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Synthesis of Alkyne-Bridged Cyclic Tripeptides toward Constrained Mimics of Vancomycin

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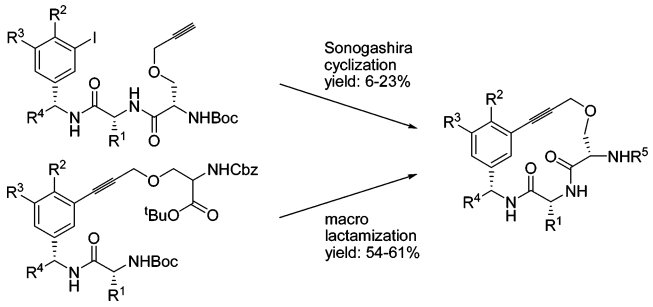

The synthesis of a range of highly constrained cyclic tripeptides has been performed using either an intramolecular Sonogashira coupling or a macrolactamization as the final ring-closing reaction. Our approach gives access to rigidified 15-membered peptidic macrocycles based on the central ring system of vancomycin. Tripeptides **3a**−**c** and dipeptide **11** were cyclized via an intramolecular Sonogashira reaction, and the cyclic peptides **4a**−**c** and **15a** were obtained in 6−23% yield. In contrast, macrolactamization of **12** and **17** resulted in the desired peptidic macrocycles **15b** and **18** with 54−61% yield. Modeling studies hint at a distorted triple bond, which explains the low yield of the Sonogashira-based cyclization. Moreover, modeling data also showed that this class of peptidic macrocycles formed a cavity-like structure in which guest molecules may bind.

Introduction

Incorporation of covalent constraints into bioactive peptides is an important design consideration to reduce the unfavorable entropy loss upon receptor-binding.[1] The resulting reduction of conformational flexibility is important to increase the affinity of the peptide for its natural receptor.[2,3] In addition, covalent constraints also play a decisive role in controlling the three-dimensional structure of a molecule, e.g., forming cavity or shell-like structures which are capable of binding other (small) molecules. In Nature many examples of covalent constraints are known, e.g., disulfide bridges, thioether bridges, lactone and lactam bridges, or biaryl ether bridges. An outstanding example of a compound having the latter constraint is the peptide antibiotic vancomycin (Figure 1).[4,5]

There is a growing interest in the development of novel synthetic methodology for conformational restriction of peptides to mimic the bioactive conformation as closely as possible. Ring-closing metathesis (RCM) has often been used for this purpose since it displays an extraordinary functional group tolerance and high yield of cyclization.[6] RCM has been used in our group previously[7,8] and more recently to synthesize pentapeptides to mimic the central ring system of vancomycin.[9] Some other C−C coupling strategies for constraining peptides from the literature include, e.g., a Suzuki coupling between two peptide fragments followed by macrolactamization[10,11] and peptide cyclizations featuring a Heck,[12,13] Suzuki,[14] Stille,[15] or Sonogashira[12b,16] reaction.

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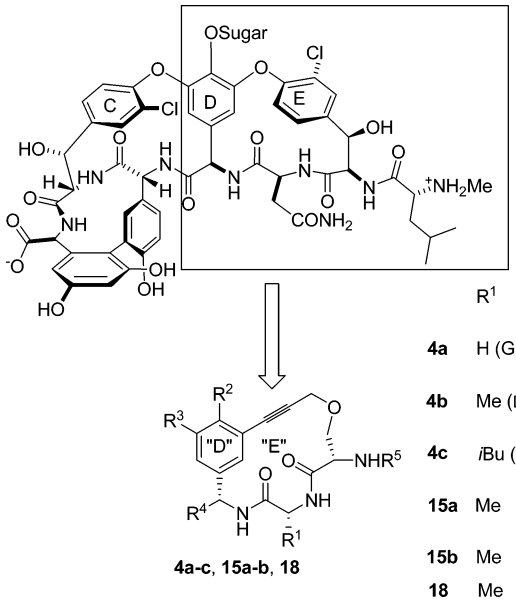

	R^1	R^2	R^3	R^4	R^5
4a	H (Gly)	OMe	I	COOMe	Boc
4b	Me (D-Ala)	OMe	I	COOMe	Boc
4c	*i*Bu (D-Leu)	OMe	I	COOMe	Boc
15a	Me	H	H	H	Boc
15b	Me	H	H	H	Cbz
18	Me	OMe	I	COOMe	Cbz

FIGURE 1. Target macrocyclic peptides inspired by the vancomycin DE ring system.

Here we describe the synthesis of highly constrained tripeptides in which an alkyne moiety is used as a cyclic constraint (Figure 1). To accomplish this goal, two approaches of tripeptide cyclization have been developed. Our first approach comprises an *intra*molecular Sonogashira reaction as the cyclization step. As an alternative, an *inter*molecular Sonogashira coupling which is followed by an *intra*molecular amide bond formation has been developed.

Similar to the ring-closing metathesis reaction, the Sonogashira coupling reaction displays also a broad functional group tolerance.[17−22] However, in contrast to an RCM-based cyclization, the Sonogashira coupling enables the stereocontrol of the newly formed cyclic system, since a triple bond can be selectively reduced to either the *E*- or *Z*-isomer.[23−25] Additionally, partial or complete reduction of the triple bond gradually releases the ring strain of the cyclic peptide. Such conformationally relaxed structures can be used to probe conformation−bioactivity relationships.

Results and Discussion

The retrosynthetic analysis of the cyclic tripeptides is depicted in Scheme 1. Three different ways of cyclization are possible: a linear tripeptide is cyclized via a Sonogashira coupling in which a new C−C bond will be formed (route I), or two amide bond formation reactions in which either a benzylic amine (route IIa) or an aliphatic amine (route IIb) forms the point of

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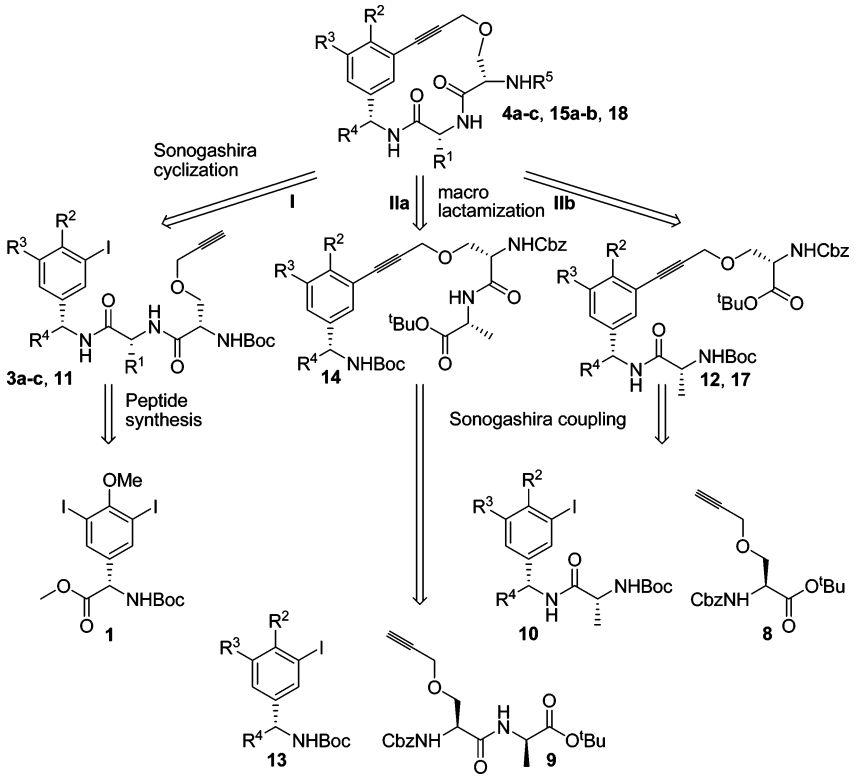
SCHEME 1. Retrosynthetic Analysis of the Peptide Macrocycles



cyclization. First, route I was explored since it did not have special requirements with respect to the choice of protecting groups (vide infra).

The syntheses started with the conversion of 4-hydroxyphenylglycine into the corresponding Boc-protected amino acid methyl ester using standard protection protocols[26] (88% over two steps). Then, iodonation was performed analogously to the method of Nishiyama et al.[27] with *N*-iodosuccinimide in acetone in 80% yield. It was found necessary to methylate the hydroxy group using MeI in the presence of K_2CO_3 to yield diiodo compound **1**. Not only did this decrease the light sensitivity of **1** but, more importantly, the electron-donating character of the methoxy functionality increased the coupling yield of the Sonogashira reaction (data not shown). The basic conditions of this reaction did not influence the chiral integrity of the phenylglycine residue, since the optical rotation of Boc-3,5-diiodo-4-hydroxyphenylglycine methyl ester was not affected by treatment with K_2CO_3 in acetone.

After removal of the Boc group by treatment with hydrochloric acid in diethyl ether, the corresponding hydrochloride of **1** was coupled with BOP/HOBt/DIPEA to Boc-Gly-OH, Boc-D-Ala-OH, and Boc-D-Leu-OH, respectively, and dipeptides **2a**−**c** were obtained in good overall yields (Scheme 2). Next, the precursors for the Sonogashira cyclization, **3a**−**c**, were obtained by coupling of the N-terminally deprotected dipeptides **2a**−**c** with BOP to alkyne **7a**. The latter compound was synthesized from Boc-Ser-OH/NaH and propargyl bromide analogously to the method of Sugano and Miyoshi[28a] (Scheme 3).

Optimization of the ring-closing conditions leading to **4a**−**c** is shown in Table 1. The first Sonogashira reactions (entries 1−3) were carried out in 1,1,2-trichloroethane (TCE) as solvent with 10 mol % $Pd(Ph_3P)_4$/CuI as the catalytic system in the presence of triethylamine (5 equiv) as a base at room temperature. According to TLC, the linear precursors were absent after 16 h. However, the cyclic products **4a**−**c** were obtained in low yields ranging from 6% (**4a**) to 23% for **4b**. Moreover, a linear diyne, as a byproduct, with general structure **5** (Figure 2) could also be isolated (**5b**, 24%; **5c**, 20%).[29] When the Sonogashira ring closure was carried out using a more diluted reaction mixture, a higher product yield was not obtained, but indeed a decreased amount of diyne **5c** was formed (entry 4).

Our experiences with Sonogashira-based dendrimer syntheses[30] made us decide to change the solvent from TCE to CH_3-CN and to run the cyclization reaction at a concentration of 10 mM (entries 5 and 6). Unfortunately, the yields of **4b,c** did not improve, but the precursors could be recovered by column chromatography.

Longer reaction times (16 → 36 h), increasing the amount of catalyst (10 → 50 mol %), running the cyclization at a higher dilution (10 → 0.5 mM), and the application of a different Pd

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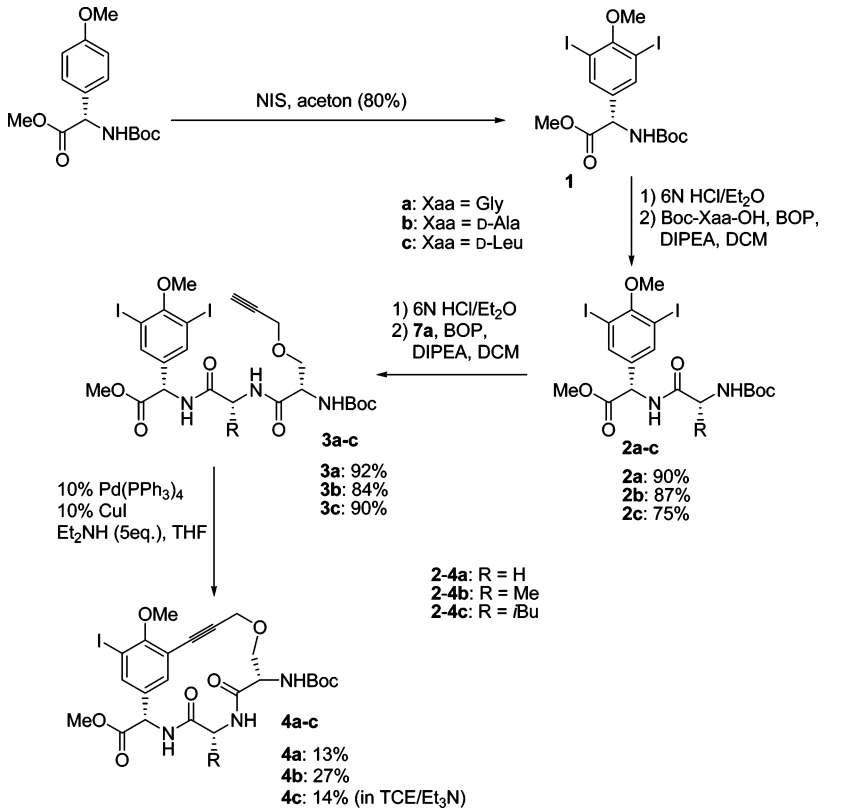
SCHEME 2. Synthesis of the Peptide Macrocycles via the Sonogashira Ring Closure



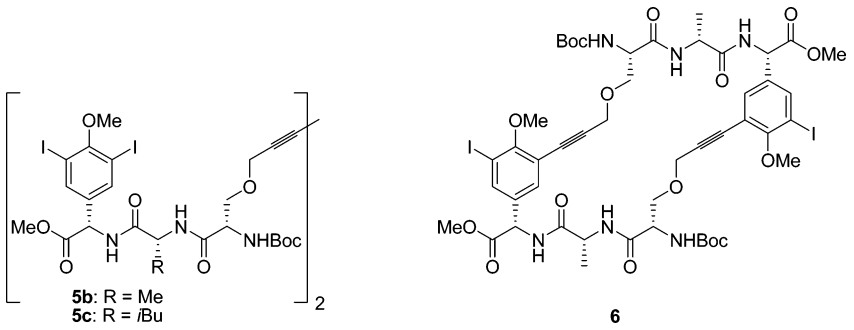
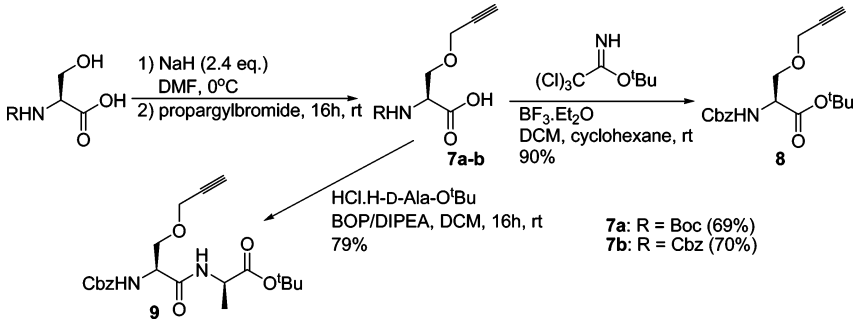


FIGURE 2. Byproducts obtained in the Sonogashira ring-closure reaction.

SCHEME 3. Synthesis of the Serine(*O*-propynyl) Derivatives



source ($Pd(Ph_3P)_2Cl_2$) were also not successful for increasing the yields of the Sonogashira ring-closing reaction.

In the literature several high-yielding Sonogashira reactions have been described with THF as solvent.[31,32] Moreover, it was also reported that THF prevented diyne formation.[32] Therefore, it was decided to use THF and the first attempts also showed that triethylamine as a base had to be replaced by diethylamine to obtain any cyclic product (entries 7 and 8). Under these conditions **4b** was obtained in 27% yield. However, formation of byproducts could not be prevented, as was apparent from isolation of cyclic dimer **6** in 26% yield.[29a]

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SCHEME 4. Synthesis of Macrocycle 15

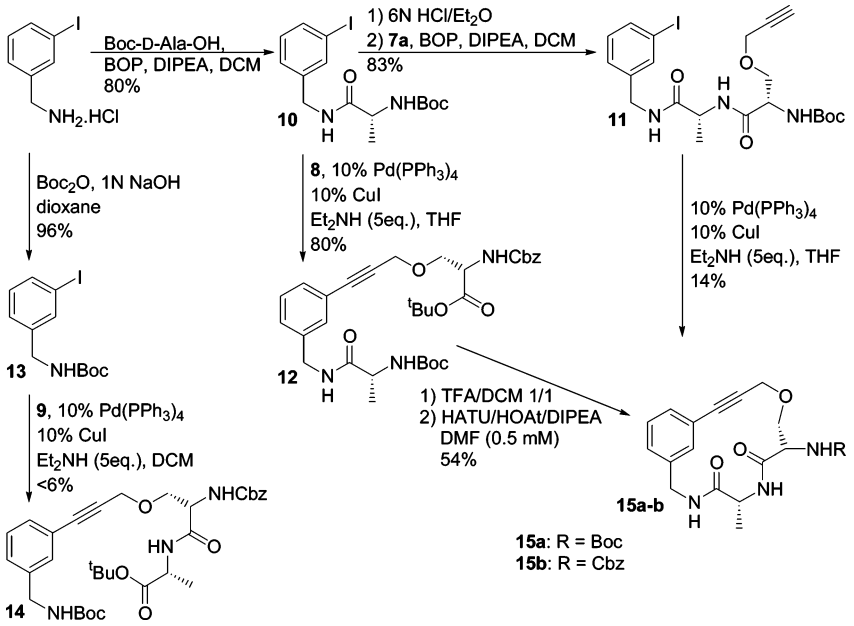

TABLE 1. Conditions for the Sonogashira Cyclization Reaction[a]

entry	precursor	concn, mM	solvent	base	product (yield, %)	byproduct[b] (yield, %)
1	**3a**	10	TCE	Et_3N	**4a** (6) [**3a** (2)][c]	
2	**3b**	15	TCE	Et_3N	**4b** (23)	**5b** (24)
3	**3c**	15	TCE	Et_3N	**4c** (14)	**5c** (20)
4	**3c**	10	TCE	Et_3N	**4c** (14)	**5c** (8)
5	**3b**	10	MeCN	Et_3N	**4b** (20) [**3b** (73)][c]	
6	**3c**	10	MeCN	Et_3N	**4c** (12) [**3c** (32)][c]	**5c** (17)
7	**3a**	10	THF	Et_2NH	**4a** (13) [**3a** (30)][c]	
8	**3b**	10	THF	Et_2NH	**4b** (27)	**6** (26)

[a] Reaction time 16 h, 10 mol % $Pd(Ph_3P)_4$/10 mol % CuI employed as the catalyst, 5 equiv of base used. [b] Byproducts were isolated by column chromatography and characterized by mass spectrometry and ^1H NMR. [c] Percent of recovered starting material.

The configuration of the chiral centers in the linear precursors was chosen as L, D, L. It is known that a D-amino acid is capable of inducing a bend in the peptide sequence. Our results showed (Table 1, entries 2, 5, and 8) that the best cyclization yield was achieved in the presence of the smallest D-amino acid, alanine (**3b**). Increasing steric bulk (leucine, **3c**) or the absence of a turn-inducing motif (glycine, **3a**) resulted in a lower cyclization yield.

A possible explanation of the low yield could also be the presence of the second iodine moiety, either electronically or sterically. Therefore, it was decided to synthesize dipeptide **11** (Scheme 4) to test this hypothesis. For this purpose, 3-iodobenzylamine was coupled to Boc-D-Ala-OH with BOP to give 3-iodobenzylamide **10**, and after removal of the Boc group by treatment with hydrochloric acid, **7a** was coupled to the corresponding hydrochloride with BOP/DIPEA to afford linear precursor **11** in 66% overall yield. The Sonogashira-based cyclization reaction was performed with the best reaction conditions as was found in previous experiments (entry 8, Table 1). After a reaction time of 16 h, the linear precursor was absent on TLC and two new spots were found and isolated. It turned out that cyclic product **15a** was formed in only 14% yield while a cyclic dimer similar to **16** (Figure 4)—having Boc instead of Cbz groups—was also present as was evidenced by ESI-MS.

Thus, not unexpectedly, the presence of the second iodine moiety was not responsible for the low yields of the Sonogashira-based macrocyclizations. More likely, the linear geometry of the triple bond hampers ring-closing. Indeed, preliminary modeling studies using MacroModel[33] of **4b** showed a distorted triple bond (Figure 3) in the resulting peptide macrocycle. The bond angle of the triple bond was ca. 170°, thus deviating considerably from linearity. As it turned out, such a strained 15-membered peptidic macrocycle was therefore difficult to synthesize, and led to formation of nonstrained byproducts **5** and **6** (Figure 2).

The conformational search for obtaining the global minimum of **4b** was carried out in chloroform, which was the solvent for recording its NMR spectrum. The observed ring-coupling constants and those calculated from the global minimum were in excellent agreement (Figure 3). This indicates that the conformation of this global minimum reflects the structure in solution.

As was shown in Scheme 1, in addition to approach I, two alternative approaches (IIa and IIb) are possible for the synthesis of the desired 15-membered peptidic macrocycles. Both routes feature an intermolecular Sonogashira reaction followed by a macrolactamization, with either a benzylic amine (approach IIa) or the peptide amine (approach IIb). Despite the outcome of modeling studies (vide supra) showing that a strained peptide macrocycle is obtained, the advantage of this approach might be that the Sonogashira reaction is carried out first, followed by a macrocyclization using a peptide coupling. Peptide couplings are among the most widely studied reactions for which many coupling reagents and conditions are available. The precursor molecules were designed in such a way that both protecting groups on the amine moiety (Boc) and on the carboxylic acid moiety (^{t}Bu) were removed simultaneously by a single acid treatment. The amino functionality that must be excluded from the reaction was protected by a Cbz group

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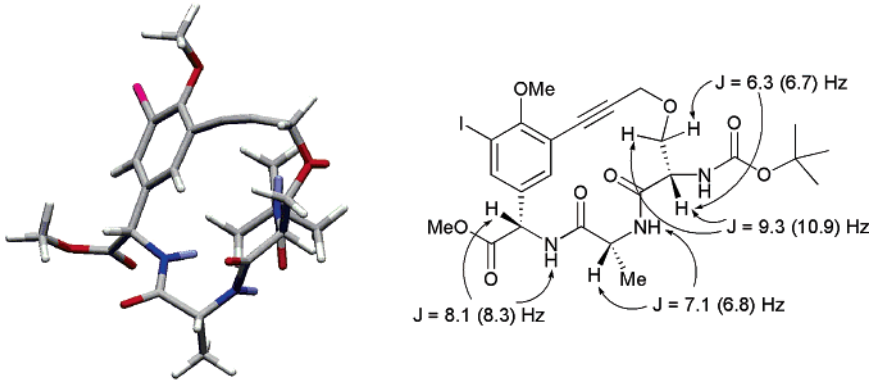
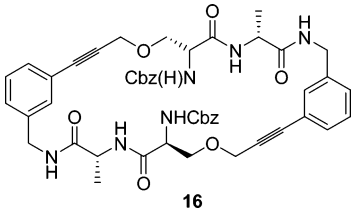


FIGURE 3. Representation of the lowest energy conformation of macrocycle **4b** and the observed and calculated (in parentheses) coupling constants.



16

FIGURE 4. Cyclic dimer **16** as a possible byproduct of macrolactamization of **12**.

TABLE 2. Conditions for the Macrolactamization[a]

Entry	concn, mM	**15b** yield, %	cyclic dimer **16**[b] yield, %
1	10	trace	95
2	5	8	79
3	1	44	50
4	0.5	54	not detected
5	0.3	10	not detected

[a] Reaction time 16 h, 1 equiv of HATU/HOAt, 2 equiv of DIPEA in DMF at rt. [b] Cyclic dimer **16** was characterized by mass spectrometry only.

(Scheme 4). Cbz-Ser-OH was converted into Cbz-Ser(*O*-propynyl)-OH (**7b**) analogously to **7a** (Scheme 3). The *tert*-butyl ester was introduced using *tert*-butyl 2,2,2-trichloroacetamidate/BF_3·etherate[34] in *tert*-butyl alcohol, since $POCl_3$/*tert*-butyl alcohol[35] gave unsatisfactory yields, and alkyne **8** was obtained in 90% yield.

First, approach IIa was attempted for the synthesis of macrocycle **15b** depicted in Scheme 4. For this purpose Boc-protected 3-iodobenzylamine (**13**) was coupled to dipeptide **9** (Scheme 3) via an intermolecular Sonogashira reaction. THF was replaced by DCM since iodo compound **13** did not dissolve in THF. Unfortunately, the coupling was very sluggish, and a yield of only 6% was obtained. Formation of coupling product **14** could only be confirmed by mass spectrometry.

This route was abandoned in favor of approach IIb, and iodo compound **10** was coupled with alkyne **8** to give linear dipeptide **12** (80%). Subsequently, dipeptide **12** was treated with acid to remove the Boc/^{t}Bu protecting groups and subjected to an HATU/HOAt-based macrolactamization at different concentrations (Table 2). The cyclic dimer **16** (Figure 4) was observed using a concentration of the deprotected starting material **12** of as low as 1 mM. Gratifyingly, at a concentration of 0.5 mM in DMF, **15b** was obtained in 54% yield, which was a significant improvement with respect to the Sonogashira-induced macrocyclization (14%), and **16** was not isolated.

Indeed, an intermolecular Sonogashira coupling followed by a macrolactamization via the peptide amine was the best approach for the synthesis of alkyne-bridged cyclic tripeptides. These cyclic tripeptides represent mimics of the right-half macrocycle of vancomycin (Figure 1). To ultimately realize the synthesis of a bicyclic system, a double Sonogashira reaction is needed. For initial approaches toward the bicyclic multiple side chain knotted framework, macrocycle **18** was synthesized (Scheme 5), which is a versatile starting compound to construct the left macrocycle mimic of vancomycin (Figure 5).

To this end, diiodo compound **2b** was coupled to alkyne **8** and linear precursor **17** was obtained in an isolated yield of 34%. It should be mentioned that the stoichiometry of the catalyst, the alkyne derivative, and the diiodo compound is an important factor to avoid diaddition of the alkyne and was found to be catalyst:**8**:**2b** = 0.1:1.5:1. After removal of both protecting groups, cyclization was performed with the same reaction conditions as described for **15**, and cyclic tripeptide **18** was obtained in 61% yield. Unexpectedly, purification of tripeptide **18** was rather difficult since it was poorly soluble in most organic solvents.

In conclusion, we designed two synthetic approaches for the preparation of alkyne-bridged cyclic tripeptides. It was found that an intramolecular Sonogashira reaction followed by a macrolactamization gave the best results to obtain these highly constrained 15-membered peptidic macrocycles. In this way a mimic of the right half of the vancomycin cavity was obtained. Incorporation of a triple bond opens an avenue to a diversity of subsequent compounds accessible by, for example, click chemistry, selective reduction, oxidation, etc. In addition, the use of diiodotyrosine (cf. the preparation of **18**) may provide an entry into a constrained mimic of vancomycin's carboxylate-binding pocket, which is under investigation.

Experimental Section

(*S*)-*N*-(*tert*-Butyloxycarbonyl)-3,5-diiodo-4-methoxyphenylglycine Methyl Ester (1). This compound was synthesized on the basis of a procedure described in the literature.[27] (*S*)-*N*-(*tert*-Butyloxycarbonyl)-4-methoxyphenylglycine methyl ester (10.6 g, 35.8 mmol) was dissolved in acetone (400 mL). The solution was cooled to −79 °C, and the reaction mixture was protected from light by aluminum foil. To this mixture was added a solution of *N*-iodosuccinimide (17.7 g, 78.8 mmol) in acetone (100 mL) dropwise over 5 h at −79 °C. After the mixture was stirred overnight at rt, the reaction was complete according to TLC and

(35) Taschner, E.; Biernat, J. F.; Rzeszotarska, B.; Wasielewski, C. *Liebigs Ann. Chem.* **1961**, *646*, 123.

SCHEME 5. Synthesis of Macrocycle 18

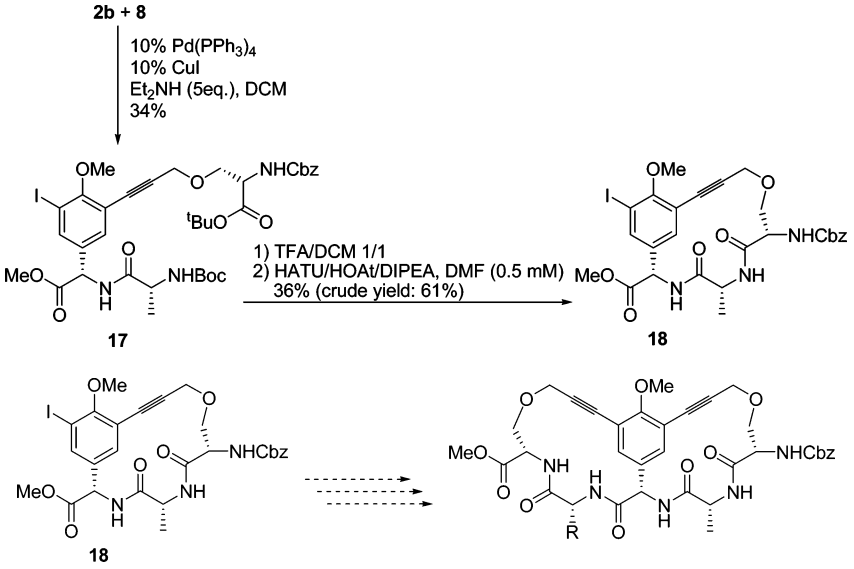


FIGURE 5. Future extension of macrocycle **18** to a bicyclic side chain knotted pentapeptide representing a constrained mimic of vancomycin's carboxylate-binding pocket.

the solvent was evaporated in vacuo. The residue was dissolved in EtOAc, washed with a saturated solution of $Na_2S_2O_3$, H_2O, and brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure, and diiodo compound **1** was obtained as a white solid after purification by column chromatography (EtOAc/hexane, 1:4, v/v) in 80% yield (15.7 g): R_f(EtOAc/hexane, 1:4, v/v) 0.50; mp 167 °C; $[\alpha]^{20}_D$ +88.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 2H), 5.77 (d, 1H, J = 6.3 Hz), 5.16 (d, 1H, J = 6.6 Hz), 3.75 (s, 3H), 3.67 (s, 3H), 1.35 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.1, 53.0, 55.4, 60.5, 80.3, 90.7, 136.5, 138.2, 154.4, 158.6, 170.4; HRMS (TOF ES$^+$) *m*/*z* 569.9236 [M + Na]$^+$, calcd for $C_{15}H_{19}I_2NO_5Na^+$ 569.9250. Anal. Calcd for $C_{15}H_{19}I_2NO_5$: C, 32.93; H, 3.50; N, 2.56. Found: C, 33.07; H, 3.46, N, 2.47.

***N*-(*tert*-Butyloxycarbonyl)-glycyl-(*S*)-3,5-diiodo-4-methoxyphenylglycine Methyl Ester (2a).** To remove the Boc group, methyl ester **1** (1.20 g, 2.2 mmol) was dissolved in DCM (10 mL), then 6 N HCl/diethyl ether (30 mL) was added, and the mixture was stirred overnight at rt. The formed precipitate was isolated by filtration, washed with diethyl ether, and subsequently dissolved in DMF (1 mmol in 25 mL). To this solution were added Boc-Gly-OH (424 mg, 1.1 equiv) and BOP (1.07 g, 1.1 equiv) followed by DIPEA (1.17 mL, 3 equiv). After 2 h the reaction was complete, and the solvent was evaporated in vacuo. The residue was dissolved in EtOAc, and the organic layer was successively washed with 1 N $KHSO_4$, saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Dipeptide **2a** was obtained as a white solid in 92% yield (1.22 g) after purification by column chromatography (EtOAc/hexane, 1:2, v/v → EtOAc/hexane, 2:1, v/v): R_f(EtOAc/hexane, 2:1, v/v) 0.54; HPLC showed that the product was more than 99% pure detected by UV and ELSD; mp 72 °C; $[\alpha]^{20}_D$ +44.9 (*c* 0.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 2H), 7.58 (br s, 1H) 5.49 (d, 2H, J = 6.9 Hz), 3.86 (m, 5H), 3.76 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.2, 44.1, 53.2, 54.2, 60.5, 80.3, 90.8, 135.8, 138.4, 156.9, 158.9, 169.2, 170.1; ESI-MS *m*/*z* calcd for $C_{17}H_{22}I_2N_2O_6$ 604, found [M + H]$^+$ 605, [M + Na]$^+$ 627, [M + Na + MeCN]$^+$ 668, [(M − C_4H_8) + H]$^+$ 549, [(M − $C_5H_8O_2$) + H]$^+$ 505, [2M + Na]$^+$ 1230; HRMS (TOF ES$^+$) *m*/*z* 626.9434 [M + Na]$^+$, calcd for $C_{17}H_{22}I_2N_2O_6Na^+$ 626.9465.

***N*-(*tert*-Butyloxycarbonyl)seryl-(*O*-propynyl)-glycyl-(*S*)-3,5-diiodo-4-methoxyphenylglycine Methyl Ester (3a).** Tripeptide **3a** (1.94 mmol) was synthesized analogously to **2a** and obtained as a white solid (1.30 g, 92%): R_f(EtOAc/hexane, 2:1, v/v) 0.53; mp 63 °C; $[\alpha]_D$ +58.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$,) δ 7.75 (s, 2H), 7.59 (d, 1H, J = 6.3 Hz), 7.16 (br s, 1H), 5.53 (m, 2H), 4.38 (m, 1H), 4.19−4.00 (m, 5H), 3.83 (s, 3H), 3.72 (m, 4H), 2.47 (m, 1H), 1.45 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.2, 43.1, 53.2, 54.3, 58.3, 60.6, 69.5, 75.5, 78.9, 80.6, 90.8, 135.7, 138.5, 155.5, 158.9, 168.1, 170.0, 170.7; ESI-MS *m*/*z* calcd for $C_{23}H_{29}N_3O_8$ 729, found [M + H]$^+$ 730, [M + Na]$^+$ 752, [(M − C_4H_8) + H]$^+$ 674, [(M − $C_5H_8O_2$) + H]$^+$ 630, [2M + H]$^+$ 1459, [2M + Na]$^+$ 1480; HRMS (TOF ES$^+$) *m*/*z* 751.9902 [M + Na]$^+$, calcd for $C_{23}H_{29}I_2N_3O_8Na^+$ 751.9942. Anal. Calcd for $C_{23}H_{29}I_2N_3O_8$: C, 37.88; H, 4.01; N, 5.76. Found: C, 37.76; H, 4.11, N, 5.70.

Cyclic Tripeptide 4a. General Procedure for the Sonogashira Cyclization Reaction. In a flame-dried nitrogen-filled flask tripeptide **3a** (93 mg, 0.13 mmol, 1 equiv) was dissolved in a dry solvent (purged with dry nitrogen gas for 25 min prior to use) to obtain a final concentration of 0.5−15 mM. The exact reaction conditions are given in Table 1. The palladium catalyst (15 mg, 0.1 equiv) was added followed by CuI (2.5 mg, 0.1 equiv) and finally the base (5 equiv). The resulting reaction mixture was stirred for 16 h. After this period of stirring, the solvent was removed under reduced pressure, and the residue was subsequently purified by column chromatography or preparative TLC. Cyclic tripeptide **4a** (10 mg, 13%) was obtained as a white solid: R_f(EtOAc/hexane, 2:1, v/v) 0.46; mp 223 °C; $[\alpha]^{20}_D$ −24.0 (*c* 0.1 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (m, 1H), 7.49 (br s, 1H), 7.31 (s, 1H), 7.23 (s, 1H), 5.55 (d, 1H, J = 6.3 Hz), 5.43 (br s, 1H), 4.54 and 4.48 (m, 1H), 4.18 and 4.12 (m, 1H), 4.41 (br s, 1H), 4.28−4.22 (m, 1H), 3.97−3.74 (m, 2H), 3.96 (s, 3H), 3.84 (s, 3H), 3.61−3.33 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.1, 45.5, 53.3, 54.2, 54.8, 59.2, 61.0, 66.8, 80.1, 85.3, 93.5, 131.3, 134.4, 138.2, 157.3, 168.3, 171.0, 173.8 (not all carbons were detectable with HMBC/HSQC); ESI-MS *m*/*z* calcd for $C_{23}H_{28}IN_3O_8$ 601, found [M + Na]$^+$ 624, [M + MeCN + Na]$^+$ 665, [(M − $C_5H_8O_2$) + H]$^+$ 502; HRMS (TOF ES$^+$) *m*/*z* 624.0839 [M + Na]$^+$, calcd for $C_{23}H_{28}IN_3O_8Na^+$ 624.0819.

Linear Tripeptide 12. In a flame-dried nitrogen-filled flask compound **10** (188 mg, 0.46 mmol) was dissolved in THF (15 mL), which was purged with dry nitrogen gas. First $Pd(PPh_3)_4$ (53 mg, 0.046 mmol) was added followed by CuI (9 mg, 0.046 mmol), Et_2-NH (320 μL, 2.3 mmol), and finally alkyne **8** (202 mg, 0.70 mmol). The reaction mixture was stirred for 16 h. Subsequently, the solvent was removed under reduced pressure, and the residue was purified

by column chromatography. Tripeptide **12** was obtained as a colorless oil in 80% yield (225 mg) after purification by column chromatography (EtOAc/hexane, 1:2, v/v → EtOAc/hexane, 1:1, v/v): R_f(EtOAc/hexane, 1:1, v/v) 0.38; $[\alpha]^{20}_D$ +11.8 (*c* 1.9 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.20 (m, 9H), 6.83 (br s, 1H), 5.68 (d, 1H, J = 6.3 Hz), 5.20 (m, 3H), 4.45−4.34 (m, 5H), 4.21 (br s, 1H), 4.04 (m, 1H), 3.78 (m, 1H), 1.46−1.36 (m, 21H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.2, 27.8, 28.1, 42.6, 50.0, 54.6, 59.0, 66.7, 69.7, 79.9, 82.2, 86.35, 122.5, 127.6, 127.8, 127.9, 128.3, 128.4, 128.5, 130.5, 136.1, 138.5, 155.5, 155.6, 169.0, 172.8; ESI-MS *m*/*z* calcd for $C_{33}H_{43}N_3O_8$ 609, found $[M + Na]^+$ 632, $[(M - C_4H_8) + Na]^+$ 576; HRMS (TOF ES$^+$) *m*/*z* 632.3097 $[M + Na]^+$, calcd for $C_{33}H_{43}N_3O_8Na^+$ 632.2948.

Cyclic Tripeptide 15b. Linear tripeptide **12** (70 mg, 0.11 mmol) was dissolved in DCM (3 mL). TFA (3 mL) was added, and the mixture was stirred overnight at rt. The reaction mixture was evaporated in vacuo, and the residue was coevaporated with DCM (twice) to remove any residual TFA. Subsequently, the residue was dissolved in DMF (220 mL); HOAt (17 mg, 0.12 mmol) and HATU (48 mg, 0.13 mmol) followed by DIPEA (50 μL, 0.32 mmol) were added, and the reaction mixture was stirred overnight. Then the solvent was removed by evaporation in vacuo, and the residue was triturated with EtOAc. The cyclic dimer was removed by filtration, and the EtOAc layer was washed with 1 N $KHSO_4$, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under reduced pressure. Compound **15b** was obtained as a white solid in 54% yield (23 mg) after purification by column chromatography (MeOH/DCM, 95:5, v/v) and preparative TLC: R_f(MeOH/DCM, 95:5, v/v) 0.64; mp 122 °C; $[\alpha]^{20}_D$ −223.5 (*c* 0.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (br s, 1H), 7.46−6.82 (m, 10H), 6.36 (br s, 1H), 5.18−5.08 (m, 2H), 4.76 (m, 1H), 4.64 (m, 1H), 4.36 (m, 1H), 4.05−3.87 (m, 2H), 3.71−3.66 (m, 1H), 3.52−3.42 (m, 2H), 1.32 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.3, 41.7, 49.5, 54.9, 58.6, 67.1, 68.0, 79.9, 87.2, 89.0, 122.6, 125.8, 128.0, 128.2, 128.3, 128.5, 131.0, 132.0, 132.1 136.3, 137.4, 156.2, 171.8, 173.2; ESI-MS *m*/*z* calcd for $C_{24}N_{25}N_3O_5$ 435, found $[M + H]^+$ 436, $[M + Na]^+$ 458; HRMS (TOF ES$^+$) *m*/*z* 458.1690 $[M + Na]^+$, calcd for $C_{24}H_{25}N_3O_5Na^+$ 458.1692. Anal. Calcd for $C_{24}H_{25}N_3O_5$: C, 66.19; H, 5.79; N, 9.65. Found: C, 66.10; H, 5.71, N, 9.61.

Linear Tripeptide 17. In a flame-dried nitrogen-filled flask, dipeptide **2b** (200 mg, 0.32 mmol) was dissolved in DCM (3 mL), which was purged with dry nitrogen gas. First $Pd(PPh_3)_4$ (37 mg, 0.032 mmol) was added followed by CuI (6 mg, 0.032 mmol), Et_2-NH (111 μL, 0.8 mmol), and finally alkyne **8** (140 mg, 0.48 mmol), and the resulting reaction mixture was stirred for 3 days. Subsequently, the solvent was removed under reduced pressure, and the residue was purified by column chromatography. Compound **17** was obtained as a colorless oil in 34% yield (90 mg) after purification by column chromatography (EtOAc/hexane, 1:4, v/v → EtOAc/hexane, 1:1, v/v): R_f(EtOAc/hexane, 1:1, v/v) 0.3; HPLC showed that the product was 97% pure detected by ELSD; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.47 (br s, 1H), 7.27 (m, 6H), 5.64 (m, 1H), 5.36 (m, 1H), 5.44 (m, 3H), 54.40 (m, 3H), 4.25 (br s, 1H), 3.94 (m, 1H), 3.88 (s, 3H), 3.84−3.80 (m, 1H), 3.73 (s, 3H), 1.45−1.33 (m, 21H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.6, 27.9, 28.2, 53.0, 54.6, 59.2, 60.9, 66.8, 70.1, 81.8, 80.3, 82.0, 82.4, 90.0, 116.8, 128.0, 128.4, 132.6, 132.8, 133.8, 136.2, 138.2, 155.9, 160.5, 168.9, 170.2, 172.1; ESI-MS *m*/*z* calcd for $C_{36}H_{46}N_3O_{11}$ 823, found $[M + Na]^+$ 846, $[(M - C_4H_8) + Na]^+$ 724, $[(M - C_4H_8) + H]^+$ 668.

Cyclic Tripeptide 18. Cyclization of linear tripeptide **17** into compound **18** was carried out as described for compound **15b**. Cyclic tripeptide **18** was obtained as a white solid in 36% yield (31 mg): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.47 (d, 1H, J = 6 Hz), 7.36−7.31 (m, 6H), 6.72 (d, 1H, J = 6 Hz), 5.74 (d, 1H, J = 9 Hz), 5.51 (d, 1H, J = 9 Hz), 5.15 (s, 2H), 4.53−4.40 (m, 2H), 4.21−4.17 (m, 1H), 4.46 (m, 1H), 4.12 (m, 1H), 3.93 (s, 3H), 3.88 (m, 1H), 3.83 (s, 3H), 1.34 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 16.6, 49.8, 52.3, 54.3, 59.2, 61.0, 67.5, 85.2, 91.5, 92.3, 115.0, 128.2, 128.4, 128.6, 131.8, 133.6, 137.9, 157.7, 169.1, 170.8, 171.3; ESI-MS *m*/*z* calcd for $C_{27}H_{28}IN_3O_8$ 649, found $[M + H]^+$ 643, $[M + Na]^+$ 672. Anal. Calcd for $C_{27}H_{28}IN_3O_8$: C, 49.93; H, 4.35; N, 6.47. Found: C, 49.86; H, 4.43; N, 6.38.

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Supporting Information Available: Experimental details of compounds **2b,c**, **3b,c**, **4b,c**, **7a,b**, **8**−**11**, and **15a**, complete characterization of compounds **1**−**4**, **7a**, **8**−**12**, **15**, **17**, and **18**, ^{1}H NMR, ^{13}C NMR, ^{1}H COSY, HMBC, HSQC, and HPLC data, and modeling data of **4b** using MacroModel together with an atom coordinates file. This material is available free of charge via the Internet at http://pubs.acs.org.

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