Di-, Tri- and Tetrapeptide-Linked Dicatechol Derivatives

Markus Albrecht,* Oliver Spieß, Matthias Schneider
Institut für Organische Chemie, Universität Karlsruhe, Richard-Willstätter-Allee, 76131 Karlsruhe, Germany
Fax +49(721)698529; E-mail: albrecht@ochhades.chemie.uni-karlsruhe.de
Received 25 August 2001

Abstract: Di-, tri- and tetrapeptide linked dicatechol derivatives are prepared by subsequent coupling of 2,3-dimethoxybenzoic acid (2), peptides 3 and 2,3-dimethoxybenzylamine (5) using classical activating conditions (EDC/HOBt or DDC/HOSu). In the final step the methyl ethers at the veratrol units are cleaved to afford the free catechol derivatives 7, which are potential ligands for metal complexes with well defined fixed conformations at the peptide spacers.

Key words: amides, peptides, ligands, supramolecular chemistry

The three-dimensional structure of a protein is crucial for its function as a molecular machine. Structurally different domains of a peptide (α-helix, turns, loops, β-sheet) combine in a well defined manner to enable the folding of the protein. However, the folding mechanisms of peptides are not very well understood.1 In some cases the coordination of metal ions to the peptides supports the formation of specific structures in natural and artificial proteins and leads to the fixation of more stable structures than hydrogen bonding alone does.2

Just recently we described the preparation of amino acid-bridged dicatechol derivatives 1-H4 which form double-stranded dinuclear titanium(IV) complexes [(1)2Ti2(OR)2]2– with two alcoholate coligands present. Despite the possible and initially observed formation of seven different isomers, only one isomer is obtained as the thermodynamically favored major species. Conformational considerations show, that the three-dimensional structure of the amino acid residue in the spacer is influenced by the stereochemistry at the metal centers.4

Figure 1 Chemical structure of the amino acid-bridged dicatechol derivative 1-H4

To go further, we now introduce di-, tri-, and tetrapeptide sequences as spacers in dicatechol derivatives. With appropriate metal ions we should be able to induce loop-, sheet-, or helix-type structures in the ligand strands. In this paper we present the straightforward synthesis of the peptide-bridged dicatechol derivatives which are highly interesting candidates for peptide/metal complex hybrid with well-defined three-dimensional structures.

The peptide-bridged dicatechol ligands 7 are prepared using standard peptide coupling strategies5 with EDC/HOBt6 or DCC/HOSu7 as activating agents. Hereby the di- 7a,b and tripeptide derivatives 7c–e are synthesized in three step procedures by coupling first 2,3-dimethoxybenzoic acid (veratryl-3-carboxylic acid = Ver-CO2 H) (2) to the N-terminus and subsequently 2,3-dimethoxybenzylamine (3-aminomethylveratrol = NH2CH2 -Ver) (5) to the C-terminus of the corresponding di- or tripeptide. In the last step the methyl ethers of 6a–e are cleaved with BBr3,8 and the deprotected peptide-bridged dicatechol ligands 7a–e are liberated (Scheme 1).

The intermediate monoveratrole substituted peptides 4a–e are not isolated in analytically pure form. However, the material, which was obtained was pure enough for further transformations and in most cases could be characterized spectroscopically. Purification can easily be performed on peptide-bridged dicatechol derivatives which are highly interesting candidates for peptide/metal complex hybrid with well-defined three-dimensional structures.

Scheme 1
the bisveratrole derivatives 6a–e by simply recrystallizing them from the appropriate solvent.

As dipeptide we have chosen to introduce alanyl-leucine and valyl-valine. Hereby the formations of the diveratrole derivatives proceed in 41% (6a) and 52% yield (6b) over two steps. The methyl ether cleavage is performed in close to quantitative yield and the dipeptide bridged dicatechols 7a and 7b are obtained as beige solids, which decompose between 80 °C and 100 °C (Figure 2).

With alanyl-leucine as spacer 7a, an amino acid sequence is present which possesses two sterically less demanding amino acids while in the valyl-valine derivative 7b two sterically highly demanding amino acids are introduced. Upon metal binding this might lead to different conformations at the strands.

Three different dicatechol derivatives 7c–e are prepared which bear tripeptide spacers (Figure 3). Again one sterically less hindered spacer with the Leu-Leu-Leu sequence 7c and one more demanding with the Val-Val-Val linker 7d is introduced. As a third example a compound with three different amino acid residues (Ala-Val-Leu) 7e is prepared.

The diveratrole ligand precursors 6c–e are synthesized in a two step sequence without purification of the intermediate monoveratrole derivatives 4c–e similar to the synthesis of the dipeptide bridged ligands. Hereby yields of 59% (6c), 49% (6d) and 39% (6e) are obtained after the two step sequence followed by recrystallization of the peptide-bridged diveratrols. The final ether cleavage again proceeds in close to quantitative yield. The Val-Val-Val linked compound 7d melts at 176–180 °C while the other derivatives decompose at 110 °C (7c) or 90 °C (7e), respectively.

As an example of a tetrapeptide derivative, we synthesized the Phe-Leu-Phe-Leu bridged dicatechol 7f in a four step procedure. The intermediate peptides with a N-terminal 2,3-dimethoxybenzoate substituent 4f and 8f are not obtained in analytically pure form. The first intermediate to be purified by crystallization is the tetrapeptide linked diveratrol 6f (Scheme 2).

Figure 2 Chemical structures of dipeptide-bridged dicatehols 7a and 7b

Figure 3 Chemical structures of tripeptide-bridged dicatechols 7c–e

Scheme 2
In the first reaction step 2,3-dimethoxybenzoic acid (2) is coupled with phenylalanyl-leucine (3f) by EDC/HOBt activation to obtain the veratryl dipeptide 4f. The same reaction step is repeated with 4f and 3f to generate the tetrapeptide 8f. Activation of the acid function of 8f (EDC/HOBt) and addition of 2,3-dimethoxybenzylamine results in the formation of the tetrapeptide bridged diterolate 6f, which after recrystallization from methanol is isolated in 36% yield over three steps. Deprotection of the methyl ethers is performed using BBr3 and the catechol 7f finally is obtained on a half gram scale in 97% yield.

Herein we presented the simple synthesis of several di-, tri- or tetrapeptide linked dicatechol derivatives 7a–f, which are potential ligands for mono- or oligonuclear metal complexes. The preparations follow straightforward peptide coupling protocols using EDC/HOBt (or DCC/HOSu) as amide coupling reagents. Different peptide linkers were used to introduce different steric demands in the ligand spacer. In future studies the ligands will be used to obtain coordination compounds in which by metal coordination either sheet will be used to obtain coordination compounds in which the multiplicity of carbon atoms. FT-IR spectra were recorded by diffuse reflection (KBr) on a Bruker IFS 113v spectrometer using DEPT techniques for the assignment of the multiplicity of carbon atoms. FT-IR spectra were recorded on a Perkin Elmer Lambda 2 spectrometer. Mass spectra were recorded on a Bruker DRX 500 spectrometer using HRMS (positive FAB; EI, 70 eV) were taken on a Finnigan MAT 90 mass spectrometer. Elemental analyses were obtained with a Heraeus CHN-O-Rapid analyzer. Solvents were purified by standard methods. Melting points: Büchi B-540 (uncorrected). Air sensitive compounds were prepared and handled under argon using Schlenk techniques.

**Coupling of Peptides 3 with 2,3-Dimethoxybenzoic Acid (2): General Procedure**

DMF was cooled to 0 °C and a mixture of EDC (ethyl(dimethylamino)propyl) carbodimide, 1 equiv), HOBt (1-hydroxybenzotriazole, 1.1 equiv) and 2,3-dimethoxybenzoic acid (2; 1 equiv) was added in one portion. Still at 0 °C, the peptide 3 (1 equiv) and a solution of NaOH (1 equiv) in H2O was added. Warming the mixture to r.t. and stirring overnight was followed by removal of the solvent in vacuum at 20 °C. The residue was dissolved in EtOAc andaq sat. NH4Cl solution. The layers were separated and the organic layer was washed twice withaq sat. NH4Cl solution. Evaporation of the solvent yielded the product, which was used for further transformations without purification.

**Coupling of N-(2,3-Dimethoxybenzoate)-Substituted Peptides 4 and 8 with 2,3-Dimethoxybenzylamine (5): General Procedure**

The crude N-(2,3-dimethoxybenzoate)-substituted peptide 4 or 8 was dissolved in DMF and the resulting solution was cooled to 0 °C. EDC, HOBt and 2,3-dimethoxybenzylamine (5) were added. The mixture was stirred overnight while warming slowly to r.t. The solvent was removed at 20 °C in vacuum and the residue was portioned between EtOAc andaq sat. NH4Cl solution. The layers were separated, the organic layer was washed twice withaq sat. NH4Cl solution, and dried (MgSO4). The solvent was distilled off in vacuum and the product was purified by recrystallization.

**Methyl Ether Cleavage: General Procedure**

The tetramethyl protected compound 6 was dissolved in CHCl3 and cooled to 0 °C. BBr3 (1 M solution in CH2Cl2, 12 equiv) was added slowly and the cooling bath was removed. After stirring overnight MeOH was added and the resulting mixture was stirred for another hour. The solvent was evaporated and the residue dissolved in EtOAc. The solution was washed two times with H2O and dried (MgSO4). Removal of the solvent provided the pure product 7.

**Preparation of Cat-CO-Ala-Leu-NHCH2-Cat (7a)**

Yield: 1.16 g; colorless oil which was used without further purification.

**Ver-CHO-Ala-Leu-4**

Yield: 989 mg [41% over two steps starting from H2N-Ala-Leu-OH (3a)], white solid after recrystallization from EtOAc; mp 168–172 °C.

**Ver-CHO-Ala-Leu-NHCH2-Cat (7a)**

Yield: 989 mg [41% over two steps starting from H2N-Ala-Leu-OH (3a)], white solid after recrystallization from EtOAc; mp 168–172 °C.
Anal. Calcd for C₃₂H₄₅N₃O₁₂ (560.9): C, 62.34; H, 7.37; N, 8.18. Found: C, 62.53; H, 7.03; N, 8.44.

**Cat-COA-Leu-NHIC₃Cat (7a)**

Yield: 421 mg (100%); beige solid; mp 80–86 °C (dec.).

1H NMR (methanol-d₄) δ = 7.39 (dd, J = 8.0, 1.8 Hz, 1 H), 7.20 (m, 1 H), 7.07 (t, J = 8.7, 1.9 Hz, 1 H), 6.93 (d, J = 8.0, 1.8 Hz, 1 H), 6.78 (d, J = 8.0, 1.8 Hz, 1 H), 6.67 (dd, J = 6.5, 1.7 Hz, 1 H), 4.47 (m, 3 H, J = 6.9, 3.8 Hz), 1.00 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.9 Hz, 3 H), 0.91 (d, J = 6.9 Hz, 3 H), 0.88 (d, J = 6.9 Hz, 3 H).

**Preparation of Cat-CO-Leu-Leu-Leu-NHCH₂-Cat (7b)**

The peptide coupling is performed with DCC/HOBt (instead of EDC/HOBt) in dioxane as coupling reagent. Yield: 360 mg (52%); white solid after recrystallization from MeOH.

**Ver-CO-Val-Val-Val-NHCH₂-Ver (6b)**

The peptide coupling is performed with DCC/HOSU (instead of EDC/HOBt) in dioxane as coupling reagent. Yield: 360 mg (52% over two steps starting from H₂N-Val-Val-OH (7a); white solid after recrystallization from MeOH.

**Preparation of Cat-CO-Leu-Leu-Leu-NHIC₃Cat (7c)**

Yield: 1.13 g of crude product, which was used without further purification; mp 86–100 °C.

**Cat-COA-Leu-NHIC₃Cat (7d)**

Yield: 421 mg (100%); beige solid; mp 80–86 °C (dec.).

1H NMR (methanol-d₄) δ = 7.39 (dd, J = 8.0, 1.8 Hz, 1 H), 7.14–7.20 (m, 2 H), 4.49 (dd, J = 8.5, 6.5 Hz, 1 H), 4.44 (dd, J = 8.5, 6.5 Hz, 1 H), 3.99 (s, 3 H), 3.91 (s, 3 H), 3.86 (s, 3 H), 2.31 (m, 1 H), 2.24 (m, 1 H), 1.02 (d, J = 6.9 Hz, 3 H), 1.00 (d, J = 6.9 Hz, 3 H), 0.89 (d, J = 6.9 Hz, 3 H), 0.84 (d, J = 6.9 Hz, 3 H).

**Preparation of Cat-COA-Leu-Leu-Leu-NHIC₃Cat (7e)**

Yield: 1.06 g (59% over two steps starting from H₂N-Val-Val-Leu-OH (3c) as a white solid after recrystallization from MeOH/H₂O; mp 175 °C (dec.).

**Ver-CO-Leu-Leu-Leu-NHIC₃Cat (7f)**

Yield: 1.13 g of crude product, which was used without further purification; mp 86–100 °C.

**Preparation of Cat-COA-Leu-Leu-Leu-NHIC₃Cat (7g)**

Yield: 1.06 g (59% over two steps starting from H₂N-Val-Val-Leu-OH (3c) as a white solid after recrystallization from MeOH/H₂O; mp 175 °C (dec.).

1H NMR (CDCl₃) δ = 7.83 (d, J = 7.7, 1.8 Hz, 1 H), 7.06–7.13 (m, 2 H), 6.94–6.97 (m, 2 H), 6.88 (dd, J = 7.7, 1.4 Hz, 1 H), 6.87 (dd, J = 8.1, 1.5 Hz, 1 H), 4.56–4.61 (m, 2 H), 4.47 (m, 2 H), 4.38 (m, 1 H), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 1.50–1.82 (m, 9 H), 0.96 (d, J = 6.1 Hz, 3 H), 0.93 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.9 Hz, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.83 (d, J = 6.5 Hz, 3 H).

**Preparation of Cat-COA-Leu-Leu-Leu-NHIC₃Cat (7h)**

Yield: 1.13 g of crude product, which was used without further purification; mp 86–100 °C.
H NMR (methanol-d4): δ = 7.73 (dd, J = 8.1, 1.5 Hz, 1 H), 6.94 (dd, J = 7.8, 1.5 Hz, 1 H), 6.60–6.74 (m, 4 H), 4.61 (dd, J = 9.4, 5.2, 1 H), 4.40–4.45 (m, 2 H), 4.34 (s, 2 H), 3.95 (m, 9 H), 0.98 (d, J = 6.1 Hz, 3 H), 0.95 (s, 3 H), 0.86–0.92 (m, 12 H).

13C NMR (methanol-d4): δ = 175.1 (C), 174.9 (C), 174.5 (C), 171.0 (C), 149.6 (C), 147.2 (C), 146.6 (C), 144.5 (C), 126.1 (C), 121.2 (CH2), 120.6 (CH), 119.8 (CH, double intensity), 119.7 (CH), 117.1 (C), 115.6 (CH), 53.7 (CH), 53.3 (CH), 53.0 (CH), 41.8 (CH2), 41.7 (CH3), 41.4 (CH2), 39.9 (CH2), 26.1 (CH), 25.8 (CH), 25.8 (CH), 23.4 (2 CH2), 23.4 (CH2), 22.1 (CH2), 21.9 (CH).

IR (KBr): 3290, 3090, 2985, 2871, 1642, 1588, 1480, 1369, 1338, 1264, 1173, 1078, 949, 822, 740, 743 cm⁻¹.

MS (+FAB, 3-NBA) m/z: 615 [MH⁺], 474, 363, 250, 222, 137.

HRMS m/z: [M⁺] calcd. for C23H30N2O3 573.2908; found, 573.2908.


**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (7c)**

Ver-CO-Val-Val-Val-NHCH2-Ver (Cat-CO-Val-Val-Val-NHCH2-Ver (7c))

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver**

Ver-CO-Val-Val-Val-NHCH2-Ver (Cat-CO-Val-Val-Val-NHCH2-Ver (7d))

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (6d)**

Ver-CO-Val-Val-Val-NHCH2-Ver (6e)

Ver-CO-Val-Val-Val-NHCH2-Ver (6e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (4e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (4e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (3e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (3e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (2e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (2e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (1e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (1e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (0e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (0e)

**Preparation of Cat-CO-Val-Val-Val-NHCH2-Ver (−e)**

Ver-CO-Val-Val-Val-NHCH2-Ver (−e)
Cat-CO-Ala-Val-Leu-NHCH₂-Cat (7e)

Yield: 613 mg (99%); slightly grey solid; mp 90 ºC (dec.).

1H NMR (methanol-d₄): δ = 7.31 (dd, J = 1.5, 7.8 Hz, 1 H), 6.93 (dd, J = 1.5, 7.8 Hz, 1 H), 6.79 (t, J = 8.1 Hz, 1 H), 6.70 (dd, J = 1.8, 7.8 Hz, 1 H), 6.65 (dd, J = 1.8, 7.8 Hz, 1 H), 6.60 (t, J = 7.8 Hz, 1 H), 4.61 (q, J = 7.1 Hz, 1 H), 4.44 (dd, J = 5.6, 9.4 Hz, 1 H), 4.32 (s, 2 H), 4.18 (d, J = 7.4 Hz, 1 H), 2.01 (m, 2 H), 1.59 (m, 2 H), 1.43 (d, J = 7.1 Hz, 3 H), 0.91 (d, J = 6.5 Hz, 6 H), 0.89 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.2 Hz, 3 H).

13C NMR (methanol-d₄): δ = 173.9 (C), 173.4 (C), 172.1 (C), 169.6 (C), 148.4 (C), 145.8 (C), 145.2 (C), 143.1 (C), 124.7 (C), 119.9 (CH), 119.2 (CH), 118.4 (CH), 118.3 (CH), 118.1 (CH), 115.5 (C), 114.2 (CH), 58.9 (CH), 51.6 (CH), 49.4 (CH), 40.4 (CH₂), 38.5 (CH₃), 30.5 (CH₄), 24.4 (CH₂), 22.0 (CH₂), 20.6 (CH₃), 18.3 (CH₃), 17.3 (CH₃), 16.5 (CH₃).

IR (KBr): 3291, 2987, 2642, 1588, 1543, 1383, 1331, 1265, 1178, 1078, 973, 843, 745 cm⁻¹.

MS (+FAB, 3-NBA) m/z: 559 [M⁺H⁺], 491, 240, 253, 208, 137, 86.

HRMS m/z: [M⁺] calcd for C₉H₁₈N₂O₄, 559.2786; found, 559.2788.

Anal. Calcd. for C₉H₁₈N₂O₄ 2 H₂O (595.64): C, 56.55; H, 7.12; N, 9.42. Found: C, 56.83; H, 7.60; N, 9.54.

Preparation of Cat-CO-Phe-Leu-Phe-NHCH₂-Cat (7f)

Ver-CO-Phe-Leu-Oh (4f)

Yield: 2.78 g crude product which was used without further purification.

1H NMR (methanol-d₄): δ = 7.07-7.39 (m, 8 H), 4.49 (dd, J = 7.8, 0.8 Hz, 1 H), 3.82 (s, 3 H), 3.56 (s, 3 H), 3.28–3.33 (m, 2 H), 3.05 (dd, J = 14.1, 7.9 Hz, 1 H), 1.64–1.75 (m, 3 H), 0.94 (d, J = 6.4 Hz, 3 H), 0.91 (d, J = 6.3 Hz, 3 H).

13C NMR (methanol-d₄): δ = 175.7 (C), 173.4 (C), 167.3 (C), 154.2 (C), 149.2 (C), 138.0 (C), 130.7 (C), 129.5 (C), 127.9 (C), 127.3 (C), 125.3 (CH), 122.7 (CH), 111.1 (CH), 61.7 (CH₃), 56.5 (CH₃), 55.7 (CH₃), 52.1 (CH), 41.7 (CH), 39.0 (CH), 26.0 (CH), 23.4 (CH), 21.9 (CH).

IR (KBr): 3291, 3065, 2958, 2871, 1641, 1587, 1530, 1480, 1456, 1359, 1208, 1031, 961, 847, 742, 700 cm⁻¹.

MS (+FAB, 3-NBA) m/z: 796 [M⁺H⁺], 657, 544, 397, 284, 256, 137, 120.

HRMS m/z: [M⁺] calcd for C₁₆H₁₅N₄O₆, 796.3922; found, 796.3951.

Anal. Calcd. for C₁₆H₁₅N₄O₆ 2 H₂O (831.9): C, 63.52; H, 6.91; N, 8.42. Found: C, 63.26; H, 6.48; N, 8.34.

Acknowledgement

This work was supported by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft.

References


