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Communication

Peptide Diyne Transformations

Synthesis of Heterocycle-Bridged Peptidic Macrocycles through 1,3-Diyne Transformations

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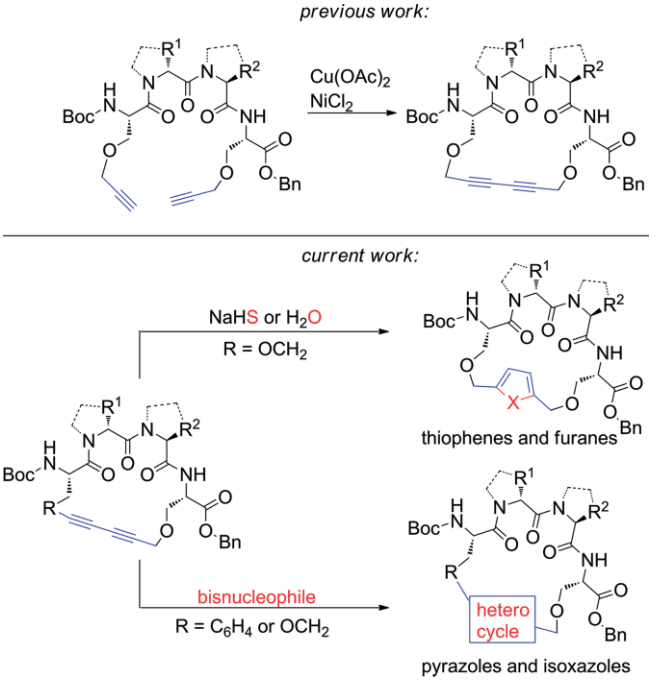
Abstract: Macrocyclic tetrapeptide analogues containing a 1,3-diyne moiety were synthesized by a Glaser–Hay-type macrocyclization. The obtained 1,3-diyne was evaluated as a handle to introduce heterocycles into the macrocyclic structure. It was shown that the macrocyclic 1,3-diynes were successfully transformed into various heterocycles by nucleophilic attack of (bis)nucleophiles to the diyne moiety. Treatment with NaHS or H_2O as nucleophiles gave rise to 2,5-bridged thiophenes or furans, whereas the use of hydrazines and hydroxylamine gave rise to the corresponding pyrazole- and isoxazole-containing macrocycles. In addition, a thermoreversible Diels–Alder cycloaddition of a cyclopeptidic bridged furan was demonstrated.

Introduction

Alkyne–alkyne coupling reactions are currently being evaluated in peptide chemistry as promising tools to cyclize or functionalize peptidic backbones.[1–4] Cyclic peptides containing a 1,3-diyne moiety can be prepared by either derivatization of peptide side chains with difunctionalized 1,3-diynes[5] or by Glaser–Hay-type oxidative alkyne–alkyne coupling reactions of α,ω-dialkynylated peptides.[1,2] To date, there are no examples in the known literature for which postcyclization transformations of the 1,3-diyne linker of a macrocyclic peptide have been applied. Interestingly, starting from one parent structure the reaction of such diynes with nucleophiles could lead to a set of different cyclic peptides that contain a heterocyclic tether, which in turn could be of value in structure–activity relationship studies. 1,3-Diynes have for a long time been considered as versatile building blocks,[6] and currently, they are receiving revived interest in organic synthesis.[7] The reaction of 1,3-diynes with S-nucleophiles can lead to thioenynes or thiophenes,[8–16] and analogous hydration or hydroamination reactions give rise to furans[15–18] or pyrroles.[12,15,17–20] Unfortunately, in many cases a high reaction temperature is needed, and these transformations are mostly reported by using arylated 1,3-diynes. It has been shown that related reactions of alkyl-substituted 1,3-diynes often result in a drastic drop in yield relative to those obtained for aromatic substituted thiophenes[8] or furans.[17,18] Also, 1,2-bisnucleophiles such as hydrazine and hydroxylamine have been used to prepare pyrazoles[21,22] and isoxazoles,[23] but such conversions have never been applied on peptidic substrates. Very recent examples that further demonstrate the potential of 1,3-diynes include reactions with guanidines or acetamidines to give amino- or alkylpyrimidines[24,25] and formal [4+2] reactions with pyrrole towards indoles.[26] In addition to these monoheterocycles, the formation of polyheterocyclic compounds has been described by intramolecular reactions between nucleophiles and 1,3-diynes.[27,28]

In continuation of our previous work regarding the synthesis of macrocyclic 1,3-diynes from tetrapeptides containing *O*-propargylated serine residues (Scheme 1),[1] we hereby report on the condensation of peptidic 1,4-dialkyl-1,3-diynes and 1-



Scheme 1. Residue incorporating R^1: D-Pro or D-Ala; residue incorporating R^2: L-Pro or L-Ala. Boc = *tert*-butoxycarbonyl.

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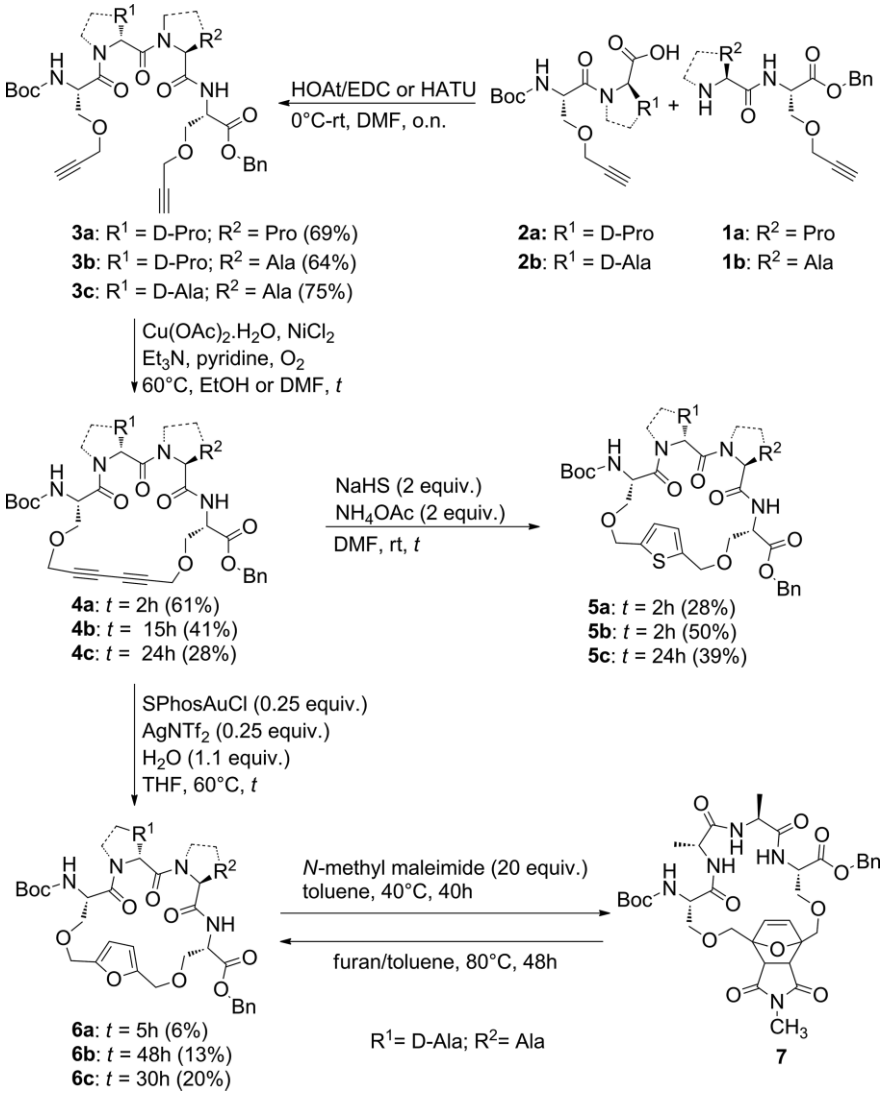
alkyl-4-aryldiynes with nucleophiles leading to a variety of heterocycle-bridged macrocycles.

Results and Discussion

To explore the possibility of forming thiophenes through double nucleophilic addition of a sulfur anion to macrocyclic 1,3-diynes, compounds **4a**–**c** were prepared from linear peptides **3a**–**c** (Scheme 2). The latter result from the coupling of corresponding dipeptides **1a**,**b** and **2a**,**b**.[1] In a first evaluation to transform macrocyclic diynes into the corresponding thiophenes, **4c** was treated with $Na_2S{\cdot}9H_2O$ (2 equiv.) in DMF at room temperature. This resulted in a complex mixture of products, and only a minor amount of the envisaged thiophene was observed after LC–MS analysis. A switch to NaHS as the nucleophile, which is more soluble in organic solvents, resulted in better conversion of the diyne into the thiophene, but in this case, concomitant *N*-Boc deprotection occurred.

The formation of a thiophene from a S-nucleophile and a diyne requires the introduction of two protons. Whereas NaHS has only one proton available, the mixture becomes more basic during the reaction. To counter this, it was decided to add NH_4OAc as a buffer instead of using H_2S. Under these conditions, clean conversion (70–92 %) towards thiophene derivatives **5a**–**c** was observed at room temperature. Whereas peptide derivatives **5a** and **5b** only required a reaction time of 2 h, **5c** required a reaction time of 24 h to reach 90 % conversion for reasons that remain unclear. Compounds **5a**–**c** were isolated by preparative reverse-phase (RP) HPLC or C18 silica gel chromatography in moderate yields (28–50 %).

In a next transformation of 1,3-diynes **4a**–**c**, water was evaluated as a nucleophile. Without activation of the alkyne moiety no reaction took place, even at elevated temperatures. However, if $SPhosAuNTf_2$ was used as an alkyne activating catalyst,[17] desired furans **6a**–**c** were obtained but more side products were formed than in the formation of thiophenes **5a**–**c**.[29]



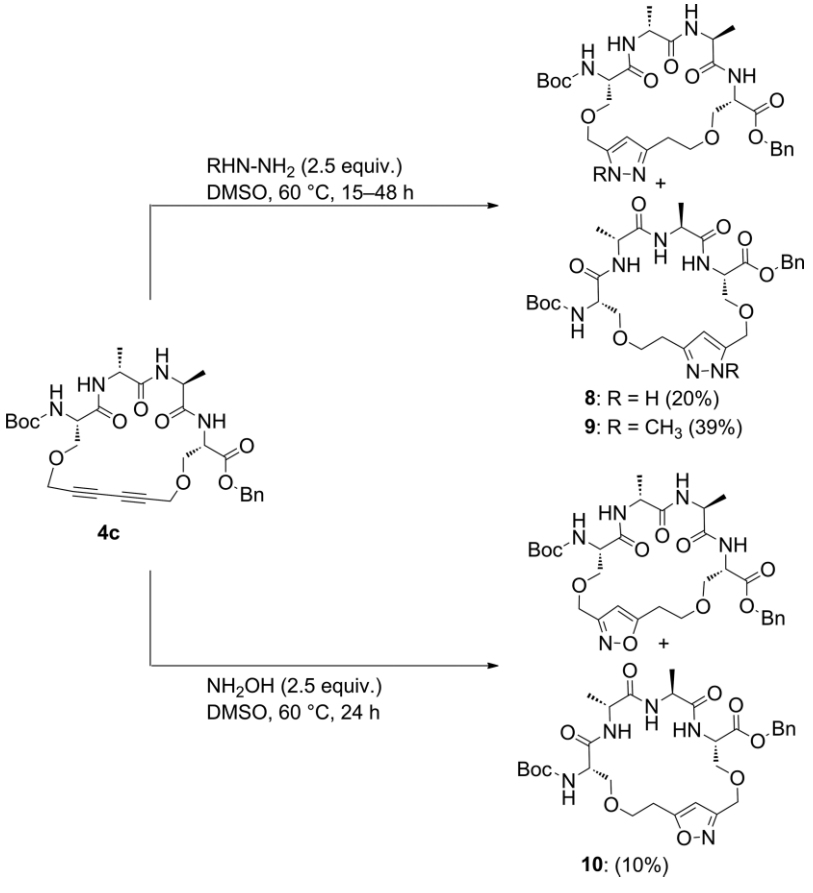
Scheme 2. Synthesis of thiophenes **5a**–**c** and furan derivatives **6a**–**c** and cycloadduct **7**. Yields of isolated products are indicated. In the case of **6a**, SPhosAuCl (1 equiv.), $AgNTf_2$ (1 equiv.), and H_2O (20 equiv.) were added. HOAt = 1-hydroxy-7-azabenzotriazole, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate, SPhos = 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl, $AgNTf_2$ = silver bis(trifluoromethanesulfonyl)imide.

The transformation of **4b** and **4c** towards **6b** and **6c** required only a catalytic amount of the gold catalyst to reach good HPLC conversions (60–90 %), but the formation of **6a** proceeded sluggishly and 1 equivalent of the catalyst and 20 equivalents of H_2O were needed to promote the reaction. This led to a rather complex mixture, from which **6a** was isolated in low yield.

The introduction of furans into peptidic structures through transformation of the 1,3-diyne linker can expand the current toolbox for reversible furan-based peptide labeling techniques.[30] Having peptide-bridged furans **6** in hand, Diels–Alder reactions with maleimides were therefore evaluated in a model study. After heating macrocycle **6c** in toluene at 40 °C in the presence of an excess amount of *N*-methylmaleimide, cycloadduct **7** (both diastereomers) was obtained after 40 h in a nearly peak-to-peak HPLC conversion. Subsequently, obtained and isolated **7** was heated to verify whether a retro-Diels–Alder transformation was possible without affecting the peptidic linker. Although at first no conversion seemed to occur upon heating **7** in toluene at 80 °C, the addition of furan as a trapping agent for the formed *N*-maleimide at this temperature completely shifted the equilibrium towards starting peptide **6c** in clean conversion, as observed by HPLC. This conversion provides proof-of-principle for the reversible linkage of macrocycle-bridged furans of type **6c** and opens a gateway for labelling studies at a distant site from the potential recognition domain within peptide macrocycles.

Transformations of diynes into pyrroles by using amines as nucleophiles have been described and could provide the nitrogen analogues of compounds **5** and **6**. Unfortunately, in many cases harsh reaction conditions are required to accomplish this.[17–20] In an attempt to reach pyrrole derivatives, the reaction of macrocycles **4a–c** with amines was evaluated (>10 different reaction conditions, see the Supporting Information), but did not result in any pyrrole formation, and only complex reaction mixtures were obtained.

All of the above-described transformations gave rise to quasi-symmetrically substituted heterocycles. In contrast, the use of hydrazines or hydroxylamines can result in different regioisomers depending on whether position 1 or 4 of the 1,3-diynes is attacked first by these bisnucleophiles. To evaluate if the nature and conformation of the macrocycles could direct the incoming nucleophile and give regioselective reactions with bisnucleophiles, compound **4c** was separately treated with hydrazine, hydroxylamine, and *N*-methylhydrazine (Scheme 3). In these cases, no or low regioselectivity was observed and mixtures of pyrazole regioisomers **8** and **9** and isoxazoles **10** were obtained. Pyrazoles **8** and isoxazoles **10** could not be separated and were obtained as a mixture of two regioisomers in 1:1 and



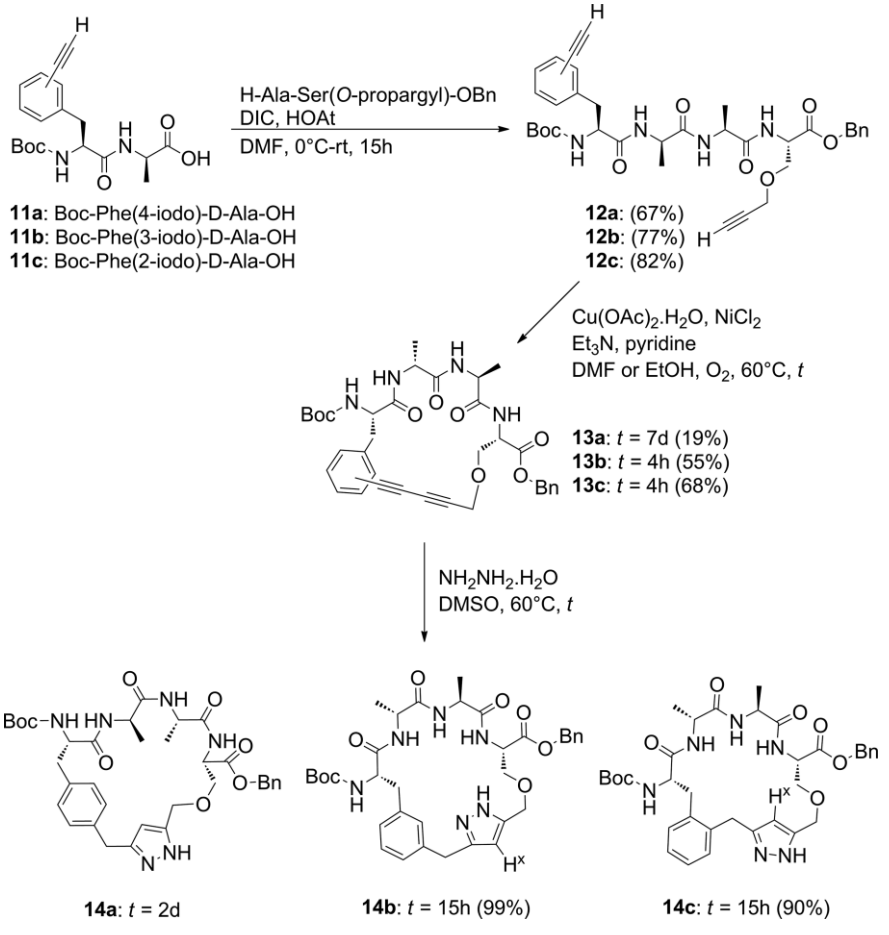
Scheme 3. Synthesis of pyrazole derivatives **8** and **9** and isoxazoles **10**. Compound **8** was isolated as a mixture of two regioisomers (1:1 ratio); compound **9** was isolated as a mixture of three regioisomers (3:1:1 ratio); compound **10** was isolated as a mixture of two regioisomers (2:1 ratio).

2:1 ratios, respectively, as shown by NMR spectroscopy. The reaction of *N*-methylhydrazine with **4c** gave rise to a mixture of three regioisomers in a 3:1:1 ratio.[31]

The reason for the observed lack of selectivity is most probably due to the quasi-symmetric nature of the 1,3-diyne moiety in substrate **4c**. This could change drastically if 1-aryl-4-alkyl-1,3-diynes are used, as an electronic bias (aryl conjugation) is present in such cases. To verify this, the synthesis of macrocyclic substrates **13a**–**c** was envisaged. Instead of using two propargylated serine residues, one serine residue was exchanged with an ethynylated phenylalanine residue. Sonogashira coupling of 2-, 3-, and 4-iodinated Boc-Phe-OMe with TMS-acetylene resulted in the corresponding trimethylsilyl-protected 2-, 3-, and 4-ethynyl-L-phenylalanine methyl esters in 54 to 92 % yield. Simultaneous silyl deprotection and saponification of the methyl esters with a 1 M NaOH solution afforded ethynylated Phe derivatives, which were used as the first amino acid in a solution-phase peptide synthesis strategy.

Coupling of ethynylated Boc-Phe-OH with H-D-Ala-OMe using HATU in DMF and subsequent saponification afforded dipeptides **11a**–**c**. Coupling of **11a**–**c** with H-Ala-Ser(*O*-propargyl)-OBn by using *N*,*N*′-diisopropylcarbodiimide (DIC)/HOAt in DMF resulted in linear tetrapeptides **12a**–**c** in good yields (67–82 %). Next, these were cyclized to corresponding peptides **13a**–**c** by using the optimized Glaser–Hay coupling reaction conditions.[1]

For the cyclization of linear peptide **12a**, DMF was used as the solvent instead of EtOH, as the use of the latter required much longer reaction times and generated more side products. Unfortunately, full consumption of **12a** still took 7 days in DMF with 5 equivalents of $Cu(OAc)_2 \cdot H_2O$ and $NiCl_2$, and concomitantly, more side products were formed than in reactions with **12b**,**c**. This long reaction time and low yield is most probably due to the large ring strain in obtained macrocycle **13a**, because both a *para*-phenylene and a 1,3-diyne structure are bridged by a peptidic macrocycle. Less strained peptide analogues **12b**,**c** cyclized within 4 h at 60 °C. Obtained arylated diynes **13a**–**c** were then treated with hydrazine in DMSO for 15 h at 60 °C, which resulted in the formation of single regioisomers **14a**–**c**, as observed by HPLC and NMR spectroscopy. ^1H–^{13}C HSQC analysis clearly shows one singlet for pyrazole proton H^x (see Scheme 4), which corresponds with one carbon atom. The fact that the two methylene units within the tether do not couple with each other in the ^1H–^{13}C HMBC and ^1H–^1H COSY NMR spectra and the fact that the newly formed CH_2 protons show cross peaks with the benzene and pyrazole ring proves the formation of only one regioisomer. This is in agreement with the expected reactivity, for which the first nucleo-



Scheme 4. Synthesis of cyclic peptides **13a**–**c** and pyrazoles **14a**–**c**. Peptide **14a** was not purified owing to the low amount of isolated **13a** but was used as an initial test reaction.

philic attack occurs at the 4-position of the 1-aryl-1,3-diyne. Although this concurs with previous literature data, often mixtures of regioisomers are reported if nonsymmetric alkyl- and aryl-substituted 1,3-diynes are used,[22,23] which is clearly not the case upon using peptides **13a**–**c**.

Conclusions

In conclusion, the synthesis of various peptide macrocycles with heterocycle-bearing tethers (thiophene **5**, furan **6**, cycloadduct **7**, pyrazole **8**, *N*-methylpyrazole **9**, and isoxazole **10**) was realized under mild reaction conditions. In addition, it was shown that a nonsymmetric arylated 1,3-diyne linker (i.e., **13a**–**c**) gave rise to the regioselective formation of the corresponding pyrazoles upon treatment with hydrazine. Further application of these methods to larger peptide sequences and the potential for reversible linking of such macrocycles through Diels–Alder reactions is currently being evaluated and will be reported in due course.

Experimental Section

General Methods: Column chromatography purifications were conducted on silica gel 60 (40–63 μm; Grace Davisil) or with a Grace Reveleris X2 Flash Chromatography System on silica gel (prepacked 40 μm; Grace Reveleris) or C18 silica gel (prepacked 40 μm, Grace Reveleris). TLC was performed on glass plates precoated with silica gel 60F254 (Merck); the spots were visualized under UV light (λ = 254 nm) and/or $KMnO_4$ (aq.) was used as the revealing system. Preparative HPLC was conducted by using a Gilson semipreparative HPLC system equipped with a Supelco Discovery Bio Wide Pore C18 column. Samples were analyzed with an Agilent 1100 Series HPLC equipped with a Supelco Discovery Bio Wide Pore C18 column (15 cm × 2.1 mm × 3 μm). The solvent system consisted of 0.1 % trifluoroacetic acid (TFA) in water and 0.1 % TFA in acetonitrile. The samples were then eluted through the column by using a gradient ranging from 3 % acetonitrile to 97 % acetonitrile over 20 min (standard gradient) at a flow rate of 0.3 mL min^{-1}. Melting points were acquired with a Büchi Melting Point B-540. IR absorption spectra were recorded with a Thermo Nicolet 700 FTIR spectrophotometer. NMR measurements were performed with a Bruker Avance II spectrometer operating at ^1H and ^{13}C frequencies of 500.13 and 125.76 MHz, respectively. The sample temperature was set to 298.2 K. The deuterated solvent is mentioned in the analysis section and tetramethylsilane was used as an internal standard. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). High-resolution mass spectrometry was conducted with a Waters Micromass QTof in ESI+ mode by using reserpine as the reference. Commercial amino acids and coupling reagents were purchased from Novabiochem and Chem-Impex. All other used reagents and chemicals were purchased from Sigma–Aldrich and were used without further purification unless otherwise specified.

Synthesis of Thiophene Peptide 5a: A representative procedure is given for the synthesis of **5a**. Compound **4a** (0.031 mmol), NaHS (0.061 mmol), and NH_4OAc (0.061 mmol) were dissolved in DMF (1 mL). The mixture was stirred at r.t. for 2 h. The mixture was concentrated in vacuo, and the obtained residue was purified by preparative RP-HPLC to obtain **5a** (5.9 mg, 28 %) as a white powder. t_R = 16.6 min. HMRS: calcd. for $[C_{34}H_{44}N_4O_9S + Na^+]$ 707.2721; found 707.2723.

Synthesis of Furan Peptide 6a: SPhosAuCl (0.03 mmol) and $AgNTf_2$ (0.03 mmol) were weighed into a vial and THF (0.2 mL) was added. The mixture was stirred for 15 min at r.t. Compound **4a** (0.03 mmol) was dissolved in THF (0.2 mL) and added to the mixture. Subsequently, MilliQ water (0.60 mmol, 33 μL) was added while stirring. The mixture was stirred at 60 °C for 5 h and concentrated in vacuo. The obtained residue was purified by preparative RP-HPLC to obtain **6a** (1.2 mg, 6 %) as a white powder. t_R = 16.7 min. HMRS: calcd. for $[C_{34}H_{44}N_4O_{10} + Na^+]$ 691.2950; found 691.2949.

Synthesis of Pyrazole Peptide 8: A representative procedure is given for the synthesis of **8**. Compound **4c** (0.033 mmol) was dissolved in DMSO (0.5 mL). $NH_2NH_2{\cdot}H_2O$ (0.083 mmol) was added, and the mixture was stirred at 60 °C for 15–48 h. DMSO was evaporated under a stream of pressurized air, and the crude mixture was purified by preparative RP-HPLC to obtain **8** (4.0 mg, 20 %) as two regioisomers as a white powder. t_R = 14.0 min. HRMS: calcd. for $[C_{30}H_{42}N_6O_9 + H^+]$ 631.3086; found 631.3084.

Synthesis of Pyrazole Peptide 14b: A representative procedure is given for the synthesis of **14b**. Compound **13b** (0.020 mmol) was dissolved in DMSO (0.5 mL) and $NH_2NH_2{\cdot}H_2O$ (0.08 mmol, 3 μL) was added. The mixture was stirred at 60 °C for 15 h. The mixture was lyophilized to obtain **10b** (12.8 mg, 99 %) as a yellow powder. ^1H NMR (500 MHz, $[D_6]$DMSO): δ = 8.10 (d, J = 8.0 Hz, 1 H), 7.79 (d, J = 8.0 Hz, 0 H), 7.74 (d, J = 7.1 Hz, 1 H), 7.27–7.40 (m, 5 H), 7.15–7.22 (m, 1 H), 7.08–7.14 (m, 1 H), 6.98–7.07 (m, 1 H), 6.94 (d, J = 7.5 Hz, 1 H), 6.65 (d, J = 6.6 Hz, 1 H), 5.99 (s, 1 H), 5.06–5.16 (m, 2 H), 4.53–4.59 (m, 1 H), 4.39 (s, 2 H), 4.22 (t, J = 1.0 Hz, 1 H), 4.06–4.14 (m, 1 H), 3.99–4.06 (m, 1 H), 3.81–3.93 (m, 3 H), 3.61–3.70 (m, 2 H), 2.85 (t, J = 6.1 Hz, 2 H), 1.25–1.38 (m, 9 H), 1.16–1.23 (m, 4 H), 0.96 ppm (d, J = 6.9 Hz, 2 H). ^{13}C NMR (126 MHz, $[D_6]$DMSO): δ = 171.9, 171.7, 171.5, 170.6, 169.8, 145.0, 139.2, 137.2, 135.8, 129.0, 128.3, 128.2, 127.9, 127.6, 127.5, 126.9, 103.7, 78.4, 68.7, 65.9, 65.6, 55.7, 52.3, 48.3, 48.1, 37.4, 32.1, 28.1, 17.2, 17.1 ppm. HRMS: calcd for $[C_{35}H_{44}N_6O_8 + Na^+]$ 699.3113; found 699.3118.

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Keywords: Cyclization · Diynes · Heterocycles · Nucleophilic addition · Peptides

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[29] HPLC chromatograms of both the crude reaction mixtures and isolated thiophenes **5** and furans **6** are included in the Supporting Information.
[30] For example, see: K. Hoogewijs, D. Buyst, J. M. Winne, J. C. Martins, A. Madder, *Chem. Commun.* **2013**, *49*, 2927–2929.
[31] See the Supporting Information for more details.

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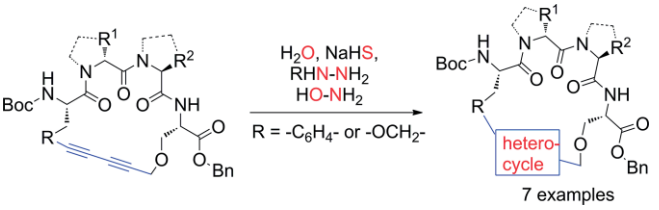

Macrocyclic tetrapeptides containing a 1,3-diyne tether are synthesized by using a mild Glaser–Hay-type coupling and are subsequently treated with nucleophiles. This results in a variety of corresponding macrocycles bearing a heterocycle in the cyclic core structure. Boc = *tert*-butoxycarbonyl.

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