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Synthesis of a tricyclic hexapeptide –via two consecutive ruthenium-catalyzed macrocyclization steps– with a constrained topology to mimic vancomycin's binding properties toward D-Ala-D-Ala dipeptide

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ABSTRACT

A ring-closing metathesis (RCM) – peptide coupling – ruthenium-catalyzed azide alkyne cycloaddition (RuAAC) strategy was developed to synthesize a tricyclic hexapeptide in which the side chain to side chain connectivity pattern resulted in a mimic with a topology that effectively mimics the bioactivity of vancomycin as a potent binder of the bacterial cell wall D-Ala-D-Ala dipeptide sequence and more importantly being an effective inhibitor of bacterial growth.

The three-dimensional folding and shape of a peptide is extremely important for being biologically active as an enzyme inhibitor as well as a receptor (ant)agonist. Well-known examples of therapeutic peptides are, among others, hirudin, cyclosporin, nisin and ziconotide are mentioned here to illustrate this fact.[1] The peptide antibiotic vancomycin (**1**, Scheme 1) is an outstanding example in which the backbone in combination with an ingenious side-chain to side-chain connectivity pattern forms a concave pocket ideally suited for binding the D-Ala-D-Ala dipeptide motif.[2] The molecular recognition of D-Ala-D-Ala by vancomycin is the mode of action as an antibiotic since it obstructs cross-linking of the bacterial cell wall, and this incomplete cell wall is sensitive to cell lysis by osmotic pressure.[3] The carboxylate binding pocket of vancomycin[4] is formed by (di)hydroxyphenylglycine and β-hydroxy-3-chlorotyrosine moieties tightly linked together via aryl carbon–carbon, aryl ether and amide bonds. The dipeptide D-Ala-D-Ala is bound through five hydrogen bonds, and three of them are directly involved in binding the carboxylate. This binding is an inspiring example of anion recognition in Nature by a preorganized and complementary host[5] since hydrogen bond donors and hydrogen bond acceptors are perfectly matched. Here, we describe the design and synthesis of a tricyclic hexapeptide (**2**, Scheme 1) as a preorganized host that can both bind D-Ala-D-Ala and inhibit bacterial growth like vancomycin. Therefore, the newly designed tricyclic hexapeptide is truly a *functional* mimic of vancomycin in which the constrained backbone conformation mimics the natural binding topology required for antimicrobial activity.

The design and retrosynthesis of tricyclic hexapeptide **2** are shown in Scheme 1. Herein, the ultimate step was the bicyclization by installing both 1,5-triazole moieties via ruthenium-catalyzed azide alkyne cycloaddition (RuAAC) starting from alkene-bridged hexapeptide **3**. This RuAAC-precursor could be obtained by stepwise elongation of cyclic dipeptide **4** with building blocks **5** and **6**,[4i] as well as commercially available **7**. Alkene-bridged dipeptide **4** was obtained by ring-closing metathesis (RCM) of bisalkene **8** in the presence of second generation Hoveyda-Grubbs catalyst, as a mixture of *E/Z* diastereoisomers in a ratio 2.3:1 in 55% yield, as described previously.[6] After Boc-group removal, the corresponding α-amine could be isolated by preparative HPLC as the pure *E*-diastereoisomer ((*E*)-**4**)[7] and was used in further synthesis as shown in Scheme 2 (vide infra). It turned out that the right choice of protecting groups R^1 and R^2 was a determining factor for the successful incorporation of D-phenylglycine derivative **5**. It was found that the trimethylsilyl (TMS) moiety was inferior toward triisoproylsilyl (TIPS) as R^1 since TMS did not resist the acidic conditions required for Boc-

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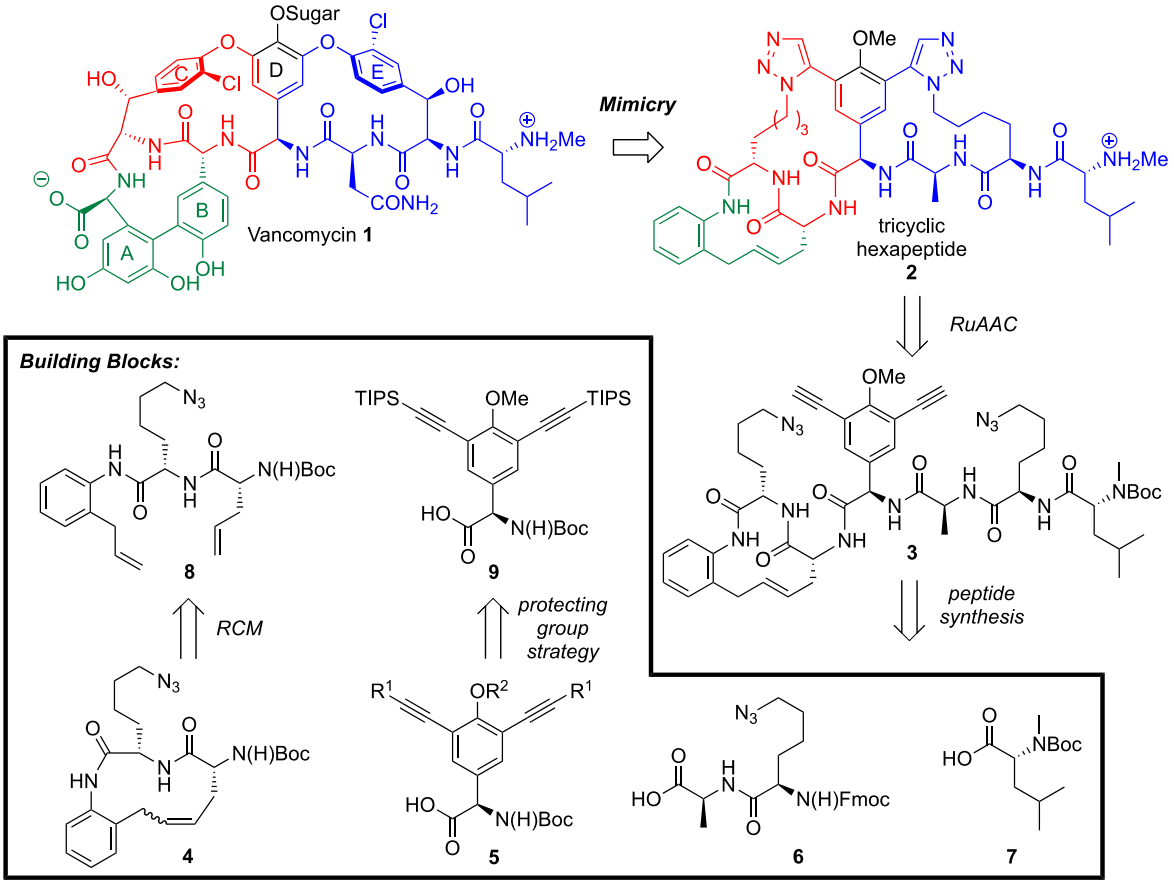
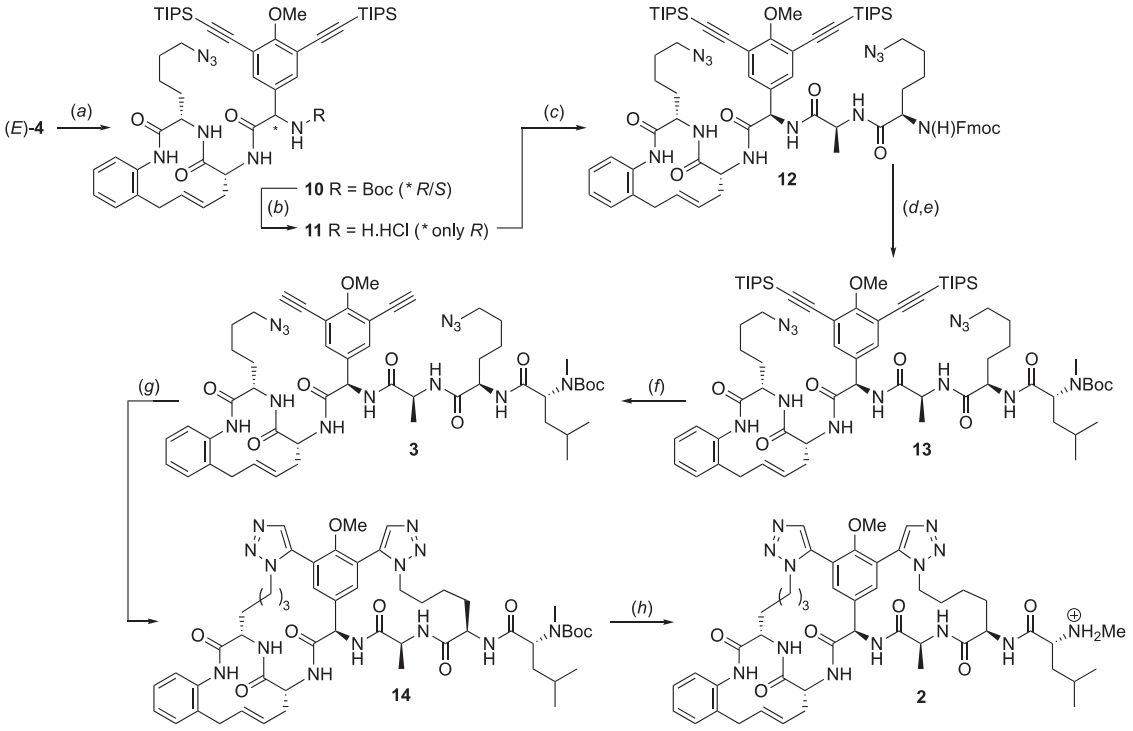


Scheme 1. Rationale for design and retrosynthetic analysis of the tricyclic hexapeptide **2**.



Scheme 2. Synthesis of tricyclic hexapeptide **2**. (*a*) **9**, DCC/HOAt, CH_2Cl_2, 2 h, 78%; (*b*) 3 M HCl/EtOAc, 1 h, 43% (only *R*-epimer); (*c*) **6**, EDCI/HOBt/DIPEA, CH_2Cl_2, 2 h, 60%; (*d*) $(CH_3)_2NH$/THF, 1 h, quant; (*e*) **7**, EDCI/HOBt/DIPEA, CH_2Cl_2, 2 h, 46%; (*f*) TBAF, THF, 1 h, 76%; (*g*) $[Cp^*RuCl]_4$, THF/MeOH (4:1 v/v), 80 °C, MW, 2 h, 16%; (*h*) TFA/CH_2Cl_2 (1:1 v/v), 1 h, quant.

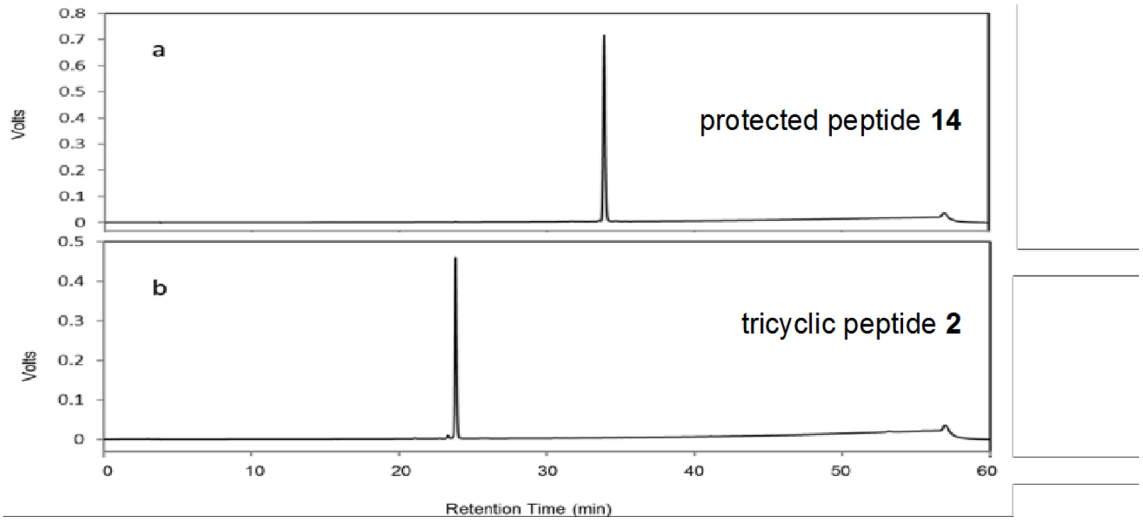


Fig. 1. HPLC traces of (a) protected tricyclic peptide **14**, and (b) title compound **2**.

group removal. Previously, a 4-acetoxy-D-phenylglycine derivative (R^2 = acetyl) was used in the synthesis, however, to avoid epimerization of the D-phenylglycine moiety after its incorporation in the peptide backbone, the stronger electron donating methoxy (R^2 = methyl) functionality was preferred to decrease the acidity of the α-proton (vide infra). These observations resulted in the design and synthesis of D-phenylglycine building block **9**[8] as shown in Scheme 1.

With these building blocks in hand, the linear assembly of hexapeptide **3** was started, as shown in Scheme 2. To minimize racemization of D-phenylglycine building block **9** during coupling with amine (*E*)-**4** three different combinations of coupling reagents were investigated.[9] A BOP/DIPEA-mediated coupling in CH_2Cl_2 resulted in a diastereomeric ratio of *S*,*R*,***R***:*S*,*R*,*S* = 1.3:1, as determined by LC-MS, while using DCC/HOAt,[10] this ratio could be slightly improved to 1.4:1. The coupling reagent 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-(3*H*)-one (DEPBT)[11] was reported to be superior to typical phosphonium and uronium-based coupling reagents in suppressing racemization during peptide fragment coupling. Unfortunately, however, applying DEPBT/Et_3N in the coupling reaction of **9**, a reasonable conversion could not be achieved.

Consequently, tripeptide **10** was isolated as a mixture of *R*/*S* diastereoisomers, and after treatment with 3 M HCl in EtOAc to remove the Boc group, the desired *R*-diastereoisomer **11** could be isolated by column chromatography in 43% yield.[12] In the next step, tripeptide amine **11** was coupled with Fmoc-D-Lys(N_3)-Ala-OH in presence of EDCI/HOBt/DIPEA and pentapeptide **12** was obtained in 60% yield. Gratifyingly, racemization of phenylglycine was absent likely due to the presence of the methoxy functionality as judged by LC-MS, which indicated that pentapeptide **12** consisted of a single diastereoisomer.[13] Next, the Fmoc group was removed by treatment with dimethylamine, which was followed by an EDCI/HOBt-mediated coupling of Boc-*N*-Me-D-Leu-OH (**7**) in CH_2Cl_2 to give fully protected hexapeptide **13** in 46% yield over two steps.[14] Finally, both TIPS groups were removed by treatment with TBAF in THF and unprotected bisalkyne **3** was obtained in 76% yield.[15].

The last crucial step was the formation of both triazole moieties starting with alkene-bridged hexapeptide **3** by a RuAAC reaction. As the first attempt the intramolecular RuAAC macrocyclization was performed with 5 mol% $[Cp^*RuCl]_4$ in THF/MeOH (4:1 v/v) for 2 h at 80 °C under microwave irradiation.[16] This catalyst was most optimal and sufficiently robust for our purpose. This catalyst was most optimal and sufficiently robust for our purpose. Three new peaks were identified by HPLC[17] and according to LC-MS these three peaks had the same mass as the precursor peptide **3** (R_t 43.02 min) which indicated that macrocyclization into the desired tricyclic compound **14** (R_t 32.97 min) had occurred and that two bicyclic compounds appeared as cyclization intermediates (mono-triazole intermediates R_t 38.25 and 39.15 min, respectively). Optimization by increasing the loading of catalyst to 10 mol%, led to an increasing amount of tricyclic hexapeptide as was shown by HPLC, while the cyclization precursor **3** and one of the bicyclic intermediates were almost absent. At a catalyst loading of 20 mol%, the bicyclization was almost complete and the desired tricyclic compound **14** was isolated by preparative HPLC in a yield of 16%.[18] After the removal of the Boc-group by treatment with TFA, the desired unprotected tricyclic hexapeptide **2** was obtained quantitatively in high purity as shown in Fig. 1.

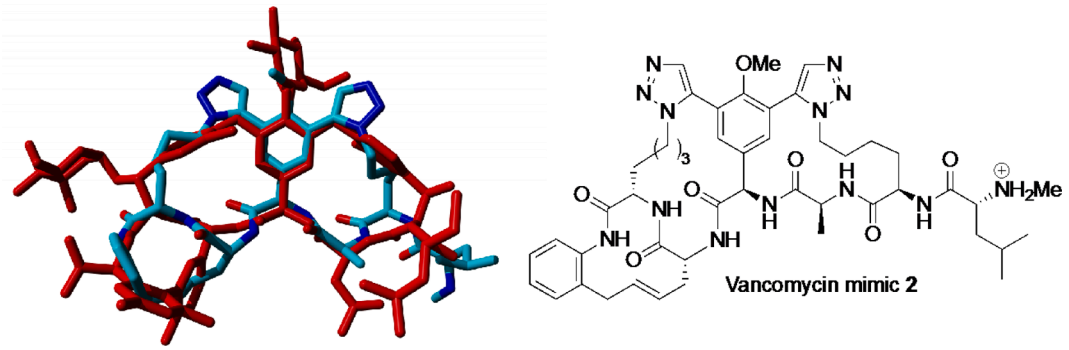


Fig. 2. Superimposition of balhimycin (red) with tricyclic hexapeptide **2**. The carbon atoms αC[1], αC[2], αC[3], αC[4], αC[5], benzylic-C[6], triazole-C[7] and -C[8] have been use as fixed coordinates for superimposition.

Table 1
The binding affinities of cell wall moieties by tricyclic mimic **2** compared to vancomycin and the MIC values of vancomycin and mimic **2**.

Compound	Ligand	K_a (M^{-1}[a]	MIC (μg/mL)[b]
Vancomycin	Ac-Lys(Ac)-D-Ala-D-Ala-OH	$(4.23 \pm 0.46) \times 10^5$	2
Vancomycin	Ac-Lys(Ac)-D-Ala-D-Lac-OH	$(2.59 \pm 0.41) \times 10^3$	
Mimic **2**	Ac-Lys(Ac)-D-Ala-D-Ala-OH	$(1.26 \pm 0.24) \times 10^4$	37.5
Mimic **2**	Ac-Lys(Ac)-D-Ala-D-Lac-OH	$(3.28 \pm 0.31) \times 10^3$	

[a]Measured in a Na-citrate/citric acid buffer (0.02 M, pH 5.1) in the presence of 5 vol% DMSO. [b] Minimal Inhibitory Concentration as obtained from a growth inhibition assay.

This unprotected tricyclic peptide was characterized by ^{1}H, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC in combination with HRMS, and was identified as the desired tricyclic peptide **2**.[19] Both unique protons of the 1,5-disubstituted-1*H*-1,2,3-triazoles could be assigned as δ_H 7.93 and δ_H 7.89 ppm with corresponding ^{13}C shifts δ_C 134.26 ppm and δ_C 133.80 ppm, which was in agreement with the literature.[20].

After energy minimization using the simulated annealing protocol employing the AMBER99 force field using the YASARA Structure 10.5.2.1 software package,[21] the tricyclic peptide **2** was superimposed on the whole crystal structure of vancomycin-related balhimycin antibiotic.[22] This superimposition indicated a high structure resemblance, with a RMSD of 1.0074 Å over eight atoms, between the tricyclic peptide backbone and balhimycin (Fig. 2).

Isothermal microcalorimetry (ITC)[23] was used to determine the binding affinities toward the natural ligand and to evaluate to what extent tricyclic peptide **2** was able to mimic vancomycin. As shown in Table 1, the binding affinities of this tricyclic peptide toward Ac-Lys(Ac)-D-Ala-D-Ala-OH was only circa 30-fold less compared to vancomycin while the binding by CDE[4] and ABC-ring[6] mimics was 100-fold less than vancomycin. The improvement of binding affinity was in agreement with the increased rigidity of the newly designed mimic and -as we expected- also favorable for its bioactivity To investigate this bioactivity of the tricyclic mimic **2**, the minimum inhibitory concentration (MIC) was evaluated against vancomycin sensitive bacterium *Staphylococcus aureus* (ATCC 49320) using an *in vitro* assay.[24] Fortunately, in line with the result of the ITC measurements, the tricyclic mimic **2** displayed a promising antibacterial activity corresponding to a MIC value of 37.5 μg/mL (the MIC value of vancomycin was 2 μg/mL as reference), a strong indication that the all three rings contributed to the antimicrobial activity.

In conclusion, the tricyclic hexapeptide **2** was designed to mimic the vancomycin cavity more closely. It was successfully synthesized following an RCM-coupling-RuAAC strategy. This newly synthesized mimic showed a good structural resemblance compared to balhymicin, a structural analog of vancomycin. Based on the binding affinity and antibacterial activity measurements, tricyclic mimic **2** was able to bind the D-Ala-D-Ala dipeptide motif by hydrogen bond formation and the highest antimicrobial activity was observed within this series of vancomycin mimics. Via this RCM-coupling-RuAAC strategy, it is expected that the synthesis of several tricyclic analogues will be possible in which the overall rigidity can be increased to form a more constrained shell-like topology as found in the native vancomycin molecule.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2022.128887.

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6 Yang X, Beroske LP, Kemmink J, Rijkers DTS, Liskamp RMJ. *Tetrahedon Lett*. 2017;58:4542.
7 The pure *E*-diastereoisomer ((*E*)-**4**) was isolated as a white solid. R_f 0.57 (CH_2Cl_2/MeOH, 95:5 v/v with 0.2% TEA); $[\alpha]_{20}^{D}$ = –92.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.98 (d, *J* = 6.9 Hz, 1H), 8.47 (s, 1H), 8.26-8.24 (m, 2H), 7.21-7.17 (m, 2H), 6.98 (t, *J* = 7.4 Hz, 1H), 5.79 (t, *J* = 12.9 Hz, 1H), 5.37-5.28 (m, 1H), 3.92 (dd, *J* = 10.9 Hz, 5.6 Hz, 1H), 3.78-3.74 (m, 1H), 3.62-3.57 (m, 2H), 2.63-2.60 (m, 1H), 2.22-2.15 (m, 2H), 2.01-1.94 (m, 3H), 1.60-1.48 (m, 2H), 1.45-1.38 (m, 1H), 1.36-1.30 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 156.4, 153.7, 150.3, 149.3, 147.7, 142.8, 84.2, 74.3, 63.0, 62.9, 60.5, 55.0, 54.2, 53.7, 51.5, 50.1; HRMS *m/z* calcd for $C_{18}H_{24}N_6NaO_2$ $[M + Na]^+$ 379.1858, found 379.1876.
8 Analytical data of bisalkyne **9**: R_f 0.48 (PE/EtOAc, 1:1 v/v with 0.5% AcOH); $[\alpha]_{20}^{D}$ = –10.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.79 (s, 1H), 7.41 (s, 2H), 5.48 (d, *J* = 5.6 Hz, 0.5H (NH)), 5.22 (d, *J* = 5.6 Hz, 0.5H (NH)), 5.00 (d, *J* = 5.6 Hz, 1H), 3.99 (s, 3H), 1.42 (s, 9H), 1.11 (s, 42H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 173.0, 162.3, 157.0, 133.5, 133.4, 133.1, 117.9, 101.8, 95.9, 82.4, 61.1, 58.1, 28.0, 18.6, 11.3; HRMS *m/z* calcd for $C_{36}H_{59}NNaO_5Si_2$ $[M + Na]^+$ 664.3829, found 664.3825.
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11 Li H, Jiang X, Ye Y, Fan C, Romoff T, Goodman M. *Org Lett*. 1999;1:91.
12 Analytical data of *R*-diastereomer **11**: R_f 0.5 (CH_2Cl_2/MeOH, 95:5 v/v with 0.3% AcOH); $[\alpha]_{20}^{D}$ = –62.3 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.42 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.07 (s, 1H), 7.48 (s, 1H), 7.20 (td, *J* = 7.9 Hz, *J* = 1.7 Hz, 1H), 7.06-6.96 (m, 2H), 6.27 (s, 1H), 5.98-5.91 (m, 1H), 5.71-5.64 (m, 1H), 4.63 (s, 1H), 4.31 (q, *J* = 4.9 Hz, 1H), 4.23-4.17 (m, 1H), 3.99 (s, 3H), 3.48 (d, *J* = 7.1 Hz, 2H), 3.12-3.09 (m, 2H), 2.80-2.74 (m, 1H), 2.54-2.50 (m, 1H), 2.06-1.98 (m, 2H), 1.50-1.31 (m, 4H), 1.07 (s. 42H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 172.6, 171.2, 168.9, 162.9, 137.5, 134.2, 131.8, 130.5, 127.9, 126.4, 124.0, 120.6, 118.8, 101.3, 97.4, 61.1, 60.4, 58.4, 54.8, 51.1, 38.3, 34.8, 29.8, 27.8, 23.0, 21.0, 18.6, 18.2, 18.0,

14.2, 11.7, 11.5, 11.3, 11.0; HRMS *m/z* calcd for $C_{49}H_{74}N_7O_4Si_2$ $[M + H]^+$ 880.5341, found 880.5332.

13 Analytical data pentapeptide **12**: R_f 0.47 (CH_2Cl_2/MeOH, 95:5 v/v); $[\alpha]_{20}^{D}$ = –23.8 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 8.66 (d, *J* = 5.0 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H), 8.54 (s, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.11 (s, 1H), 7.97 (d, *J* = 7.4 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.58-7.54 (m, 4H), 7.41-7.33 (m, 3H), 7.26-7.15 (m, 4H), 6.96 (td, *J* = 7.4 Hz, *J* = 1.3 Hz, 1H), 6.06-5.99 (m, 1H), 5.48 (d, *J* = 7.8 Hz, 1H), 5.38-5.31 (m, 1H), 4.42 (q, *J* = 7.0 Hz, 1H), 4.26-4.14 (m, 3H), 4.04 (d, *J* = 5.2 Hz, 1H), 3.99-3.90 (m, 2H), 3.84 (s, 3H), 3.47 (d, *J* = 7.4, 2H), 3.27-3.22 (m, 4H), 2.40-2.22 (m, 2H), 1.94-1.85 (m, 2H), 1.80-1.78(m, 2H), 1.59-1.40 (m, 6H), 1.35-1.22 (m, 2H), 1.18 (d, *J* = 7.0 Hz, 3H), 1.04 (s. 42H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ = 172.1, 171.8, 171.6, 170.0, 169.9, 162.3, 156.4, 144.4, 144.1, 141.1, 138.0, 134.7, 133.7, 131.9, 131.2, 128.0, 127.8, 127.7, 127.4, 125.7, 125.6, 123.9, 120.5, 120.0, 117.4, 105.0, 102.4, 96.0, 66.1, 61.3, 55.4, 54.9, 50.9, 48.2, 47.1, 31.8, 28.3, 27.9, 23.1, 18.9, 11.1; HRMS *m/z* calcd for $C_{73}H_{99}N_{12}O_8Si_2$ $[M + H]^+$ 1327.7247, found 1327.7242; calcd for $C_{73}H_{98}N_{12}NaO_8Si_2$ $[M + Na]^+$ 1349.7069, found 1349.7060.

14 Analytical data hexapeptide **13**: R_f 0.6 (CH_2Cl_2/MeOH, 95:5 v/v); $[\alpha]_{20}^{D}$ = –87.5 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 8.90 (d, *J* = 3.2 Hz, 1H), 8.78 (d, *J* = 12.6 Hz, 1H), 8.55 (s, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.86 (s, 1H), 7.67 (s, 1H), 7.50 (s, 2H), 7.20-7.16 (m, 2H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.10-6.04 (m, 1H), 5.46 (d, *J* = 6.9 Hz, 1H), 5.42-5.35 (m, 1H), 4.60-4.56 (m, 1H), 4.45-4.43 (m, 2H), 4.23-4.19 (m, 2H), 3.87 (s, 3H), 3.48-3.47 (m, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 3.05-2.92 (m, 3H), 2.64 (s, 3H), 2.41-2.36 (m, 2H), 1.83-1.79 (m, 2H), 1.77-1.43 (m, 11H), 1.37 (s, 9H), 1.15 (d, *J* = 6.9 Hz, 3H), 0.95-0.85 (m, 8H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ = 172.5, 171.6, 171.5, 171.2, 170.7, 169.7, 169.6, 166.4, 162.0, 138.0, 134.7, 133.1, 132.0, 131.2, 128.9, 127.8, 123.9, 119.7, 117.3, 102.5, 95.8, 79.5, 61.2, 60.2, 55.3, 54.8, 52.8, 51.0, 50.9, 37.6, 31.9, 28.4, 28.2, 27.7, 24.9, 22.8, 21.2, 18.9, 14.5, 11.2; HRMS *m/z* calcd for $C_{70}H_{110}N_{13}O_9Si_2$ $[M + H]^+$ 1332.8088, found 1332.8097; calcd for $C_{70}H_{109}N_{13}NaO_9Si_2$ $[M + Na]^+$ 1354.7909, found 1354.7917.

15 Analytical data of bisalkyne **3**: R_f 0.45 (CH_2Cl_2/MeOH, 95:5 v/v); $[\alpha]_{20}^{D}$ = –57.3 (*c* 1.0, $CHCl_3$/MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 8.86 (s, 1H), 8.67 (s, 1H), 8.55 (s, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 8.05 (s, 1H), 7.90 (s, 1H), 7.70-7.66 (m, 1H), 7.51 (s, 2H), 7.20-7.15 (m, 2H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.10-6.04 (m, 1H), 5.46 (d, *J* = 6.9 Hz, 1H), 5.39-5.32 (m, 1H), 4.62-4.56 (m, 1H), 4.46-4.37 (m, 4H), 4.21-4.17 (m, 2H), 3.84 (s, 3H), 3.57-3.42 (m, 2H), 3.22 (t, *J* = 6.6 Hz, 2H), 3.14-3.07 (m, 2H), 2.63 (s, 3H), 2.40-2.29 (m, 2H), 1.83-1.73 (m, 2H), 1.66-1.41 (m, 7H), 1.37 (s, 9H), 1.15 (d, *J* = 6.9 Hz, 3H), 1.05-0.85 (m. 8H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ = 172.5, 171.7, 171.6, 170.1, 169.8, 162.1, 138.0, 134.4, 133.6, 131.9, 131.2, 128.7, 127.9, 127.7, 124.0, 123.9, 111.9, 116.4, 86.0, 79.4, 61.3, 55.3, 52.8, 50.9, 49.0, 37.4, 34.9, 31.9, 30.2, 28.4, 28.2, 27.8, 25.0, 22.9; HRMS *m/z* calcd for $C_{52}H_{70}N_{13}O_9$ $[M + H]^+$ 1020.5419, found 1020.5416.

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17 Analytical HPLC was performed on an automated HPLC system equipped with a UV/Vis detector operating at 220/254 nm and an evaporative light scattering detector using a C18 column (pore size: 100 Å, particle size: 5 μm; 250 × 4.6 mm) at a flow rate of 1 mL/min using a linear gradient of 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 50 min. Preparative RP-HPLC was performed on an automated preparative HPLC system equipped with a UV/Vis detector operating at 214 nm using a C18 column (pore size: 100 Å, particle size: 10 μm; 250 × 22 mm) at a flow rate of 12.0 mL/min using a linear gradient of 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 50 min.

18 Characterized as the tricyclic hexapeptide **14**: HRMS *m/z* calcd for $C_{52}H_{70}N_{13}O_9$ $[M + H]^+$ 1020.5419, found 1020.5415; calcd for $C_{52}H_{69}N_{13}NaO_9$ $[M + Na]^+$ 1042.5239, found 1042.5238.

19 Analytical data of tricyclic hexapeptide **2**: ^{1}H-NMR (500 MHz, DMSO-d_6) δ = 8.94 (s, 1H), 8.92 (s, 1H), 8.83 (s, 1H), 8.82 (s, 1H), 8.43 (s, 1H), 8.38(d, *J* = 7.9 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.94 (s, 1H), 7.90 (s, 1H), 7.52 (s, 1H), 7.48 (s, 1H), 7.24-7.20 (m, 2H), 7.00 (t, *J* = 7.5 Hz, 1H), 5.67 (d, *J* = 7.9 Hz, 1H), 5.43-5.39 (m, 1H), 4.44 (s, 1H), 4.17-4.04 (m, 6H), 3.77 (s, 1H), 3.50 (s, 2H), 3.18 (m, 3H), 3.02 (s, 1H), 2.48 (s, 3H), 2.37-2.08 (m, 2H), 1.79-1.49 (m, 8H), 1.30 (d, *J* = 6.9 Hz, 3H), 1.24-1.11 (m. 5H), 0.99-0.82 (m, 8H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 134.3, 133.8, 132.9, 132.6, 132.3, 131.4, 128.0, 124.0, 119.8, 61.5, 59.6, 56.4, 55.6, 55.1, 54.2, 51.6, 48.2, 48.1, 44.4, 34.3, 31.8, 31.1, 30.4, 30.2, 29.5, 29.2, 25.1, 24.4, 23.3, 22.9, 22.4, 22.8, 22.3,17.5, 14.1; HRMS *m/z* calcd for $C_{47}H_{62}N_{13}O_7$ $[M + H]^+$ 920.4890, found 920.4904.

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21 Modeling has been performed with using the YASARA software: www.yasara.org.

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