Formation of a cyclic tetrapeptide mimic by thermal azide-alkyne 1,3-dipolar cycloaddition

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CONTENT

Synthesis of 9 .............................................................................................................................. S2

1H NMR and 13C NMR Spectra of 5 .................................................................................. S7

1H NMR and 13C NMR Spectra of 6 .................................................................................. S8

1H NMR Spectrum of the Cyclodimerisation Product of 6 at 25°C and at 100°C .................. S9

1H NMR and 13C NMR Spectra of 7 .................................................................................. S10

1H NMR and 13C NMR Spectra of 8 .................................................................................. S11

1H NMR Spectrum of 9 at 25°C and at 100°C ..................................................................... S12

13C NMR Spectrum of 9 ..................................................................................................... S13

ESI MS Spectrum of 9 ......................................................................................................... S14

2D NOESY NMR Spectrum of 9 .......................................................................................... S15

Single crystal structure X-ray analysis of (R,R)-9·2(DMSO) ............................................. S16

Single crystal structure X-ray analysis of anhydrous (S,S)-10 ........................................... S18
**Synthesis of Cyclic Pseudotetrapeptide 9:**

**General details.** Analyses were carried out as follows: melting points, Müller SPM-X 300; NMR, Bruker Avance 600, Bruker DPX 400; EI-MS: GCT-Premier CAB 163, Waters; MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; ESI-MS, Bruker Esquire 3000; IR, FT-IR System Spectrum BX, Perkin-Elmer; elemental analysis, Elementar vario Micro cube. The following abbreviations are used: Epa, 2-amino-6-ethynyl-2-pyridine; Lac, lactic acid; Tri, triazole; Ala, alanine.

![Chemical Structure](image)

6-[(Trimethylsilyl)ethynyl]pyridine-2-amine. 2-Amino-6-bromopyridine (1.5 g, 8.7 mmol), (Ph3)PdCl2 (240 mg, 345 μmol, 4 mol%), bis(2-diphenylphosphinophenyl)ether (DPEphos) (185 mg, 345 μmol, 4 mol%) and CuI (165 mg, 870 μmol, 10 mol%) were stirred in freshly distilled NEt3 (30 mL) for 30 min at room temperature. To this yellow solution, ethynyltrimethylsilane (1.3 mL, 9.6 mmol, 1.2 eq) was added dropwise. The resulting black solution was stirred for additional 12 h. After removal of the solvent, the residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, v/v) to give the product as an off white solid, which was pure enough for the next step. Analytical pure material was obtained by sublimation (100 °C, 5 · 10⁻² mbar). Yield 1.6 g (97%); mp. 126°C; ¹H NMR (600 MHz, [D₆]DMSO, 25°C) δ = 0.21 (s, 9H, Si(CH₃)₃), 6.11 (s, 2H, NH₂), 6.43 (d, 1H, ³J(H,H) = 8.3 Hz, EpaH(3)), 6.63 (d, 1H, ³J(H,H) = 7.9 Hz, EpaH(5)), 7.33 (t, 1H, ³J(H,H) = 8.2, EpaH(4)); ¹³C NMR (151 MHz, [D₆]DMSO, 25°C) δ = –0.2 (Si(CH₃)₃), 91.4 (Si-C≡C), 105.5 (Si-C≡C), 108.9 (EpaC(3)), 115.6 (EpaC(5)), 137.3 (EpaC(4)), 139.7 (EpaC(6)), 159.7 (EpaC(2)); MS (70 eV): m/z (%): 175.06 (100%) [M⁺–CH₃], 190.09 (83%) [M⁺]; elemental analysis calcd (%) for C₁₀H₁₄N₂Si: C 63.11, H 7.41, N 14.72; found C 63.32, H 7.39, N 14.80.
TMS-Epa-(S)-Lac-OMs (5). (S)-1-Chloro-1-oxopropan-2-yl methanesulfonate (4.0 g, 19.8 mmol) (obtained in three steps from (S)-methyl lactate by mesylation,\textsuperscript{1} ester hydrolysis,\textsuperscript{2} and conversion of the free acid into the acid chloride\textsuperscript{3} by following the described procedures) in dry dichloromethane (3 mL) was added dropwise to a solution of 6-[(trimethylsilyl)ethynyl]pyridine-2-amine (2.5 g, 13.2 mmol), pyridine (1.5 mL, 18.5 mmol) and DMAP (8 mg, 0.5 mol%) in dry dichloromethane (15 mL) at 0 °C. The solution was stirred overnight while it was allowed to reach room temperature. Afterwards, it was washed with 10% aqueous K$_2$CO$_3$ (20 mL) and water (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, v/v) to give the crude product. Recrystallisation from hexane/ethyl acetate afforded it in analytical pure form as white needles. Yield 3.7 g (83 %); mp. 117-118°C; $[\alpha]_D^{20} = -46.7$ (c = 1, CHCl$_3$); $^1$H NMR (600 MHz, [D$_6$]DMSO, 25°C) $\delta$ = 0.24 (s, 9H, Si(CH$_3$)$_3$), 1.52 (d, 3H, $^3$J(H,H) = 6.7 Hz, LacCH$_3$), 3.25 (s, 3H, SO$_2$CH$_3$), 5.23 (q, 1H, $^3$J(H,H) = 6.7 Hz, LacCH), 7.30 (d, 1H, $^3$J(H,H) = 7.7 Hz, EpaH(5)), 7.83 (t, 1H, $^3$J(H,H) = 7.9, EpaH(4)), 8.07 (d, 1H, $^3$J(H,H) = 7.9 Hz, EpaH(3)), 11.00 (s, 1H, NH); $^{13}$C NMR (151 MHz, [D$_6$]DMSO, 25°C) $\delta$ = -0.4 (Si(CH$_3$)$_3$), 18.6 (LacCH$_3$), 38.1 (SO$_2$CH$_3$), 75.2 (LacCH), 94.1 (Si-C=C), 103.5 (Si-C=C), 114.1 (EpaC(3)), 123.1 (EpaC(5)), 139.3 (EpaC(4)), 140.4 (EpaC(6)), 151.4 (EpaC(2)), 168.2 (LacC=O); MS (70 eV): $m/z$ (%): 217.08 (52%) [M$^+$-CH(CH$_3$)$_2$OSO$_2$CH$_3$], 261.11 (100%) [M$^+$-SO$_2$CH$_3$], 340.09 (11%) [M$^+$]; elemental analysis calcd (%) for C$_{14}$H$_{20}$N$_2$O$_4$SSi: C 49.39, H 5.92, N 8.23, S 9.27; found C 49.49, H 5.95, N 8.16, S 9.42.

TMS-Epa-(R)-Ala-N₃ (6). Compound 5 (500 mg, 1.47 mmol) and NaN₃ (115 mg, 1.77 mmol, 1.2 equiv) were stirred in DMF (3 mL) at 50 °C for 30 min. Ethylacetate (10 mL) was added and the mixture was washed with water (10 mL). The solvent was evaporated and the crude product subjected to column chromatography (silica gel; hexane/ethyl acetate, 3:1, v/v) to give 6 as a slightly yellow oil. Yield 380 mg (90 %); ¹H NMR (600 MHz, [D₆]DMSO, 25°C) δ = 0.23 (s, 9H, Si(CH₃)₃), 1.43 (d, 3H, ³J(H,H) = 6.9 Hz, LacCH₃), 4.09 (q, 1H, ³J(H,H) = 6.9 Hz, LacCH), 7.28 (d, 1H, ³J(H,H) = 7.3 Hz, EpaH(5)), 7.82 (t, 1H, ³J(H,H) = 7.9, EpaH(4)), 8.09 (d, 1H, ³J(H,H) = 8.4 Hz, EpaH(3)), 11.03 (s, br, 1H, NH); ¹³C NMR (151 MHz, [D₆]DMSO, 25°C) δ = −0.4 (Si(CH₃)₃), 16.6 (LacCH₃), 57.1 (LacCH), 94.0 (Si-C≡C), 103.5 (Si-C≡C), 114.0 (EpaC(3)), 123.0 (EpaC(5)), 139.2 (EpaC(4)), 140.1 (EpaC(6)), 151.6 (EpaC(2)), 170.1 (LacC=O); IR (KBr): ν bar = 2123 cm⁻¹ (azide).

H-Epa-(S)-Lac-OMs (7). To a solution of 5 (1.24 g, 3.65 mmol) in THF (10 mL) was added a solution of nBu₄NF·3 H₂O (1.73 g, 7.29 mmol, 1.5 equiv) in THF (10 mL) dropwise at 0 °C. The reaction mixture was stirred at this temperature for 30 min and then diluted with ethyl acetate (20 mL). The resulting mixture was washed with water (40 mL). The water phase was extracted twice with ethyl acetate (20 mL), and the combined organic layers were concentrated in vacuo. The residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, v/v) to give 7 as a white solid. Recrystallisation from hexane/ethyl acetate afforded the pure compound as colourless needles. Yield 840 mg (86 %); [α]₀²⁰ = −48.0 (c = 1, CHCl₃); mp. 104°C; ¹H NMR (600 MHz, [D₆]acetone, 25°C) δ = 1.68 (d, 3H, ³J(H,H) = 6.8 Hz, LacCH₃), 3.28 (s, 3H, SO₂CH₃), 3.78 (s, 1H, HC≡C), 5.34 (q, 1H, ³J(H,H) = 6.8 Hz, LacCH), 7.33 (d, 1H, ³J(H,H) = 7.5 Hz, EpaH(5)),
7.84 (t, 1H, $^3\text{J}$(H,H) = 8.0, EpaH(4)), 8.29 (d, 1H, $^3\text{J}$(H,H) = 8.4 Hz, EpaH(3)), 9.48 (s, br, 1H, NH);

$^{13}$C NMR (151 MHz, [D$_6$]acetone, 25°C) $\delta$ = 19.1 (LacCH$_3$), 38.7 (SO$_2$CH$_3$), 76.7 (HC≡C), 78.8 (LacCH), 83.2 (HC≡C), 114.9 (EpaC(3)), 124.6 (EpaC(5)), 139.7 (EpaC(4)), 141.5 (EpaC(6)), 152.3 (EpaC(2)), 169.0 (LacC=O); MS (70 eV): $m/z$ (%): 145.04 (100%) [M$^-$–CH(CH$_3$)OSO$_2$CH$_3$], 189.07 (45%) [M$^-$–SO$_2$CH$_3$], 268.05 (11%) [M$^-$]; elemental analysis calcd (%) for C$_{11}$H$_{12}$N$_2$O$_4$S: C 49.24, H 4.51, N 10.44, S 11.95; found C 49.53, H 4.59, N 10.32, S 11.75.

**H-Epa-(R)-Ala-N$_3$ (8).** Compound 7 (750 mg, 2.80 mmol) and NaN$_3$ (200 mg, 3.08 mmol, 1.1 equiv) were stirred in DMF (3 mL) at 50 °C for 30 min. Ethylacetate (10 mL) was added and the mixture was washed with water (10 mL). The solvent was evaporated and the crude product subjected to column chromatography (silica gel; hexane/ethyl acetate, 1:1, v/v) to give 8 as an almost colourless oil. Yield 580 mg (95 %); $^1$H NMR (600 MHz, [D$_6$]DMSO, 25°C) $\delta$ = 1.44 (d, 3H, $^3\text{J}$(H,H) = 6.9 Hz, LacCH$_3$), 4.11 (q, 1H, $^3\text{J}$(H,H) = 6.9 Hz, LacCH), 4.35 (s, 1H, HC≡C), 7.33 (d, 1H, $^3\text{J}$(H,H) = 7.6 Hz, EpaH(5)), 7.84 (t, 1H, $^3\text{J}$(H,H) = 7.9, EpaH(4)), 8.10 (d, 1H, $^3\text{J}$(H,H) = 8.2 Hz, EpaH(3)), 11.00 (s, br, 1H, NH); $^{13}$C NMR (151 MHz, [D$_6$]DMSO, 25°C) $\delta$ = 16.6 (LacCH$_3$), 57.1 (LacCH), 80.3 (HC≡C), 82.3 (HC≡C), 114.1 (EpaC(3)), 123.4 (EpaC(5)), 139.3 (EpaC(4)), 139.9 (EpaC(6)), 151.5 (EpaC(2)), 170.1 (LacC=O); IR (KBr): v bar = 2122 cm$^{-1}$ (azide).

**Cyclic Pseudotetrapeptide (9).** Compound 8 (500 mg, 2.33 mmol) was dissolved in CHCl$_3$ (5 mL) in a 20 mL vial. The cap of the vial was kept ajar to allow the solvent to evaporate slowly. After
product started to precipitate (after 2 days), the cap was closed and the reaction mixture was kept at ambient temperature for two weeks. The white solid was filtered off and washed thoroughly with CHCl₃. The product was dried \textit{in vacuo} and recrystallised from DMSO/water. Yield 220 mg (44%). HPLC analysis indicated that the material thus obtained comprises a product mixture containing 10% of an impurity, which could not be removed by recrystallisation. The NMR data of the major product were taken from the spectra of the mixture. Mp. > 350°C; $[\alpha]_D^{20} = -490.0$ (c = 0.1, DMSO); $^1$H NMR (600 MHz, [D₆]DMSO, 25°C) $\delta = 2.14$ (d, 6H, $^3$J(H,H) = 7.0 Hz, LacCH₃), 6.39 (q, 2H, $^3$J(H,H) = 7.0 Hz, LacCH), 7.44 (d, 2H, $^3$J(H,H) = 8.2 Hz, EpaH(3)), 7.71 (d, 2H, $^3$J(H,H) = 7.9 Hz, EpaH(5)), 7.86 (t, 2H, $^3$J(H,H) = 7.9, EpaH(4)), 8.55 (s, 2H, TriCH), 10.82 (s, br, 2H, NH); $^{13}$C NMR (151 MHz, [D₆]DMSO, 25°C) $\delta = 16.7$ (LacCH₃), 61.1 (LacCH), 115.1 (EpaC(3)), 118.1 (EpaC(5)), 134.1 (TriC(4)), 134.5 (TriC(5)), 139.7 (EpaC(4)), 145.0 (EpaC(2)), 150.0 (EpaC(6)), 170.4 (LacC=O); MS (MALDI, positive mode): $m/z$ (%): 431.1 (2%) [M+H⁺], 453.1 (100%) [M+Na⁺], 469.2 (36%) [M+K⁺]; elemental analysis calcd (%) for C$_{20}$H$_{18}$N$_{10}$O$_2$·0.5H$_2$O: C 54.66, H 4.36, N 31.87; found C 54.77, H 4.50, N 31.90.
\(^1\)H NMR Spectrum: \(\text{5 in [D}_6\text{]DMSO (600 MHz, 25°C, Bruker Avance 600).}\)

\[^{13}\text{C NMR Spectrum: 5 in [D}_6\text{]DMSO (151 MHz, 25°C, Bruker Avance 600).}\)

This compound is prone to oligomerisation. The spectra were thus recorded of freshly prepared samples.

$^1$H NMR Spectrum: Cyclodimerisation product of 6 in [D$_6$]DMSO at 25°C (a) and at 100°C (b) (600 MHz, Bruker Avance 600).
$^1$H NMR Spectrum: 7 in [D$_6$]acetone (600 MHz, 25°C, Bruker Avance 600).

\[ ^1H \text{ NMR Spectrum: } 8 \text{ in } [D_6]DMSO (600 MHz, 25°C, Bruker Avance 600). \]

This compound is prone to oligomerisation. The minor signals visible in the spectrum of this freshly prepared sample derive from the products thus formed.

\[ ^{13}C \text{ NMR Spectrum: } 8 \text{ in } [D_6]DMSO (151 MHz, 25°C, Bruker Avance 600). \]
$^1$H NMR Spectrum: Cyclodimerisation product of 8, compound 9, in [D$_6$]DMSO at 25°C (a) and at 100°C (b) (600 MHz, Bruker Avance 600). The blue dots in the spectrum at 100°C indicate signals of the side product.
$^{13}$C NMR Spectrum: Cyclodimerisation product of 8, compound 9, in [D$_6$]DMSO (151 MHz, 25°C, Bruker Avance 600).
ESI-MS Spectrum: Cyclodimerisation product of \(8\), compound \(9\), \((c = 1 \cdot 10^{-4} \text{ M})\) in 5\% \([\text{D}_6]\)DMSO in H\(_2\)O/MeOH 1:1 (\(v/v\)) (Bruker Esquire 3000, negative mode).

<table>
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<th>(m/z) calcd.</th>
<th>(m/z) exp.</th>
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NOESY NMR Spectrum: Cyclodimerisation product of 8, compound 9, (1.0 mM) in [D₆]DMSO (mixing time 1 s) (600 MHz, 25°C, Bruker Avance 600).
Single crystal structure X-ray analysis of \((R,R)\)-9·2(DMSO): Crystals of \((R,R)\)-9·2(DMSO) grow from dimethyl sulfoxide/water in the form of prisms. The cyclopeptide molecules sit on a crystallographic 2-fold axis of symmetry, passing through the centre of the ring. The crystals contain two independent dimethyl sulfoxide molecules, which are disordered about 2-fold axes of symmetry.

Figure S1. Crystal structure of \((R,R)\)-9·2(DMSO), crystallised from dimethyl sulfoxide/water. Anisotropic displacement parameters are drawn at the 50% probability level and hydrogen atoms omitted for clarity. Atoms labelled with a star are related to those without by the symmetry operation [1-x, -y, z].

X-ray Crystal Data for \((R,R)\)-9·2(DMSO): \([C_{20}H_{18}N_{10}O_2]\)·2[C\(_2\)H\(_6\)O\(_S\)], \(M_r = 586.7\) g \(\cdot\) mol\(^{-1}\), colourless prism, crystal size 0.021 x 0.040 x 0.045 mm\(^3\), orthorhombic, space group P2\(_1\)2\(_1\)2\(_1\), \(a = 12.2734(12)\) Å, \(b = 12.7812(13)\) Å, \(c = 8.6196(9)\) Å, \(U = 1352.1(2)\) Å\(^3\), \(T = 100(2)\) K, \(Z = 2\), \(D_{calc} = 1.441\) g\-cm\(^3\), \(\lambda = 0.71073\) Å, \(\mu = 0.249\) mm\(^{-1}\), Gaussian absorption correction \((T_{min} = 0.99192, T_{max} = 0.99527)\), scaling SADABS, Bruker Kappa Mach3 Apex2 diffractometer, 3.19 \(<\ \theta \ <36.50^\circ\), 89526 measured reflections, 6518 independent reflections, 6260 reflections with \(I > 2\sigma(I)\), \(R_{int} = 0.0388\). Structure solved by direct methods and refined by full-matrix least-squares against \(F^2\) to \(R_f\).
= 0.0751 \[I > 2\sigma(I)\], \(wR_2 = 0.1819\), 217 parameters.\(^4\) There are two independent half dimethyl sulfoxide solute molecules in the asymmetric unit which are disordered about two independent 2-fold axes. Since C11A and the symmetry related C11B of one of the disordered dimethyl sulfoxide solute molecules are very close to one another the S1-C11A and S1-C11B distances were restrained to be equal with an effective standard deviation of 0.02. The Flack parameter is -0.06(15).\(^5\) The Hooft factor \(y\) based on 2840 Bijvoet pairs is -0.064(14).\(^6\) H atoms riding, \(S = 1.226\), residual electron density +0.55 / -0.52 e Å\(^{-3}\). CCDC 773683.


Single crystal structure X-ray analysis of anhydrous (S,S)-10: Fine acicular crystals of anhydrous (S,S)-10 were obtained by sublimation on a hot-stage microscope at 260°C (Figure S2). The cyclopeptide molecules are solely linked by N-H···O=C hydrogen bonds (N···O 3.006(4) Å).

Figure S2. Sublimation of (S,S)-10. View of the fine needles of (S,S)-10 through crossed-polarizers on the hot-stage microscope.

Figure S3. Crystal structure of anhydrous (S,S)-10 (sublimate). Anisotropic displacement parameters are drawn at the 50% probability level and hydrogen atoms omitted for clarity.
X-ray Crystal Data for anhydrous (S$_2$S)$_{-10}$: [C$_{22}$H$_{22}$N$_6$O$_4$], sublimed at 260 °C, $M_r = 434.46$, colourless needle, crystal size: 0.005 x 0.005 x 0.550 mm$^3$; $a = 9.138(4)$, $b = 11.325(5)$, $c = 19.715(8)$ Å, $U = 2040.3(1)$ Å$^3$, $T = 293$ K, orthorhombic, space group P2$_1$2$_1$2$_1$ (No. 19), $Z = 4$, $D_{calc} = 1.41$ g cm$^{-3}$, $F(000) = 912$, Bruker-AXS Apex 2 diffractometer on the SCD beamline at the ANKA synchrotron facility Karlsruhe, $\lambda$(synchrotron) = 1.0 Å, $\mu = 0.83$ mm$^{-1}$, 19076 measured and 2848 independent reflections ($R_{int} = 0.087$), 2661 with $I > 2\sigma(I)$, $\theta_{max} = 33.9^\circ$, apparent $T_{min/max} = 0.6322$ (SADABS), direct methods solution (SHELXS-97) and least-squares refinement (SHELXL-97) on $F_0^2$ using merged data from several measurements, programs from G. Sheldrick, University of Göttingen, 1997. Due to the small size of the crystal (Ø ca. 5 μm), only data in the measured range had a significant signal-to-noise ratio. The mean I/sigma ratio fell off quite sharply at higher theta-values, hence the relatively high $R_{int}$ of 0.087. Data were measured with an average redundancy of 12 in the range $\infty - 0.95$ Å in order to increase the mean $I/\sigma(I)$ ratio (16.28 in the same range). Chebyshev type weights, 298 parameters, H atoms riding, Friedel pairs insignificantly different hence merged, $R_f = 0.0496$ ($I > 2\sigma(I)$), $wR_2 = 0.1342$ (all data), $\Delta\rho_{max/min} = 0.270/-0.209$ eÅ$^{-3}$. CCDC 773685.

The supplementary crystallographic data for (R,R)-9-2(DMSO) (CCDC 773683), and 10 (CCDC 773685) can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Table S1 contains a comparison of the torsion angles (°) in the ring and the dihedral angles (°) between the pyridyl groups and between the amide groups of the cyclic pseudotetrapeptide 9 with those of the tetrapeptide 10 in the crystal structures of (R,R)-9-2(DMSO), anhydrous 10 (sublimated), tetragonal (Form I) and orthorhombic (Form II) 10·H$_2$O.\footnote{S. Kubik, R. Goddard, S. Otto, S. Pohl, C. Reyheller and S. Stüwe, Biosens. Bioelectr., 2005, 20, 2364-2375}
Table S1. Comparison of the torsion angles (°) in the ring and the dihedral angles (°) between the pyridyl groups and between the amide groups of the cyclic pseudotetrapeptide 9 with those of the tetrapeptides 10a) in the crystal structures of (R,R)-9·2(DMSO), anhydrous 10 (sublimated), tetragonal (Form I) and orthorhombic (Form II) 10·H2O.

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<th>(R,R)-9</th>
<th>anhydrous 10 (sublimated)a)</th>
<th>tetragonal 10·H2Oa)</th>
<th>orthorhombic 10·H2Oa)</th>
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angle between pyridyl groups 79.94 87.79 61.15 81.64

angle between amide groups 45.42 34.51 33.97 34.71

a) 9 and 10 are enantiomers, so equivalent torsion angles (α) are related by (360-α)°.

b) Δ is the largest difference in the torsion angles (shown in italics), taking account of stereochemistry.a)