Supporting information for

Oxidative $\alpha,\omega$-diyne coupling as an approach towards novel peptidic macrocycles

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1. Model study

A model study to evaluate the optimal reaction conditions for the intermolecular dimerisation of alkynylated amino acids 1a and 1b was performed (Table 1). The general reaction procedure consisted of adding the solvent (0.5 ml) to a mixture of 1a (0.08 mmol) or 1b (0.08 mmol) with a Cu-catalyst (0.5 equiv.) and/or Ni-catalyst (0.5 equiv.). The reaction mixture was stirred at rt and the base (3 equiv.), ligand (5 equiv.) and oxidant were added. In case of O₃ as oxidant, the reaction mixture was stirred under O₂-atmosphere using a O₂-balloon. In case of MnO₂, pyridine N-oxide or H₂O₂ (35 wt-% in H₂O) as oxidants, these reagents were added in excess (5 equiv.) to the reaction mixture and stirred open to the atmosphere, but using a plug of cotton wool to prevent excessive loss of solvents in case of long reaction times. Also in case of air as oxidant, the reaction mixture was stirred open to the atmosphere as described above. The reaction temperature was increased to 60°C when no or slow conversion towards the corresponding diyne was observed. Conversions were determined via HPLC by direct sampling of the reaction mixture.

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Table 1: Model study
Synthesis of starting materials 1a and 1b

**tert-Butyl (S)-(1-(benzylamino)-1-oxopent-4-yn-2-yl)carbamate 1a**

(S)-2-((tert-Butyloxycarbonyl)amino)pent-4-ynoic acid (1.41 mmol), 1-hydroxy-7-azabenzotriazole (HOAt) (4.22 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) (1.41 mmol) were dissolved in CH₂Cl₂ (40 ml). DIPEA (N,N-diisopropylethylamine) (4.22 mmol) was added dropwise and the mixture was stirred at rt for 5 min. The reaction was cooled to 0 °C and benzylamine (1.41 mmol) in CH₂Cl₂ (5 ml) was added dropwise. The reaction mixture was stirred at rt for 26 h protected from atmospheric moisture via a CaCl₂-tube, concentrated in vacuo and the resulting residue was triturated in EtOAc (15 ml) and filtered. The filtrate was washed with a saturated aqueous citric acid solution (15 ml), a saturated aqueous KHCO₃ solution (2x15 ml) and brine (2x15 ml). The organic phase was dried over MgSO₄, filtered and concentrated in vacuo to afford tert-butyl (S)-(1-(benzylamino)-1-oxopent-4-yn-2-yl)carbamate as a white solid in 71% yield (0.31 g); mp: 89.5 – 91.1 °C; IR (neat): 3230, 2074, 1736, 1675 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ 7.22 – 7.38 (m, 5 H), 6.62 (br. s, 1 H), 5.28 (br. s, 1 H), 4.44 – 4.53 (m, 2 H), 4.25 – 4.39 (m, 1 H), 2.79 – 2.91 (m, 1 H), 2.56 – 2.69 (m, 1 H), 2.06 (t, J = 2.7 Hz, 1 H), 1.43 (s, 9 H); ¹³C NMR (163 MHz, CDCl₃) δ 175.5, 155.6, 137.8, 128.9, 127.9, 127.8, 81.0, 79.6, 72.0, 53.3, 43.9, 28.4, 22.6; HRMS Calcd for [C₁₇H₂₂N₂O₃ – Boc + H⁺]: 203.1179. Found 203.1183.

**tert-Butyl (S)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate 1b**

N-(tert-Butyloxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine (21.46 mmol), HOAt (64.40 mmol) and EDC.HCl (64.40 mmol) were dissolved in CH₂Cl₂ (150 ml). DIPEA (64.40 mmol) was added dropwise and the mixture was stirred at rt for 5 min. The reaction mixture was cooled to 0 °C and benzylamine (64.40 mmol) in CH₂Cl₂ (5 ml) was added dropwise. After stirring for 36 h at rt protected from atmospheric moisture via a CaCl₂-tube, the reaction mixture was concentrated in vacuo. The resulting residue was triturated with EtOAc (50 ml) and filtered. The filtrate was washed with an aqueous saturated citric acid solution (50 ml), an aqueous saturated KHCO₃ solution (2x50 ml) and brine (2x50 ml). The organic phase was dried over MgSO₄, filtered and concentrated in vacuo. The residue was purified via column silica gel chromatography (EtOAc/hexane 1/1) to afford tert-butyl (S)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate as a white solid in 45% yield (3.18 g); mp: 100.0 – 102.0 °C; IR (neat): 3300, 2178, 1742, 1095 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ 7.20 – 7.36 (m, 5 H), 6.81 (t, J = 5.4 Hz, 1 H), 5.36 – 5.55 (m, 1 H), 4.41 – 4.51 (m, 2 H), 4.33 (br. s., 1 H), 4.15 (m, 2 H), 3.94 (dd, J = 4.4 and 3.8 Hz, 1 H), 3.69 (dd, J = 6.3 and 4.4 Hz, 1 H), 2.45 (t, J = 2.4 Hz, 1 H), 1.42 (s, 9 H); ¹³C NMR (63 MHz, CDCl₃): δ 170.0, 155.5, 137.9, 128.6,
HRMS Calcd for [C_{19}H_{24}N_{2}O_{4} – Boc + H^+]: 233.1284. Found 233.1270. The obtained spectroscopic data were in accordance with literature data.¹

**Synthesis of diynes 2a and 2b**

**Di-tert-butyldi-(2S,9S)-1,10-bis(benzylamino)-1,10-dioxodeca-4,6-diyn-2,9-diyl)dicarbamate 2a**

![Image of diyne 2a](image)

tert-Butyl (S)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yl)propan-2-yl)carbamate (1a) (0.083 mmol), Cu(OAc)₂·H₂O (0.04 mmol) and NiCl₂ (0.04 mmol) were added to EtOH (0.5 ml). Subsequently, Et₃N (0.25 mmol) and pyridine (0.41 mmol) were added dropwise to the mixture. The reaction mixture was stirred under O₂-atmosphere at rt for 4d. Afterwards, the reaction mixture was concentrated in vacuo. EtOAc (2 ml) was added and the mixture was acidified to pH 5 with an aqueous saturated citric acid solution and extracted with EtOAc (2x5 ml). The combined organic phases were washed with a saturated NH₄Cl solution (5 ml), brine (2x5 ml), dried with MgSO₄, filtered and concentrated in vacuo. The resulting mixture was purified via column silica gel chromatography (EtOAc/hexane 1/1) to afford di-tert-butyldi-(2S,9S)-1,10-bis(benzylamino)-1,10-dioxodeca-4,6-diyn-2,9-diyl)dicarbamate as a yellow solid in 36% yield (18 mg); mp: 82.0–82.9 °C; IR (neat): 2217, 2143, 1729, 1681 cm⁻¹;¹ H NMR (250 MHz, CDCl₃): δ 7.15 – 7.41 (m, 10 H), 6.78 – 6.88 (m, 2 H), 5.45 (d, J = 7.8 Hz, 2 H), 4.26 – 4.50 (m, 6 H), 2.77 (ddd, J = 19.6, 6.2, 6.2 Hz, 4 H), 1.41 (s, 18 H);¹³C NMR (63 MHz, CDCl₃) δ 170.0, 155.3, 137.7, 128.7, 127.6, 127.5, 80.7, 73.3, 67.9, 53.0, 43.5, 28.2, 23.4; HRMS Calcd for [C₃₄H₄₂N₄O₆ + Na⁺]: 625.2997. Found 625.3010.

**Di-tert-butyldi-(4S,15S)-3,16-dioxo-1,18-diphenyl-6,13-dioxa-2,17-diazaoctadeca-8,10-diyn-4,15-diyl)dicarbamate 2b**

![Image of diyne 2b](image)
tert-Butyl (S)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (1b) (6.72 mmol) and Cu(OAc)₂·H₂O (3.36 mmol) were added to EtOH (50 ml). Triethylamine (20.15 mmol) and pyridine (33.6 mmol) were added dropwise to the reaction mixture and the reaction mixture was stirred for 22h under O₂-atmosphere at rt. The reaction mixture was concentrated in vacuo and EtOAc (30 ml) was added to the residue. The mixture was acidified to pH 5 with an aqueous saturated citric acid solution and extracted with EtOAc (2x30 ml). The combined organic phases were washed with an aqueous saturated NH₄Cl solution (10 ml), brine (2x20 ml), dried over MgSO₄, filtered and concentrated in vacuo. The resulting residue was purified via column silica gel chromatography (hexane/EtOAc 1/2) to afford di-tert-butyl ((4S,15S)-3,16-dioxo-1,18-diphenyl-6,13-dioxa-2,17-diazaoctadeca-8,10-diyn-4,15-diyl)dicarbamate as yellow solid in 61% yield (2.69 g); mp: 64.1 – 66.2 °C; IR (neat): 2236, 2156, 1753, 1666, 1070 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ 7.19 – 7.40 (m, 10H), 6.68 (t, J = 5.2 Hz, 2H), 5.37 (br. S., 2H), 4.48 (d, J = 5.9 Hz, 4H), 4.32 (br. S., 2H), 4.25 (d, J = 4.8 Hz, 4H), 3.95 (dd, J = 4.4 and 3.8 Hz, 2H), 3.69 (dd, J = 6.3 and 4.4 Hz, 2H), 1.43 (s, 18H); ¹³C NMR (63 MHz, CDCl₃) δ 169.8, 155.4, 137.9, 128.7, 127.5, 80.5, 75.0, 70.9, 69.8, 59.1, 54.1, 43.5, 28.3; HRMS Calcd for [C₃₆H₄₆N₄O₈+H]⁺: 663.3399. Found: 663.3389.

2. Synthesis of Fmoc-AA(O-propargyl)-OH 18 and 20

Scheme 1: Synthesis of Fmoc protected amino acids 18 and 20.
**N-(**tert**-butoxycarbonyl)**-O-(prop-2-yn-1-yl)-L-serine 17:

![Chemical structure](image)

*N-(**tert**-Butoxycarbonyl)**-O-(prop-2-yn-1-yl)-L-serine 17 was synthesised according to literature.² (**tert**-Butoxycarbonyl)-L-serine (24.37 mmol) was dissolved in DMF (150 ml). The solution was cooled to 0 °C and NaH (58.50 mmol, 60 wt-% suspension in mineral oil) was added in portions. After stirring for 2h protected from atmospheric moisture with a CaCl₂-tube, propargylbromide (34.10 mmol, 9 wt-% in toluene) was added dropwise. After stirring for 2h at rt, the excess NaH was destroyed by addition of H₂O (50 ml), where the first 2 ml were carefully dropped. The resulting reaction mixture was concentrated in vacuo, dissolved in H₂O (150 ml), washed with Et₂O (50 ml) and acidified with 1M KHSO₄ to pH 1-2. The acidic aqueous phase was extracted with EtOAc (2x50 ml) and the combined organic phases were washed with brine (5x20 ml). The organic layer was dried over MgSO₄, filtered and concentrated in vacuo to afford *N-(**tert**-butoxycarbonyl)**-O-(prop-2-yn-1-yl)-L-serine as a orange oil in 89% yield (6.80 g). IR (neat): 3336, 2078, 1736, 1559, 1123 cm⁻¹;¹ **H NMR (CDCl₃, 250 MHz): δ 5.39 (d, J = 8.2 Hz, 1H), 4.49 (d, J = 8.2 Hz, 1H), 4.16 – 4.23 (m, 2H), 4.00 (dd, J = 9.3 and 3.0 Hz, 1H), 3.81 (dd, J = 9.4 and 3.5 Hz, 1H), 2.47 (t, J = 2.4 Hz, 1H), 1.47 (s, 9 H);¹³ **C NMR (CDCl₃, 63 MHz): δ 175.2, 156.0, 80.7, 78.9, 75.5, 69.7, 58.9, 53.9, 28.5; HRMS Calcd for [C₁₁H₁₇NO₅ + Na⁺]: 266.0999. Found: 266.0992. The obtained spectroscopic data were in accordance with literature data.²

**N-((**9H-fluoren-9-yl**)-methoxy)carbonyl)**-O-(prop-2-yn-1-yl)-L-serine 18:

![Chemical structure](image)

**tert**-Butyl (S)-(1-(benzylamino)-1-oxo-3-(prop-2-yn-1-yl oxy)propan-2-yl)carbamate (21.71 mmol) was dissolved in CH₂Cl₂ (50 ml), followed by the addition of an excess of TFA (40 ml). The reaction mixture was stirred at rt for 2h and concentrated in vacuo. The obtained oil was dissolved in H₂O (50 ml) and the pH of the solution was adjusted to pH 10 with an aqueous saturated NaHCO₃ solution (30 ml). This basic aqueous solution was added to a solution of Fmoc-OSu (N-(9-fluorenylmethoxy carbonyloxy)succinimide) (23.88 mmol) in 1,4-dioxane (75 ml). Afterwards, the reaction mixture was stirred at rt for 16h. The reaction mixture was concentrated in vacuo and the residual oil was dissolved in EtOAc (140 ml). The organic phase was extracted with an aqueous 1% NaHCO₃ solution (2x100 ml). The pH of the combined aqueous layers were adjusted to pH 1 with an aqueous 6M HCl, followed by extraction with EtOAc (4x10 0ml). The combined organic phases were washed with brine (2x50 ml), dried over MgSO₄, filtered and the obtained solids were dried (0.05 mmHg) to
afford N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(prop-2-yn-1-yl)-L-serine as a white solid in 52% yield (4.10 g); mp: 156.1 – 157.9 °C; IR (neat): 3300, 2166, 1753, 1427, 1070 cm\(^{-1}\); \(^1\)H NMR (250 MHz, DMSO-d\(_6\)); \(\delta\) 12.81 (br. S, H), 7.88 (d, \(J = 7.3\) Hz, 2H), 7.74 (d, \(J = 7.1\) Hz, 2H), 7.67 (d, \(J = 8.2\) Hz, 1H), 7.36 (ddd, \(J = 23.5\) and 7.8 and 1.4 Hz, 4H), 4.17 – 4.30 (m, 4H), 4.15 (d, \(J = 2.3\) Hz, 2H), 3.70 (d, \(J = 5.6\) Hz, 2H), 3.46 (t, \(J = 2.3\) Hz, 1H); \(^{13}\)C NMR (63 MHz, DMSO-d\(_6\)) \(\delta\) 171.4, 156.0, 144.4, 141.4, 131.3, 130.8, 128.3, 127.7, 126.0, 120.8, 115.2, 79.8, 77.5, 68.6, 65.8, 57.7, 53.9, 46.6; HRMS Calcd for \([C_21H_{19}NO_5 + H]^+\): 366.1348. Found: 366.1336.

(S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid 20

![Chemical structure](image)

(S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid was synthesised according to literature.\(^3\) \(K_2CO_3\) (64.0 mmol) was added to a solution of (tert-butoxycarbonyl)-L-tyrosine (21.33 mmol) in DMF (30 ml). Propargylbromide (64.0 mmol, 80 wt-% in toluene) was added dropwise to the reaction mixture and stirred at rt for 20h closed from the atmospheric moisture with a CaCl\(_2\)-tube. The formed precipitate was filtered and concentrated in vacuo. The reaction mixture was diluted with \(H_2O\) (150 ml) and extracted with EtOAc (2x100 ml). The combined organic phases were dried over MgSO\(_4\), filtered and concentrated in vacuo to obtain a white solid. The obtained solid was dissolved in 1,4-dioxane/H\(_2\)O mixture (1/1) (80 ml), followed by the addition of \(NaHCO_3\) (5.50 mmol). After stirring for 30min at rt, Fmoc-OSu (5.50 mmol) was added and the resulting reaction mixture was stirred for 20h at rt. The reaction mixture was concentrated in vacuo and the obtained residue was purified via column silica gel chromatography (CH\(_2\)Cl\(_2\)/MeOH/AcOH 100/10/1) to afford (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid as a white solid in 64% yield (2.38 g); mp: 132.4 – 137.2 °C; IR (neat): 3283, 3039, 2134, 1740 cm\(^{-1}\); \(^1\)H NMR (250 MHz, DMSO-d\(_6\)); \(\delta\) 12.68 (br. S, 1H), 7.87 (d, \(J = 7.3\) Hz, 2H), 7.66 (m, 3H), 7.40 (t, \(J = 7.4\) Hz, 2H), 7.29 (q, \(J = 7.5\) Hz, 2H), 7.19 (d, \(J = 8.6\) Hz, 2H), 6.87 (d, \(J = 8.6\) Hz, 2H), 4.72 (d, \(J = 2.5\) Hz, 2H), 4.03 – 4.24 (m, 4H), 3.52 (t, \(J = 2.6\) Hz, 1H), 3.01 (dd, \(J = 6.9\) and 3.8 Hz, 1 H), 2.80 (dd, \(J = 10.0\) and 7.5 Hz, 1H); \(^{13}\)C NMR (126 MHz, DMSO-d\(_6\)) \(\delta\) 174.0, 156.6, 156.5, 144.4, 141.4, 131.3, 130.8, 128.3, 127.7, 126.0, 120.8, 115.2, 80.1, 78.7, 86.3, 56.4, 56.0, 47.3, 36.3; HRMS Calcd for \([C_{22}H_{23}NO_5 + Na]^+\): 464.1469. Found 464.1466.
3. Solution phase synthesis of peptides 13a-c, 14a-c and 15a-c

Scheme 2: Synthesis of dipeptides 11a-b and 12a-b and tetrapeptides 13a-c, 14a-c and 15a-c

Synthesis of dipeptides 12a-b and 24a-b:

Benzyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serinate 23

Benzyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serinate 23 was synthesised according to literature. ( tert-Butoxycarbonyl)-L-serine (21.10 mmol) was dissolved in DMF (150 ml). NaH (42.20 mmol, 60 wt-% suspension in mineral oil) was added slowly. After stirring for 30min, propargyl bromide (29.50 mmol, 80 wt-% in toluene) was added. The reaction mixture was stirred at rt for 15h closed for atmospheric moisture with a CaCl₂-tube. The reaction mixture was diluted with brine (200 ml) and extracted with EtOAc (2x100 ml). The aqueous layer was acidified to pH 1-2 with 1M KHSO₄ and extracted with EtOAc (3x150 ml). The
combined organic layers were dried over MgSO₄, filtered, concentrated in vacuo and the resulting residue was dissolved in DMF (150 ml). NaHCO₃ (42.20 mmol) and benzyl bromide (52.80 mmol) were added dropwise. The reaction mixture was stirred at rt for 21h, diluted with brine (150 ml) and extracted with EtOAc (3x100 ml). The combined organic layers were washed with brine (2x100 ml), dried over MgSO₄, filtered and concentrated in vacuo. The obtained mixture was purified via column silica gel chromatography (hexane/EtOAc 1/1) to afford benzyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serinate as a colourless liquid in 30% yield (2.07 g). IR (neat): 3289, 2134, 1746 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ 7.29 – 7.44 (m, 5 H), 5.41 (d, J = 8.8 Hz, 1 H), 5.23 (dd, J = 12.5 Hz, 2 H), 4.46 – 4.57 (m, 1 H), 4.12 (d, J = 2.5 Hz, 2 H), 4.01 (dd, J = 5.0, 2.5 Hz, 1 H), 3.78 (dd, J = 5.0, 2.5 Hz, 1 H), 2.42 (t, J = 2.3 Hz, 1 H), 1.46 (s, 9 H); ¹³C NMR (CDCl₃, 126 MHz,): δ= 170.6, 155.7, 135.7, 128.8, 128.5, 128.3, 80.3, 79.0, 75.3, 70.0, 67.4, 58.8, 54.2, 28.5; HRMS Calcd for [C₁₈H₂₃NO₅ + Na⁺]: 356.1469. Found 356.1475. The obtained spectroscopic data were in accordance with literature data.⁴

**tert-Butyl (S)-2-(((S)-1-(benzylloxy)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate 24a**

[Diagram of the molecule]

Benzyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serinate (3.0 mmol) was dissolved in TFA (5 ml) and CH₂Cl₂ (5 ml) and stirred for 1h at rt protected from atmospheric moisture with a CaCl₂-tube. The reaction mixture was concentrated in vacuo and dissolved in CH₂Cl₂ (20 ml). The reaction mixture was cooled to 0 °C and N-Boc-Proline (3.0 mmol) and HOAt (6.0 mmol) were added. EDC.HCl (6.0 mmol) and DIPEA (6.0 mmol) were added next and the reaction mixture was stirred for 1h at rt protected from atmospheric moisture with a CaCl₂-tube. The reaction mixture was diluted with EtOAc (20 ml) and washed with H₂O (3x20 ml) and brine (3x20 ml). The organic layer was dried over MgSO₄, filtered and purified via column silica gel chromatography (petroleumether/EtOAc 5/1) to afford tert-butyl (S)-2-(((S)-1-(benzylloxy)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate as a yellow oil in 80% yield (1.03 g). IR (neat): 3288, 2114, 1744, 1674 cm⁻¹; ¹H NMR (250 MHz, CDCl₃): δ = 7.32 – 7.43 (m, 5 H), 6.88 (br. S, 1 H), 5.22 (dd, J = 12.5 Hz and 12.5 Hz, 2 H), 4.81 (br. S, 1 H), 4.31 (br. S, 1 H), 4.11 (d, J = 2.5 Hz, 2 H), 4.00 (dd, J = 2.5 Hz and 2.5 Hz, 1 H), 3.79 (dd, J = 2.5 Hz and 2.5 Hz, 1 H), 3.34 – 3.58 (m, 2 H), 2.40 (t, J = 2.4 Hz, 1 H), 2.17 (br. S, 2 H), 1.83 – 1.90 (m, 4 H), 1.47 (s, 9 H); ¹³C NMR (126 MHz, CDCl₃): δ 172.8, 169.8, 149.9, 135.6, 128.8, 128.6, 128.4, 79.0, 78.1, 75.3, 69.6, 67.5, 61.2, 58.7, 52.7, 47.2, 31.0, 28.6, 23.9; HRMS Calcd for [C₂₃H₃₀N₂O₆ + Na⁺]: 453.1996. Found 453.1991.
Benzyl \(N-(\text{tert-butoxycarbonyl})-O-(\text{prop-2-yn-1-yl})-L\)-serinate 24b

\[
\begin{align*}
\text{Benzyl } N-(\text{tert-butoxycarbonyl})-O-(\text{prop-2-yn-1-yl})-L\)-serinate (12.6 mmol) was dissolved in TFA (40 ml) and CH\(_2\)Cl\(_2\) (40 ml) and stirred for 1h at rt protected from atmospheric moisture with a CaCl\(_2\)-tube. The reaction mixture was concentrated in vacuo and dissolved in DMF (100 ml), followed by the addition of DIPEA (12.6 mmol). This mixture was added to a mixture of \(N\)-Boc-Alanine (11.5 mmol), HATU (13.7 mmol) and DIPEA (12.6 mmol), dissolved in DMF (100 ml) and stirred for 30min at 0 °C. The resulting mixture was stirred for 15h protected from atmospheric moisture with a CaCl\(_2\)-tube at rt. The reaction mixture was concentrated in vacuo and the obtained residue was dissolved in EtOAc (100 ml) and washed with an aqueous 1M KHSO\(_4\) solution (100 ml), an aqueous 1M NaOH solution (100 ml) and brine (3x100 ml). The organic layer was dried over MgSO\(_4\), filtered, concentrated in vacuo and purified via column silica gel chromatography (petroleumether/EtOAc 3/1) to afford benzyl \(N-(\text{tert-butoxycarbonyl})-O-(\text{prop-2-yn-1-yl})-L\)-serinate as a yellow liquid in 75% yield (3.5 g). IR (neat) 3351, 2173, 1723, 1699 \text{ cm}^{-1}; \text{\(^1\)H NMR (250 MHz, CDCl}_3\): \(\delta = 7.30 - 7.38 \text{ (m, 5 H)}, 6.78 (d, } J = 8.3 \text{ Hz, 1 H}), 5.20 (dd, } J = 7.5 \text{ and } 5.0 \text{ Hz, 2 H), 5.05 (br. s, 1 H), 4.78 - 4.81 \text{ (m, 1 H)}, 4.21 (br. s, 1 H), 4.09 (d, } J = 2.4 \text{ Hz, 2 H), 4.01 (dd, } J = 9.4 \text{ and } 3.2 \text{ Hz, 1 H}, 3.76 (dd, } J = 9.4 \text{ and } 3.2 \text{ Hz, 1 H), 2.40 (t, } J = 2.4 \text{ Hz, 1 H), 1.44 (s, 9 H), 1.36 \text{ (d, } J = 7.1 \text{ Hz, 3 H); } \text{\(^{13}\)C NMR (126 MHz, CDCl}_3\): } \delta = 172.8, 169.8, 155.5, 135.5, 128.8, 128.6, 128.4, 80.3, 79.0, 75.4, 69.5, 67.6, 58.8, 52.8, 50.3, 28.5, 18.9; \text{HRMS Calcd for [C}_{21}\text{H}_{28}\text{N}_2\text{O}_6 + Na}^+: 427.1840. \text{Found 427.1836.}

Methyl D-prolinate hydrochloride 21b

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\begin{align*}
\text{Methyl D-prolinate hydrochloride 21b was synthesised according to literature.}^5 \text{ To a cooled solution of D-proline (4.34 mmol) in MeOH (5 ml) was added SOCl}_2 \text{ (5.65 mmol) dropwise over 1.5h. The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a CaCl}_2\text{-tube. The solution was concentrated in vacuo to obtain a yellow oil. After high vacuum (0.05 mmHg) drying methyl D-prolinate hydrochloride was obtained as a gray oil in quantitative yield (0.72 g). IR (neat): 3391, 1739, 1232 \text{ cm}^{-1}; \text{\(^1\)H NMR (250 MHz, CDCl}_3\): } \delta 10.90 \text{ (br. s, 1 H), 9.13 \ (br. s, 1 H), 4.50 \ (br. s, 1 H), 3.86 \ (s, 3 H), 3.42 - 3.74 \ (m, 2 H), 2.35 - 2.56 \ (m, 1 H), 1.99 - 2.31 \ (m, 3 H); } \text{\(^{13}\)C NMR (126 MHz, CDCl}_3\): } \delta 169.2, 59.4, 53.9, 46.3, 29.0, 23.8; \text{HRMS Calcd for [C}_6\text{H}_{11}\text{NO}_2 + H}^+: 130.0863. \text{Found 130.0868. The obtained spectroscopic data were in accordance with literature data.}^5

S10
Methyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-proline 22a

A solution of N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine (0.82 mmol) in CH₂Cl₂ (10 ml) was cooled to 0°C and treated with (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (HATU) (1.15 mmol). Methyl D-proline hydrochloride (0.99 mmol) was added, followed by the addition of N-methylmorpholine (NMM) (3.29 mmol). The reaction mixture was stirred for 15 h at 0°C closed from atmospheric moisture with a CaCl₂-tube. The reaction mixture was concentrated in vacuo and the obtained residue was dissolved in EtOAc (10 ml). The organic phase was washed with H₂O (2×10 ml), 1 M HCl (10 ml), saturated aqueous NaHCO₃ solution (10 ml) and brine (10 ml). The organic layer was dried over MgSO₄, filtered and concentrated in vacuo. The obtained residue was purified via column silica gel chromatography (petroleum ether/EtOAc 2/1) to afford methyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-proline as a yellow oil in 76% yield (0.22 g). IR (neat): 3276, 2976, 2114, 1744, 1709, 1647; ¹H NMR (500 MHz, CDCl₃): δ 5.38 (d, J = 8.5 Hz, 1 H), 4.68 – 4.75 (m, 1 H), 4.48 (dd, J = 2.5 Hz and 2.5 Hz, 1 H), 4.09 – 4.20 (m, 2 H), 3.60 – 3.80 (m, 7 H), 2.42 (t, J = 2.5 Hz, 1 H), 2.17 – 2.28 (m, 1 H), 1.89 – 2.13 (m, 3 H), 1.45 (s, 9 H); ¹³C NMR (126 MHz, CDCl₃): δ 172.4, 169.1, 155.1, 79.9, 79.2, 74.8, 70.3, 59.1, 58.6, 52.2, 51.5, 47.2, 29.2, 28.3, 24.7; HRMS Calcd for [C₁₇H₂₆N₂O₆ + H⁺]: 355.1864. Found 355.1859.

Methyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-alaninate 22b

A solution of N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine (20.6 mmol) in DMF (125 ml) was cooled to 0°C and treated with benzotriazol-1-ol (HOt) (24.7 mmol), EDC.HCl (24.7 mmol), DIPEA (22.6 mmol) and a solution of methyl D-alaninate hydrochloride (22.6 mmol) and DIPEA (22.6 mmol) in DMF (125 ml). The reaction mixture was stirred for 15 h at 0°C closed from atmospheric moisture with a CaCl₂-tube. The reaction mixture was concentrated in vacuo and the obtained residue was dissolved in EtOAc (100 ml). The organic phase was washed with an aqueous 1M KHSO₄ solution (100 ml), an aqueous 1M NaOH solution (100 ml) and brine (3×100 ml). The organic layer was dried over MgSO₄, filtered and concentrated in vacuo. The obtained residue was purified via column silica gel chromatography (petroleum ether/EtOAc 2/1) to afford methyl N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-alaninate as a yellow oil in 34% yield (2.32 g). IR (neat): 3294, 2156, 1735, 1654; ¹H NMR (500 MHz, CDCl₃): δ 6.86 (br. s, 1 H), 5.35 (br. s, 1 H), 4.56
(quin, $J = 7.2$ Hz, 1 H), 4.24 - 4.32 (m, 1 H), 4.09 - 4.21 (m, 2 H), 3.89 (br. s, 1 H), 3.72 (s, 3 H), 3.66 (dd, $J = 9.4$, 6.0 Hz, 1 H), 2.43 (t, $J = 1.0$ Hz, 1 H), 1.43 (s, 9 H), 1.39 ppm (d, $J = 6.8$ Hz, 3 H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 173.3, 169.7, 155.8, 80.6, 79.2, 75.4, 69.4, 58.8, 54.1, 52.7, 48.4, 28.5, 18.5 ppm; HRMS Calcd for [C$_{15}$H$_{24}$N$_2$O$_6$ + H$^+$]: 329.1701. Found 329.1709.

$N$-(tert-Butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-proline $12a$

To a solution of $N$-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-prolinate (0.71 mmol) in a mixture of H$_2$O (10 ml) and THF (10 ml) was LiOH.H$_2$O (1.06 mmol) added. The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a CaCl$_2$-tube. The reaction mixture was diluted with H$_2$O (10 ml) and EtOAc (10 ml). The aqueous phase was separated and the pH was adjusted to pH 1-2 with a 1M HCl solution. The resulting mixture was extracted with EtOAc (3x20 ml). The organic phase was washed with brine (3x20 ml), dried over MgSO$_4$, filtered and concentrated in vacuo. After high vacuum (0.05mm Hg) drying $N$-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-proline was obtained as a yellow solid in 61% yield (0.18 g). mp: 49.2 – 52.3 °C; IR (neat): 3275, 2115, 1704, 1635, 1633 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 12.36 (s, 1 H), 6.87 and 7.29 (d, $J = 8.2$ Hz, 1 H)*, 4.45 – 4.54 and 4.77 – 4.83 (m, 1 H)*, 4.16 – 4.21 and 4.45 – 4.54 (m, 1 H)*, 4.07 – 4.08 and 4.13 – 4.15 (m, 2 H)*, 3.46 – 3.52 and 3.53 – 3.70 (m, 2 H)*, 3.40 – 3.41 and 3.44 – 3.47 (t, $J = 2.0$ Hz, 1 H)*, 3.33 – 3.37 and 3.53 – 3.70 (m, 2 H)*, 2.07 – 2.19 (m, 1 H), 1.65 – 1.74 and 1.82 – 1.95 (m, 3 H)*, 1.39 and 1.37 (2×s, 9 H)*; $^{13}$C NMR (126 MHz, DMSO-d$_6$): $\delta$ $^{13}$C NMR (DMSO-d$_6$,126MHz): $\delta$ 173.6 + 173.0*, 168.7 + 168.1*, 155.4 + 154.9*, 80.0, 78.3, 77.3 + 77.1*, 68.9 + 68.6*, 58.7, 57.7, 51.8 + 51.3*, 46.4 + 46.1*, 30.6 + 28.6*, 28.1 + 27.7*, 24.3; HRMS Calcd for [C$_{16}$H$_{24}$N$_2$O$_6$ –Boc + H$^+$]: 241.1183. Found 241.1178.

*Double signals due to rotamerism across the C-N bond of the amide as well as the carbamate.

$N$-(tert-Butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-alanine $12b$
To a solution of methyl \(N\)-(tert-butoxycarbonyl)-\(O\)-(prop-2-yn-1-yl)-L-seryl-D-alaninate (1.5 mmol) in a mixture of \(\text{H}_2\text{O}\) (10 ml) and \(\text{THF}\) (10 ml) was \(\text{LiOH}\) (3.8 mmol) added. The reaction mixture was stirred for 2h at rt protected from atmospheric moisture with a \(\text{CaCl}_2\)-tube. The reaction mixture was diluted with \(\text{H}_2\text{O}\) (20 ml) and \(\text{EtOAc}\) (20 ml). The aqueous phase was separated and the pH was adjusted to pH 1-2 with an aqueous 1M HCl solution. The resulting mixture was extracted with \(\text{EtOAc}\) (3x25 ml). The organic phase was washed with brine (3x25 ml), dried over \(\text{MgSO}_4\), filtered and concentrated in vacuo to afford \(N\)-(tert-butoxycarbonyl)-\(O\)-(prop-2-yn-1-yl)-L-seryl-D-alanine as a yellow solid in 99% yield (0.48 g). mp: 95.2 - 97.3 °C; IR (neat): 3281, 2135, 1714, 1655 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta = 7.02\) (br. s, 1 H), 5.45 - 5.56 (m, 1 H), 4.58 (br. s, 1 H), 4.47 (br. s, 1 H), 4.09 - 4.22 (m, 2 H), 3.86 (dd, \(J = 9.2, 4.3\) Hz, 1 H), 3.68 (br. s, 1 H), 2.44 (t, \(J = 2.4\) Hz, 1 H), 1.43 ppm (s, 12 H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta 175.5, 170.3, 156.2, 81.0, 79.1, 75.5, 69.6, 58.8, 54.0, 54.0, 48.4, 28.5, 18.3\) ppm; HRMS Calcd for [\(\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_6\) + H\(^+\)]: 315.1551. Found 315.1556.

**Difficult D-Pro Pro coupling:**

The coupling of Boc-Pro-OH with dipeptide \(11\), after Boc deprotection of \(24\), appeared to be problematic due to a difficult D-Pro Pro coupling. Several coupling reagents were tried and the conversions were rather poor (Table 2). Therefore, instead of a linear synthesis, a convergent synthesis was chosen towards tetrapeptide \(13\), where HATU as coupling reagent in DMF afforded the highest conversion (>80%).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Coupling reagent</th>
<th>solvent</th>
<th>t (h)</th>
<th>Conv. (%)</th>
</tr>
</thead>
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<td>HOBt/EDC</td>
<td>\text{CH}_2\text{Cl}_2</td>
<td>48</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>HOAt/EDC</td>
<td>\text{CH}_2\text{Cl}_2</td>
<td>48</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>HATU</td>
<td>\text{CH}_2\text{Cl}_2</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>TBTU</td>
<td>DMF</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
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<td>EtOAc</td>
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<tr>
<td>6</td>
<td>BOP-Cl</td>
<td>\text{CH}_2\text{Cl}_2</td>
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<td>0</td>
</tr>
<tr>
<td>7(^a)</td>
<td>PyBOP</td>
<td>\text{CH}_3\text{CN}</td>
<td>72</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 2:** Evaluation of the D-Pro Pro coupling with several coupling reagentia (\(^a\) a complex reaction mixture was obtained)

**Synthesis of tetrapeptides 13b-c, 14b-c and 15b-c:**

Benzyl \(N\)-(tert-butoxycarbonyl)-\(O\)-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-\(O\)-(prop-2-yn-1-yl)-L-serinate \(13\)
Benzyl $N$-((tert-butoxycarbonyl)-L-alanyl)-$O$-(prop-2-yn-1-yl)-L-serinate (2.7 mmol) was dissolved in a mixture of TFA (5 ml) and CH$_2$Cl$_2$ (5 ml). The reaction mixture was stirred for 1h closed from atmospheric moisture with a CaCl$_2$-tube and concentrated in vacuo to obtain 11b as a TFA salt. To a cooled solution of $N$-((tert-butoxycarbonyl)-$O$-(prop-2-yn-1-yl)-L-seryl-D-proline (2.6 mmol) in CH$_2$Cl$_2$ (10 ml) was added HOBT (3.1 mmol), EDC.HCl (3.1 mmol) and DIPEA (5.7 mmol) and the reaction mixture was stirred for 30min at 0 °C. The TFA salt (11b), dissolved in CH$_2$Cl$_2$ (100 ml), was added to the reaction mixture, followed by the addition of DIPEA (2.9 mmol). The reaction mixture was stirred for 15h at rt protected from atmospheric moisture with a CaCl$_2$-tube. The reaction mixture was concentrated in vacuo and the obtained residue was purified via column silica gel chromatography (CHCl$_3$/iPrOH 30/1) to afford benzyl $N$-((tert-butoxycarbonyl)-$O$-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-$O$-(prop-2-yn-1-yl)-L-serinate as a yellow oil in 64% yield (1.08 g). HRMS Calcd for [C$_{32}$H$_{42}$N$_4$O$_9$ + Na$^+$]: 649.2844. Found 649.2865.

Benzyl $N$-$N$-((tert-butoxycarbonyl)-$O$-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-$O$-(prop-2-yn-1-yl)-L-serinate 13c

Benzyl $N$-((tert-butoxycarbonyl)-L-alanyl)-$O$-(prop-2-yn-1-yl)-L-serinate (8.80 mmol) was dissolved in a mixture of TFA (100 ml) and CH$_2$Cl$_2$ (100 ml) and stirred for 2h. The reaction mixture was evaporated in vacuo, dissolved in DMF (150 ml), followed by the addition of DIPEA (8.80 mmol). This mixture was added to a mixture of $N$-((tert-butoxycarbonyl)-$O$-(prop-2-yn-1-yl)-L-seryl-D-alanine (7.98 mmol), HOAt (9.57 mmol), EDC.HCl (9.57 mmol) and DIPEA (8.80 mmol), dissolved in DMF (150 ml) and stirred for 30min at 0 °C. The resulting reaction mixture was stirred for 15h at rt, evaporated in vacuo and the resulting crude mixture was purified via column silica gel chromatography (CHCl$_3$/MeOH 99/1) to afford benzyl $N$-$N$-((tert-butoxycarbonyl)-$O$-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-$O$-(prop-2-yn-1-yl)-L-serinate as a yellow solid in 75% yield (3.60 g). HRMS Calcd for [C$_{30}$H$_{40}$N$_4$O$_9$ + Na$^+$]: 623.2687. Found 623.2681.

Benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-tetrahydropyrrolo-[2,1-i]-6-methyl-5,8,11-trioxo-1,14-dioxa-4,7,10-triazacycloicosa-16,18-diyne-3-carboxylate 14b
Benzyl N-N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-prolyl-L-alanyl-O-(prop-2-yn-1-yl)-L-serinate (1.5 mmol), Cu(OAc)$_2$H$_2$O (1.5 mmol) and NiCl$_2$ (1.5 mmol) were added to EtOH (750 ml). Et$_3$N (4.5 mmol) and pyridine (7.5 mmol) were added and the reaction mixture was stirred at 60 °C under O$_2$-atmosphere for 15h. The reaction mixture was concentrated in vacuo and purified via column silica gel chromatography (CH$_2$Cl$_2$/iPrOH 97/3) to afford benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-10-pyrolo-6-dimethyl-5,8,11-trioxo-1,14-dioxoa-4,7,10-triazacycloicaosa-16,18-diyne-3-carboxylate as a red solid in 41% yield (0.38 g). HRMS Calcd for [C$_{32}$H$_{40}$N$_4$O$_9$ + Na$^+$]: 647.2687. Found 647.2694.

Benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxoa-4,7,10-triazacycloicaosa-16,18-diyne-3-carboxylate 14c

Benzyln N-N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-seryl-D-alanyl-L-alanyl-O-(prop-2-yn-1-yl)-L-serinate (2.50 mmol), Cu(OAc)$_2$H$_2$O (2.50 mmol) and NiCl$_2$ (2.50 mmol) were added to DMF (1200 ml). Et$_3$N (7.49 mmol) and pyridine (12.49 mmol) were added and the reaction mixture was stirred at 60 °C under O$_2$-atmosphere for 24h. The reaction mixture was concentrated in vacuo and purified via column silica gel chromatography (CHCl$_3$/MeOH 99/1) to afford benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxoa-4,7,10-triazacycloicaosa-16,18-diyne-3-carboxylate as a white solid in 28% yield (410 mg). HRMS Calcd for [C$_{30}$H$_{38}$N$_4$O$_9$ + H$^+$]: 599.2712. Found 599.2718.

(3S,6S,17S,22aR)-17-((tert-Butoxycarbonyl)amino)-3-methyl-1,4,18-trioxooctadecahydro-1H,16H-pyrrolo[2,1-i][1,14]dioxoa-4,7,10-triazacycloicosine-6-carboxylic acid 15b
To a mixture of benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-10-pyrolo-6-dimethyl-5,8,11-trioxo-1,14-dioxo-4,7,10-triazacycloicos-16,18-dyne-3-carboxylate (0.032 mmol) in MeOH (1 ml) was added palladium on carbon (10 wt-% Pd on C, 10 mg). The reaction mixture was stirred under H₂ (1 atm) at rt for 24h, filtered over Celite and concentrated in vacuo to afford (3S,6S,17S,22aR)-17-((tert-butoxycarbonyl)amino)-3-methyl-1,4,18-trioxo-octadecahydro-1H,16H-pyrrolo[2,1-i][1,14]dioxo[4,7,10]triazacycloicosine-6-carboxylic acid as a white solid in 92% yield (16.0 mg). HRMS Calcd for [C₃₁H₄₈N₅O₉ + Na⁺]: 565.2844. Found 565.2830.

(3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxo-4,7,10-triazacycloicosane-3-carboxylic acid 15c

To a mixture of benzyl (3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxo-4,7,10-triazacycloicos-16,18-dyne-3-carboxylate (0.017 mmol) in MeOH (1 ml) was added palladium on carbon (10 wt-% Pd on C, 5 mg). The reaction mixture was stirred under H₂ (1 atm) at rt for 24h, filtered over Celite and concentrated in vacuo to afford (3S,6S,17S,22aR)-17-((tert-butoxycarbonyl)amino)-3-methyl-1,4,18-trioxooctadecahydro-1H(3S,6S,9R,12S)-12-((tert-butoxycarbonyl)amino)-6,9-dimethyl-5,8,11-trioxo-1,14-dioxo-4,7,10-triazacycloicosane-3-carboxylic acid as a white solid in 85% yield (7.5 mg). HRMS Calcd for [C₂₅H₄₀N₅O₉ + Na⁺]: 539.2687. Found 539.2693.

4. Solid Phase Peptide Synthesis of 5a-k, 7 and 9

5a: 39% yield; HPLC (standard gradient): t_{ret} = 10.27 min.; HRMS Calcd for [C₂₃H₃₃N₅O₇ + H⁺]: 504.2453. Found 504.2444.

5b: 43% yield; HPLC (standard gradient): t_{ret} = 8.62 min.; HRMS Calcd for [C₂₁H₂₉N₅O₇ + H⁺]: 464.2140. Found 464.2132.
Cyclization and purification of peptides 5a-k, 7 and 9

a. Purification using RP preparative HPLC

The obtained ethanolic reaction mixtures containing the diyne, pyridine, triethylamine and catalysts were evaporated in vacuo and directly loaded on the RP HPLC column to give analytically pure samples as white solids after lyophilization.

6a: 9% yield (3.5 mg); HPLC (standard gradient): t_{ret} = 9.46 min.; HRMS Calcd for [C_{24}H_{34}N_{5}O_{7} + H^+]: 502.2267. Found 502.2296.

6b: 39% yield; HPLC (standard gradient): t_{ret} = 8.74 min.; HRMS Calcd for [C_{21}H_{27}N_{5}O_{7} + H^+]: 462.1983. Found 462.1982.
6c: 14% yield; HPLC (standard gradient): \( t_{\text{ret}} = 9.02 \text{ min.} \); HRMS Calcd for \([C_{22}H_{29}N_{5}O_{7} + H^+]: 476.2140\). Found 476.2122.

6d: 40% yield; HPLC (standard gradient): \( t_{\text{ret}} = 9.11 \text{ min.} \); HRMS Calcd for \([C_{22}H_{29}N_{5}O_{7} + H^+]: 476.2140\). Found 476.2136.

6e: 4% yield; HPLC (standard gradient): \( t_{\text{ret}} = 8.68 \text{ min.} \); HRMS Calcd for \([C_{21}H_{29}N_{5}O_{7} + H^+]: 464.2140\). Found 464.2145.

6f: 4% yield; HPLC (standard gradient): \( t_{\text{ret}} = 9.16 \text{ min.} \); HRMS Calcd for \([C_{23}H_{31}N_{5}O_{7} + H^+]: 490.2296\). Found 490.2292.

6g: 10% yield; HPLC (standard gradient): \( t_{\text{ret}} = 8.21 \text{ min.} \); HRMS Calcd for \([C_{20}H_{27}N_{6}O_{7} + H^+]: 450.1983\). Found 450.1985.

6h: 25% yield; HPLC (standard gradient): \( t_{\text{ret}} = 7.46 \text{ min.} \); HRMS Calcd for \([C_{20}H_{27}N_{6}O_{7} + H^+]: 450.1983\). Found 450.1980.

6i: 4% yield; HPLC (standard gradient): \( t_{\text{ret}} = 7.32 \text{ min.} \); HRMS Calcd for \([C_{20}H_{26}N_{6}O_{8} + H^+]: 479.1885\). Found: 479.1862.

6j: 5% yield; HPLC (standard gradient): \( t_{\text{ret}} = 11.55 \text{ min.} \); HRMS Calcd for \([C_{28}H_{33}N_{5}O_{9} + H^+]: 584.2351\). Found: 584.2299.

6k: 17% yield; HPLC (standard gradient): \( t_{\text{ret}} = 12.00 \text{ min.} \); HRMS Calcd for \([C_{31}H_{40}N_{6}O_{9} + H^+]: 641.2930\). Found: 641.2910.

8: 5% yield; HPLC (standard gradient): \( t_{\text{ret}} = 12.18 \text{ min.} \); HRMS Calcd for \([C_{36}H_{38}N_{5}O_{7} + H^+]: 578.2609\). Found 578.2607.

10: 4% yield; HPLC (standard gradient): \( t_{\text{ret}} = 14.45 \text{ min.} \) (90% pure); HRMS Calcd for \([C_{36}H_{39}N_{5}O_{7} + Na^+]: 676.2742\). Found 676.2738.

b. Purification using silica gel column chromatography

As stated in the manuscript, the silica gel column chromatography of poorly soluble peptides (in CH$_2$Cl$_2$) resulted in green coloured compounds because of the presence of co-eluted copper salts. Therefore, different chelators were tested as plugs over a silica column to verify whether these salts can be retained during chromatography. Conditions were evaluated where the reaction mixture was treated (a) with a blank (no scavenger), (b) 5 equiv Na$_4$EDTA, (c) 5 equiv Na$_3$citrate and (4) 5 equiv Na$_3$PO$_4$. The mixture was stirred for a few minutes and silica was added before the solvent was evaporated. This resulted in blue-green coated silica which was afterwards brought onto the purification column. As can be seen from the figure below, only the use of Na$_3$PO$_4$ as scavenger gave good results.
Scheme 0.1. Different scavengers tested to complex the Cu- and Ni-salts: A = blank, B = Na₄EDTA, C = Na₃citrate and D = Na₃PO₄.

In a control experiment, the effect of Na₃PO₄ on complexing the copper salts was demonstrated. In the figure below (left) the reaction mixture containing the Cu- and Ni-salts is compared to the same solution but treated with 5 equiv of Na₃PO₄. It can be seen that the Na₃PO₄ is responsible for clearing the supernatant solution from the excess of coloured salts.

The use of silica gel column chromatography as purification method was demonstrated for peptide derivatives 6a, 6c, 6e, 6g, 6i, 8 and 10 which gave low yield via RP preparative HPLC. The mixture was treated with 5 equiv of Na₃PO₄ (as aq. sat. solution) during 15 minutes. Afterwards the mixture was concentrated, loaded on a plug of silica gel (2cm, diameter 0.5cm) and eluted using 20% MeOH in CH₂Cl₂. After evaporation in vacuo, 5mL of hexane
was added and the mixture was left without stirring to remove traces of pyridine. After decanting the hexane, the remaining solids were further evaporated at high vacuum (0.1 mbar) resulting in cyclic peptides as white solids.

6a 68% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.29. HPLC (standard gradient): tₑₑₜ = 9.46 min.

6c 66% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.22. HPLC (standard gradient): tₑₑₜ = 9.02 min.

6e 48% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.15. HPLC (standard gradient): tₑₑₜ = 8.68 min.

6g 45% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.12. HPLC (standard gradient): tₑₑₜ = 8.21 min.

6i 61% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.05. HPLC (standard gradient): tₑₑₜ = 7.32 min.

8 54% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.29. HPLC (standard gradient): tₑₑₜ = 12.18 min.

10 76% yield; TLC (silica, 20% MeOH in CH₂Cl₂): R₇ = 0.31. HPLC (standard gradient): tₑₑₜ = 14.45 min.
c. Solid phase cyclisation of peptide 5a

Compound 5a was synthesised on solid phase via the general procedure for SPPS. However, instead of acetylation of the amine terminus, cleaving the peptide from the resin and purifying the acyclic peptide via preparative HPLC, the solid supported N-Fmoc protected peptide was directly cyclised on solid phase using a BIOTAGE Initiator® SP Microwave Synthesizer. Cu(OAc)$_2$·H$_2$O (1 equiv.), NiCl$_2$ (1 equiv.), Et$_3$N (3 equiv.) and pyridine (5 equiv.) were dissolved in CHCl$_3$ (1 ml) and added to the resin (50 mg). After reaction for 30min at 50 °C in the microwave, 90% conversion towards the cyclic peptide is observed according to HPLC and LC-MS. As a sideproduct, dimer 6a’ is formed in 10%, according to HPLC and LC-MS.

Figure 1a: HPLC chromatogram of crude starting material 5a

Figure 1b: HPLC chromatogram of the crude reaction mixture of the solid phase cyclisation for peptide 5a
6. Copies of $^1$H and $^{13}$C NMR of compounds 1, 2, 12, 13a, 14a, 15a, 17, 18, 20-24 and ROESY NMR of compounds 13a, 14a and 15a

Figure 2: $^1$H NMR spectrum of 1a (250 MHz, CDCl$_3$)

Figure 3: $^{13}$C NMR spectrum of 1a (126 MHz, CDCl$_3$)
Figure 4: $^1\text{H}$ NMR spectrum of $1b$ (250 MHz, CDCl$_3$)

Figure 5: $^{13}\text{C}$ NMR spectrum of $1b$ (63 MHz, CDCl$_3$)
Figure 6: $^1$H NMR spectrum of 2a (250 MHz, CDCl$_3$)

Figure 7: $^{13}$C NMR spectrum of 2a (63 MHz, CDCl$_3$)
Figure 8: $^1$H NMR spectrum of 2b (250 MHz, CDCl$_3$)

Figure 9: $^{13}$C NMR spectrum of 2b (63 MHz, CDCl$_3$)
Figure 10: $^1$H NMR spectrum of 17 (250 MHz, CDCl$_3$)

Figure 11: $^{13}$C NMR spectrum of 17 (63 MHz, CDCl$_3$)
Figure 12: $^1$H NMR spectrum of 18 (250 MHz, DMSO-d$_6$)

Figure 13: $^{13}$C NMR spectrum of 18 (63 MHz, DMSO-d$_6$)
Figure 14: $^1$H NMR spectrum of 20 (250 MHz, DMSO-$d_6$)

Figure 15: $^{13}$C NMR spectrum of 20 (126 MHz, DMSO-$d_6$)
Figure 16: $^1$H NMR spectrum of 23 (250 MHz, CDCl$_3$)

Figure 17: $^{13}$C NMR spectrum of 23 (126 MHz, CDCl$_3$)
Figure 18: $^1$H NMR spectrum of 24a (250 MHz, CDCl$_3$)

Figure 19: $^{13}$C NMR spectrum of 24a (126 MHz, CDCl$_3$)
Figure 20: $^1$H NMR spectrum of 24b (250 MHz, CDCl$_3$)

Figure 21: $^{13}$C NMR spectrum of 24b (126 MHz, CDCl$_3$)
Figure 22: $^1$H NMR spectrum of 21 (250 MHz, CDCl$_3$)

Figure 23: $^{13}$C NMR spectrum of 21 (63 MHz, CDCl$_3$)
Figure 24: $^1$H NMR spectrum of 22a (500 MHz, CDCl$_3$)

Figure 25: $^{13}$C NMR spectrum of 22a (126 MHz, CDCl$_3$)
Figure 26: $^1$H NMR spectrum of 22b (500 MHz, CDCl$_3$)

Figure 27: $^{13}$C NMR spectrum of 22b (126 MHz, CDCl$_3$)
Figure 28: $^1$H NMR spectrum of 12a (500 MHz, DMSO-d$_6$)

Figure 29: $^{13}$C NMR spectrum of 12a (126 MHz, DMSO-d$_6$)
12b

Figure 30: $^1$H NMR spectrum of 12a (500 MHz, CDCl$_3$)

Figure 31: $^{13}$C NMR spectrum of 12a (126 MHz, CDCl$_3$)
Figure 32: $^1$H spectrum of 13a (700 MHz, CD$_3$CN)

Figure 33: $^{13}$C DEPT 135 spectrum of 13a (176 MHz, CD$_3$CN)
Figure 33: $^1$H-$^1$H ROESY spectrum of 13a (700 MHz, 250 ms mixing time, CD$_3$CN)

The main isomer (trans-trans) of 13a is exchanging slowly on the NMR time scale, as evidenced by the exchange cross peaks in the $^1$H-$^1$H ROESY spectrum. A cross peak between e.g. the trans-trans isomer and the cis-cis isomer is not witnessed due to the low probability of two peptide bonds changing geometry at the same time.
Figure 34: $^1$H spectrum of 14a (700 MHz, CD$_3$CN)

Figure 35: $^{13}$C DEPT 135 spectrum of 14a (176 MHz, CD$_3$CN)
Figure 36: $^1$H-$^1$H ROESY spectrum of $^{14}$a (700 MHz, 250 ms mixing time, CD$_3$CN)
Figure 37: $^1$H spectrum of 15a (700 MHz, CD$_3$CN)

Figure 38: $^{13}$C DEPT 135 spectrum of 15a (176 MHz, CD$_3$CN)
Figure 39: $^1$H-$^1$H ROESY spectrum of 15a (700 MHz, 250 ms mixing time, CD$_3$CN)
7. Copies of HPLC chromatograms of purified peptides 5a-k, 7 and 9 and crude reaction mixtures of peptides 6a-k, 8 and 10

**Figure 40:** HPLC chromatogram of 5a purified via preparative RP-HPLC.
Figure 41: HPLC chromatogram of the crude reaction mixture of 6a.
Figure 42: HPLC chromatogram of 5b purified via preparative RP-HPLC.
Figure 43: HPLC chromatogram of the crude reaction mixture of 6b.
Figure 44: HPLC chromatogram of 5c purified via preparative RP-HPLC.
Figure 45: HPLC chromatogram of the crude reaction mixture of 6c.
Figure 46: HPLC chromatogram of 5d purified via preparative RP-HPLC.
Figure 47: HPLC chromatogram of the crude reaction mixture of 6d.
Figure 48: HPLC chromatogram of 5e purified via preparative RP-HPLC.
Figure 49: HPLC chromatogram of the crude reaction mixture of 6e.
Figure 50: HPLC chromatogram of 5f purified via preparative RP-HPLC.
Figure 51: HPLC chromatogram of the crude reaction mixture of 6f.
Figure 52: HPLC chromatogram of 5g purified via preparative RP-HPLC.
Figure 53: HPLC chromatogram of the crude reaction mixture of 6g.
Figure 54: HPLC chromatogram of 5h purified via preparative RP-HPLC.
Figure 55: HPLC chromatogram of the crude reaction mixture of 6h.
Figure 56: HPLC chromatogram of 5i purified via preparative RP-HPLC.
Figure 57: HPLC chromatogram of the crude reaction mixture of 6i.
Figure 58: HPLC chromatogram of 5j purified via preparative RP-HPLC.
Figure 59: HPLC chromatogram of the crude reaction mixture of 6j.
Figure 60: HPLC chromatogram of 5k purified via preparative RP-HPLC.
Figure 61: HPLC chromatogram of the crude reaction mixture of 6k.
Figure 64: HPLC chromatogram of 7 purified via preparative RP-HPLC.
Figure 65: HPLC chromatogram of the crude reaction mixture of 8.
Figure 62: HPLC chromatogram of 9 purified via preparative RP-HPLC.
Figure 63: HPLC chromatogram of the crude reaction mixture of 10.
8. References


