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Mimicking the Biologically Active Part of the Cyclopeptides Segetalin A and B by "Clipping" of a Linear Tripeptide Derivative by Metal Coordination

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A new method for the conformational fixation of bioactive loop-type peptide structures is presented. Hereby, ligand moieties are attached to the termini of a linear peptide sequence. Upon metal complexation, a macrocyclic structure with a loop-type conformation of the peptide is formed. As a representative example, the preparation of a WAG-bridged dicatechol derivative is described which mimics the active part of the natural products Segetalin A and Segetalin B.

Keywords: Peptide coupling; Cyclopeptide; Coordination compound; Segetalin

The Future of Supramolecular Chemistry

Supramolecular chemistry is the "chemistry beyond the molecule". Thus, during the last 30 years or so, research in the field of supramolecular chemistry has provided us with a large amount of information on non-covalently linked molecular architectures. Many complicated and, in some cases, aesthetically appealing structures have been reported, and we have learned how to specifically build up large structures which are sometimes comparable in size with nature's proteins or viruses. However, in the early days of supramolecular chemistry, the function of the supermolecule was important.

Now that we have learned how to use non-covalent interactions, we have to focus more thoroughly on the function of the supramolecular aggregates. In our paper, we describe the use of metal complexation for a conformational fixation of a linear peptide in a cyclic structure. Hereby, we potentially transform an artificial inactive random-coil peptide into a biologically active cyclopeptide. In principle, we use a non-covalent interaction (metal coordination) to induce a specific biological function.

Markus Albrecht was born in 1964 and studied Chemistry in Würzburg and Münster. He obtained his Dr rer. nat. in 1992 for his work on organometallic planar-tetracoordinate carbon compounds (research group of Professor Gerhard Erker). After one year as a postdoctoral fellow in the laboratories of Professor Kenneth N. Raymond in Berkeley (bioinorganic chemistry), he moved to the Institute of Organic Chemistry of the University of Karlsruhe and received his habilitation in 1997. His work on the metal-directed self-assembly of metallo-supramolecular aggregates was honoured with the "ADUC-Jahrespreis für Habilitanden", and between 1998 and 2001, he was a Heisenberg-fellow of the DFG. In 2002, his teaching was awarded with the "Landeslehrpreis 2002 des Landes Baden-Württemberg". Since spring 2002, he has been Professor of Organic Chemistry at the RWTH Aachen.

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INTRODUCTION

In 1994, Itokawa *et al.* isolated a series of cyclic peptides from the seeds of *Vaccaria segetalis* (Caryophyllaceae) and named them Segetalins. The seeds are used in Chinese natural medicine to activate blood flow and promote milk secretion, and to treat amenorrhoea and breast infections [1–3].

In biological tests with ovariectomized rats, the cyclo-hexapeptide Segetalin A (**1**) and the cyclo-pentapeptide Segetalin B (**2**) were shown to possess an oestrogen-like activity [3]. A sequence of four amino acids of the Segetalins A and B (Trp–Ala–Gly–Val) is similar. Therefore, it is supposed that this sequence represents the biologically active part of the molecules [4]. Comparison of the solution structures of Segetalin A and B derived from NMR spectroscopy indicates that the valine residue might be of minor influence (the spatial orientation of the isopropyl groups of **1** vs. **2** seems to be different) [1–3]. In 2001, Sonnet *et al.* described the first total synthesis of Segetalin A (**1**) and the preparation of two analogues with alanine or valine substituting the tryptophane residue of the natural product [4]; see Fig. 1.

Recently, we described a method to use metal coordination for the fixation of short peptide sequences in a loop-type conformation. Therefore, we prepared peptides with 2,3-dihydroxybenzoic acid attached to the N-terminus of the peptide and 2,3-dihydroxybenzylamine bound to the C-terminus [5]. Reaction of a Val–Val–Val-bridged dicatechol ligand **3**-H_4 with *cis*-dioxomolybdenum bis(acetylacetonate) in the presence of base (e.g. potassium carbonate) led to the metallamacrocycle $[(\mathbf{3})MoO_2]^{2-}$ with a conformationally highly restricted loop-type peptide moiety [6–17].

To show the versatility of our method, we present herein the preparation of a WAG (=Trp–Ala–Gly)-bridged dicatechol ligand **4**-H_4, and we describe complexation studies of this ligand with *cis*-dioxomolybdenum bis(acetylacetonate) to obtain metallamacrocyclic $K_2[(\mathbf{4})MoO_2]$, which contains a part of the biologically active segment of the Segetalins A and B. Although metal complex stabilized peptides are already described in the literature [7–11], the concept of switching on the biological activity of a random coil peptide by conformational fixation by metal coordination, to the best of our knowledge, is new.

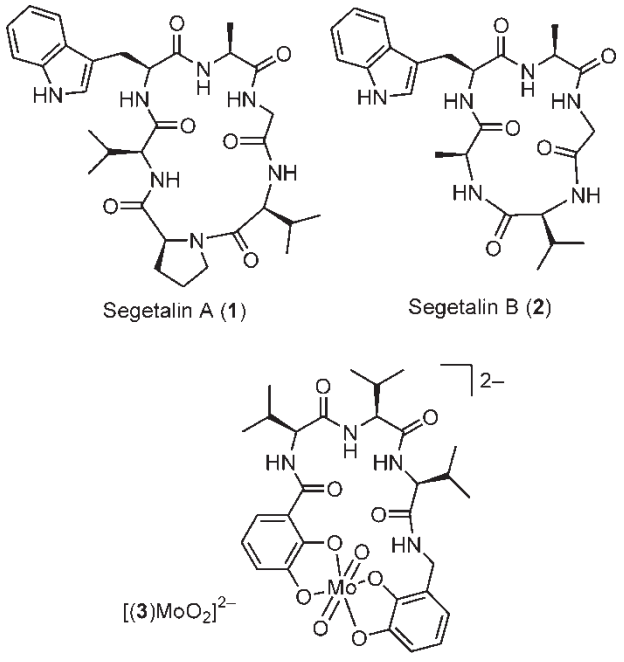


FIGURE 1 Segetalins A (**1**) and B (**2**) and a molybdenum(VI) dioxo containing metalla-cyclopeptide ($[(\mathbf{3})MoO_2]^{2-}$).

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 or AM 400 spectrometer using DEPT techniques for the assignment of the multiplicity of carbon atoms. FT-IR spectra were recorded by diffuse reflection (KBr) on a Bruker IFS spectrometer. Mass spectra (EI, 70 eV; positive FAB with DMSO/3-NBA as matrix) were recorded on a Finnigan MAT 90 mass spectrometer. ESI-MS were detected using a Bruker Bioapex II FTMS equipped with a 7 T magnet. Elemental analyses were obtained with a Heraeus CHN-O-Rapid analyser. UV–vis spectra were recorded on a Perkin Elmer Lambda 2 spectrometer. Melting points: Büchi B-540 (uncorrected). Solvents were purified by standard methods.

Preparation of 6

Fmoc-Gly-OH (1.00 g, 3.36 mmol) was dissolved in a dichloromethane (33 mL)/DMF (6.7 mL) mixture, and ethyldiisopropylamine (Hünigs base, 0.64 mL, 3.70 mmol) and HBTU (1.53 g, 4.03 mmol) in 11 mL of DMF were added. After 20 min, 2,3-dimethoxy-benzylamine **5** (0.50 mL, 3.36 mmol) was added, and the mixture was stirred overnight, then successively washed with sat. aq. NH_4Cl, aq. $NaHCO_3$ and water. Solvent was removed *in vacuo* to obtain **6** as a yellow solid in 93% yield (1.40 g).

Mp: 150°C decomp. ^1H NMR ($CDCl_3$): $\delta = 7.75$ (d, $J = 7.5$ Hz, 2H), 7.57 (d, $J = 7.3$ Hz, 2H), 7.39 (t, $J = 7.4$ Hz, 2H), 7.32–7.26 (m, 2H), 6.98 (pseudo t, $J = 7.4$, 7.9 Hz, 1H), 6.84 (t, $J = 8.5$ Hz, 2H), 6.49 (b, 1H, NH), 5.56 (b, 1H, NH), 4.46 (d, $J = 5.8$ Hz, 2H), 4.41 (d, $J = 7.6$ Hz, 2H), 4.19 (t, $J = 6.8$ Hz, 1H), 3.99–3.84 (m, 2H), 3.38 (s, 3H), 3.82 (s, 3H). ^{13}C NMR ($CDCl_3$): $\delta = 168.5$ (C), 156.5 (C), 152.6 (C), 147.1 (C), 143.7 (2 C), 141.3 (2 C), 131.3 (C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 124.3 (CH), 121.3 (CH), 120.0

(2 CH), 112.1 (CH), 67.1 (CH_2), 60.1 (CH_3), 55.7 (CH_3), 47.1 (CH), 44.5 (CH_2), 39.0 (CH_2). MS (FAB, DMSO/3-NBA): $m/z = 447.3$ $[M + H]^+$, 469.3 $[M + Na]^+$. IR (KBr): ν (cm^{-1}) = 3261, 3071, 1656, 1553, 1485, 1277. UV–vis ($CHCl_3$): λ_{max} (nm) = 227, 267, 289, 301. Analysis calcd. for $C_{26}H_{26}N_2O_5 \cdot 1/4\,H_2O$: C 69.24, H 5.92, N 6.21; found: C 69.27, H 5.69, N 6.49.

Cleavage of the Fmoc-protecting Group of 6

Piperidine (0.32 mL, 3.23 mmol) was added to a solution of **6** (1.20 g, 2.69 mmol) in 30 mL of dichloromethane. The mixture was stirred overnight, and then solvent was removed under vacuum. The residue vigorously was washed with hexane. The resulting deprotected compound was used without characterization and without further purification.

Preparation of 7

Fmoc-Ala-OH (837 mg, 2.69 mmol) was dissolved in dichloromethane (30 mL), and Hünigs base (0.51 mL, 2.96 mmol) and a solution of HBTU (1.22 g, 3.23 mmol) in 10.5 mL of DMF were added in succession. After 20 min of activation, a suspension of deprotected **6** (2.69 mmol) in 5 mL of dichloromethane and 2.5 mL of DMF was added. After 16 h of stirring, the mixture was washed with saturated aq. NH_4Cl, aq. $NaHCO_3$, water and saturated aq. NaCl. The solution was dried over $MgSO_4$, and solvent was removed under vacuum to yield **7** as a slightly yellow solid in 90% yield (2.41 g). The product can be recrystallized from methanol.

Mp: 166°C decomp. ^{1}H NMR ($CDCl_3$): $\delta = 8.12$ (br., 1H, NH), 7.81 (br., 1H, NH), 7.71 (d, $J = 7.5$ Hz, 2H), 7.54 (pseudo t, $J = 10.2$, 7.9 Hz, 2H), 7.36 (d, $J = 5.9$ Hz, 1H, NH), 7.32 (t, $J = 7.3$ Hz, 2H), 7.23 (t, $J = 7.3$ Hz, 2H), 6.81 (t, $J = 7.1$ Hz, 1H), 6.74 (d, $J = 7.4$ Hz, 1H), 6.67 (d, $J = 7.7$ Hz, 1H), 4.33–4.18 (m, 2H), 4.16 (b, 1H), 4.08–4.03 (m, 3H), 3.81 (s, 2H), 3.70 (s, 3H), 3.67 (s, 3H), 1.25 (d, $J = 7.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$): $\delta = 173.5$ (C), 169.1 (C), 156.5 (C), 152.4 (C), 146.6 (C), 144.0 (C), 143.9 (C), 141.1 (C), 141.0 (C), 132.3 (C), 127.8 (2 CH), 127.2 (2 CH), 125.4 (2 CH), 123.9 (CH), 120.4 (CH), 120.2 (2 CH), 111.5 (CH), 66.2 (CH_2), 60.3 (CH_3), 55.7 (CH_3), 51.1 (CH), 47.1 (CH), 42.9 (CH_2), 37.5 (CH_2), 17.8 (CH_3). MS (FAB, DMSO/3-NBA): $m/z = 518.3$ $[M + H]^+$, 540.2 $[M + Na]^+$. IR (KBr): ν (cm^{-1}) = 3289, 1660, 1554, 1260. UV–vis ($CHCl_3$): λ_{max} (nm) = 227, 267, 289, 301. Analysis calcd. for $C_{29}H_{31}N_3O_6 \cdot 1/2\,H_2O$: C 66.15, H 6.12, N 7.98; found: C 65.98, H 5.91, N 8.20.

Preparation of 8

Compound **7** was deprotected as described for **6**. Hünigs base (0.38 mL, 2.20 mmol) and HBTU (912 mg, 2.40 mmol) dissolved in 10 mL of DMF were added to a solution of Fmoc-Trp(Boc)-OH (1.55 g, 2.00 mmol) in 30 mL of dichloromethane. After 20 min, a suspension of deprotected **7** (2.00 mmol) in 11 mL of DMF and 6 mL dichloromethane was added. The mixture was stirred overnight and washed with saturated aq. NH_4Cl, aq. $NaHCO_3$, water and saturated aq. NaCl. Drying ($MgSO_4$) and removal of the solvent *in vacuo* yielded **8** in 87% (1.40 g). **8** can be recrystallized from methanol.

Mp: 143°C decomp. ^{1}H NMR (DMSO-d_6): $\delta = 8.36$ (d, $J = 6.8$ Hz, 1H, NH), 8.18 (t, $J = 5.8$ Hz, 1H, NH), 8.13 (t, $J = 5.8$ Hz, 1H, NH), 8.00 (d, $J = 8.0$ Hz, 1H, NH), 7.84 (d, $J = 7.6$ Hz, 2H), 7.74 (d, $J = 7.7$ Hz, 1H), 7.65 (d, $J = 8.5$ Hz, 1H), 7.57 (m, 3H), 7.35 (m, 2H), 7.30 (m, 1H), 7.24–7.19 (m, 3H), 6.95 (t, $J = 7.8$ Hz, 1H), 6.88 (d, $J = 7.6$ Hz, 1H), 6.80 (d, $J = 7.5$ Hz, 1H), 4.39 (m, 1H), 4.28 (m, 3H), 4.30–4.11 (m, 3H), 3.76–3.72 (m, 2H), 3.74 (s, 3H), 3.70 (s, 3H), 3.08 (m, 1H), 2.90 (m, 1H), 1.54 (s, 9H), 1.24 (d, $J = 7.0$ Hz). ^{13}C NMR (DMSO-d_6): $\delta = 172.9$ (C), 172.0 (C), 169.1 (C), 156.4 (C), 152.6 (C), 149.5 (C), 146.6 (C), 144.1 (C), 141.1 (C), 141.0 (C), 135.1 (C), 132.8 (C), 130.8 (C), 128.0 (2 CH), 127.4 (2 CH), 125.7 (CH), 125.6 (CH), 124.7 (CH), 124.5 (CH), 124.2 (CH), 122.8 (CH), 120.5 (CH), 120.4 (2 CH), 117.2 (C), 115.1 (CH), 112.1 (CH), 83.9 (C), 66.2 (CH_2), 60.4 (CH_3), 56.1 (CH_3), 54.8 (CH), 49.1 (CH), 47.0 (CH), 42.6 (CH_2), 38.7 (CH_3), 37.4 (CH_2), 28.1 (3 CH_3), 27.7 (CH_2), 18.4 (CH), one C was not observed. MS (FAB, DMSO/3-NBA): $m/z = 803.4$ $[M]^+$, 804.4 $[M + H]^+$, 826.4 $[M + Na]^+$. IR (KBr): ν (cm^{-1}) = 3284, 1636, 1541, 1452, 1371, 1259, 742. UV–vis ($CHCl_3$): λ_{max} (nm) = 201, 264, 293. Analysis calcd. for $C_{45}H_{49}N_5O_9 \cdot H_2O$: C 66.49, H 6.32, N 8.62; found: C 66.43, H 6.10, N 9.02.

Preparation of the Protected Ligand 10

Compound **8** was deprotected as described for the deprotection of **6**. Hünigs base (185 µl, 1.08 mmol) and HBTU (448 mg, 1.18 mmol) in 3.2 mL of DMF were added to 2,3-dimethoxybenzoic acid **9** (179 mg, 0.98 mmol) in 9 mL of dichloromethane, and the mixture was activated for 20 min. Deprotected **8** (0.98 mmol) in 8 mL of dichloromethane and 1.7 mL of DMF was added, and the mixture was stirred overnight. After washing the mixture with sat. aq. NH_4Cl, $NaHCO_3$, water and sat. aq. NaCl and drying over $MgSO_4$, the solvent was removed under vacuum. The residue was purified by column chromatography using ethyl acetate for the removal of impurities and ethyl acetate/methanol 2:1 v/v to obtain the product in 82% (601 mg) as a white solid.

Mp: 67–78°C decomp. ^{1}H NMR ($CDCl_3$): $\delta = 8.73$ (d, $J = 5.9$ Hz, 1H, NH), 8.07 (m, 1H, NH),

7.54 (m, 3H), 7.26 (s, 1H), 7.17 (t, $J = 7.2$ Hz, 1H), 7.01–7.15 (m, 4H), 7.00 (m, 1H, NH), 6.95 (t, $J = 8.0$ Hz, 1H), 6.79 (d, $J = 1.3$ Hz, 1H), 6.77 (d, $J = 1.4$ Hz, 1H), 4.95 (q, $J = 6.4$ Hz, 1H), 4.51–4.41 (m, 2H), 4.33 (pseudo t, $J = 7.2$, 6.8 Hz, 1H), 4.02 (dd, $J = 16.7$, 6.0 Hz, 1H), 3.89 (dd, $J = 16.9$, 5.4 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.62 (s, 3H), 3.24 (d, $J = 6.5$ Hz, 2H), 1.62 (s, 9H), 1.24 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR ($CDCl_3$): $\delta = 172.6$ (C), 171.9 (C), 169.1 (C), 166.4 (C), 152.93 (C), 152.91 (C), 149.9 (C), 148.4 (C), 147.4 (C), 132.2 (C), 130.5 (C), 130.8 (C), 125.6 (C), 125.2 (CH), 124.8 (CH), 124.5 (CH), 123.1 (CH), 123.0 (CH), 121.3 (CH), 119.4 (CH), 116.6 (CH), 115.7 (CH), 112.0 (CH), 84.2 (C), 61.5 (CH_3), 61.0 (CH_3), 56.5 (CH_3), 56.1 (CH_3), 54.8 (CH), 50.1 (CH), 43.6 (CH_2), 39.0 (CH_3), 38.8 (CH_2), 28.5 (3 CH_3), 27.9 (CH_2), 18.1 (CH), one C was not observed. MS (FAB, DMSO/3-NBA): $m/z = 745.4$ $[M]^+$, 746.3 $[M + H]^+$, 768.3 $[M + Na]^+$. IR (KBr): ν (cm^{-1}) = 3292, 1733, 1631, 1522, 1371, 1265. UV–vis ($CHCl_3$): λ_{max} (nm) = 229, 285, 294. Analysis calcd. for $C_{39}H_{47}N_5O_{10}\cdot H_2O$: C 61.33, H 6.47, N 9.17; found: C 60.95, H 6.40, N 9.59.

Deprotection of the Ligand 4-H_4

A solution of **10** (126 mg, 0.17 mmol) in chloroform (3 mL) was added to an ice-cooled 1 molar BBr_3 solution in CH_2Cl_2 (4.24 mL). After 5 days at room temperature in the dark, 20 mL of methanol were added. The solution was reduced in vacuum, dissolved several times in methanol and evacuated to dryness. The remaining oil was dried under vacuum, and the addition of water led to precipitation of **4**-H_4 in 67% yield (66.3 mg) as a grey solid.

Mp: 135°C decomp. ^{1}H NMR (methanol-d_4): $\delta =$ 7.57 (d, $J = 7.9$ Hz, 1H, NH), 7.31 (dd, $J = 7.4$, 0.7 Hz, 1H, NH), 7.23 (dd, $J = 8.2$, 1.5 Hz, 1H, NH), 7.20 (s, 1H), 7.07 (t, $J = 1$ Hz, 2H), 6.98 (t, $J = 0.9$ Hz, 1H), 6.93 (m, 1H), 6.72–6.60 (m, 6H), 4.82 (t, $J = 6.8$ Hz, 1H), 4.30 (s, 2H), 4.22 (q, $J = 7.0$ Hz, 1H), 3.68 (d, $J = 11.6$ Hz), 3.35 (hidden under solvent peak), 1.24 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (methanol-d_4): $\delta = 174.3$ (C), 173.8 (C), 171.0 (C), 169.6 (C), 148.1 (C), 146.1 (C), 145.7 (C), 143.4 (C), 136.9 (C), 127.6 (C), 125.2 (C), 124.0 (CH), 121.6 (CH), 120.4 (CH), 119.7 (CH), 119.1 (CH), 119.0 (CH), 118.9 (CH), 118.8 (CH), 118.4 (CH), 116.5 (C), 114.7 (CH), 111.4 (CH), 109.4 (C), 55.2 (CH), 50.1 (CH), 42.3 (CH_2), 38.9 (CH_2), 38.1 (CH_3), 27.6 (CH_2). MS (FAB, DMSO/3-NBA): $m/z =$ 589.2 $[M]^+$, 590.2 $[M + H]^+$. High-resolution MS calcd. for $C_{30}H_{32}N_5O_8$: 590.2251, found: 590.2241. IR (KBr): ν (cm^{-1}) = 3376, 1643, 1530, 1264, 743. UV–vis ($CHCl_3$): λ_{max} (nm) = 201, 218, 251, 280. Analysis calcd. for $C_{30}H_{31}N_5O_8\cdot H_2O$: C 59.30, H 5.47, N 11.53, found: C 59.23, H 5.45, N 10.57.

Preparation of K_2[(4)MoO_2]

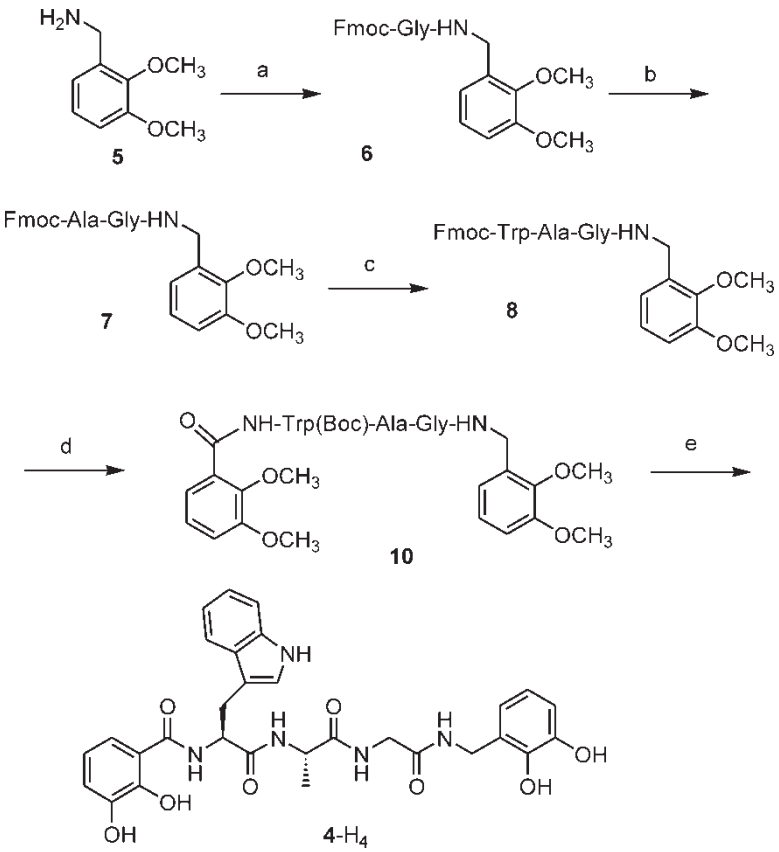
$(acac)_2MoO_2$ (2.6 mg, 0.01 mmol) and K_2CO_3 (0.8 mg, 0.01 mmol) were dissolved in 30 mL of methanol, and ligand **4**-H_4 (4.7 mg, 0.01 mmol) in 5 mL of methanol was added. The mixture was stirred overnight, and solvent was removed under vacuum to obtain the complex K_2[(**4**)MoO_2] as a red–orange solid.

^{1}H NMR (methanol-d_4): $\delta = 7.63$ (d, $J = 7.0$ Hz, 1H), 7.26 (d, $J = 8.0$ Hz, 1H), 7.16 (dd, $J = 1.6$ Hz, 8.9 Hz, 1H), 7.08 (pseudo-t, $J = 6.8$ Hz, 8.0 Hz, 1H), 7.03 (pseudo-t, $J = 6.8$ Hz, 7.9 Hz, 1H), 6.88 (s, 1H), 6.72 (dd, $J = 1.6$ Hz, 7.6 Hz, 1H), 6.61 (t, $J = 7.6$ Hz, 1H), 6.55 (m, 1H), 6.47 (dd, $J = 1.6$ Hz, 7.6 Hz, 1H), 6.42 (t, $J = 7.8$ Hz, 1H), 5.07 (m, 1H), 4.64 (d, $J = 13.7$ Hz, 1H), 4.33 (d, $J = 13.7$ Hz, 1H), 3.97 (q, $J = 7.2$ Hz, 1H), 3.73 (d, $J = 17.1$ Hz), 2.91 (m, 1H), 2.68 (m, 1H), 1.28 (d, $J = 7.2$ Hz, 3H); the signal of the missing proton was not detected and probably was hidden under a solvent peak. ^{13}C NMR (methanol-d_4): $\delta = 178.9$ (C), 175.0 (C), 174.4 (C), 167.9 (C), 159.3 (C), 157.8 (C), 157.3 (C), 155.5 (C), 135.6 (C), 127.0 (C), 124.1 (CH), 122.0 (C), 121.1 (CH), 119.2 (CH), 118.6 (CH), 117.8 (CH), 116.6 (CH), 115.6 (C), 115.3 (CH), 113.9 (CH), 111.9 (CH), 111.0 (CH), 108.6 (C), 66.7 (CH), 52.4 (CH), 51.6 (CH), 41.9 (CH_2), 35.6 (CH_2), 28.8 (CH_2), 15.3 (CH_3). Negative ESI-MS: $m/z = 357.5$ $[M]^{2-}$, 754 $[M + K]^-$. IR (KBr): ν (cm^{-1}) = 3421, 1626, 1454, 1266, 853, 750. UV–vis (methanol): λ_{max} (nm) = 198, 275.

RESULTS AND DISCUSSION

The synthesis of the dicatechol ligand **4**-H_4 is outlined in Scheme 1. The preparation of the WAG-bridged dicatechol ligand **4**-H_4 follows a repetitive synthetic procedure using the Fmoc-strategy with deprotection of the N-terminal Fmoc-protected derivatives by reaction with piperidine in dichloromethane [18,19]. The intermediately obtained unprotected amines were not characterized and were used without further purification.

In the first step, 2,3-dimethoxybenzylamine (**5**) was coupled with Fmoc-Gly-OH using HBTU as a coupling reagent [20–22] to obtain **6** in 93% yield. The Fmoc protecting group was removed with piperidine in dichloromethane. In the following very similar reaction sequences, an Fmoc-alanyl (90% yield of **7**) and a Fmoc/Boc-protected tryptophan residue were introduced successively to obtain **8** in 68%. The derivative **8** already contains the desired WAG-sequence [1–3]. In the next step, after removal of the Fmoc-moiety, 2,3-dimethoxybenzoic acid (**9**) was coupled to the N-terminus [23] of the WAG sequence, and the protected ligand **10** was obtained in 82% yield. Cleavage of the methyl ethers



SCHEME 1 (a) Fmoc-Gly-OH, HBTU, DMF/CH_2Cl_2 (93%); (b) 1) piperidine, CH_2Cl_2 (quant.), 2) Fmoc-Ala-OH, HBTU, DMF/CH_2Cl_2 (90%); (c) 1) piperidine, CH_2Cl_2 (quant.), 2) Fmoc-Trp(Boc)-OH, HBTU, DMF/CH_2Cl_2 (87%); (d) 1) piperidine, CH_2Cl_2 (quant.), 2) 2,3-dimethoxybenzoic acid (**9**), HBTU, DMF/CH_2Cl_2 (82%); (e) BBr_3 (25 eq.), $CHCl_3$ (67%).

proceeded by reaction with BBr_3 [24], and the Boc-protecting group at the tryptophane was removed simultaneously to yield the dicatechol ligand **4**-H_4 in 67% after recrystallization from water.

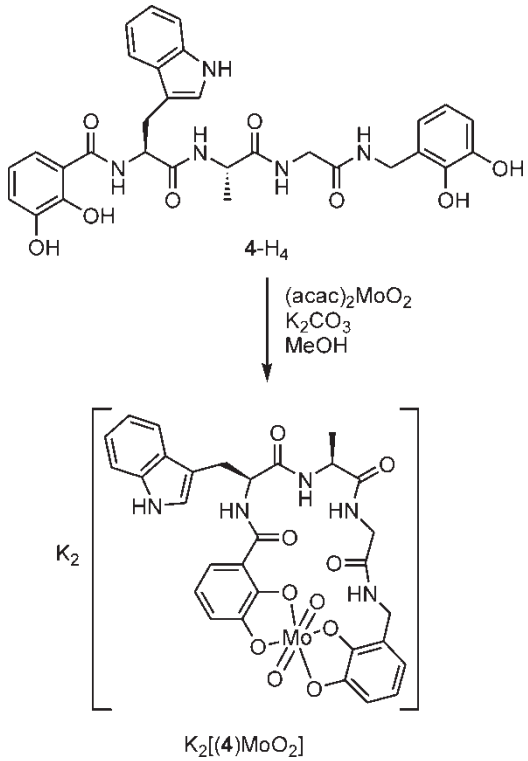
The derivative **4**-H_4 was characterized by standard spectroscopic methods and showed characteristic ^{1}H NMR signals in methanol-d_4 at $\delta = 6.72–6.60$ (m, 6 H) for the catechol units, at $\delta = 7.20$ (s, 1 H), 7.07 (m, 2 H), 6.98 (t, $J = 0.9$ Hz, 1 H), 6.93 (m, 1 H), 4.82 (t, $J = 6.8$ Hz, 1 H, δ-H) for the tryptophane-, at $\delta = 4.22$ (q, $J = 7.0$ Hz, 1 H, α-H), 1.24 (d, $J = 7.0$ Hz, 3 H, β-H) for the alanyl-, and at $\delta = 3.68$ (d, $J = 11.6$ Hz, 1 H) for the glycyl residue. The benzylic protons were observed at 4.30 ppm (2 H), while the β-protons of the tryptophan and one proton of the glycine were hidden under the solvent, respectively water peak. Corresponding ^{13}C NMR signals were observed in methanol-d_4 at $\delta = 174.3$ (C), 173.8 (C), 171.0 (C), 169.6 (C), 148.1 (C), 146.1 (C), 145.7 (C), 143.4 (C), 136.9 (C), 127.6 (C), 125.2 (C), 124.0 (CH), 121.6 (CH), 120.4 (CH), 119.7 (CH), 119.1 (CH), 119.0 (CH), 118.9 (CH), 118.8 (CH), 118.4 (CH), 116.5 (C), 114.7 (CH), 111.4 (CH), 109.4 (C), 55.2 (CH), 50.1 (CH), 42.3 (CH_2), 38.9 (CH_2), 38.1 (CH_3), 27.6 (CH_2).

Positive FAB mass spectrometry of **4**-H_4 in DMSO/3-NBA showed the molar peak at $m/z = 589$ $[M]^+$ and the peak of the monoprotonated species at $m/z = 590$ $[M + H]^+$.

In compound **4**-H_4, the WAG-sequence, which is a part of the biologically active moiety of the Segetalins A and B [1–3], is connected to two catechols. Reaction of this ligand **4**-H_4 with *cis*-dioxomolybdenum bis(acetylacetonate) in methanol in the presence of potassium carbonate resulted in the formation of K_2[(**4**)MoO_2] [6,25,26]—a 19-membered metallamacrocycle—with one dicatechol molybdenum(VI)dioxo complex unit and a conformationally fixed Trp–Ala–Gly tripeptide front. The complex was purified by filtration over Sephadex LH20 (methanol) and was obtained in 43% yield.

The complex salt, K_2[(**4**)MoO_2], was characterized by NMR spectroscopy as well as ESI mass spectrometry.

The ^{1}H NMR spectrum of K_2[(**4**)MoO_2] in methanol-d_4 showed chemical shifts which are significantly different to the signals of the linear ligand **4**-H_4 (discussed above). For K_2[(**4**)MoO_2], the peaks of the amino acid residues were observed at $\delta = 7.63$, 7.26, 7.16, 7.08, 6.88, 5.07 (m, 1 H, α-H), and 2.91/2.68 (each m, 1H, β-H) for tryptophan, at $\delta = 3.97$ (q, $J = 7.2$ Hz, 1 H, α-H) and 1.28 (d, $J = 7.2$ Hz, 3 H, β-H) for alanine, and at $\delta = 3.73$ (d, $J = 17.1$ Hz, 1 H) for glycine; see Scheme 2.



SCHEME 2 (acac = acetylacetonate).

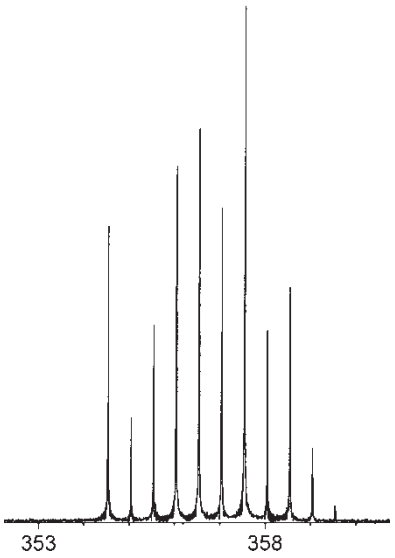


FIGURE 2 Part of the negative ESI-MS showing the expected peak pattern of the dianionic metallacyclopeptide $[(\mathbf{4})MoO_2]^{2-}$ with the typical peak separation of 0.5 units and the maximum at $m/z = 357.5$ (corresponding to the dominant ^{98}Mo isotope).

The second glycine proton was hidden under the solvent peak. Further signals were observed for the catechol units ($\delta = 7.03$, 6.72, 6.61, 6.55, 6.47, and 6.42) and for the diastereotopic protons of the benzyl group [$\delta = 4.64$ and 4.33 (2 d, $J = 13.7$ Hz)]. The ^{13}C NMR resonances were detected in methanol-d_4 at $\delta = 178.9$ (C), 175.0 (C), 174.4 (C), 167.9 (C), 159.3 (C), 157.8 (C), 157.3 (C), 155.5 (C), 135.6 (C), 127.0 (C), 124.1 (CH), 122.0 (C), 121.1 (CH), 119.2 (CH), 118.6 (CH), 117.8 (CH), 116.6 (CH), 115.6 (C), 115.3 (CH), 113.9 (CH), 111.9 (CH), 111.0 (CH), 108.6 (C), 66.7 (CH), 52.4 (CH), 51.6 (CH), 41.9 (CH_2), 35.6 (CH_2), 28.8 (CH_2), 15.3 (CH_3).

In the negative ESI-MS in methanol, dominating peaks were observed at $m/z = 754$ $\{K[(\mathbf{4})MoO_2]^-\}$ and at $m/z = 357.5$. The latter signal showed a peak separation of 0.5 units, which was typical for doubly charged species and which was assigned to $[(\mathbf{4})MoO_2]^{2-}$. The isotopic pattern is shown in Fig. 2 and is in accordance with the calculated pattern.

CONCLUSIONS

In this paper, we presented the first metal ion-directed clipping [27–29] of a linear peptide to adopt a structure, which is a segment of a naturally occurring biologically active class of cyclopeptides: the Segetalins A and B. The tripeptide derivative **4**-H_4, which bears two terminal catechol ligand units, is prepared in a straightforward synthetic procedure using HBTU amide coupling and Fmoc-protecting group strategies.

Clipping of the linear derivative proceeds smoothly by addition of $(acac)_2MoO_2$ and K_2CO_3 in methanol. The *cis*-dioxomolybdenum(VI) complex, $K_2[(\mathbf{4})MoO_2]$, is characterized by spectroscopic methods. Further studies will be directed towards the synthesis of similar ligands using peptide sequences of other biologically active cyclopeptides or peptidic turn structures (Bouvardin [30–32], Somatostatin [33] and so on), and the conformation at the metallacyclopeptides will be controlled by use of different metal ions. In addition, activity tests with the metallacyclopeptides are being planned for the future.

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