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Synthesis of bicyclic tripeptides inspired by the ABC-ring system of vancomycin through ruthenium-based cyclization chemistries

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ABSTRACT

The synthesis of a bicyclic tripeptide that mimics the ABC ring system of vancomycin is described by using a ring closing metathesis (RCM) – peptide coupling – ruthenium-catalyzed azide-alkyne cycloaddition (RuAAC) strategy.

Introduction

Cyclic peptides are increasingly important and serve as relevant lead structures and bioactive molecules in medicinal chemistry and drug design since they offer a plethora of biological activities and are especially interesting for the modulation of protein-protein interactions.[1] Their (total) synthesis, however, is still a highly challenging task and the development of novel and efficient cyclization approaches is an active field of research.[1f] Within this class, multicyclic as well as side-chain knotted cyclic peptides, including the conotoxins and cyclotides,[2] lantibiotics,[3] and glycopeptide antibiotics,[4] are of special interest since they combine extreme potency with shape persistent folding of the peptide backbone. The most classical and outstanding example of the effects of macrocyclization and side-chain knotting is found in the heptapeptide vancomycin.[5] These lead to an almost absolute control of shape and folding of this glycopeptide antibiotic and allows for a very strong binding of the rather flexible natural target ~Lys-D-Ala-D-Ala-OH.[6]

Over the years we have been inspired by cyclized peptides such as vancomycin as well as nisin and have explored alternative approaches for peptide cyclization, among others, Sonogashira cross-coupling,[7] ring closing metathesis,[8] and Cu^{+} as well as Ru^{2+} catalyzed azide-alkyne cycloadditions,[9] to obtain highly constrained side-chain to side-chain knotted peptides. Previously, we have shown that alkyne-, alkene- and triazole-tethered cyclic tri-, hexa- and heptapeptides could be synthesized that mimic vancomycin by binding ~Lys-D-Ala-D-Ala-OH and ~Lys-D-Ala-D-Lac-OH. To further increase the rigidity of these mimics, we were looking for complementary cyclization approaches for introducing an additional cyclic constraint to control the sequence of cyclization and thereby the folding topology of the peptide backbone. So far, most vancomycin mimics, including our own, have focused mainly on the CDE-ring system.[10] However, the ABC-ring system probably provides the additional needed rigidity in the form of a lid, making vancomycin more of a clam to hold ~Lys-D-Ala-D-Ala-OH more permanently by reducing the off-rate. Herein, we describe our efforts toward effective mimicry of the ABC-ring system,[10h,11,12] which ultimately combined with DE-mimicry should lead to potent vancomycin mimics.

Results and discussion

Previously, we have reported the synthesis of triazole-containing vancomycin mimics like **14** (Fig. 1).[9b] Despite their bicyclic framework these structures are still relatively flexible, since mimics such as **14** do bind Ac-Lys(Ac)-D-Ala-D-Ala-OH albeit with

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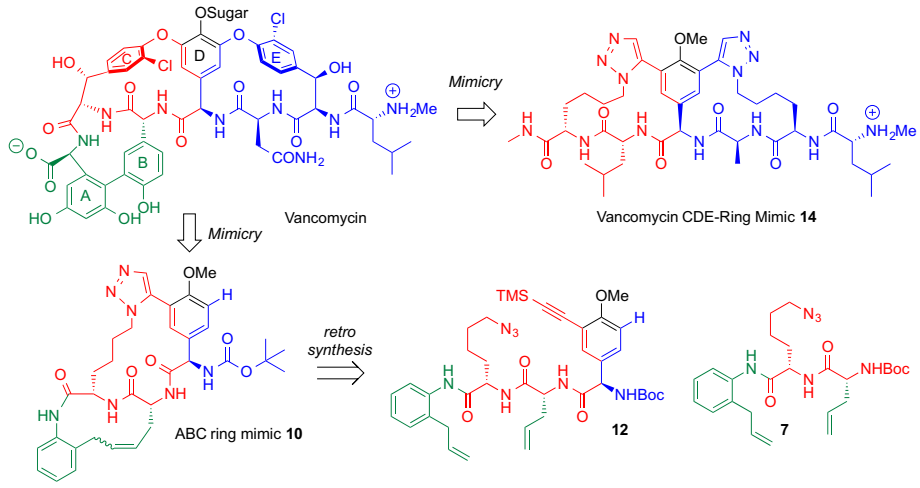


Fig. 1. Design of vancomycin mimics.

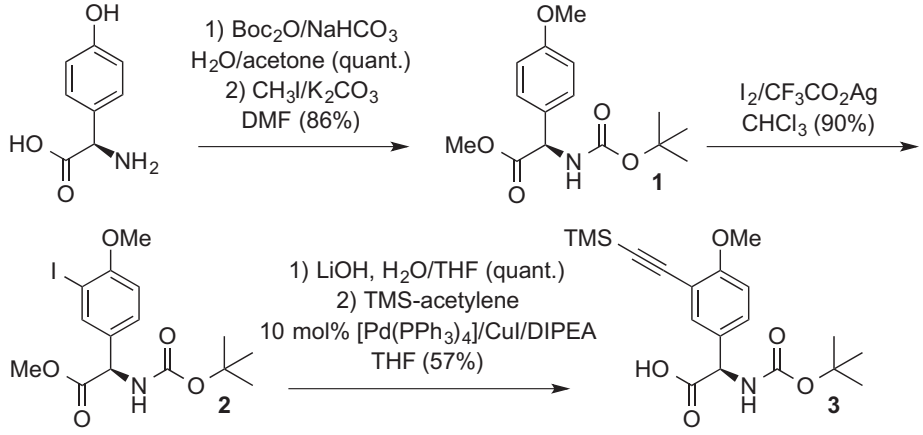
a lower affinity than vancomycin as judged by isothermal microcalorimetry (ITC). In order to include the AB-ring system,[11] we wished to apply RCM, which we and others have successfully reported on several occassions.[13] This led to the target bicyclic tripeptide **10**. Retrosynthesis showed that bicycle **10** might be accessible through two consecutive macrocyclization steps starting from **12** (Fig. 1). Since the preferred order of the macrocyclization steps, by RCM or RuAAC,[14] was not known (*vide infra*), precursor dipeptide **7** was proposed, in order to optimize the RCM in the presence of an azide moiety (Fig. 1).

TMS-protected alkyne **3** was conveniently accessible from commercially available 4-hydroxy-D-phenylglycine (Scheme 1). After protection of the amine group, conversion of the phenolic hydroxy group to a methyl ether and preparation of the methyl ester, amino acid derivative **1** was subjected to iodination, according to Nicolaou and co-workers,[15] to give mono-iodo compound **2** in high yield (90%). After saponification of the methyl ester, the alkyne was installed by a Pd-catalyzed Sonogashira cross-coupling reaction to give protected phenylglycine building block **3** in an acceptable yield of 57%.[16]

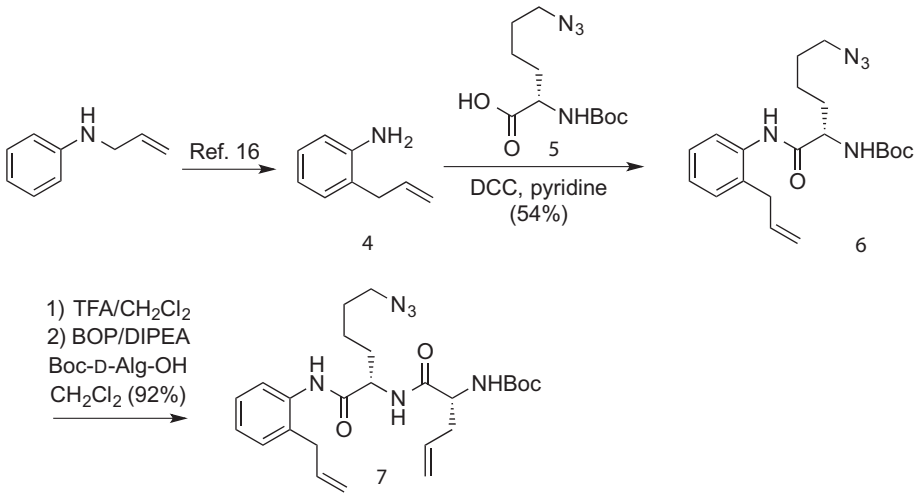
The synthesis of the required RCM-precursor **7** is shown in Scheme 2. To this end 2-allylaniline **4** was obtained by a Claisen rearrangement according to Brucelle and Renaud.[17] In a first attempt to couple aniline **4** to azido acid **5**,[18] BOP/DIPEA as a coupling reagent did not afford anilide **6**. Therefore, DCC in pyridine was used to form the anilide **6** in 54% yield since this combination of coupling reagent/solvent was effective in the coupling of the poor nucleophile 4-nitroaniline with amino acids.[19] After deprotection of anilide **6** by treatment with TFA, Boc-D-Alg-OH was coupled using BOP/DIPEA, and bisalkene dipeptide **7** was obtained in 92% yield over two steps.

As RCM precursor peptide **7** contained an azide moiety, the first[20] and second[21] generation Grubbs catalysts could not be used since the tricyclohexylphosphine ligands would likely reduce the azide into an amine *via* a Staudinger reduction.[22] Therefore, the second generation Hoveyda-Grubbs catalyst[23] was used in refluxing CH_2Cl_2 and macrocyclic peptide **8** was isolated in 55% yield as a mixture of the *E*/*Z* diastereoisomers, as shown in Scheme 3 (**8a** (*Z*):**8b** (*E*) = 1:2.3). After Boc removal both diastereoisomers **8a**, **b** could be separated by preparative HPLC and were characterized by NMR and LC-(HR)MS.

In its protected form, both diastereoisomers (R_t 32.97 and 33.36 min) of macrocycle **8** could not be separated (see, ESI Fig. SI-18). However, when an aliquot of **8** was treated with TFA to remove the Boc functionality, this resulted in a base line separation of both diastereoisomers **8a**, **b** (R_t 22.24 and 22.98 min, Fig. SI-19). The ^{1}H NMR spectrum of diastereoisomer **8a** gave broad peaks at 25 °C; fortunately at higher temperature, peak splitting was observed and at 80 °C a well-defined *J* coupling of the olefinic protons could be derived (Fig. SI-11). This value, ~8 Hz, corresponded to the *Z*-configuration of the double bond. The proton spectrum of diastereoisomer **8b** had resulted already at 25 °C in a well-defined *J* coupling of ~13 Hz for the alkene bond indicating an *E*-geometry of **8b** (Fig. SI-12).[24]



Scheme 1. Synthesis of phenylglycine building block **3**.

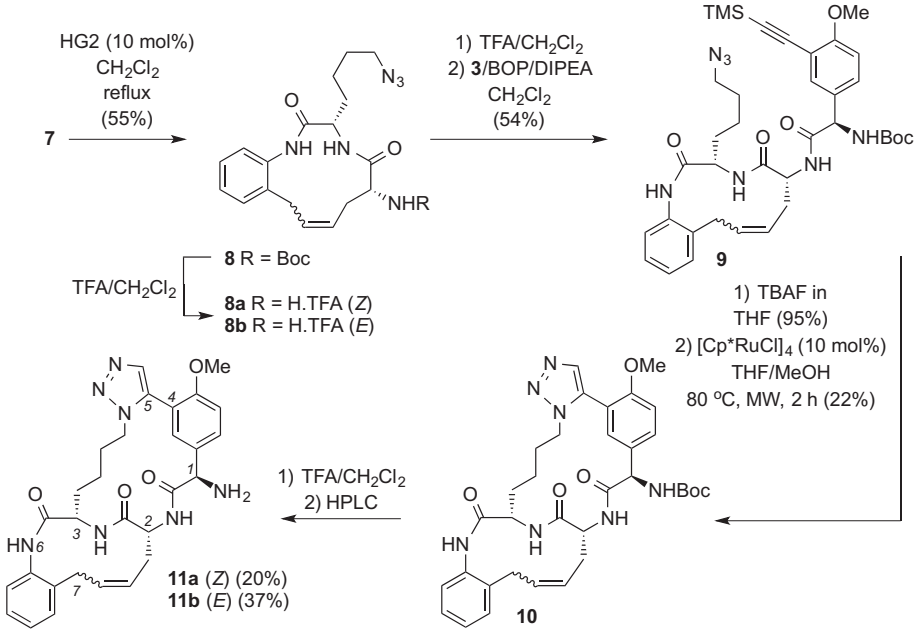


Scheme 2. Synthesis of RCM precursor dipeptide **7**.

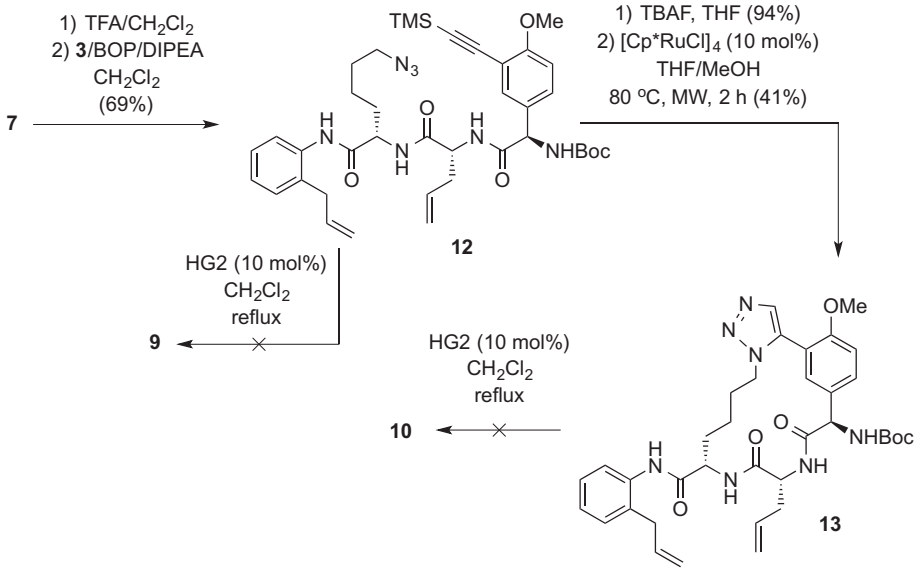
Thus, macrocycle **8** was treated with TFA and the resulting free α-amine coupled to alkyne derivative **3** in the presence of BOP/DIPEA to afford the protected click precursor **9** in 54% overall yield (Scheme 3). Then, tripeptide **9** was treated with TBAF to remove the TMS functionality, and the unprotected alkyne which was isolated in 95% yield after column chromatography, subjected to RuAAC in THF/MeOH at 80 °C under microwave irradiation in the presence of 10 mol% $[Cp^*RuCl]_4$. Bicyclic tripeptide **10** was isolated in 22% yield after column chromatography as a mixture of *E*/*Z* diastereoisomers. Heating at 80 °C under microwave irradiation in the absence of $[Cp^*RuCl]_4$ did not lead to any conversion. To improve the yield of cyclization, lower and higher catalyst loadings (5 and 15 mol%, respectively) were used which turned out to be ineffective since incomplete conversion of the starting material (at 5 mol%) or extensive formation of baseline compounds (at 15 mol%) were observed. As a control experiment, the unprotected alkyne was also subjected to regular CuAAC[25] in the presence of either CuI or $[Cu(CH_3CN)_4]PF_6$ as the Cu^+ species; these reaction conditions did not lead to the formation of bicyclic tripeptide **10**. This experiment showed that a 1,5-triazole moiety with a curved geometry was essential for ring closure since the more linear geometry of the 1,4-triazole was clearly incompatible with the topology of the bicyclic framework of tripeptide **10**. Similar to macrocycle **8**, the individual diastereoisomers of bicyclic tripeptide **11** (*Z*-**11a** and *E*-**11b**, respectively) could be obtained after Boc removal of **10** and purification by preparative HPLC. It is interesting to note that the *Z*:*E* ratio during the conversion of **8a**, **b** into **11a**, **b** shifted from 1:2.3 to 2:1, an indication that the *Z*-geometry of the alkene was favored in the bicyclic tripeptide topology.

Since bicyclic tripeptide **11** can be used as a versatile building block in the synthesis of tricyclic heptapeptides to mimic the side-chain to side-chain connectivity pattern of vancomycin, hydrogenation of the double bond to an alkane bridge would be desirable to obtain a single isomer instead of an *E*/*Z* mixture of diastereoisomers. Therefore, several hydrogenation conditions in the presence of Pd/C, Raney Ni, and $Pd(OH)_2$ were investigated, unfortunately all were unsuccessful.

Two reverse reaction sequences to arrive at the desired bicyclic tripeptide **10** were also investigated, both starting from linear dipeptide **7** (Scheme 4). As the first step, Boc-protected **7** was trea-



Scheme 3. RCM-coupling-RuAAC strategy for the synthesis of bicyclic tripeptide **10**.



Scheme 4. Alternative RCM-coupling-RuAAC strategies for the synthesis of bicyclic tripeptide **10**.

ted with TFA and the resulting amine coupled to alkyne derivative **3** in the presence of BOP/DIPEA to afford tripeptide **12** in 69% yield after purification by column chromatography. RCM of precursor peptide **12** in the presence of second generation Hoveyda-Grubbs catalyst in CH_2Cl_2 or 1,2-dichloroethane did not result in the formation of alkene-bridged macrocycle **9**. The progress of the reaction was monitored by LCMS, and although starting material had disappeared, the desired bicyclic peptide **9** could not be observed. In hindsight, it was assumed that the desired ene-ene RCM pathway was possibly overrun by the thermodynamically favored ene-yne reactivity to yield 2-silyl-substituted 1,3-dienes, which, however, were not identified.[26] Alternatively, TMS-protected precursor peptide **12** was treated with TBAF and subsequently subjected to RuAAC to install the triazole moiety as the cyclic constraint. *Via* this route, macrocycle **13** was obtained in an improved 41% yield compared to the above preparation of **10** (Scheme 3). Unfortunately, RCM of **13** did not result in the successful isolation of bicyclic tripeptide **10**. TLC analysis indicated that conversion of the starting compound was incomplete and some baseline material was present, additionally the formation of bicyclic tripeptide **10** could not be observed by LCMS.

The structures of bicyclic tripeptides **11a** (as the *Z*-diastereoisomer) and **11b** (as the *E*-diastereoisomer) were energy minimized using the simulated annealing protocol employing the AMBER99 force field using the YASARA Structure 10.5.2.1 software package.[27] The peptides were superimposed with the left half of the vancomycin-related balhimycin antibiotic comprising the ABC-ring system.[28] An RMSD of 0.76 and 0.58 Å over seven atoms was calculated (see Scheme 3 for atom numbering) of the superimpositions of **11a** and **11b**, respectively (Fig. 2). To evaluate if this structural

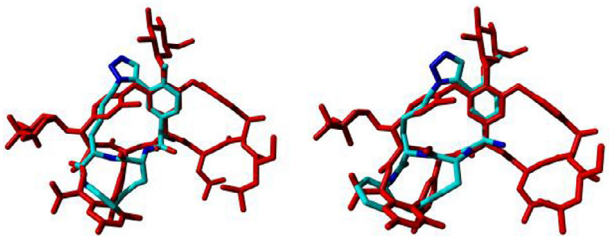


Fig. 2. Superimposition of balhimycin (in red) with bicyclic tripeptide **11a** (left) and **11b** (right), respectively. The carbon atoms αC^1, αC^2, αC^3, arom-C^4, triazole-C^5, N^6, benzylic-C^7 have been used as fixed coordinates for superimposition.

Table 1
The binding affinity as measured using ITC.

Compound	Ligand	K_a (M^{-1})[a]
VM	Ac-Lys(Ac)-D-Ala-D-Ala-OH	$(3.95 \pm 0.41) \times 10^5$
VM	Ac-Lys(Ac)-D-Ala-D-Lac-OH	$(2.38 \pm 0.23) \times 10^3$
11a	Ac-Lys(Ac)-D-Ala-D-Ala-OH	$(2.35 \pm 0.36) \times 10^3$
11a	Ac-Lys(Ac)-D-Ala-D-Lac-OH	$(2.17 \pm 0.30) \times 10^3$
11b	Ac-Lys(Ac)-D-Ala-D-Ala-OH	$(4.06 \pm 0.84) \times 10^3$
11b	Ac-Lys(Ac)-D-Ala-D-Lac-OH	$(1.21 \pm 0.86) \times 10^3$

[a] Measured in a Na-citrate/citric acid buffer (0.02 M, pH 5.1), VM: vancomycin.

resemblance correlates with binding affinity toward Ac-Lys(Ac)-D-Ala-D-Ala-OH and Ac-Lys(Ac)-D-Ala-D-Lac-OH, isothermal microcalorimetry (ITC) was performed, as shown in Table 1.[29,30] Based on these data, mimics **11a** and **11b** still bind Ac-Lys(Ac)-D-Ala-D-Ala-OH appreciably, considering that a large part of the 'clam' is missing, albeit at least 100-fold less compared to vancomycin. Bicycle **11b** is somewhat more active than **11a**, while binding toward Ac-Lys(Ac)-D-Ala-D-Lac-OH was comparable for all three receptor molecules. This was in line with the MIC-values obtained from a growth inhibition assay[31] of the *Staphylococcus aureus* ATCC 49320 strain, where values of 300 μg/mL (**11b**), >300 μg/mL (**11a**) and 2 μg/mL (VM) were found, respectively. Although a reasonable structural resemblance was found, not unexpectedly, for efficient binding (and activity) some extra factors need to be addressed such as the proper alignment of hydrogen bonding and further rigidification, possibly in attempts to combine the ABC and CDE-ring systems.[9b]

Conclusion

In conclusion, bicyclic tripeptide **10** as a mimic of the ABC-ring system was successfully synthesized starting from precursor dipeptide **7** following an RCM-coupling-RuAAC strategy. Mimics of this part of the vancomycin structure are less explored as only a single hydrogen bond contributes to the binding of Ac-Lys(Ac)-D-Ala-D-Ala-OH *via* the carbonyl oxygen of the lysine residue. The mixture of double bond isomers could be separated by HPLC to give each individual *E*/*Z* diastereoisomer as the free amine as judged by NMR and LC-MS. Bicyclic tripeptide **10** represents an important building block to ultimately arrive at a series of tricyclic

heptapeptides for possible effective mimicry of vancomycin in which ruthenium-based cyclization approaches will be used to control the topology and rigidity of the peptide backbone.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2017.10.046.

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