Approaches Towards Enkephalin Analogs Exhibiting Two Catechol Units

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Abstract: Peptide-bridged dicatechols were prepared by a combination of solid- and solution-phase synthesis. The target compounds are potential precursors for constrained metal containing enkephalin analogs. While a simple model compound selectively forms a metallamacrocycle with the *cis*-molybdenum(VI)dioxo unit, this goal could not be achieved with the corresponding peptide bridged derivatives.

Key words: peptides, solid-phase synthesis, enkephalin, macrocycles, phenols

Enkephalins are pentapeptides, which act as neurotransmitters as well as neuromodulators. They are endogenous ligands for the opiate receptor in the brain. Methionine enkephalin as well as leucine enkephalin were first isolated from porcine brain and have the Tyr-Gly-Gly-Phe-sequence in common, followed either by Met or Leu.[1]

Due to the interesting biological activity of the enkephalins, numerous analogs were prepared.[2] In this context, cyclic enkephalin analogs **1** were obtained, e.g. by the group of Goodman (Figure 1). In vivo, the cyclic compounds are up to 100 times more potent than morphine.[3] This high activity is probably due to a higher conformational preorganization of the macrocycles compared to the more flexible open chain enkephalins.

Recently we introduced catechol units at the termini of short naturally occurring peptide sequences and by metal coordination fixed the peptides in a macrocyclic conformation.[4–6] We were now interested to use this concept for the formation of metallamacrocycles which contain a constrained enkephalin unit, similar as in **1**. Therefore, we expected the ligands **2a**,**b** to be good candidates for the formation of metallacyclopeptides. In **2a**,**b** one of the glycine units of the enkephalins is substituted by aspartic acid to enable the side-chain attachment of a catechol moiety (Figure 1). This work was performed in order to test, whether short peptides, which bear catechol units at the side chain are able to specifically form metal complexes. In future studies the gained knowledge should help to design metallacyclopeptides with desired conformations.

Initially, we prepared a very simple model system **3,** which possesses two 2,3-dihydroxybenzamide units bound to azelic acid (nonanedicarboxylic acid). The benzyl amides resemble the ligand units of **2a**,**b** while the heptamethylene chain is a very much simplified model for the peptidic spacer, due to its similar chain length. Here, we introduced one atom less, than is found in the peptide spacer, to mimic the constrain of the amide units.

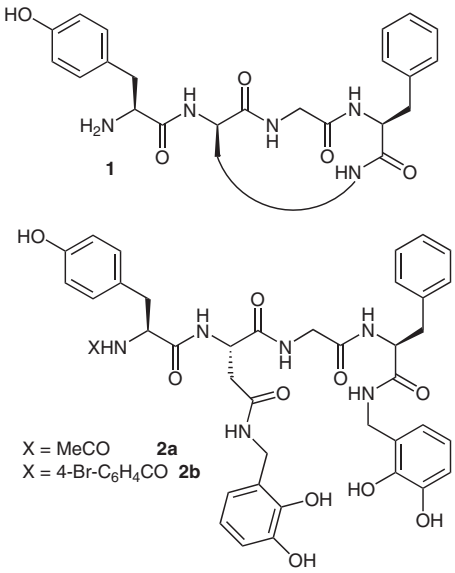

Figure 1 General representation of cyclic enkephalin analogs **1** as described by Goodman and the dicatechol analogs **2** proposed by us.

The dicatechol derivative **3** was prepared starting from azelic acid (**4**), which was activated by DIPEA (*N*,*N*-diisopropylethylamine) and HBTU [*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate] in acetonitrile and then was coupled with two equivalents of 2,3-dimethoxybenzylamine (**5**) (Scheme 1).[7] The protected ligand precursor was isolated in 77% and was then demethylated in 90% yield by reaction with BBr_3 to obtain **3**.[8] The dicatechol **3** formed a metal complex $K_2[\mathbf{3}MoO_2]$ by reaction with $MoO_2(acac)_2$ in methanol in the presence of K_2CO_3. Ligand **3** as well as the molybdenum(VI) complex could be characterized by spectroscopic methods.[9] Hereby negative ESI-MS of $K_2[\mathbf{3}MoO_2]$ showed, that it has the 1:1 stoichiometry. In the ^1H NMR spectra, the benzylic protons can be considered as a spectroscopic probe. In **3**, they appear as a singlet at $\delta = 4.48$ (in CD_3OD) while for $K_2[\mathbf{3}MoO_2]$ a broad multiplet is observed at $\delta = 4.19–4.10$ (D_2O). This is due to the diastereotopicity of the protons close to the newly introduced stereocenter at the metal complex unit.

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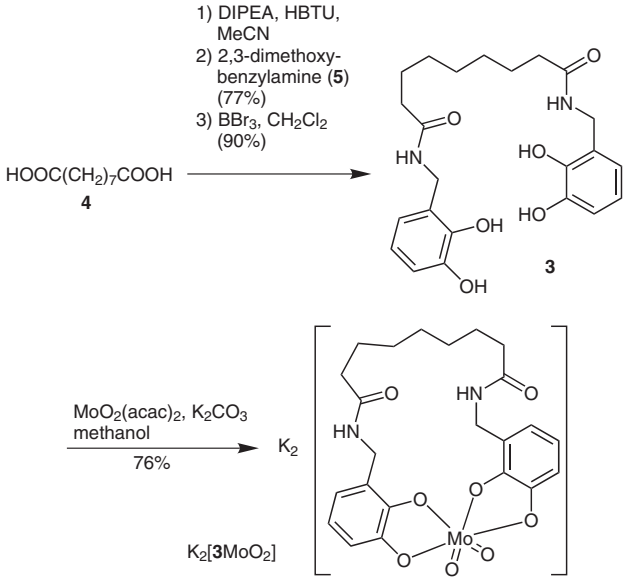
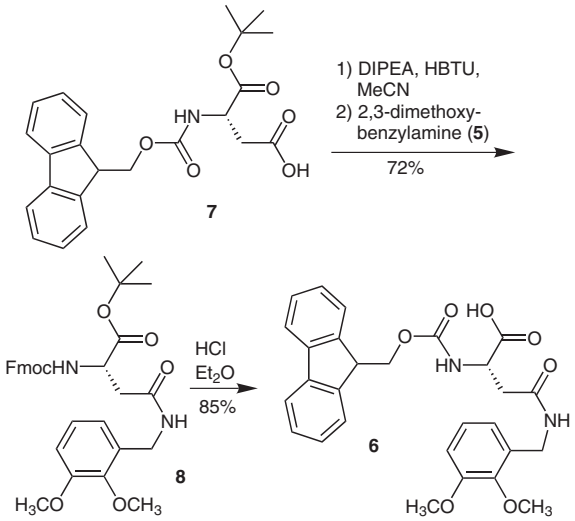


Scheme 1 Preparation of a simple model ligand **3** and its molybdenum(VI)dioxo complex.

For the synthesis of the peptide ligand **2a**, the 9-fluorenylmethyloxycarbonyl-(Fmoc-)-protected aspartic acid derivative **6** had to be prepared first. As shown in Scheme 2, the side chain carboxylic acid of Fmoc-Asp-OBu-*t* (**7**) was activated with DIPEA/HBTU and was then reacted with 2,3-dimethoxybenzylamine (**5**), to obtain the fully protected amino acid derivative **8** in 72% yield.[7] The *tert*-butyl group of **8** could be selectively removed by reaction with HCl in diethyl ether[10] and the Fmoc-protected amino acid derivative **6** was obtained in 85% yield.



Scheme 2 Synthesis of the aspartic acid derivative **6**.

The enkephalin analogs were assembled by solid-phase synthesis. Therefore, the 4-Fmoc-hydrazinobenzoyl resin **9** was deprotected with piperidine in DMF.[5,11] The resin **10** was the starting point for the successive introduction of the amino acid residues. In a first reaction sequence Fmoc-Phe-OH was activated with DIPEA/HBTU and was then coupled to the hydrazine units of the resin. The Fmoc-groups were removed by reaction with piperidine in DMF. The same protocol of activation, coupling and deprotection was performed successively with Fmoc-Gly-OH, with Fmoc-Asp(CH_2Ver)-OH (**6**) (Ver = 3-veratryl = 2,3-dimethoxybenzene), and with Fmoc-Tyr(Bu-*t*)-OH. After removal of the Fmoc-protecting group at the N-terminus, the amino group was N-protected by reaction with acetic anhydride (Scheme 3).

An advantage of the hydrazinobenzoyl resin was that amide coupling at the C-terminus of the peptide could be performed simultaneously with the cleavage from the resin. Therefore, the hydrazine unit was oxidized in the presence of copper(II) acetate and air and the activated C-terminus was trapped with an appropriate amine.[11] In this study 2,3-dimethoxybenzyl amine (**5**) was used, to obtain the protected ligand precursor **11** over 11 steps in 69% yield.

Finally, the *tert*-butyl group at tyrosine and the methyl ethers of the veratryl unit were cleaved in one step using the $AlCl_3$/ethanethiol reaction conditions.[12] The reaction mixture was worked up by quenching with methanol, removal of volatile components in vacuum and extraction of the residue with water. The remaining solid material was isolated by filtration and was dried in vacuum.

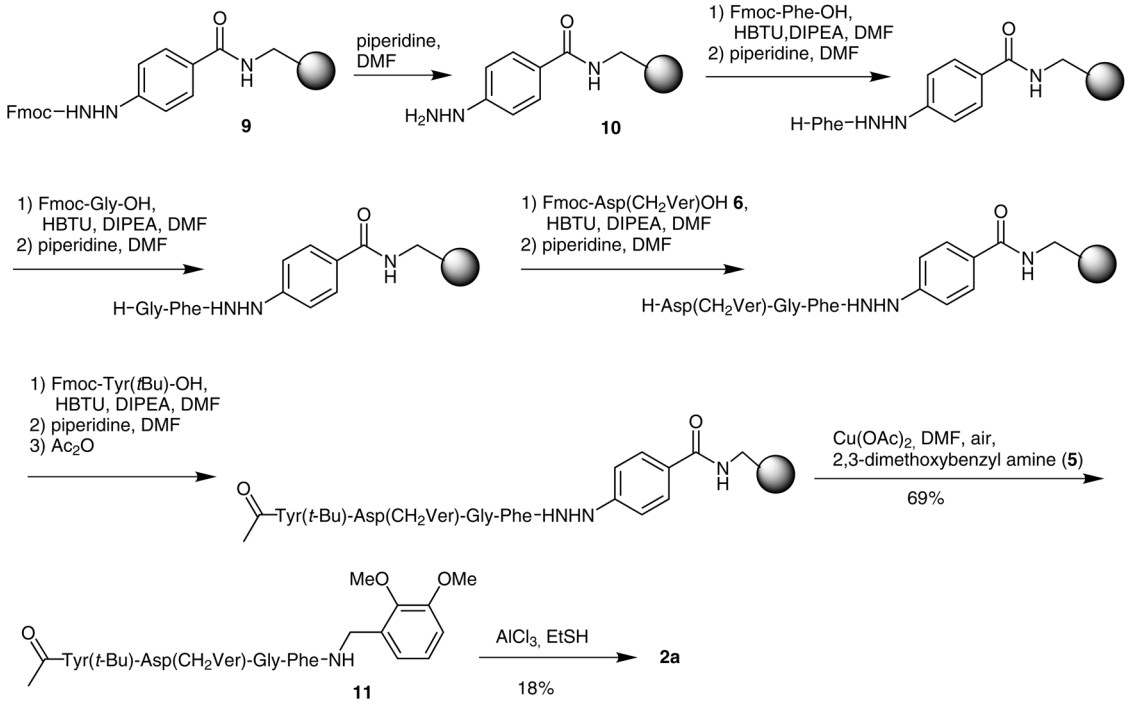
The desired dicatechol functionalized peptide Ac-Tyr-Asp(CH_2Cat)-Gly-Phe-CH_2Cat (**2a**) (Cat = 3-catechyl = 2,3-dihydroxyphenyl) was obtained in 18% yield. Compound **2a** was characterized by ESI-MS, showing molar peaks in the positive mode at m/z = 807.6 $[M + Na]^+$ and 785.4 $[M + H]^+$. In the negative mode the corresponding peak of $[M - H]^-$ was found at m/z = 783.5. Due to the low solubility of **2a**, the ^{1}H NMR spectrum was recorded in a mixture of CD_3OD and DMSO-d_6. The spectrum was characteristic for **2a** (see Experimental Section).

However, the low yield of **2a** was not satisfying. We supposed that it was due to the low solubility of **2a** in common solvents but a reasonable solubility in water, which results in the loss of product during work up. To overcome this problem, we synthesized the dicatechol **2b** in a somewhat modified procedure (Scheme 4).

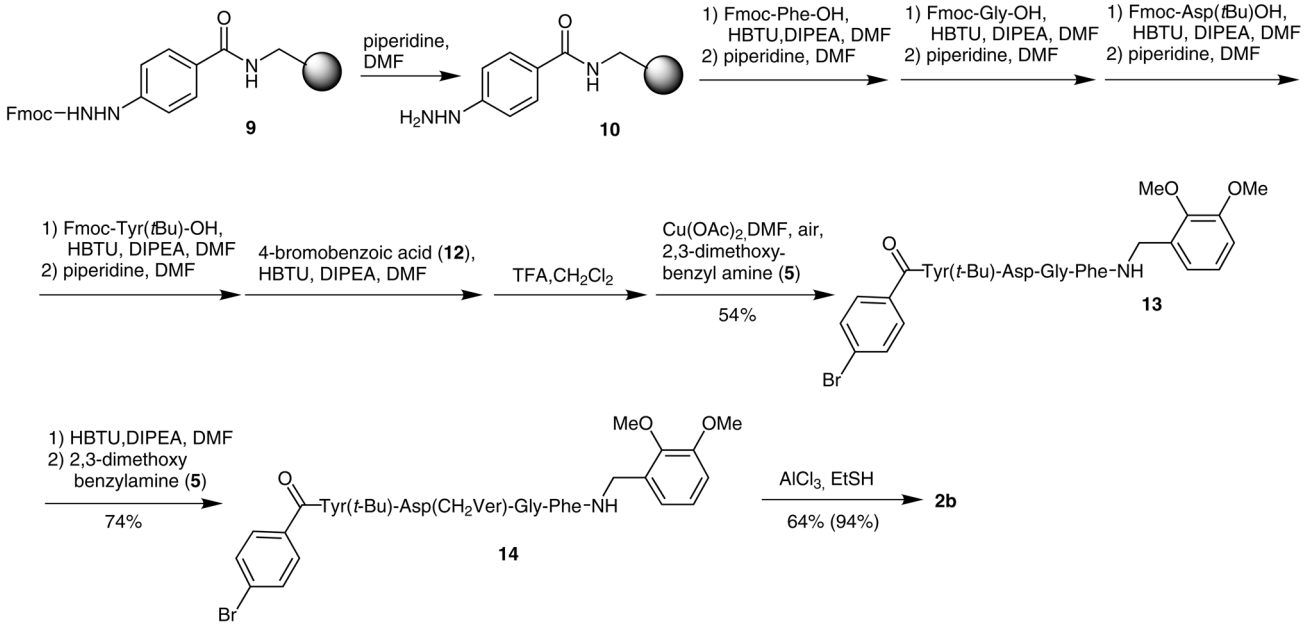
Following the above described solid-phase protocol, we deprotected the resin **9** and successively activated, coupled and deprotected Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Asp(Bu-*t*)-OH, and Fmoc-Tyr(Bu-*t*)-OH. Finally, 4-bromoacetic acid (**12**) was attached to the N-terminus.

Prior to cleavage of the peptide from the resin, the *tert*-butyl protecting group at the aspartic acid residue was selectively (vide infra) removed by reaction with 20% trifluoroacetic acid (TFA) in dichloromethane for 20 minutes.[10] Finally the peptide was cleaved with simultaneous attachment of 2,3-dimethoxybenzylamine (**5**) as was described before, to yield **13** in 54% over 12 steps.

The obtained peptide **13** was characterized by negative and positive ESI-MS, ^{1}H NMR spectroscopy, and elemental analysis. The data showed that, as desired, one *tert*-bu-



Scheme 3 Solid-phase synthesis of the enkephalin analogue dicatechol derivative **2a** (Ver = 3-veratryl = 2,3-dimethoxybenzene).



Scheme 4 Solid-phase synthesis of the enkephalin analogue dicatechol derivative **2b**.

tyl group was still attached. However, spectroscopy did not allow to distinguish, whether the remaining group is bound to tyrosine or to aspartic acid.

Activation of the free carboxylic acid function of the aspartic acid residue with DIPEA/HBTU and amide formation with 2,3-dimethoxybenzylamine (**5**) finally showed that the *t*-butyl group was bound to the phenol of tyrosine. The protected ligand precursor **14** was obtained in 74% yield as a colorless solid. The protecting groups were cleaved in one reaction step with $AlCl_3$/ethanethiol,[12] to obtain the dicatechol functionalized enkephalin analog **2b** in 94% yield as crude product in good purity. However, it could further be purified by recrystallization from methanol–water to obtain a yield of 64% of very pure **2b**. Compound **2b** was characterized by positive ESI-MS, ^{1}H and ^{13}C NMR, IR spectra, and elemental analysis (see experimental section).

The synthesis of **2b** described herein has major advantages over the one described for the preparation of **2a**: Selective cleavage of the aspartic acid protecting group and attachment of 2,3-dimethoxybenzylamine (**5**) to this position avoids the preparation of the building block **6**.

The 4-bromobenzoyl group induces solubility properties, which enable a simple and effective removal of the protecting group in the final step.

Finally a series of coordination studies of **2b** with $MoO_2(acac)_2$ were performed in methanol in the presence of potassium or sodium carbonate. However, in contrast to the simple model compound **3**, compounds **2a** and **2b** did not yield defined mononuclear macrocyclic complexes. Probably mixtures of oligomeric coordination compounds were formed.

Our described results showed, that the chain length of the dicatechol compounds **2a** and **2b** (as well as **3**) should be appropriate for coordination of both catechols to one metal center. However, in the peptidic systems, the preferred conformation of the peptide chain seems to disfavor a cyclic arrangement, which would be present in a 1:1 complex.

In conclusion, we have presented a synthetic entry to dicatechol functionalized enkephalin analogues, which have the potential to form conformationally constrained metallacyclopeptides. Although no defined coordination compounds are observed with the ligand **2b**, we have established the preparative methods, which we need to systematically screen this system. The L-aspartic acid residue can be substituted by D-aspartic acid or by glutaric acid. Side-chain catechol-functionalized lysine or ornithine residues could be introduced in this position. The respective studies will be performed in our laboratories and finally should result in the desired metallamacrocycles.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 spectrometer. FT-IR spectra were recorded on a Bruker IFS spectrometer (drift = diffuse reflection FT-IR spectroscopy). Mass spectra (EI, 70 eV; FAB) were taken on a Finnigan MAT 95 or 212 mass spectrometer. Elemental analyses were obtained with a Heraeus CHN-O-Rapid analyzer. Melting points: Büchi B-540 (uncorrected). The 4-Fmoc-hydrazinobenzoyl AM resin **9** and the protected amino acid building blocks were purchased from Novabiochem.

Azelic Acid Bis(2,3-dimethoxybenzylamide)

Azelic acid (**4**; 1.00 g, 5.31 mmol, 1 equiv) was dissolved in MeCN (60 mL), and DIPEA (2.00 mL, 11.7 mmol, 2.2 equiv) and HBTU (4.84 g, 12.8 mmol, 2.4 equiv) were added. After 15 min, 2,3-dimethoxybenzylamine (**5**; 1.95 mL, 11.7 mmol, 2.2 equiv) was added. The mixture was stirred for 2 h and the precipitate was removed by filtration and washed with MeCN to give 1.98 g (77%) of a colorless solid; mp 128 °C.

IR (drift, KBr): 3432, 3293, 2928, 1631, 1541, 1482, 1276, 1086, 1007, 751 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.04 (t, *J* = 8.0 Hz, 2 H), 6.90–6.86 (m, 4 H), 4.45 (d, *J* = 5.8 Hz, 4 H, benzyl CH_2), 3.87 (s, 6 H, OCH_3), 3.86 (s, 6 H, OCH_3), 2.15 (t, *J* = 7.6 Hz, 4 H), 1.64–1.56 (m, 4 H), 1.27 (br s, 6 H).

^{13}C NMR (CD_3OD, 100 MHz): δ = 172.5 (2 C), 152.4 (2 C), 131.8 (2 C), 124.1 (2 CH), 121.4 (2 CH), 111.7 (2 CH), 60.6 (2 CH_3), 55.7 (2 CH_3), 38.9 (2 CH_2), 36.7 (2 CH_2), 29.0 (2 CH_2), 28.8 (CH_2), 25.5 (2 CH_2).

MS (EI-DIP,70 eV): *m*/*z* (%) = 466.2 (6.35 [M]$^+$), 166.0 (100).

Anal. Calcd for $C_{27}H_{38}N_2O_6$: C, 66.64; H, 7.87; N, 5.76. Found: C, 66.21; H, 7.76; N, 5.72.

Azelic Acid Bis(2,3-dihydroxybenzylamide) (3)

At –15 °C, the protected ligand precursor azelic acid bis(2,3-dimethoxybenzylamide) (298 mg, 0.61 mmol, 1 equiv) was dissolved in CH_2Cl_2 (30 mL) and BBr_3 (590 μL, 6.12 mmol, 10 equiv) was added. After stirring overnight, MeOH (20 mL) was added and the volatile components were removed in vacuum. The residue was washed with H_2O to afford 236 mg (90%) of a slightly brown solid; mp 143 °C.

IR (drift, KBr): 3395, 2933, 2859, 1623, 1552, 1480, 1376, 1259, 1223, 1077, 1043, 741 cm^{-1}.

^{1}H NMR (CD_3OD, 400 MHz): δ = 6.97 (dd, *J* = 9.4, 1.7 Hz, 2 H), 6.71 (dd, *J* = 7.7, 1.9 Hz, 2 H), 6.68–6.64 (m, 4 H), 4.48 (s, 4 H, benzyl CH_2), 2.47 (t, *J* = 7.7 Hz, 4 H), 1.68–1.59 (br m, 4 H), 1.31 (br s, 6 H).

MS (EI-DIP, 70 eV): *m*/*z* (%) = 430.1 (10.73 [M]$^+$), 128.0 (100).

Anal. Calcd for $C_{23}H_{30}N_2O_6 \cdot 2.5H_2O$: C, 58.04; H, 6.57; N, 5.89. Found: C, 58.48; H, 6.81; N, 5.58.

Metal Complex K_2[3MoO_2]

Ligand **3** (28 mg, 0.07 mmol, 1 equiv), K_2CO_3 (35 mg, 0.25 mmol, 4 equiv) and $MoO_2(acac)_2$ (25 mg, 0.08 mmol, 1.2 equiv) were dissolved in MeOH (10 mL). The mixture was stirred overnight, the solvent was removed in vacuum and the residue was filtered over Sephadex LH to give 20.3 mg (76%) of a red solid.

IR (drift, KBr): 3395, 2928, 1632, 1535, 1457, 1278, 1252, 1211, 890 cm^{-1}.

^{1}H NMR (D_2O, 400 MHz): δ = 6.52–6.40 (br m, 6 H), 4.19–4.10 (br m, 4 H, benzyl CH_2), 2.20–1.62 (m, 4 H), 1.50–1.18 (m, 4 H), 1.14–0.98 (br, 6 H).

Negative ESI-MS: *m*/*z* = 557.3 [{**3**MoO_2} + H]$^-$.

Anal. Calcd for $C_{23}H_{26}K_2MoN_2O_8 \cdot 3H_2O$: C, 40.23; H, 4.70; N, 4.08. Found: C, 40.23; H, 4.68; N, 3.92.

Fmoc-Asp(CH_2Ver)-OBu-*t* (8)

Fmoc-Asp-OBu-*t* (**7**; 850 mg, 2.07 mmol, 1 equiv) was dissolved in MeCN (15 mL) and DIPEA (0.39 mL, 2.27 mmol, 1.2 equiv) as well as HBTU (940 mg, 2.48 mmol, 1.2 equiv) were added. The mixture was activated for 30 min and then benzylamine **5** (0.34 mL, 2.27 mmol, 1.1 equiv) was added and the mixture was stirred for 3 days. The precipitate was collected by filtration and dried in vacuum to yield a colorless solid (837 mg, 72%); mp 157 °C.

IR (drift, KBr): 3345, 3259, 2977, 2938, 1728, 1699, 1649, 1546, 1481, 1451, 1369, 1347, 1296, 1273, 1231, 1157, 1079, 1036, 1007, 743 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.74 (d, *J* = 7.4 Hz, 2 H, Fmoc), 7.59 (d, *J* = 7.4 Hz, 2 H, Fmoc), 7.39 (t, *J* = 7.4 Hz, 2 H, Fmoc), 7.33–7.28 (m, 2 H, Fmoc), 6.97 (t, *J* = 8.0 Hz, 1 H, Ver), 6.87-6.81 (m, 2 H, Ver), 6.12–6.03 (m, 2 H, NH), 4.50–4.46 (m, 1 H, α-H), 4.44 (d, *J* = 5.8 Hz, 2 H, CH_2 Fmoc), 4.37 (dd, *J* = 10.2, 7.4 Hz, 1 H, benzyl CH_2), 4.31–4.25 (m, 1 H, benzyl CH_2), 4.20 (t, *J* = 7.1 Hz, 1 H, CH Fmoc), 3.85 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 2.91–2.84 (m, 1 H, CH_2 Asp), 2.74–2.67 (m, 1 H, CH_2 Asp), 1.43 (s, 9 H, *t*-C_4H_9).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 143.6 (C), 141.1 (C), 131.2 (C), 127.5 2 (CH), 126.9 (2 CH), 125.1 (2 CH), 124.1 (CH), 121.3 (CH), 119.7 (2 CH), 112.0 (CH), 82.2 (C), 67.1 (2 CH), 60.6 (3 CH), 55.7 (CH_3), 51.3 (CH), 47.1 (CH), 39.1 (CH_2), 38.0 (CH_2), 27.8 (3 CH_3); the missing (C) can not be observed.

MS (ESI): *m*/*z* = 583.3 $[M + Na]^+$.

Anal. Calcd for $C_{32}H_{36}N_2O_7$: C, 68.55; H, 6.47; N, 5.00. Found: C, 68.62; H, 6.62; N, 4.98.

Fmoc-Asp(CH_2Ver)-OH (8)

Fmoc-Asp(CH_2Ver)-OH (**6**; 750 mg, 1.34 mmol) was stirred for 30 min with Et_2O saturated with gaseous HCl (30 mL). The solvent was removed in vacuum and the residue was washed with Et_2O to give 576 mg (85%) of a white solid; mp 163 °C.

IR (drift, KBr): 3300, 2940, 1702, 1642, 1542, 1481, 1448, 1430, 1275, 1226, 1172, 1084, 1050, 1004, 740 cm^{-1}.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.32 (t, *J* = 5.8 Hz, 1 H, NH), 7.94 (d, *J* = 7.2 Hz, 2 H, Fmoc), 7.76 (d, *J* = 7.4 Hz, 2 H, Fmoc), 7.65 (d, *J* = 8.2 Hz, 1 H, NH), 7.47 (t, *J* = 7.4 Hz, 2 H, Fmoc), 7.38 (t, *J* = 7.4 Hz, 2 H, Fmoc), 7.04–6.95 (m, 2 H, Ver), 6.88 (d, *J* = 7.2 Hz, 1 H, Ver), 4.50–4.42 (m, 1 H, α-H), 4.35–4.31 (m, 3 H, CH_2 + CH Fmoc), 4.30–4.25 (m, 2 H, benzyl CH_2), 3.83 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 2.76–2.70 (m, 1 H, CH_2 Asp), 2.61 (dd, *J* = 10.9, 8.0 Hz, 1 H, CH_2 Asp).

^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.5 (C), 169.5 (C), 156.2 (C), 152.2 (C), 146.6 (C), 144.2 (2 C), 141.1 (2 C), 132.9 (C), 128.1 (2 CH), 127.5 (2 CH), 125.7 (2 CH), 124.2 (CH), 120.6 (3 CH), 112.1 (CH), 66.3 (CH_2), 60.6 (CH_3), 45.2 (CH_3), 51.2 (CH), 47.2 (CH), 37.6 (CH_2), 37.5 (CH_2).

MS (ESI): *m*/*z* = 504.2 $[M - H]^-$.

Anal. Calcd for $C_{28}H_{28}N_2O_7 \cdot 0.75H_2O$: C, 64.92; H, 5.74; N, 5.41. Found: C, 64.98; H, 5.82; N, 5.26.

Solid-Phase Synthesis of the Peptide Sequences; General Procedure

The synthesis of dicatechol peptide precursors was performed on the 4-Fmoc-hydrazinobenzoyl resin **9** (4-Fmoc-hydrazinobenzoyl AM resin, Novabiochem)[11] by using N-Fmoc-protected amino acids. Prior to use, the resin was swollen in CH_2Cl_2 and after washing with DMF, the Fmoc-group was removed with a 20% solution of piperidine in DMF to obtain **10**.

For the preparation of the peptide sequences, the following protocol was used: The C-terminal-unprotected *N*-Fmoc amino acid (2 equiv) was activated with DIPEA (4 equiv) and HBTU (2 equiv) in DMF. After 10 min, this solution was added to **10** and the mixture was shaken for 1 h. Before attaching the next residue, the Fmoc group had to be removed by treating the resin with a 20% solution of piperidine in DMF for 15 min. The N-terminus of the peptide was either capped by reaction with Ac_2O in DMF or by attachment of 4-bromobenzoic acid (**12**) which was activated with DIPEA/HBTU. Finally, the resin was successively washed with DMF, CH_2Cl_2, and MeOH. After drying under vacuum, the peptide was cleaved from the resin with simultaneous attachment of **5** to the C-terminus. This was achieved by treatment with $Cu(OAc)_2$ (1 equiv) in DMF and bubbling air through the mixture for 4 h in the presence of **5**. The resin was removed by filtration and washed with CH_2Cl_2 (in the case of **2a** with DMF), and then the combined organic layers were washed with aq 1 M $KHSO_4$, H_2O, and brine. After drying (Na_2SO_4), the organic solvents were removed by distillation in vacuum.

Ac-Tyr(*t*Bu)-Asp(CH_2Ver)-Gly-Phe-CH_2Ver (11)

Following the general procedure for solid-phase peptide coupling, compound **9** (843 mg (0.67 mmol) was treated with piperidine in DMF, and Fmoc-Phe-OH (522 mg, 1.35 mmol), Fmoc-Gly-OH (401 mg, 1.35 mmol), Fmoc-Asp(CH_2Ver)-OH (**6**; 510 mg, 1.01 mmol), Fmoc-Tyr(Bu-*t*)-OH (619 mg, 1.35 mmol) and Ac_2O (3.5 mL)–DMF (3.5 mL) were attached successively, and if appropriate deprotected. The peptide was cleaved using $Cu(OAc)_2$ (61 mg, 0.34 mmol) in DMF (6 mL) in the presence of **5** (2.2 mL, 14.9 mmol). The obtained crude product was washed with Et_2O to obtain 419 mg (69%) of a colorless solid; mp 216 °C.

IR (drift, KBr): 3288, 1638, 1544, 1482, 1275, 1233, 1168, 1083 cm^{-1}.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.42 (t, *J* = 5.5 Hz, 1 H, NH), 8.31 (d, *J* = 7.4 Hz, 1 H, NH), 8.21–8.13 (m, 3 H, NH), 8.07 (d, *J* = 8.2 Hz, 1 H, NH), 7.21 (s, 5 H, Phe), 7.11 (d, *J* = 8.5 Hz, 2 H, Tyr), 7.01–6.96 (m, 1 H, Cat.), 6.95–6.94 (m, 1 H, Ver), 6.93–6.91 (m, 2 H, Ver), 6.83 (d, *J* = 8.2 Hz, 2 H, Tyr), 6.82–6.80 (m, 1 H, Ver), 6.65 (dd, *J* = 7.0, 1.7 Hz, 1 H, Ver), 4.58–4.43 (m, 3 H, α-H), 4.28 (d, *J* = 6.3 Hz, 2 H, benzyl CH_2), 4.25 (d, *J* = 6.8 Hz, 2 H, benzyl CH_2), 3.78 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 3.38 (d, *J* = 7.1 Hz, 2 H, CH_2 Gly), 3.07–3.01 (m, 1 H, CH_2), 2.95–2.90 (m, 1 H, CH_2), 2.90–2.83 (m, 1 H, CH_2), 2.71–2.63 (m, 2 H, CH_2), 2.58–2.52 (m, 1 H, CH_2), 1.74 (s, 3 H, $OCOCH_3$), 1.25 (s, 9 H, *t*-C_4H_9).

MS (ESI): *m*/*z* = 919.6 $[M + Na]^+$, 897.3 $[M + H]^+$.

Anal. Calcd for $C_{48}H_{60}N_2O_{11} \cdot 2H_2O$: C, 61.79; H, 6.91; N, 9.01. Found: C, 61.38; H, 6.68; N, 8.94.

Ac-Tyr-Asp(CH_2Cat)-Gly-Phe-CH_2Cat (2a)

At 0 °C, $AlCl_3$ (186 mg, 1.39 mmol, 25 equiv) was dissolved in ethanethiol (5 mL) and **11** (50 mg) was added and the mixture was stirred overnight. After hydrolysis with MeOH (15 mL), the volatile components were removed in vacuum the residue was suspended in H_2O and then filtered to obtain 8 mg (18%) of a grey solid; mp 180 °C (dec.).

IR (drift, KBr): 3331, 2930, 1653, 1518, 1478, 1343, 1259, 742 cm^{-1}.

^{1}H NMR (CD_3OD + DMSO-d_6, 300 MHz): δ = 7.20 (br s, 5 H, Phe), 7.00 (d, *J* = 8.7 Hz, 2 H, Tyr), 6.72–6.67 (br m, 3 H, Tyr + Cat), 6.67–6.56 (m, 5 H, Cat), 4.60–4.56 (br m, 1 H, α-H), 4.51–4.42 (br m, 2 H, α-H), 4.35–4.25 (br m, 4 H, benzyl CH_2), 3.70 (br s, 2 H, CH_2 Gly), 3.22–3.17 (br m, 1 H, CH_2), 3.05–2.90 (br m, 2 H, CH_2), 2.81–2.70 (br m, 2 H, CH_2), 2.67–2.65 (br m, 1 H, CH_2), 1.88 (s, 3 H, $OCOCH_3$).

Positive ESI-MS: *m*/*z* = 807.6 $[M + Na]^+$, 785.4 $[M + H]^+$.

Negative ESI-MS: *m*/*z* = 783.5 $[M - H]^-$.

(4-Bromobenzoyl)-Tyr(Bu-*t*)-Asp-Gly-Phe-CH_2Ver (13)

Following the general procedure for the solid-phase peptide coupling, the compound **9** (1.33 g, 1.06 mmol) was treated with piperidine in DMF, and Fmoc-Phe-OH (821 mg, 2.12 mmol), Fmoc-Gly-OH (630 mg, 2.12 mmol), Fmoc-Asp(Bu-*t*)-OH (974 mg, 2.12 mmol), Fmoc-Tyr(Bu-*t*)-OH (974 mg, 2.12 mmol), and 4-bromobenzoic acid (**12**; 426 mg, 2.12 mmol) were succesively attached, and if appropriate were deprotected. Prior to cleavage, the resin was treated with 20% TFA in CH_2Cl_2 (10 mL) for 20 min and then was washed with DIPEA in DMF. The peptide was cleaved using $Cu(OAc)_2$ (96 mg, 0.53 mmol) in DMF (12 mL) in the presence of **5** (1 mL, 10.8 mmol). The obtained crude product was washed with Et_2O to obtain 508 mg (54%) of a yellow solid; mp 210 °C (dec.).

IR (drift, KBr): 3287, 1636, 1518, 1482, 1273, 1227, 1157, 750 cm^{-1}.

^{1}H NMR (CD_3OD, 300 MHz): δ = 7.66 (d, *J* = 8.7 Hz, 2 H, benzoyl), 7.56 (d, *J* = 8.7 Hz, 2 H, benzoyl), 7.25 (s, 5 H, Phe), 7.11 (d,

J = 8.4 Hz, 2 H, Tyr), 6.99–6.90 (m, 2 H, Ver), 6.71 (d, *J* = 8.4 Hz, 2 H, Tyr), 6.70–6.66 (m, 1 H, Ver), 4.74 (t, *J* = 7.4 Hz, 1 H, α-H), 4.69–4.61 (m, 2 H, α-H), 4.33 (s, 2 H, benzyl CH_2), 3.93 (d, *J* = 16.8 Hz, 1 H, CH_2 Gly), 3.86 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.71 (d, *J* = 16.8 Hz, 1 H, CH_2 Gly), 3.24–3.13 (m, 2 H, CH_2 Tyr/Phe), 3.07–2.96 (m, 2 H, CH_2 Tyr/Phe), 2.92–2.83 (m, 1 H, CH_2 Asp), 2.64 (dd, *J* = 16.4, 7.0 Hz, 1 H, CH_2 Asp), 1.45 (s, 9 H, *t*-C_4H_9).

MS (FAB, 3-NBA/DMSO); Positive Mode: *m*/*z* = 913.1 $[M + Na]^+$, 891.0 $[M + H]^+$.

MS (FAB, 3-NBA/DMSO); Negative Mode: *m*/*z* = 888.8 $[M - H]^-$.

Anal. Calcd for $C_{44}H_{50}BrN_5O_{10}\cdot H_2O$): C, 58.28; H, 5.78; N, 7.72. Found: C, 58.33; H, 5.86; N, 7.86.

(4-Bromobenzoyl)-Tyr(Bu-*t*)-Asp(CH_2Ver)-Gly-Phe-CH_2Ver (14)

A solution of **13** (505 mg, 0.57 mmol, 1 equiv) and DIPEA (107 μL, 0.63 mmol, 1.1 equiv) in DMF (5 mL) and CH_2Cl_2 (25 mL) was combined with a solution of HBTU (259 mg, 0.68 mmol, 1.2 equiv) in DMF (3 mL). After 20 min of activation, **5** (190 μL, 1.14 mmol, 2 equiv) was added and the mixture was stirred overnight. EtOAc was added and the mixture was washed with sat. aq NH_4Cl, $NaHCO_3$, H_2O, and brine. The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product can be recrystallized from hexane–*i*-PrOH; yield: 431 mg (74%); colorless solid; mp 208 °C.

IR (drift, KBr): 3419, 3298, 2936, 1633, 1519, 1481, 1275, 1227, 1159, 1079, 1009, 846 cm^{-1}.

^1H NMR (DMSO-d_6, 400 MHz): δ = 8.63 (d, *J* = 8.5 Hz, 1 H, NH), 8.45 (d, *J* = 7.7 Hz, 1 H, NH), 8.36 (t, *J* = 5.9 Hz, 1 H, NH), 8.10 (d, *J* = 8.2 Hz, 1 H, NH), 7.92 (m, 2 H, NH), 7.72 (d, *J* = 8.7 Hz, 2 H, benzoyl), 7.64 (d, *J* = 8.7 Hz, 2 H, benzoyl), 7.22 (s, 5 H, Phe), 7.12–7.05 (m, 4 H), 6.95–6.91 (m, 3 H), 6.63–6.59 (m, 3 H), 4.67–4.50 (br m, 3 H), 4.28 (d, *J* = 5.8 Hz, 2 H, benzyl CH_2), 4.25–4.20 (m, 2 H, benzyl CH_2), 3.81 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 3.71–3.60 (m, 2 H, CH_2 Gly), 3.04–2.96 (m, 2 H, CH_2 Tyr/Phe), 2.85–2.80 (m, 2 H, CH_2 Tyr/Phe), 2.74–2.66 (m, 2 H, CH_2 Asp), 1.34 (s, 9 H, *t*-C_4H_9).

MS (ESI): *m*/*z* = 968.4 $[M - (OBu\text{-}t)]^+$, 912.4 $[M - Ver + Na]^+$.

Anal. Calcd for $C_{53}H_{61}BrN_5O_{11}\cdot 4H_2O$: C, 57.35; H, 6.27; N, 7.57. Found: C, 57.54; H, 6.01; N, 7.98.

(4-Bromobenzoyl)-Tyr-Asp(CH_2Cat)-Gly-Phe-CH_2Cat (2b)

At 0 °C, $AlCl_3$ (481 mg, 3.61 mmol, 25 equiv) was dissolved in ethanethiol (12 mL) and **14** (150 mg, 0.14 mmol) was added and the mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 (9 mL) and under cooling was quenched with MeOH (30 mL). The volatile components were removed in vacuum and the residue was suspended in H_2O and then filtered to obtain 125 mg (94%) of crude product, which can be recrystallized from MeOH–H_2O; yield: 78 mg (64%); mp 185 °C (dec.).

IR (drift, KBr): 3307, 3089, 2931, 1652, 1594, 1518, 1480, 1229, 745 cm^{-1}.

^1H NMR (CD_3OD, 400 MHz): δ = 7.64 (d, *J* = 8.5 Hz, 2 H, benzoyl), 7.55 (d, *J* = 8.8 Hz, 2 H, benzoyl), 7.22–7.15 (m, 6 H, Phe + Cat), 7.09 (d, *J* = 8.5 Hz, 2 H, Tyr), 6.73–6.68 (m, 4 H, Tyr + Cat), 6.60 (t, *J* = 7.7 Hz, 2 H, Cat), 6.52 (dd, *J* = 7.7, 1.7 Hz, 1 H, Cat), 4.74–4.69 (m, 1 H, α-H), 4.65 (t, *J* = 6.3 Hz, 1 H, α-H), 4.58 (dd, *J* = 8.5, 6.0 Hz, 1 H, α-H), 4.26 (br s, 4 H, benzyl CH_2), 3.88 (d, *J* = 17.0 Hz, 1 H, CH_2 Gly), 3.70 (d, *J* = 17.0 Hz, 1 H, CH_2 Gly), 3.19–3.15 (m, 1 H, CH_2), 3.14–3.11 (m, 1 H, CH_2), 3.02 (d, *J* = 8.0 Hz, 1 H, CH_2), 2.97–2.92 (m, 1 H, CH_2), 2.91–2.88 (m, 1 H, CH_2), 2.81–2.74 (m, 1 H, CH_2).

^{13}C NMR (CD_3OD, 100 MHz): δ = 173.0 (C), 172.4 (C), 172.3 (C), 171.9 (C), 169.9 (C), 167.9 (C), 163.4 (C), 155.9 (C), 136.9 (C), 132.8 (C), 131.3 (2 CH), 130.1 (2 CH), 129.0 (4 CH), 128.9 (2 CH), 128.1 (2 CH), 127.4 (C), 126.3 (CH), 125.9 (C), 124.6 (C), 119.8 (CH), 119.2 (CH), 114.9 (CH_3), 114.2 (CH), 55.9 (CH), 55.2 (CH), 50.2 (CH), 42.6 (CH_2), 38.6 (CH_2), 37.4 (CH_2), 36.4 (CH_2), 35.7 (CH_2), 35.1 (CH_2); four carbon atoms cannot be observed.

MS (ESI): *m*/*z* = 844.1 $[M - Br]^+$.

Anal. Calcd for $C_{45}H_{45}BrN_6O_{11}\cdot 6H_2O$: C, 52.28; H, 5.56; N, 8.13. Found: C, 52.45; H, 5.09; N, 7.67.

References

(1) Sewald, N.; Jakubke, H.-D. *Peptides: Chemistry and Biology*; VCH: Weinheim, **2002**.

(2) Evans, S. M.; Lenz, G. R. *Chemtracts: Org. Chem.* **1995**, *8*, 122.

(3) (a) Baker, T. J.; Rew, Y.; Goodman, M. *Pure. Appl. Chem.* **2000**, *72*, 347. (b) Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Cassel, J. A.; DeHaven, R. N.; Goodman, M. *J. Med. Chem.* **2002**, *45*, 3746. (c) Rew, Y.; Goodman, M. *J. Org. Chem.* **2002**, *67*, 8820.

(4) (a) Albrecht, M.; Stortz, P.; Nolting, R. *Synthesis* **2003**, 1307. (b) Albrecht, M.; Stortz, P.; Weis, P. *Supramol. Chem.* **2003**, *15*, 477.

(5) (a) Albrecht, M.; Stortz, P.; Weis, P. *Synlett* **2003**, 867. (b) Albrecht, M.; Stortz, P.; Runsink, J.; Weis, P. *Chem. Eur. J.* **2004**, *10*, 3657.

(6) For other metal stabilized peptide microstructures, see: (a) Torrado, A.; Imperiali, B. *J. Org. Chem.* **1996**, *61*, 8940. (b) Kelso, M. J.; Hoang, H. N.; Appleton, T. G.; Fairlie, D. P. *J. Am. Chem. Soc.* **2000**, *122*, 10488. (c) Constable, E. C.; Housecroft, C. E.; Mundwiler, S. *J. Chem. Soc., Dalton Trans.* **2003**, 2112.

(7) (a) Li, P.; Xu, J.-C. *Tetrahedron* **2000**, *56*, 9949. (b) Speicher, A.; Eicher, T. *J. Prakt. Chem.* **1998**, *340*, 581.

(8) McOmie, J. F. W.; West, D. E. *Org. Synth. Collect. Vol. V.*; Wiley: New York, **1973**, 412.

(9) Duhme, A.-K. *J. Chem. Soc., Dalton Trans.* **1997**, 773.

(10) Jones, J. *Synthese von Aminosäuren und Peptiden*; VCH: Weinheim, **1995**.

(11) (a) Millington, C. R.; Quarrell, R.; Lowe, G. *Tetrahedron Lett.* **1998**, *39*, 7201. (b) Rosenbaum, C.; Waldmann, H. *Tetrahedron Lett.* **2001**, *42*, 3677. (c) Peters, C.; Waldmann, H. *J. Org. Chem.* **2003**, *68*, 6053.

(12) Node, M.; Nishide, K.; Fuji, K.; Fujita, E. *J. Org. Chem.* **1980**, *45*, 4275.