 3.96 – 3.86 (m, 1H), 3.19 (s, 3H), 2.89 (s, 3H), 2.69 (s, 3H), 2.06 – 1.77 (m, 5H), 1.75 – 1.63 (m, 6H), 1.57 – 1.49 (m, 3H), 1.47 (s, 9H), 1.42 (d, *J* = 7.3 Hz, 3H), 1.29 (s, 3H), 1.23 – 1.04 (m, 2H), 0.96 (d, *J* = 6.5 Hz, 11H), 0.90 (d, *J* = 6.4 Hz, 6H); MS (ESI) *m/z* calcd for $C_{47}H_{72}N_{13}O_9$ $[M+H]^+$ 962.56, found 963.05; calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.54, found 984.60; MALDI-TOF MS *m/z* calcd for $C_{47}H_{71}N_{13}NaO_9$ $[M+Na]^+$ 984.540, found 984.740; calcd for $C_{47}H_{71}KN_{13}NO_9$ $[M+K]^+$ 1000.513, found 1000.701.

Bicyclic compound 43: Bicyclic compound **42** (20 mg, 0.021 mmol) was treated with TFA (1 mL) in DCM (1 mL) for 1 h, after which the volatiles were removed under reduced pressure. The residue was purified with preparative RP-HPLC. Pure compound **43** was obtained as a white solid after lyophilization (10.2 mg, 55%); HPLC analysis, t_R = 18.9 min; MS (ESI) *m/z* calcd for $C_{42}H_{64}N_{13}O_7$ $[M+H]^+$ 862.51, found 862.85; MALDI-TOF MS *m/z* calcd for $C_{42}H_{64}N_{13}O_7$ $[M+H]^+$ 862.505, found 862.685; calcd for $C_{42}H_{63}N_{13}NaO_7$ $[M+Na]^+$ 884.487, found 862.685.

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Chapter 5

Structural Characterization, Molecular Modeling and Biological Evaluation of the Triazole-Bridged Vancomycin Mimics

5.1 Introduction

Vancomycin (**1**) (Figure 1) is a representative member of the family of glycopeptide antibiotics which are the most important class of drugs for the treatment of resistant bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA).[1] Vancomycin inhibits bacterial cell wall synthesis by binding to the peptidoglycan peptide terminus D-Ala-D-Ala found in cell wall precursors. The constrained structure of vancomycin forms a hydrophobic binding pocket and is complexed with the D-Ala-D-Ala sequence through van der Waals contacts, and this complex is further stabilized by forming five hydrogen bonds lining in the pocket (Figure 1).[2] The design and synthesis of simplified vancomycin mimics must target the constrained cyclic structure to mimic the binding pocket of vancomycin. In this thesis, a series of 1,4- or 1,5-triazole bridged vancomycin DE-ring mimics was designed and synthesized employing CuAAC and RuAAC macrocyclization. Furthermore, a 1,5-triazole bridged bicyclic vancomycin CDE-ring mimic using RuAAC macrocyclization was also synthesized to achieve a more constrained mimic than the monocyclic compound. To evaluate their mimicry properties as vancomycin mimics, this chapter will describe their structural characterization, molecular modeling, binding affinity and *in vitro* biological evaluation.

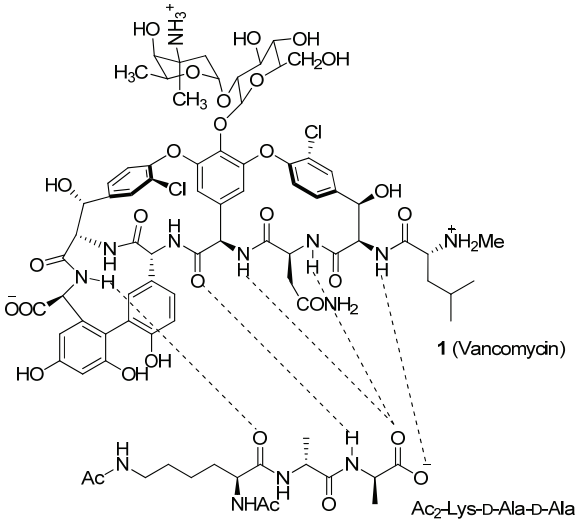
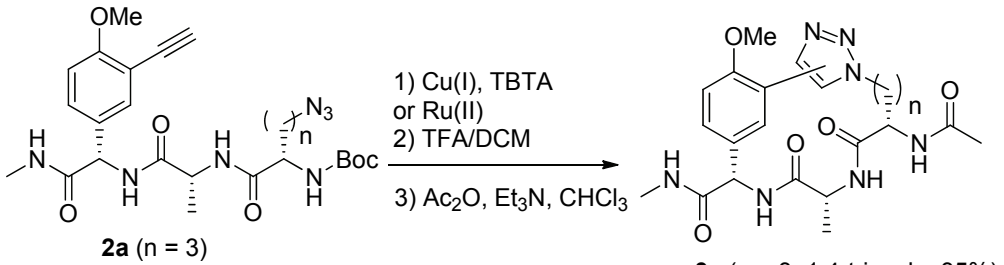


Figure 1. Structure of vancomycin and its binding with the peptidoglycan peptide terminus Ac_2-Lys-D-Ala-D-Ala.

5.2 Structural characterization by NMR

With the TBTA-assisted CuAAC macrocyclization and RuAAC macrocyclization as described in **Chapter 2** and **Chapter 3**, respectively, cyclic monomers **3a-d** have been synthesized in three steps from the linear tripeptides **2a-b**. By removing the Boc-group, the N-terminus of the monocyclic peptide was converted to an acetyl amide (Scheme 1), which could participate in hydrogen bond formation with the natural ligand of vancomycin.

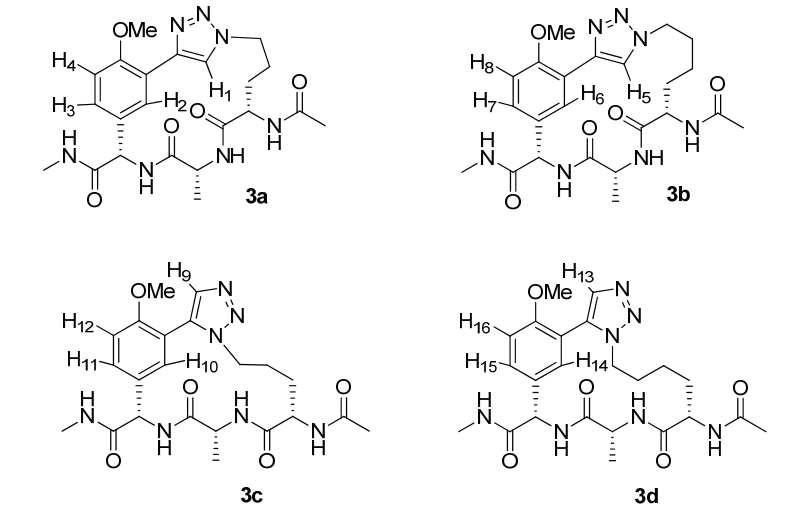


2a (n = 3)
2b (n = 4)

3a (n = 3, 1,4-triazole, 35%)
3b (n = 4, 1,4-triazole, 45%)
3c (n = 3, 1,5-triazole, 56%)
3d (n = 4, 1,5-triazole, 53%)

Scheme 1. Synthesis of the monocyclic vancomycin DE-ring mimics with N-terminus protected as an acetyl amide.

The structure of cyclic monomers **3a-d** could be confirmed by ^{1}H-NMR. The signals correlating to the single proton at position 5 or 4 of the 1,4- or 1,5-disubstituted triazole moiety of compounds **3a-d** were found in a different aromatic region (Figure 2). For compounds **3a-b**, the proton signals H_1 and H_5 of the 1,4-disubtituted triazole were appeared in the aromatic region between δ = 8.36-8.62 ppm (due to a low solubility in CD_3OD, the proton NMR of compound **3a** was measured in $CD_3OD/CDCl_3$ (v/v, 1:1)), while in sharp contrast, the proton signals H_9 and H_{13} of the 1,5-disubstituted triazole in compounds **3c-d** were found around δ = 7.6 ppm (Figure 2). This large difference may be caused by the different conformation of the triazole ring in the cyclic peptide. As seen in the modeling structure (vide infra) of the 1,4-triazole containing cyclic tripeptides **3a-b**, the protons H_1 and H_5 are directed to the inside of the macrocyclic ring, while the protons H_9 and H_{13} in cyclic tripeptides **3c-d** are pointing to the periphery of the macrocyclic ring (see Figure 5).



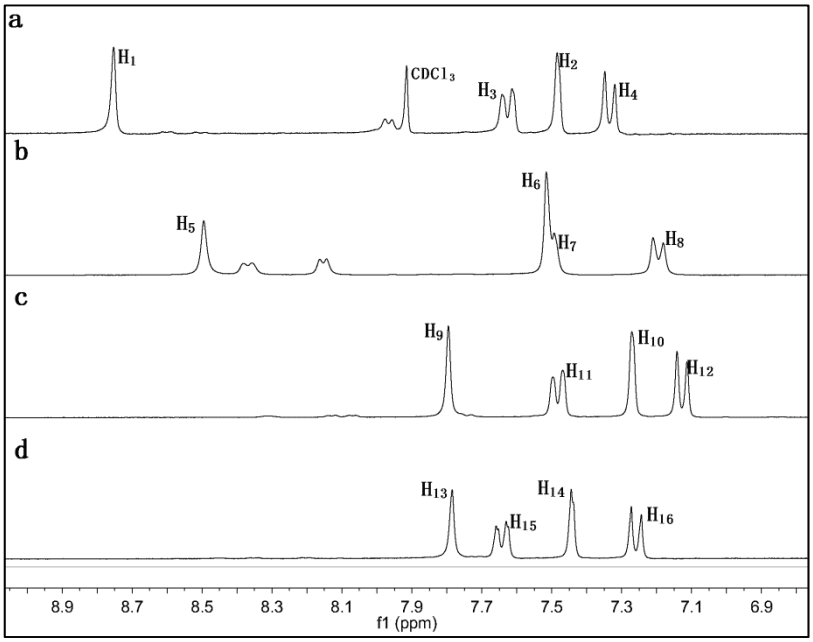
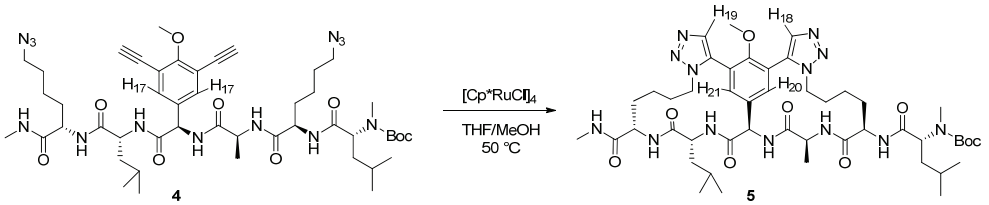


Figure 2. Partial ^{1}H NMR spectrum of compounds **3a-d** (300 MHz, **3a** in $CD_3OD/CDCl_3$ (1:1) and **3b-d** in CD_3OD).

By employing RuAAC macrocyclization chemistry, a bicyclic peptidomimetic **5** of the vancomycin CDE-ring was synthesized from the linear hexapeptide **4** (Scheme 2). The formation of the bicyclic structure could be confirmed by a TCEP-reduction experiment (**Chapter 4**). The reduction of the azide groups in

linear hexapeptide **4** with TCEP led to the formation of the corresponding free amines with loss in molecular weight of 26 and 52 determined by MALDI-TOF MS. The reduction experiment was also followed by HPLC analysis indicating large shifts in retention time between azide and amine derivatives. However, treatment of the bicylic compound **5** with TCEP did not show any change with respect to the molecular weight and druing HPLC analysis.



Scheme 2. Synthesis of the bicyclic peptidomimetic **5**.

Characterization of bicyclic compound **5** by ^{1}H NMR indicated the presence of proton signals of the triazole moieties in the bicyclic compound (Figure 3). Compared to linear peptide **4** with only one proton peak H_{17} in the aromatic region, there were four proton peaks (H_{18}, H_{19}, H_{20} and H_{21}) present in case of bicyclic compound **5**, which was consistent with the bicyclic structure. The four protons could be assigned to the protons of the two triazole moieties and the protons of the aromatic ring of hydroxyphenylglycine. Due to the formation of the bicyclic structure, the two protons on the aromatic ring of hydroxyphenylglycine were in a different environment and therefore could be observed as two separated signals. For a further confirmation, 2D HSQC NMR was also performed to assign the proton signals, and four ^{1}H-^{13}C coupling signals ((7.57, 134.2), (7.85, 134.5), (7.89, 134.3), and (8.04, 135.4)) were observed in the aromatic region (Figure 4), indicating the presence of the desired bicyclic structure.

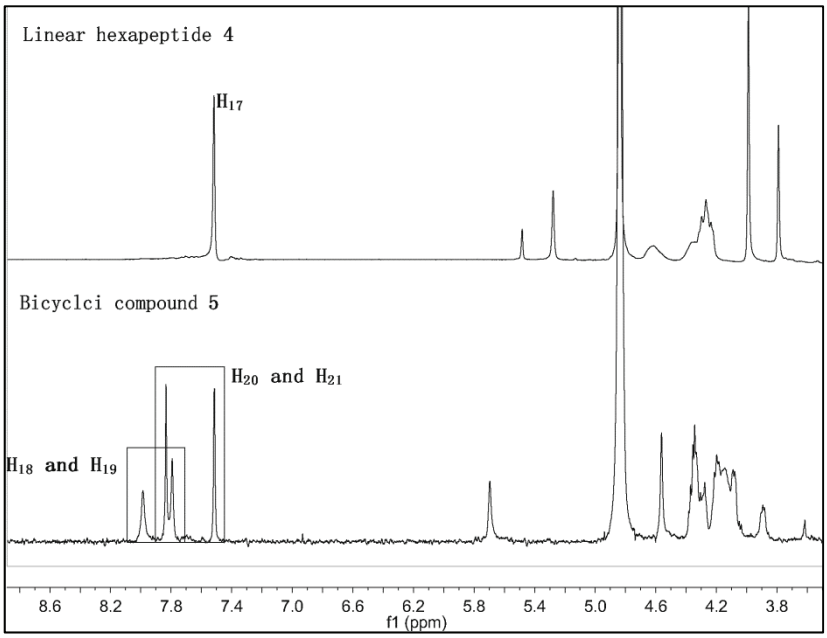


Figure 3. Partial ^{1}H NMR spectrum of the linear hexapeptide **4** and bicyclic compound **5** (500 MHz, in CD_3OD).

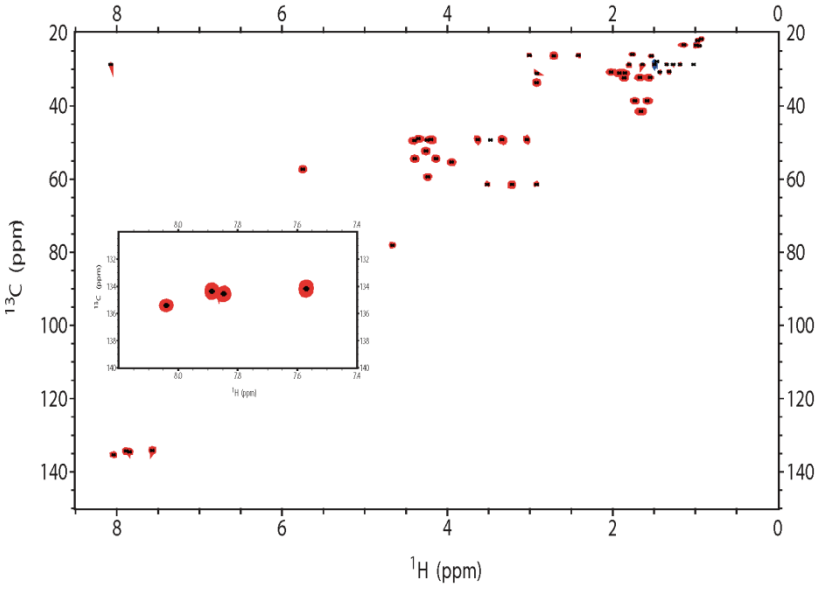


Figure 4. 2D HSQC NMR spectrum of the bicyclic compound **5** (500 MHz, in CD_3OD).

5.3 Molecular modeling

The newly synthesized triazole-bridged cyclic monomers and bicyclic compound were subjected to molecular modeling to obtain additional structural information. After energy minimization, the cyclic structures of these compounds were superimposed with the corresponding part of the crystal structure of balhimycin, which is a vancomycin related glycopeptides antibiotic, in complex with Lys-D-Ala-D-Ala,[3] by minimizing the RMSD between the selected atoms in the ring as shown in Figure 5 and Figure 6.

The Orn-derived 1,4-triazole-bridged cyclic monomer **3a** has a 16-membered ring, the same as the DE-ring of vancomycin, and its superimposition showed a good resemblance with a RMSD value of 0.7442 Å over five superposed atoms. The Orn-derived 1,5-triazole-bridged cyclic monomer **3c** has a 15-membered ring, which is one atom less than the DE-ring of vancomycin. However, it also indicated a good resemblance with a RMSD of 0.6048 Å.

The superimposition of the Lys-derived cyclic monomer **3d** indicated a high structural resemblance, with a RMSD of 0.4501 Å, since the number of atoms in its ring system equals that of the DE-ring of vancomycin (16 atoms), while superimposition of **3b** had a more distorted appearance with a RMSD of 2.8600 Å and a bended conformation of the peptide backbone.

As observed in the modeling structures of cyclic monomer **3a**, **3c** and **3d**, the peptide backbone showed in all cases a linear alignment with the corresponding peptide backbone of balhimycin, which made them in potential good structural mimics of the DE-ring system of vancomycin.

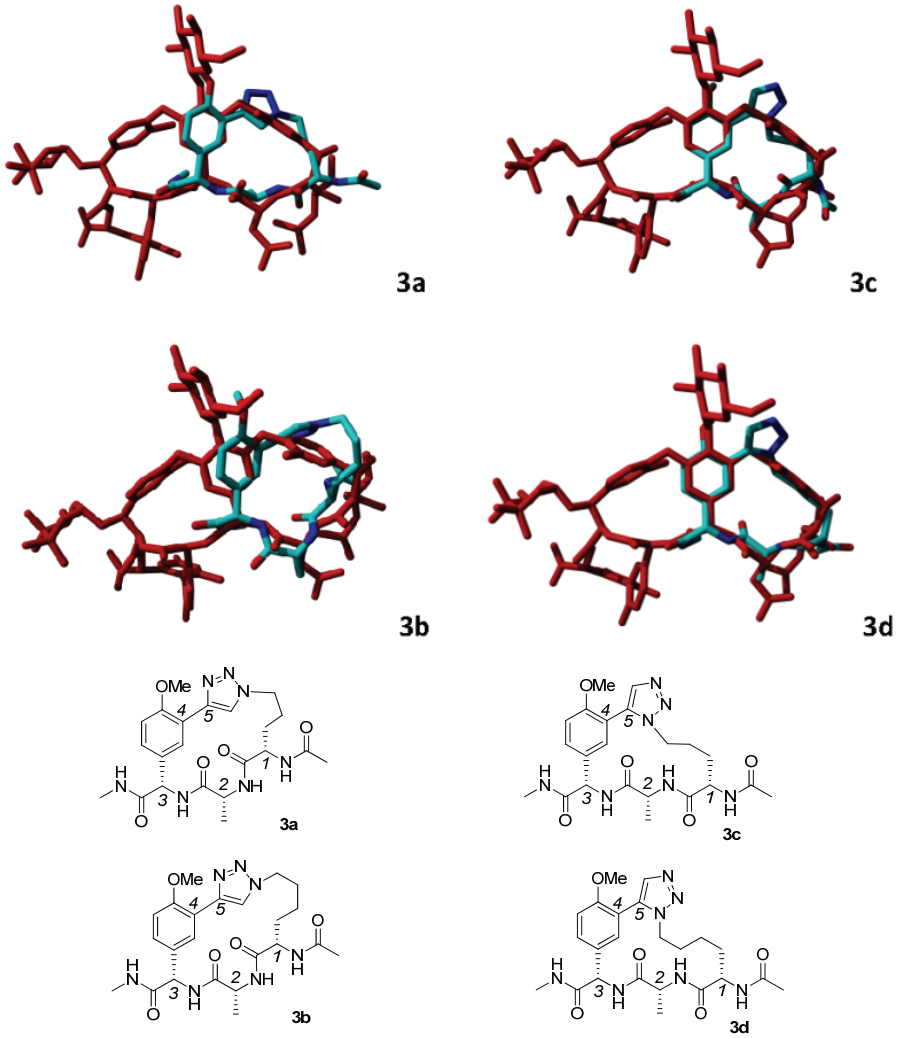
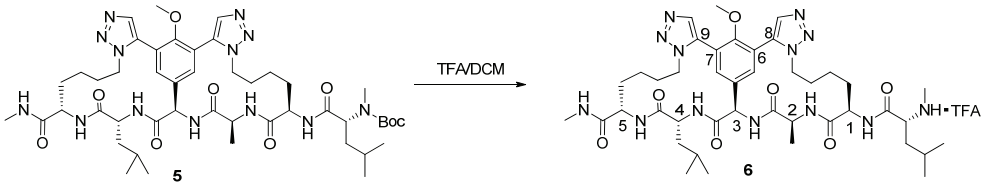


Figure 5. Superimposition of balhimycin (in red) with 1,4-triazole **3a** and **3b** (left) and 1,5-triazole **3c** and **3d** (right). The carbon atoms αC^1, αC^2, αC^3, arom-C^4, and triazole-C^5 have been used as fixed coordinates for superimposition.



Scheme 3. Deprotection of the Boc-group to afford the final bicyclic peptidomimetic **6**.

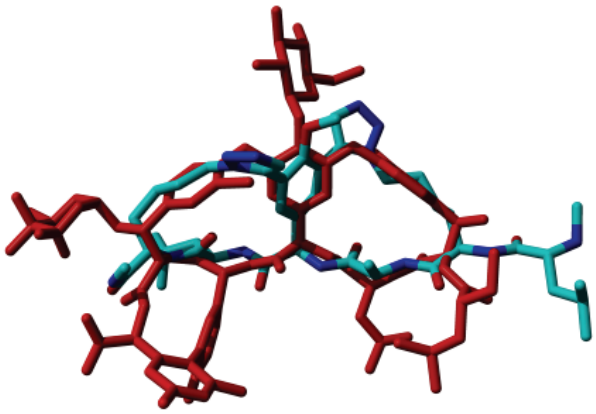


Figure 6. Superimposition of balhimycin (in red) with bicyclic compound **2**. The carbon atoms αC^1, αC^2, αC^3, αC^4, αC^5, arom-C^6, arom-C^7 and triazole-C^8 and C^9 have been used as fixed coordinates for superimposition.

After removal of the Boc-group, bicyclic compound **6** was obtained as the desired vancomycin CDE-ring peptidomimetic (Scheme 3). After energy minimization, the bicyclic structure was also superimposed with the crystal structure of the vancomycin-related balhimycin antibiotic by minimizing the RMSD between the nine selected atoms in the ring (Figure 6). A total RMSD of 1.4150 Å over nine atoms was calculated, which demonstrated very good resemblance of the bicyclic peptide backbone with the corresponding CDE-ring part of vancomycin. The peptide backbone of the bicyclic compound showed a very good alignment with the backbone of balhimycin, which makes it a very promising structural mimic of the vancomycin CDE-ring system.

5.4 Binding affinity with the natural ligand of vancomycin

To evaluate the mimicry properties of the synthesized DE-ring and CDE-ring mimics, the most important and straightforward method is to determine its binding affinities toward the natural ligand of vancomycin. As mentioned above, vancomycin binds to the peptidoglycan peptide terminal D-Ala-D-Ala sequence. For the CD/CDE-ring mimics, the binding affinity with D-Ala-D-Ala will be a direct evidence for their mimicry properties.

Microcalorimetry[4] (ITC) as measured by ITC was used to determine the binding affinity of the synthesized DE-ring mimics **3a-d** and CDE-ring mimic **6**.

Since compounds **3a-d** were synthesized as the enantiomers of vancomycin peptide backbone stereochemistry, their binding was measured with Ac-Ala-Ala instead of Ac-D-Ala-D-Ala. Compound **6** could serve as vancomycin CDE-ring mimic and its binding affinity toward Ac-D-Ala-D-Ala and Ac_2-Lys-D-Ala-D-Ala was determined. For comparison, the binding affinity between vancomycin and its natural ligands was also measured as reference, and the affinity as measured by ITC was consistent with the reported data in the literature.[5]

As shown in Table 1, vancomycin binds to the D-Ala-D-Ala with high affinity, while the affinity toward Ac_2-Lys-D-Ala-D-Ala is 30-fold higher than Ac-D-Ala-D-Ala, which may resulted from the additional hydrophobic interactions of the Lys residue with the vancomycin binding pocket. In agreement with the literature, the binding affinity of vancomycin toward Ac_2-Lys-D-Ala-D-Lac was found to be 100-fold lower, in accordance with the reduced activity of vancomycin against resistant bacteria.

Table 1. The binding affinity of vancomycin, the synthesized mimetics **3a-d** and **6** with the ligand measured by ITC.[a]

Entry	Compound	Ring Size (DE-ring)	Ligand	Ka (M^{-1})
1	**1**	16	Ac-D-Ala-D-Ala	$1.16 \pm 0.09 \times 10^4$
2	**1**	16	Ac_2-Lys-D-Ala-D-Ala	$3.47 \pm 0.12 \times 10^5$
3	**1**	16	Ac_2-Lys-D-Ala-D-Lac	$2.19 \pm 0.12 \times 10^3$
4	**3a**	16	Ac-Ala-Ala	$1.85 \pm 0.22 \times 10^3$
5	**3b**	17	Ac-Ala-Ala	$2.20 \pm 0.29 \times 10^3$
6	**3c**	15	Ac-Ala-Ala	$2.27 \pm 0.35 \times 10^3$
7	**3d**	16	Ac-Ala-Ala	$2.18 \pm 0.22 \times 10^3$
8	**6**	16	Ac-D-Ala-D-Ala	$2.15 \pm 0.24 \times 10^3$
9	**6**	16	Ac_2-Lys-D-Ala-D-Ala	$2.46 \pm 0.29 \times 10^3$
10	**6**	16	Ac_2-Lys-D-Ala-D-Lac	$3.14 \pm 0.38 \times 10^3$

[a] The binding affinity of **3a-d** with its ligand were measured in 0.02M Na citrate buffer with 5% DMSO and the binding affinity of **1** and **6** with its ligand were measured in 0.02M Na citrate buffer.

The triazole bridged DE-ring mimics **3a-d** all showed binding affinity toward Ac-Ala-Ala around same level, however, about 5-fold lower affinity compared to the binding affinity between vancomycin and Ac-D-Ala-D-Ala. From this point, it was concluded that the synthesized DE-ring mimics were not as effective as vancomycin, which could form a cavity-like binding pocket to bind the ligand

more tightly. From Table 1, it became also clear that the differences in binding affinity for these synthetic mimics **3a-d** were not very obvious. This might indicate that the cyclic structure of **3a-d** was not constrained enough to form a very rigid binding cavity, while in the present case the binding to the Ac-Ala-Ala could be originated from the non-specfic binding to the terminal carboxylic acid.

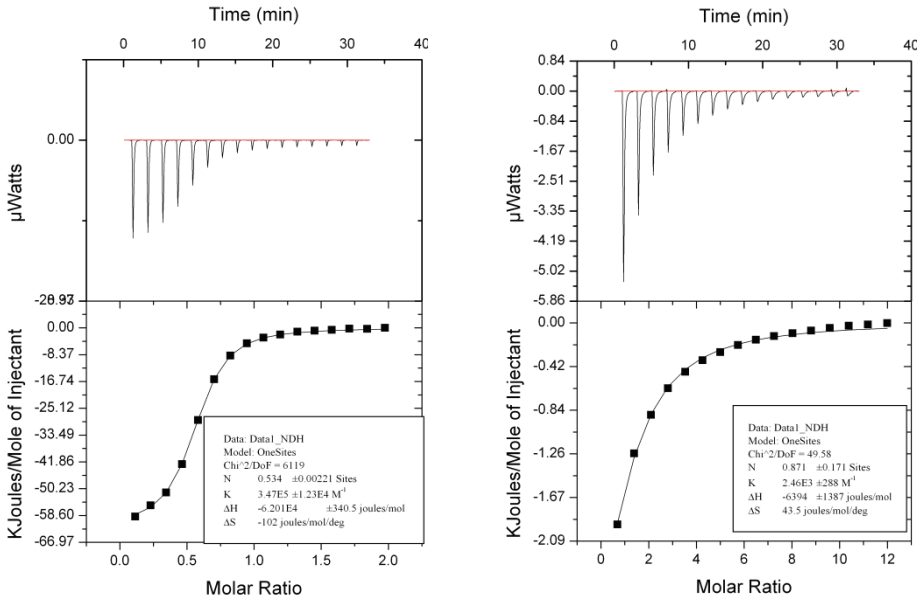


Figure 7. Binding affinity measurements of vancomycin (left, Entry 2) and bicyclic compound **6** (right, Entry 9) with Ac_2-Lys-D-Ala-D-Ala.

Although the bicyclic peptidomimetic **6** was expected to have a higher binding affinity, it was found that its affinity for Ac-D-Ala-D-Ala was at the same level as the DE-ring mimics **3a-d**. Its binding affinity toward Ac-D-Ala-D-Ala and Ac_2-Lys-D-Ala-D-Ala was in the same order of magnitude, which might indicate that the CD-fragment of the bicyclic structure did not contribute to the binding with the ligand. Surprisingly, the bicyclic peptidomimetic **6** even had a somewhat higher affinity to Ac_2-Lys-D-Ala-D-Lac compared to Ac_2-Lys-D-Ala-D-Ala, which may reflect non-specific binding to the carboxylic acid.

Based on these binding affinity measurements, it was concluded that the vancomycin-inspired peptidomimetics **3a-d** and **6** were able to bind the D-Ala-D-Ala peptide sequence through hydrogen bond formation via the carboxylate and the cyclic peptide backbone more or less nonspecific way. The

binding affinity was of a similar magnitude as previously reported cyclic mimics of the vancomycin DE-ring.[6] A possible explanation for the low binding affinity maybe found in less constrained structure of the synthetic mimics, which could not form a cavity-like binding pocket as in vancomycin. The CD-fragment of the bicyclic peptidomimetic **6** did not increase the rigidity any further and showed no improvement with respect to the binding affinity compared to its monocyclic compounds. This observation may reflect the importance of the rigid AB-fragment in vancomycin, which could induce the CDE-ring of vancomycin in a more rigid conformation to form a cavity-like structure.

5.5 Biological evaluation of the bicyclic peptidomimetic 6

The ultimate goal of this research is to find biologically active peptidomimetics of vancomycin. Therefore, the antibacterial activity of the bicyclic peptide **6** was examined using an *in vitro* assay against vancomycin sensitive bacteria *Staphylococcus aureus* (ATCC 49230).

The activity of vancomycin was first tested as a reference. Indeed, vancomycin showed an LC99.9 (lethal concentration 99.9%) of 3.12 μg/mL after 24 h incubation with the bacteria (Figure 8). Next, the antibacterial activity of bicyclic peptide **6** was tested employing the same assay. Unfortunately, compound **6** did not show inhibition of the bacterial growth up to 400 μg/mL (Figure 9). The disappointing biological activity of bicyclic peptide **6** was consistent with the observed low binding affinity toward D-Ala-D-Ala as found in the ITC measurement.

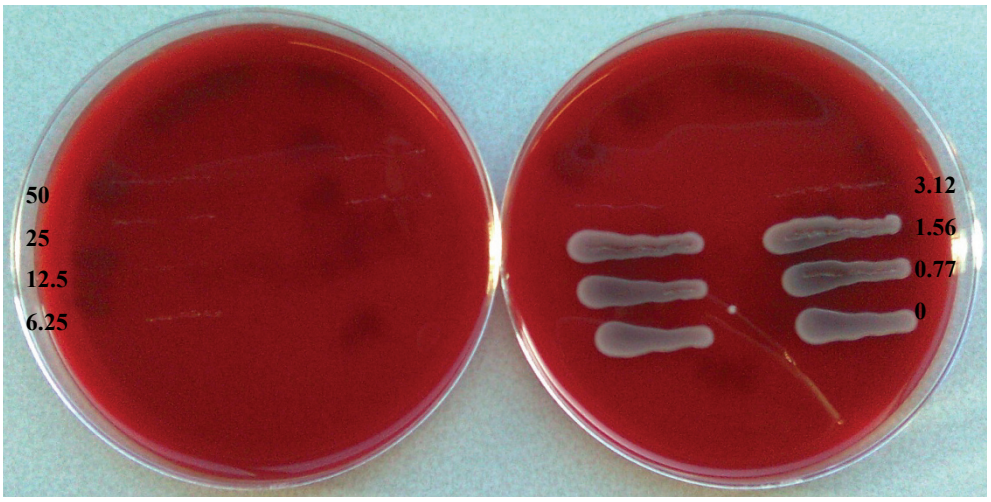


Figure 8. LC99.9% assay of **vancomycin** vs. ***S. aureus*** *(ATCC 49230)* (t = 24 h). Concentration of **Vancomycin**: 50, 25, 12.5 and 6.25 μg/mL (left agar, in duplicate); 3.12, 1.56, 0.77 and 0 μg/mL (right agar, in duplicate).

Figure 9. LC99.9% assay of bicyclic compound **6** vs. ***S. aureus*** *(ATCC 49230)* (t = 24 h). Concentration of bicyclic compound **6**: 400, 200, 100, and 50 μg/mL (left agar, in duplicate); 25, 12.5, 6.25, and 0 μg/mL (right agar, in duplicate).

5.6 Conclusion

In this chapter, the structural characterization by NMR, structural information obtained by molecular modeling, binding affinity measurements by ITC and the *in vitro* biological evaluation of the triazole bridged vancomycin mimics were described. The NMR characterization confirmed the successful synthesis of the triazole bridged mono- and bicyclic vancomycin peptidomimetics employing either CuAAC or RuAAC macrocyclization strategy. Molecular modeling was performed to obtain structural information of the synthesized cyclic peptidomimetics. The energy minimized structures were compared to the crystal structure of a vancomycin-related glycopeptides antibiotic. The pocket-like structure of these cyclic mimics could make them potential mimics of vancomycin. Next, the binding affinity of these cyclic peptidomimetics with the natural ligand of vancomycin was measured by ITC experiments. Binding affinity toward D-Ala-D-Ala was observed, however, with lower affinity compared to vancomycin. Finally, the biological evaluation of the bicyclic peptidomimetic was performed. Unfortunately, the compound did not show inhibition of bacterial growth. To conclude, further optimization of these cyclic peptidomimetics is required, especially the incorporation of extra constrints to increase rigidity would be taken into account in the newly designed 'next generation' vancomycin-inspired peptidomimetics.

5.7 Experimental section

5.7.1 Synthesis and analyses of compound 3a-d

Compounds 3a-d were synthesized from the linear tripeptide **2a-b**, for which the synthesis has been described in **Chapter 2**. After following the procedures of the TBTA-promoted CuAAC macrocyclization (**Chapter 2**) or RuAAC macrocyclization (**Chapter 3**), the obtained Boc-protected compounds were directly treated with TFA in DCM. After Boc-deprotection, the crude products were dissolved in $CHCl_3$ and treated with Ac_2O and Et_3N as base to acetylate the N-terminus. The desired compounds **3a-d** were isolated by preparative RP-HPLC.

Compound 3a was obtained as white solid in 35% yield after 3 steps; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (v/v, 1:1)) δ = 8.48 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.21 (s, 1H), 7.06 (d, J = 8.6 Hz, 1H), 5.22 (s, 1H), 4.64 – 4.43 (m, 2H), 4.35 (d, J

= 6.9 Hz, 2H), 3.92 (s, 3H), 2.76 (s, 4H), 2.28 – 2.07 (m, 1H), 2.03 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 5H); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD (v/v, 1:1)) δ = 177.8, 177.2, 175.9, 175.1, 160.4, 150.2, 134.1, 132.4, 132.2, 128.6, 123.2, 115.5, 105.0, 60.9, 59.2, 55.3, 54.8, 32.8, 29.8, 29.5, 26.0, 20.3; MS (ESI) *m/z* calcd for $C_{22}H_{30}N_7O_5$ $[M+H]^+$ 472.23, found 472.50.

Compound 3b was obtained as white solid in 45% yield after 3 steps; ^{1}H NMR (300 MHz, CD_3OD) δ = 8.36 (s, 1H), 8.24 (d, *J* = 6.9 Hz, 1H), 8.02 (d, *J* = 5.8 Hz, 1H), 7.37 (d, *J* = 6.7 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 1H), 5.31 (s, 1H), 4.55 (s, 2H), 4.34 – 4.14 (m, 2H), 3.86 (s, 3H), 2.73 (s, 3H), 2.06 (s, 3H), 1.91 (s, 2H), 1.60 (s, 1H), 1.39 (d, *J* = 7.3 Hz, 5H), 1.25 (d, *J* = 20.9 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ = 177.4, 176.5, 175.1, 160.4, 148.8, 133.7, 132.9, 132.8, 129.2, 122.7, 115.1, 60.3, 58.8, 57.2, 54.8, 54.2, 33.7, 33.3, 32.9, 29.1, 26.7, 25.4, 19.8; MS (ESI) *m/z* calcd for $C_{23}H_{32}N_7O_5$ $[M+H]^+$ 486.25, found 486.46.

Compound 3c was obtained as white solid in 56% yield after 3 steps; ^{1}H NMR (300 MHz, CD_3OD) δ = 7.80 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.27 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 5.41 (s, 1H), 4.29 (t, *J* = 6.3 Hz, 2H), 4.14 (t, *J* = 6.1 Hz, 1H), 4.04 (q, *J* = 7.3 Hz, 1H), 3.81 (s, 3H), 2.78 (s, 3H), 1.98 (s, 3H), 1.68 (d, *J* = 7.0 Hz, 2H), 1.60 – 1.46 (m, 1H), 1.42 (d, *J* = 7.3 Hz, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ = 177.0, 176.8, 176.1, 174.9, 162.7, 161.0, 136.6, 135.2, 134.7, 134.1, 121.0, 117.1, 115.0, 59.6, 59.0, 57.9, 55.8, 32.7, 30.2, 29.0, 25.1, 19.2; MS (ESI) *m/z* calcd for $C_{22}H_{30}N_7O_5$ $[M+H]^+$ 472.23, found 472.35.

Compound 3d was obtained as whithe solid in 53% yield after 3 steps; ^{1}H NMR (300 MHz, CD_3OD) δ = 7.65 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.31 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 5.42 (s, 1H), 4.27 (dd, *J* = 14.2, 6.8 Hz, 3H), 4.02 (t, *J* = 9.3 Hz, 1H), 3.81 (s, 3H), 2.77 (s, 3H), 2.04 – 1.90 (m, 4H), 1.87 – 1.69 (m, 2H), 1.41 (dd, *J* = 27.3, 9.4 Hz, 4H), 1.12 – 0.82 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ = 176.7, 176.4, 176.3, 175.3, 161.2, 139.2, 136.3, 135.5, 133.8, 120.1, 115.0, 59.8, 58.9, 57.3, 54.3, 34.7, 33.8, 29.0, 25.2, 25.0, 19.5; MS (ESI) *m/z* calcd for $C_{23}H_{32}N_7O_5$ $[M+H]^+$ 486.25, found 486.50.

5.7.2 Molecular modeling

Molecular modeling was accomplished using the Yasara Structure 10.5.2.1 software package. The models were energy minimized using a simulated annealing protocol employing the Amber99 forcefield. The crystal structure of balhimycin (a vancomycin-related glycopeptide antibiotic) in complex with Lys-D-Ala-D-Ala (Protein Data bank accession code: 1GO6)[3] was used as target structure for superimposition. The superimposition was carried out by minimizing the RMSD (root mean square deviations) between the selected atoms in the ring.

5.7.3 Binding affinity measurement

Binding affinity measurement was determined by using microcalorimetry, which was performed on an automated MicroCal Auto-iTC_{200} instrument. A typical ITC (isothermal titration calorimetry) experiment was carried out by injection the ligand solution (10-15 mM) into the cell containing the solution of the synthesized mimics (0.3 mM) or vancomycin (0.1 mM). A typical experiment contained 16 injections in 60 minutes and the resulting data was analyzed by non-linear fitting program in Origin software.

5.7.4 Biological evaluation of the synthetic peptidomimetic 6

A *Staphylococcus aureus* strain (ATCC 49230) was obtained from Medische Microbiologie/AMC on bloodagar. TSB (Tryptic Soy Broth) culture medium was made in house. Bloodagar plates were purchased from FischerSci Oxoid (art PB5008A). Sterile flatbottom microplate (96 wells) was used for incubation.

Antibacterial activity of vancomycin and the synthetic peptidomimetic **6** was evaluated employing an LC99.9 assay, which can determine the lowest sample concentration to give 99.9% bacterial death.

One colony of bacteria (*S. aureus*, ATCC 49230) was first incubated in 5 mL of TSB culture at 37 °C and 150 rpm for 18 h. Then this bacterial culture was diluted 100-fold and incubated for 3 h at 37 °C and 150 rpm. The CFU/mL of this bacterial culture was determined by measurement of the OD_{630} and this bacterial culture was diluted to 2×10^6 CFU/mL. A certain amount of compound was dissolved in TSB to make a stock solution. The stock solution of the compound was diluted to different concentration in the microplate well with a final volume of

50 μL. To each well, 50 μL of bacterial culture (2×10^6 CFU/mL) was added. The microplate was incubated for 24 h at 37°C and 120 rpm. The CFU/mL of each well was determined by measurement of the OD_{630} and the LC99.9 was defined as the lowest concentration of the sample at which less than 0.1% of an inoculums of 10^6 CFU/mL survived. The bacteria survival was examined by adding 10 μL of the sample on bloodagar plate and the plate was incubated for 24 h at 37°C.

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Summary

Cyclic peptides are an important class of compounds with broad biological activities. Both natural as well as synthetic cyclic peptides have been recognized as a great resource and inspiration for drug discovery. Macrocyclization is recognized as an efficient way to restrict the conformational freedom of a peptide which often leads to increased affinity and selectivity. However, only a limited number of approaches for efficient peptide macrocyclization are presently available and the development of synthetic methods toward cyclic peptides is a challenging task for the medicinal chemist.

Vancomycin is the most representative member of a family of glycopeptide antibiotics, which are the most important class of drugs for the treatment of resistant bacterial infections. Because of the emerging resistance to vancomycin, it is of utmost importance to design and synthesize novel vancomycin mimics as potential peptide-based antibiotics. The intriguing structure of vancomycin consists of a tricyclic structure including two biaryl ether bridges and one biaryl bridge, which make its structural modification very challenging. The ongoing development of novel synthetic methodology to access conformationally restricted cyclic peptides encouraged us to develop effective approaches to mimic the bioactive conformation of vancomycin as closely as possible. One of the central goals in our group is to uncover promising alternatives for the biaryl ether bridge in vancomycin leading to attractive mimics. This thesis describes the application of CuAAC and RuAAC macrocyclization for the synthesis of vancomycin mimics.

In **Chapter 1**, a general introduction of cyclic peptides and their synthetic approaches were given as the background of the research described in this thesis. Vancomycin as inspiring and interesting target, its structural characteristics and action mechanism against bacteria, was generally described. The application of CuAAC and RuAAC macrocyclization in the synthesis of cyclic peptides was described to provide a proof of feasibility for carrying out the research in this thesis. Finally, a general outline was provided for obtaining a preview of the research performed in this thesis.

In **Chapter 2**, the synthesis of cyclic 1,4-triazole-bridged tripeptides as vancomycin DE-ring mimics was described. Based on the successful synthesis of a series of linear tripeptides functionalized with alkyne and azide groups, the macrocyclization using CuAAC chemistry was successfully achieved. With the

TBTA-promoted CuAAC macrocyclization strategy, two cyclic monomeric tripeptides, derived from ornithine and lysine, were successfully synthesized. For comparison, the macrolactamization strategy was also applied to the synthesis of these 1,4-triazole-bridged cyclic tripeptides, which was, however, not advantageous compared to the CuAAC based macrocyclization.

As a relevant follow-up study after the successful application of CuAAC for the synthesis of the vancomycin DE-ring mimics, **Chapter 3** described a RuAAC click-type macrocyclization protocol, and a series of cyclic 1,5-triazole-bridged tripeptides as vancomycin DE-ring mimics was synthesized in good yield. The most constrained cyclic tripeptide obtained had only 13 atoms in the macrocyclic ring, while the lysine-derived tripeptide was of the same size as the vancomycin DE-ring containing 16 atoms. The RuAAC macrocyclization proved to be effective for the synthesis of 1,5-disubstituted triazole-containing cyclic tripeptides, and was superior compared to the CuAAC-based click cyclization with respect to the prevalent synthesis of monomeric cyclic peptides.

With the successful application of the RuAAC macrocyclization for the synthesis of the DE-ring mimics of vancomycin, the synthesis of 1,5-triazole-bridged bicyclic vancomycin CDE-ring peptidomimetic using RuAAC macrocyclization was successfully achieved in **Chapter 4**. The synthesis of a linear hexapeptide functionalized with alkyne and azide groups was carefully optimized and the synthesis could be accomplished within 10 steps with good to excellent yield. The RuAAC macrocyclization was successfully developed to synthesize the 1,5-triazole-bridged bicyclic peptidomimetic. The protocol could be generally applied for the synthesis of a small library of bicyclic peptidomimetic for screening the biological activity as vancomycin mimetics. The methodology developed in this study proved that the RuAAC macrocyclization had excellent intramolecular selectivity and could in principle be applied in the synthesis of more complicated bicyclic compounds with biological relevance.

In **Chapter 5**, the structure of the synthesized triazole-bridged vancomycin mimics was further investigated by NMR spectroscopy. The triazole proton signal observed by NMR confirmed the formation of the desired DE- and CDE-ring mimics of vancomycin. Molecular modeling was performed to obtain structural information of the synthesized mimics. By comparing the modeling structure and the crystal structure of vancomycin in complex with Ac_2-Lys-D-Ala-D-Ala, three of the synthesized cyclic tripeptides and the bicyclic compound were proved to be good structural mimics of the corresponding part of vancomycin. The binding

affinity of the obtained mimics with the natural ligand of vancomycin was measured by ITC experiments. Unfortunately, a lower binding affinity to D-Ala-D-Ala peptide sequence was observed compared to vancomycin. The antibacterial activity of the synthesized bicyclic compound was investigated, but no obvious inhibition of the bacterial growth was observed.

In general, the investigation as described in this thesis showed the successful application of CuAAC and RuAAC macrocyclization strategy for the synthesis of vancomycin DE- and CDE-ring mimics. Especially, the RuAAC chemistry turned out to be a very efficient strategy for the synthesis of small cyclic peptides with excellent intramolecular selectivity. Although the synthesized peptidomimetics were not biologically active, the methodology developed here could be applied for the structural optimization of cyclic peptides that lead to biologically interesting targets.

Nederlandse Samenvatting

Cyclische peptiden zijn een belangrijke klasse van verbindingen met een groot aantal biologische activiteiten. Zowel natuurlijke alsook synthetische cyclische peptiden worden gezien als een rijke hulpbron en dienen tevens als inspiratie voor het ontwerpen van nieuwe geneesmiddelen. Macrocyclisatie wordt gezien als een efficiënte manier om de conformationele vrijheid van een peptide te beperken omdat hiermee in het algemeen zowel de affiniteit en selectiviteit van het peptide verhoogd kunnen worden. Echter, tot op de dag van vandaag is er maar een beperkt aantal benaderingen voor een efficiënte peptide macrocyclisatie in de wetenschappelijke literatuur beschreven. Daarom is het ontwikkelen van nieuwe benaderingen voor peptide macrocyclisatie een uitdagende taak voor de organisch chemicus in het algemeen en voor de geneesmiddelchemicus in het bijzonder om hiermee betere, op peptiden gebaseerde, geneesmiddelen te ontwikkelen.

Vancomycine is de meest representatieve verbinding van een klasse van glycopeptide antibiotica. Deze vormen een zeer belangrijke klasse van geneesmiddelen voor de chemotherapeutische behandeling van bacteriële infecties die veroorzaakt worden door resistente bacteriën. Er zijn reeds bacteriën die een resistentie voor vancomycine ontwikkeld hebben. Dit is een zeer gevaarlijke ontwikkeling omdat infecties met deze resistente bacteriën niet meer met de gangbare antibiotica behandeld kunnen worden. Het is daarom van groot belang dat nieuwe antibiotica ontwikkeld worden, bijvoorbeeld geïnspireerd door de structuur en werking van vancomycine. Deze zogenaamde vancomycine-mimetica kunnen dan in het meest gunstigste scenario ingezet worden ter bestrijding van vancomycine-resistente bacteriën.

De intrigerende moleculaire structuur van vancomycine bestaat uit een verknoping van twee biaryl ether bruggen en één biaryl eenheid resulterend in een tricyclische structuur. De complexiteit van deze structuur maakt het niet eenvoudig om gedetailleerde structuur-activiteits-relaties met vancomycine uit te voeren om zo tot een verbeterd antibioticum te komen. Echter, deze complexe tricyclische structuur is wel na te bootsen met kleine cyclische peptiden, met het ultieme doel de antimicrobiële activiteit van vancomycine te behouden of te verbeteren. In dit proefschrift is een aantal benaderingen onderzocht om kleine cyclische vancomycine-gerelateerde peptiden te synthetiseren met recent ontwikkelde macrocyclisatie technieken. Het doel van het onderzoek zoals dat in dit proefschrift beschreven wordt is tweeledig. Ten eerste, het toepassen en verder optimaliseren

van moderne macrocyclisatie chemie, met name de zogenaamde koper-gekatalyseerde alkyn-azide cycloadditie (CuAAC) en de ruthenium-gekatalyseerde alkyn-azide cycloadditie (RuAAC), uitgevoerd met precursor-peptiden afgeleid van vancomycine, en ten tweede, het testen van deze vancomycine-mimetica op hun biologische activiteit als potentieel nieuw antibioticum.

In **Hoofdstuk 1** wordt een introductie gegeven over peptiden en de synthese van peptiden, welke dient als algemene achtergrond voor het onderzoek beschreven in dit proefschrift. Vervolgens wordt de structuur en het biologisch werkingsmechanisme van vancomycine beschreven, en wordt nader uitgelegd op welke wijze de complexe structuur van vancomycine onderzoekers inspireert deze met eenvoudigere moleculen na te bootsen. Daarna wordt de toepassing van CuAAC en RuAAC in macrocyclisatie reacties voor de synthese van cyclische peptiden aan de hand van een aantal voorbeelden geïllustreerd. Bovendien wordt het aannemelijk gemaakt waarom gekozen is voor CuAAC respectievelijk RuAAC macrocyclisaties voor de synthese van vancomycine-mimetica. Tot slot worden inhoud en resultaten van de volgende hoofdstukken kort benoemd.

In **Hoofdstuk 2** werd de synthese van cyclische 1,4-triazool-gebrugde tripeptiden als vancomycine-DE-ring mimetica beschreven. Gebaseerd op een succesvolle synthese van een aantal lineaire tripeptiden, welke voorzien waren van een alkyn en een azide eenheid, werden deze tripeptiden onderworpen aan CuAAC macrocyclisatie condities. Uiteindelijk werd met behulp van een TBTA-geassisteerde CuAAC macrocyclisatie twee cyclische tripeptiden verkregen, waarin hetzij een ornithine residu, hetzij een lysine residu, participeerde in de cyclische structuur. Daarnaast werd een macrolactamisatie uitgevoerd als een alternatieve cyclisatiereactie ter verkrijging van de gewenste 1,4-triazool-gebrugde cyclische tripeptiden, maar deze benadering bleek niet efficiënter te verlopen dan de CuAAC macrocyclisatie.

Een logische vervolgstudie nadat de gewenste 1,4-triazool-gebrugde cyclische tripeptiden succesvol gesynthetiseerd zijn, maakt gebruik van RuAAC macrocyclisatie chemie ter verkrijging van 1,5-triazool-gebrugde cyclische tripeptiden als vancomycine-DE-ring mimetica. Deze resultaten werden in **Hoofdstuk 3** beschreven. Het meest compacte cyclische tripeptide dat via RuAAC macrocyclisatie verkregen werd bevatte slechts 13 atomen in de ring, terwijl indien er een lysine residu aanwezig was, de ringstructuur 16 atomen omvatte, precies overeenkomend met de DE-ring van vancomycine. De RuAAC macrocyclisatie

bleek zeer effectief voor de synthese van 1,5-digesubstitueerde-triazool bevattende cyclische tripeptiden, en was bovendien superieur ten opzichte van CuAAC-gebaseerde cyclisatie reacties met betrekking tot het verkrijgen van de gewenste monocyclische tripeptiden.

Nadat RuAAC macrocyclisatie succesvol is toegepast voor de synthese van vancomycine-DE-ring mimetica, is vervolgens onderzocht in hoeverre RuAAC macrocyclisatie chemie zich leent voor de succesvolle synthese van een vancomycine-CDE-ring mimeticum. Deze resultaten staan beschreven in **Hoofdstuk 4**. De synthese van een lineair hexapeptide, voorzien van twee alkyn en twee azide functionaliteiten, is na een zorgvuldige optimalisatie teruggebracht tot tien afzonderlijke reactiestappen, waarbij het gewenste hexapeptide in hoge opbrengst werd verkregen. RuAAC macrocyclisatie resulteerde uiteindelijk in de succesvolle synthese van een 1,5-triazool-gebrugd bicyclisch vancomycine peptidomimeticum. Tevens is uit dit onderzoek gebleken dat RuAAC macrocyclisatie een hoge mate van intramoleculaire cyclisatie-selectiviteit vertoonde waarmee compacte ringsystemen met hoge opbrengsten verkregen kunnen worden. Het ontwikkelde syntheseprotocol is in het algemeen ook geschikt om met behulp van combinatoriële chemie bibliotheken van biologisch actieve verbindingen te synthetiseren.

In **Hoofdstuk 5** wordt de structuur van de gesynthetiseerde triazool-gebrugde vancomycine mimetica nader bestudeerd met behulp van NMR spectroscopie. Het karakteristieke signaal van het triazool-gebonden proton was in alle gevallen zichtbaar: een belangrijke indicatie dat de gewenste DE- en CDE-ring mimetica van vancomycine daadwerkelijk gesynthetiseerd zijn. Tevens is met behulp van moleculaire mechanica berekeningen aanvullende structuurdata, en inzicht in de driedimensionele oriëntatie van de vancomycine-mimetica verkregen. Zo is van alle mimetica de conformatie met de laagste energie vergeleken met de kristalstructuur van een vancomycine-analogon – ligand complex, waarbij een viertal mimetica goede structuurovereenkomsten vertoonden. Daarnaast zijn affiniteitsmetingen uitgevoerd met behulp van isothermale titratiecalorimetrie en hieruit bleek dat de bindingsaffiniteit voor het natuurlijke ligand belangrijk lager was vergeleken met vancomycine. Tot slot is de *in vitro* antibacteriële activiteit van het bicyclische CDE-ring mimeticum onderzocht waaruit bleek dat deze inactief was.

Samenvattend, het onderzoek zoals dat beschreven is in dit proefschrift toont de succesvolle toepassing van CuAAC en RuAAC macrocyclisatie chemie aan

voor de synthese van vancomycine-gerelateerde DE- en CDE-ring mimetica. Opvallend genoeg bleek dat de RuAAC macrocyclisatie benadering een zeer efficiënte manier te zijn om zeer compacte ringsystemen te synthetiseren. Ondanks het feit dat de gesynthetiseerde vancomycine-mimetica geen biologische activiteit vertoonden, is de ontwikkelde synthese-strategie in het algemeen geschikt om compacte cyclische peptiden te synthetiseren voor biologisch interessante doeleinden.

Appendices

List of Abbreviation

Curriculum vitae

List of Publications

International Symposia

Acknowledgement

List of Abbreviation

Ac	acetyl
Ac_2O	acetic anhydride
ACE	angiotensin-converting-enzyme
Ala	Alanine
BF_3	trifluoroborane
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
^{t}Bu	*tert*-butyl
CFU	colony-forming unit
Cbz	benzyloxycarbonyl
Cp*	pentamethylcyclopentadienyl
Cys	Cysteine
Dab	2,4-diaminobutanoic acid
Dap	2,3-diaminopropionic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
D*i*PEA	diisopropylethylamine
DKP	diketopiperazine
DMF	*N,N*-dimethylformamide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ESI-MS	electrospray ionization mass spectroscopy
Et_2NH	diethylamine
Et_3N	triethylamine
EtOAc	ethyl acetate
Et_2O	diethyl ether
Fmoc	9-fluorenylmethyloxycarbonyl
HOAt	7-aza-1-hydroxybenzotriazole
HOBt	1-hydroxybenzotriazole

HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum coherence
ITC	isothermal titration calorimetry
Lac	Lactic acid
LC-MS	liquid chromatography mass spectroscopy
Leu	Leucine
Lys	Lysine
MALDI-TOF MS	matrix assisted laser desorption ionization time-of-flight mass spectroscopy
MIC	minimum inhibition concentration
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
OD_{630}	optical density at 630 nm
Orn	Ornithine
Pro	Proline
RMSD	root-mean-square-deviation
RP	reverse phase
S_NAr	nucleophilic aromatic substitution
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TIPS	triisopropylsilyl
TIS	triisopropylsilane
TMS	trimethylsilyl
TSB	tryptic soy broth
Tyr	Tyrosine
Val	Valine
Xaa	amino acid

Curriculum vitae

The author of this thesis, Jinqiang Zhang, was born on March 4 (lunar calendar), 1983. He grew up in a small village in the northwest of China. After 4 years of study at the College of Chemistry and Chemical Engineering, Lanzhou University, China, he got a Bachelor's degree in chemistry in 2006. Then he was recommended to study in Zhejiang University, China, for his master's degree. During the 2 years of his master's study, he focused on Organic Synthesis, Molecular Recognition & Self-assembly and Supramolecular Polymers under the supervision of Prof. Feihe Huang. After receiving his Master's degree in 2008, he got a scholarship from the Chinese Scholarship Council for supporting a PhD study abroad and moved to the Department of Medicinal Chemistry & Chemical Biology, Utrecht University, the Netherlands, in December 2008. As a PhD student, he majored in the synthesis of constrained peptidomimetics towards new antibiotics under the supervision of Dr. Dirk Rijkers and Prof. Rob Liskamp. The research results are described in this thesis. After finishing the PhD study, he will move to the group of Prof. William Lubell in Université de Montréal. He is going to start as a post-doctoral fellow in the beginning of 2013.

List of Publications

Publications during the PhD study:

Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J., Cu(I) and Ru(II) Mediated 'Click' Cyclization of Tripeptides Towards Vancomycin-Inspired Peptidomimics, *Org. Lett.* **2011**, 13, 3438−3441.

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Posters:

NWO-CW Symposium, **2010**, Lunteren, the Netherlands:
Zhang, J.; Rijkers, D. T. S.; Liskamp, R. M. J., Synthesis of Constrained Cyclic Peptides by Cu(I)-catalyzed Click Cyclization: Toward the Synthesis of Vancomycin Mimics.

American Peptide Symposium, **2011**, San Diego, CA, USA:
Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J., Cu(I)- and Ru(II)-Mediated 'Click' Cyclization of Tripeptides: Towards Vancomycin-Inspired Mimics.

32nd European Peptide Symposium, **2012**, Athens, Greece:
Zhang, J.; Longin, O.; Rijkers, D. T. S.; Liskamp, R. M. J., Cu(I)- and Ru(II)-catalyzed 'Click' Cyclization of Tripeptides: Toward Vancomycin-Inspired Mimics.

Presentations:

NWO-CHAINS Symposium, **2011,** Maarssen, the Netherlands:
Zhang, J.; Kemmink, J.; Rijkers, D. T. S.; Liskamp, R. M. J., Cu(I)- and Ru(II)-Mediated 'Click' Cyclization of Tripeptides Towards Vancomycin-Inspired Mimics.

32nd European Peptide Symposium, **2012**, Athens, Greece:
Zhang, J.; Rijkers, D. T. S.; Liskamp, R. M. J., Synthesis of Bicyclic 1,5-Triazole-Bridged Vancomycin CDE-ring Peptidomimemics via RuAAC Macrocyclization.

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Jinqiang Zhang
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