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Ionic complexes of 1,1′-dimethyltitanocene(IV) dichloride with simple α-amino acids: synthesis, structural characterisation and investigation on hydrolytic stability in aqueous solution†

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Five cationic complexes of the general formula $[Cp'_2Ti(A)_2]^{2+}$ $[Cl^-]_2$ [Cp′ = η^5-$(CH_3)C_5H_4$ and A = glycine, 1; 2-methylalanine, 2; *N*-methylglycine, 3; L-alanine, 4; and D-alanine 5] were prepared by the reaction of Cp'_2TiCl_2 and the appropriate α-amino acid in 1 : 2 molar ratio from methanol–water solution in high yield. Air-stable crystalline solids, highly soluble in water, were characterized by means of elemental analysis, IR, Raman, ^1H, ^{13}C and ^{14}N NMR spectroscopy. The structure of compound 3 was determined by single crystal X-ray crystallography: orthorhombic P*bca No. 61*, $a = 9.5310(3)$, $b = 18.2980(5)$, $c = 26.6350(5)$ Å, $V = 4654$ Å^3, $Z = 8$. Hydrolytic stability of all compounds in D_2O was investigated using ^1H NMR spectroscopy within the pD interval of 2.9–6.5. All compounds slowly decomposed during 24 h at pD = 2.94, forming a mixture of hydrolytic products $[Cp'_2Ti(A)(D_2O)]^{2+}$, $[Cp'_2Ti(D_2O)_2]^{2+}$ and respective α-amino acids. By elevating pD to 4.0 and up to 6.5, a yellowish precipitate was formed, which indicates decomposition of the complexes. These compounds were characterized using elemental analyses, IR and Raman spectroscopy and attributed to oligomeric and/or polymeric structures described empirically by the formula $Ti(Cp')_xO_y(OH)_z$ ($x = 0.65$; $y = 0.3$, $z = 1.9$). Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: 1,1′-dimethyltitanocene dichloride; metallocenes; amino acids; NMR spectroscopy; IR spectroscopy; hydrolysis

INTRODUCTION

Bent metallocenes Cp_2MCl_2 (where Cp = η^5-C_5H_5; M = early transition metal, Ti, V, Mo, Nb) and in particular titanocene dichloride, Cp_2TiCl_2 (TDC), were extensively studied after the discovery of their antitumour properties.[1] Despite the fact, that TDC has entered phase II of clinical trials, the mechanism of its antitumour action is not well understood.

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Direct interactions with DNA and/or with DNA-processing proteins were proposed as two complementary mechanisms.[2,3] Prompted by several literature reports[4–7] and reviews[5,8,34–38] supporting the latter mechanism, we became interested in the synthesis, structural characterization and investigation of hydrolytic stability of the model titanocene (IV) compounds, containing protein building blocks, TDC-A complexes (where A = Gly, *N*-Me-Gly, 2-Me-Ala, L, D- or L, D-Ala, Phe, Val, nVal, Leu, Ile, Cys, Met, *S*-Ph-Cys, Pro, Trp, Ser).[9–11] During the study it was found that limited hydrolytic stability of TDC and, in particular, its α-amino acid complexes, at elevated pH is the main disadvantage for further investigations, especially those conducted under physiological conditions.[11,12] Searching for more hydrolytically stabile titanocene (IV) complexes the

scope was extended to Cp'_2TiCl_2[$Cp' = \eta^5 - (CH_3)C_5H_4$], 1,1′-dimethyltitanocene dichloride (DMTDC), whose hydrolytic behaviour was studied by means of ^{1}H NMR spectroscopy[6] and claimed to possess enhanced stability at physiological pH in comparison with TDC. DMTDC has been stated to be stabile at pH 2–7.5 for at least 24 h. Nevertheless, the presence of precipitate formed during experiments, clearly indicative for decomposition of the metallocene unit, was ommited not studied and not characterized.[6] No studies in order to characterize the solution at different pH (e.g. ^{1}H NMR spectra) were presented. Herein, synthesis and structural characterization are reported of a series of new cationic DMTDC-A complexes containing first group α-amino acids,[39] Gly, *N*-MeGly, 2-MeAla, L-Ala and D-Ala. DMTDC reacts easily with the appropriate α-amino acid at room temperature in a 1 : 2 molar ratio in methanol–water solution, affording air-stable crystalline solids, where the A-ligands are bonded to the titanium atom solely via the oxygen of the carboxylic group. This bonding situation was elucidated using a combination of vibrational (IR, Raman), ^{1}H, ^{13}C and ^{14}N NMR spectroscopy and also by X-ray crystallography.[14] However, all compounds were found to undergo partial decomposition in aqueous solution during 24 h at low pD values (pD = 2.9), which process is further accelerated raising pD to ca. 4 and resulting in complete decomposition at pD = 6–7. Hydrolytic stability and the process of decomposition was therefore studied in detail by means of ^{1}H NMR spectroscopy in D_2O within the pD interval of 2.9–6.5.

In the light of our investigation, it is aimed to complete and somewhat revise the results of Mokdsi and Harding.[6] It is hoped that the results will help to clarify some facts related to the hydrolytic behaviour of titanocene (IV) compounds, that are of much importance for understanding the mechanism of their antitumour action.

EXPERIMENTAL SECTION

All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Reaction yields are reported for pure products as an average from three consecutive runs and are based on DMTDC. All listed melting points are uncorrected. DMTDC was prepared and purified according to the literature procedure.[15] The α-amino acids of analytical grade were used as received (Fluka, Aldrich) without further purification. D_2O (99.98% isotope purity) was used as purchased (Aldrich), whereas methanol (Merck) and other solvents (Fluka) were purified and dried using standard methods and distilled prior to use. The monomer of methylcyclopentadiene was cracked from its dimer (93% Aldrich) and purified according to the literature method[6] and stored at −78 °C until use. The IR spectra (KBr, 4000–300 cm^{-1}, resolution 2 cm^{-1}) were recorded on a Perkin-Elmer 684 instrument. Raman spectra were measured on a Bruker FT-spectrometer IFS 55 with an FRA-106 accessory (diode pumped Nd : Yag laser, 1064 nm, Ge detector cooled by liquid nitrogen; solid samples, power of incident light 80 mW/mm^2, 50–3500 cm^{-1}, resolution 2 cm^{-1}, 100–200 scans were averaged). For complexes **1–5** only characteristic vibrations are given, for the decomposition product of DMTDC and complex **1** a full set of vibrations is listed. The ^{1}H, ^{13}C and ^{14}N NMR spectra were recorded on a Bruker AMX 360 spectrometer in D_2O at 298 K; typical sample: ∼100 mg of **1–5** in 600 µl of D_2O. Chemical shifts were referenced as follows: for ^{1}H to the signal of residual HDO (δ = 4.80 ppm at 298 K), ^{13}C to DSS (sf: 90.556 MHz) and for ^{14}N to external CH_3NO_2 (sf: 26.025 MHz). The pDs of resulting solutions [pD = pH(*meter reading*) + 0.4][16] were measured at the start, at given time intervals and at the end of the acquisitions using a glass combination pH electrode for NMR (Aldrich) with inner Ag–AgCl reference electrode connected to GRYF 107 pH-meter at 298 K; accuracy ±0.02 of pD unit [calibrated on Hydrion© dry buffers (Aldrich), pH = 2.00, 4.00, 7.00 dissolved prior to use in 500 ml of distilled water]. The pD values vary during the acquisition in ±0.05 unit. The X-ray crystallography intensity data were collected at 150 K on Nonius Kappa CCD area detector diffractometer for red block of 0.15 × 0.20 × 0.35 mm^3. Suitable single crystals were prepared by slow evaporation of the solution of complex **3** (100 mg/2 ml of methanol) maintained at 0 °C for several days. Programs used for processing of collected data were: audit creation method-SHELXL-97, PLATON for Windows and ORTEP III.[17,18] Elemental analyses (C/H/N/Cl) of all compounds gave significant results and were performed by micro-analytical laboratory of the Department of Organic Chemistry, University of Pardubice, Czech Republic.

Compound 1—general procedure for all compounds

DMTDC (1.000 g, 3.61 mmol), glycine (0.542 g, 7.22 mmol) and water (150 µl, 8.33 mmol) were stirred in 5 ml of dry methanol in a 10 ml one necked, round bottom Schlenk flask, while maintaining the temperature below 20 °C. After dissolution (8–10 h), remaining small amounts of insoluble solids were filtered off and the solvent volume was reduced by 1/2 of original. The resulting slurry was vigorously stirred with 20 ml of a dry diethylether/dichloromethane (5 : 1 v/v) mixture for 1 h. The precipitated material was separated, washed with dry dichloromethane (3 × 5 ml) and dried in vacuum; 1.295 g (84.1%) of light orange solid was obtained, m.p. >201 °C dec.

Anal. calcd, for $C_{16}H_{24}Cl_2N_2O_4Ti$ (M_r = 427.04): C, 44.96; H, 5.67; N, 6.57; Cl, 16.60. Found C, 44.69; H, 5.66; N, 6.51; Cl, 16.65; (lit. [6] C, 47.2; H, 6.2; N, 6.1; Cl, 15.6 for $C_{16}H_{24}Cl_2N_2O_4Ti \cdot 0.5\,H_2O$). IR (KBr, cm^{-1}): 3449 vs, b [$\nu_{as}(NH_3)$], 3112 s, b [ν(C-H), Cp], 3001w, 2960w, 2936s [$\nu(CH_3)$], 1679, 1675 vs [ν_{as}(COO)], 1505 vs [ν(C-CH_3)], 1378 m-s [ν_s(COO)], 1259 m [ν(C-C), Cp], 939 w, 1055 m [δ(C-H)], 860, 853 s [γ(C-H), Cp]. Raman (cm^{-1}): 3115

[ν(C-H), Cp], 2993, 2958, 2936 [$\nu(CH_3)$], 1678, 1673 [ν_{as}(COO)], 1507 [ν(C-CH_3)], 1378, 1373 [ν_s(COO)], 1258 [ν(C-C), Cp], 939, 1056 [δ(C-H), Cp], 851, 849 [γ(C-H), Cp)], 254 (a_1-tilting, Cp′-Ti-Cp′). ^{1}H NMR (ppm): 2.10 (s, C_5H_4-C$\underline{H}_3$, 6H), 3.74 (s, α-CH_2, 4H), 6.41 and 6.76 [2 × m, C_5H_4, 8H, $J^3(H_2, H_3) = J(H_4, H_5) = 5.08$ Hz; $J^4(H_2, H_4) = J(H_3, H_5) = 2.64$ Hz]; ^{13}C NMR (ppm): 18.63 (C_5H_4-$\underline{C}H_3$), 44.84 (CH_2), 121.22, 125.84, 142.82 (C_5H_4), 175.20 (COO); ^{14}N NMR (ppm): −353.43.

Compound 2

DMTDC (1.000 g, 3.61 mmol), 2-methylalanine (0.745 g, 7.22 mmol) and water (150 µl, 8.33 mmol) were stirred in 5 ml of dry methanol; 1.553 g of orange solid (89%) were obtained, m.p. >190 °C dec.

Anal. calcd, for $C_{20}H_{32}Cl_2N_2O_4Ti$ ($M_r = 483.14$): C, 49.67; H, 6.62; N, 5.79; Cl, 14.67. Found C, 49.45; H, 6.57; N, 5.78; Cl, 14.70. IR (KBr, cm^{-1}): 3445 vs, b [$\nu_{as}(NH_3)$], 3115 s, b [ν(C-H), Cp], 3002, 2977, 2922 s, b [$\nu(CH_3)$], 1658, 1651 vs [ν_{as}(COO)], 1505 s [ν(C-CH_3)], 1382 s [ν_s(COO)], 1086 m [ν(C-C), Cp], 937 w, 1054 w [δ(C-H)], 852 s [γ(C-H), Cp]. Raman (cm^{-1}): 3115 [ν(C-H), Cp], 3000, 2975, 2940 [$\nu(CH_3)$], 1648 [ν_{as}(COO)], 1505 [ν(C-CH_3)], 1375 [ν_s(COO)], 1077 [ν(C-C), Cp], 1061, 940 [δ(C-H), Cp], 855 [γ(C-H), Cp], 258 (a_1-tilting, Cp′-Ti-Cp′). ^{1}H NMR (ppm): 1.51 (s, C_5H_4-C$\underline{H}_3$, 6H), 1.60 (s, CH_3, 6H), 2.07 (s, CH_3, 6H), 6.37 and 6.63 [2 × m, C_5H_4, 8H, $J^3(H_2, H_3) = J(H_4, H_5) = 5.08$ Hz; $J^4(H_2, H_4) = J(H_3, H_5) = 2.42$ Hz]. ^{13}C NMR (ppm): 27.45, 27.71 (2 × CH_3), 62.30 [$(CH_3)_2\underline{C}$], 121.41, 125.85, 141.30 (C_5H_4), 181.58 (COO). ^{14}N NMR (ppm): −329.68.

Compound 3

DMTDC (1.000 g, 3.61 mmol), *N*-methylglycine (0.643 g, 7.22 mmol) and water (150 µl, 8.33 mmol) were stirred in 10 ml of dry methanol; 1.516 g (92.3%) of a hygroscopic orange solid, which crystallises as a solvate with methanol, were obtained, m.p. >143 °C dec.

Anal. calcd, for $C_{18}H_{28}Cl_2N_2O_4Ti \cdot 1\,CH_3OH$ (M_r = 455.08): C, 46.79; H, 6.61; N, 5.75; Cl, 14.55. Found C, 46.88; H,6.50; N, 5.81; Cl, 14.59. IR (KBr, cm^{-1}): 3440 vs, b [$\nu_{as}(NH_3)$], 3125 s, b [ν(C-H), Cp], 2998, 2968, 2931 s, b [$\nu(CH_3)$], 1665, 1659 vs [ν_{as}(COO)], 1502 vs [ν(C-CH_3)], 1379 m-s [ν_s(COO)], 1082 m [ν(C-C), Cp], 939 w, 1056 w [δ(C-H)], 858, 850 s [γ(C-H), Cp]. Raman (cm^{-1}): 3124 [ν(C-H), Cp], 2990, 2964, 2931 [$\nu(CH_3)$], 1665, 1659 [ν_{as}(COO)], 1507 [ν(C-CH_3)], 1378, 1372 [ν_s(COO)], 1080 [ν(C-C), Cp], 1059, 936 [δ(C-H), Cp], 853, 848 [γ(C-H), Cp], 254 (a_1-tilting, Cp′-Ti-Cp′). ^{1}H NMR (ppm): 2.09 (s, C_5H_4-C$\underline{H}_3$, 6H), 2.76 (s, *N*-CH_3, 6H), 3.19 (C$\underline{H}_3$OH), 3.79 (s, α-CH_2, 4H), 6.40 and 6.75 (2 × m, C_5H_4, 8H, $J^3(H_2, H_3) = J(H_4, H_5) = 4.84$ Hz; $J^4(H_2, H_4) = J(H_3, H_5) = 2.91$ Hz). ^{13}C NMR (ppm): 18.59 (CH_3), 36.81 (*N*-CH_3), 53.91 (CH_2), 121.28, 125.81, 141.30 (C_5H_4), 174.31 (COO). ^{14}N NMR (ppm): −335.68.

Compound 4

DMTDC (1.000 g, 3.61 mmol), L-alanine (0.643 g, 7.22 mmol) and water (150 µl, 8.33 mmol) were stirred in 10 ml of dry methanol; 1.516 g (95.4%) of light orange solid were obtained, m.p. >203 °C dec.

Anal. calcd, for $C_{18}H_{28}Cl_2N_2O_4Ti$ (M_r = 455.08): C, 47.46; H, 6.20; N, 6.15; Cl, 15.58. Found C, 47.48; H, 6.13; N, 6.15; Cl, 15.51. IR (KBr, cm^{-1}): 3440 vs, b [$\nu_{as}(NH_3)$], 3125 s, b [ν(C-H), Cp], 2998, 2968, 2931 s, b [$\nu(CH_3)$], 1665, 1659 vs [ν_{as}(COO)], 1502 vs [ν(C-CH_3)], 1379 m-s [ν_s(COO)], 1082 m [ν(C-C), Cp], 939 w, 1056 w [δ(C-H)], 858, 850 s [γ(C-H), Cp]. Raman (cm^{-1}): 3117 [ν(C-H), Cp], 2978, 2966, 2931 [$\nu(CH_3)$], 1657 [ν_{as}(COO)], 1502 [ν(C-CH_3)], 1381, 1377 [ν_s(COO)], 1079 [ν(C-C), Cp], 1057, 937 [δ(C-H), Cp], 845, 832 [γ(C-H), Cp)], 252 (a_1-tilting, Cp′-Ti-Cp′). ^{1}H NMR (ppm): 1.58 [d, CH_3, 6H J(H, H) = 7.27 Hz], 2.05 (s, C_5H_4-C$\underline{H}_3$, 6H), 4.12 (α-CH), 6.33 and 6.68 [2 × m, C_5H_4, 8H, $J^3(H_2, H_3) = J(H_4, H_5) = 5.57$ Hz; $J^4(H_2, H_4) = J(H_3, H_5) = 2.42$ Hz]. ^{13}C NMR (ppm): 18.79 (CH_3), 19.82 (C_5H_4-$\underline{C}H_3$), 53.79 (CH), 120.70, 125.49, 142.05 (C_5H_4), 178.41 (COO). ^{14}N NMR (ppm): −340.37.

Compound 5

DMTDC (1.000 g, 3.61 mmol), D-alanine (0.643 g, 7.22 mmol) and water (150 µl, 8.33 mmol) were stirred in 10 ml of dry methanol; 1.516 g (90.3%) of light orange solid were obtained, m.p. >200 °C dec.

Anal. calcd, for $C_{18}H_{28}Cl_2N_2O_4Ti$ (M_r = 455.08): C, 47.46; H, 6.20; N, 6.15; Cl, 15.58. Found C, 47.43; H, 6.15; N, 6.15; Cl, 15.56. **IR** (KBr, cm^{-1}): 3440 vs, b [$\nu_{as}(NH_3)$], 3125 s, b [ν(C-H), Cp], 2998, 2968, 2941, s, b [$\nu(CH_3)$], 1665, 1659 νs[ν_{as}(COO)], 1504 s [ν(C-CH_3)], 1378 s [ν_s(COO)], 1080 w-m [ν(C-C), Cp], 939 w, 1056 w [δ(C-H)], 858, 851 s [γ(C-H), Cp]. Raman (cm^{-1}): 3121 [ν(C-H), Cp], 2983, 2974, 2931 [$\nu(CH_3)$], 1663 [ν_{as}(COO)], 1506 [ν(C-CH_3)], 1381 [ν_s(COO)], 1083 [ν(C-C), Cp], 1056, 940 [δ(C-H), Cp], 845 [γ(C-H), Cp)], 253 (a_1-tilting, Cp′-Ti-Cp′). ^{1}H NMR (ppm): 1.49 [d, CH_3, 6H J(H,H) = 7.27 Hz], 2.07 (s, C_5H_4-C$\underline{H}_3$, 6H), 4.11 (α-CH), 6.32 and 6.68 [2 × m, C_5H_4, 8H, $J^3(H_2, H_3) = J(H_4, H_5) = 5.57$ Hz; $J^4(H_2, H_4) = J(H_3, H_5) = 2.90$ Hz]. ^{13}C NMR (ppm): 18.72 (CH_3), 19.77 (C_5H_4-$\underline{C}H_3$), 53.73 (CH), 120.61, 125.45, 142.03 (C_5H_4), 178.39 (COO). ^{14}N NMR (ppm): −340.84.

^{1}H, ^{13}C NMR spectra of DMTDC in D_2O

In a 2 ml Schlenk flask, immersed in an ultrasound bath, was during 10 h dissolved DMTDC (100 mg, 0.36 mmol) in D_2O (600 µl). The pD of the resulting solution (pD = 1.49) was adjusted to pD = 2.94 ± 0.02 (adding small portions of NaOD/D_2O solution) and ^{1}H, ^{13}C NMR spectrum were recorded under the same conditions as in the case of **1–5**.

^{1}H NMR (ppm): 2.10 (s, CH_3, 6H), 6.61 (s, broad, α-H = H_2, H_5, 4H), 6.65 (s, broad, β-H = H_3, H_4, 4H). ^{13}C NMR (ppm): 18.67 (CH_3), 123.42 (C_2, C_5), 124.01 (C_3, C_4), 142.02 (C_1).

Decomposition of DMTDC or complex 1

A weighted quantity of complex **1** (0.500 g, 1.17 mmol) or DMTDC (0.500 g, 1.80 mmol) was dissolved in 5 ml of distilled water in a 10 ml Schlenk flask. The vigorously stirred orange solution was slowly neutralized by dropwise addition of NaOH solution (ca. 0.05 M) during 20–30 min to

pH = 6–7 (on pH paper). After further 30 min of stirring, precipitated material was filtered off, washed with ice-cold water (2 × 5 ml), diethylether (3 × 10 ml) and dried in vacuo to yield 101 mg and/or 156 mg of (yield over ~95% based on Ti) of yellowish polycrystalline solid with m.p. >170 °C (dec.) insoluble in water and in the most of common organic solvents, e.g. alcohols, C_6H_6, toluene, $CHCl_3$, THF, acetone, soluble partially only in DMSO.

Anal. calcd, for Ti $[\eta^5\text{-}C_5H_4(CH_3)]_{0.65}O_{0.3}(OH)_{1.9}$: C, 29.26; H, 4.06; Ti, 54.12; (N, 0.00; Cl, 0.00); found DMTDC: C, 29.54; H, 4.07, Ti, 54.09; (Cl, <0.2); complex **1**: C, 29.52; H, 4.32, Ti, 54.01; (N, 0.3; Cl, <0.3). IR, cm^{-1}: 3419vs, b (fine structure), 3117w-s, 3027sh, 2964m-s, 2926m, 2866w, 2735w, sh, 1635m, b (fine structure), 1558m, 1539m, 1496sh, 1456w-m, 1448m, 1418m, 1394w, 1377m, 1353sh, 1339w, 1261w, 1243w, 1161w, 1072s, 1053s, 1036s, 935vw, 797s,b and 625vs,b (fine structure), 550s,b, 396s, 323sh. Raman, cm^{-1}: 3109, 3089, 3062, 3047, 2955, 2925, 2870, 2737, 1602 (broad, fine structure), 1499, 1449, 1416, 1392, 1375, 1354, 1263, 1241, 1072, 1054, 936, 852, 828, 808, 633, 386, 346, 234 (broad), 150, 122.

RESULTS AND DISCUSSION

During all experiments we have not observed any significant difference between complexes **4** and **5** containing L-Ala and D-Ala optical isomers.

Synthesis

Preparation of DMTDC-A complexes can be described by the following scheme, where α-amino acids are used in their dipolar (zwitterion) form $RCHNH_3^+COO$:

$$Cp'_2TiCl_2 + 2\ AA \longrightarrow [Cp'_2Ti\ (AA)_2]^{2+}[Cl^-]_2 \qquad (1)$$

AA = Gly, 2-Me-Gly, 2-Me-Ala, L-Ala, D-Ala;

MeOH; ≤ 20 °C

The glycine complex was previously prepared by Mokdsi and Harding[6] and presented as a hydrate of the formula $C_{16}H_{24}Cl_2N_2O_4Ti \cdot 1.5H_2O$. In accord with our microanalytical results, the proposed hydrate structure of such a complex does not match that of complex **1**, which was found to contain no water molecules in its structure. This discrepancy could be most likely explained by the fact that we add the exactly calculated amount of water to the reaction mixture (compare with Mokdsi and Hardings), which then could not be built into the molecular structure of **1**. These authors ref.[6] worked with much smaller quantities of materials than we have (about 1/6 of our charge) in 20 ml of methanol containing 1.5 vol% of water. Unfortunately, we have not been able to grow suitable crystals of compound **1** in order to verify this statement by X-ray work. Furthermore, the synthetic route described in their report for glycine complex was found unsuccessful due to inconsistent reaction yields and impure products. According to our experience this variability was closely connected with the final step of preparation (following the literature procedure), employing evaporation of solvent and cooling of the residue overnight.[6]

Further crystallization from methanol was generally unsuccessful. The modified method for compounds **1–5**, outlined in the experimental section, yielded analytically pure products in very good or high yields without any additional purification being necessary. The complexes prepared are air-stabile and not sensitive towards moisture, except complex **3**, which is hygroscopic. All complexes are highly water-soluble, which is understandable assuming that introduction of the ionic α-amino acid fragment into the molecule greatly enhances water solubility. Compounds **1–5** are also soluble in methanol and DMSO, but are not soluble in other organic solvents, e.g. chlorinated solvents, DMF, THF, diethylether, C_6H_6 etc.

We have found a very important role of water during the course of reaction. Preparations performed without the presence of water showed a slowing-down the dissolution process and reaction rate by a factor of more than three to five times, consequently, which fact was analogously observed by Ali and Burgess[19] for nucleophilic substitution of chloride ions for CN^- and SCN^- anions in CH_3CN. Molecules of water can accelerate chloride ion cleavage, during dissolution of DMTDC, which is presumably the rate-limiting step of this reaction followed by fast combination of the resultant intermediate with the α-amino acid zwitterion. The role of water in DMTDC dissolution came out of the mechanism proposed by Toney and Marks[13] for hydrolysis of bent metallocenes, where cleavage of the first chloride ligand has been found to be the rate-limiting step. Even though the concentration of α-amino acids at the first stage of reaction is not very high, due to their limited solubility in methanol, it is sufficient to succesfully allow the reaction to proceed. However, an excess larger than approximately 2 mol of water per 1 mol of DMTDC has no effect on the reaction rate and, on the contrary, makes itself felt in decomposition of either DMTDC or complexes formed, which observation was demonstrated by the change of colour of the reaction mixture to light brown or brown, a decrease in reaction yield of approximately 20–25% and in the case of complexes **4** and **5** also through apparently lower yields and formation of oily mixtures, from which products were solidified only by long term cooling at 0 °C (Table 1).

Vibrational spectroscopy

The vibrational spectra of **1–5** show pronounced shift of carboxyl group stretching modes [ν_{as}(COO), ν_s(COO)] towards higher and lower wave-numbers, respectively, owing to co-ordination of the carboxyl group to the central titanium atom, in comparison with free amino acid ligands; IR cm^{-1}, ν_{as}(COO)-complex: 1679, 1675 **1**, 1658, 1651 **2**, 1665, 1659 **3**, 1657, 1652 **4**, 1657, 1654 **5**; ν_{as}(COO)-amino acid: 1596 (Gly), 1613 (2-Me-Ala), 1626 (*N*-Me-Gly), 1624 (L-Ala), 1626 (D-Ala); ν_s(COO)-complex: 1378, 1373 **1**, 1382 **2**, 1379 **3**, 1368 **4**, 1365 **5**; ν_s(COO)-amino acid: 1369 (Gly), 1412 (2-Me-Ala), 1407

Table 1. Influence of water on the reaction yield

	Water amount (μl/mmol)[a]							
	0/0		150/8.33		300/16.67		500/27.78	
Complex	*t* (h)	Yield (%)	*t* (h)	Yield (%)	*t* (h)	Yield (%)	*t* (h)	Yield (%)
1	26	80.2	5	84.1	5	66.8	5	61.0
2	24	85.1	5	89	5	65.2	5	65.8
3	21	94.2	6	92.3	6	68.5	6	53.2
4	20	91.1	8	95.4	8	70.1	8	36[b]
5	24	87.2	8	90.3	8	70.1	8	45[b]

[a] Water density was based on 1 g cm^{-3}.
[b] Solids obtained from oily solution after 14 day cooling at 0 °C.

(*N*-Me-Gly), 1414 (L-Ala), 1414 (D-Ala).[20,21] Observed values of carboxyl group stretching modes reflect the increase in ketone-like character of the carboxyl group in complexes **1–5**, rather than the delocalized arrangement of the same group found for zwitterion forms of α-amino acids. Non-equivalence of carboxyl groups in the solid state belonging to both of the ligands can be seen as two ν_{as}(COO) stretchings with the same intensity differing in approximately 5 cm^{-1}. The untouched NH_3^+ groups were observed through the very strong band of $\nu(NH_3)$ at 3440 ± 10 cm^{-1} and the so called 'indicator band', which is typically found in IR-spectra of α-amino acids at 2000–2100 cm^{-1}.[22] Shift of the $\nu(NH_3)$ of 20–35 cm^{-1} compared with the zwitterion is affected by the change of counter ion COO^- for Cl^-.

Intensities of all bands in Raman spectra, related to the carboxyl group stretching modes, nicely comply with those found in IR spectra [Raman, cm^{-1}, ν_{as}(COO)-complex: 1678, 1673 **1**, 1648 **2**, 1665, 1659 **3**, 1657 **4**, 1663 **5**; ν_{as} (COO)-amino acids: 1595 (Gly), 1615 (2-Me-Ala), 1626 (*N*-Me-Gly), 1625 (L-Ala), (D-Ala); ν_s(COO)-complex: 1378, 1373 **1**, 1375 **2**, 1378, 1372 **3**, 1381, 1377 **4**, 1381 **5**; ν_s(COO)-amino acid: 1365 (Gly), 1420 (2-Me-Ala), 1405 (*N*-Me-Gly), 1421 (L-Ala), 1418 (D-Ala). The a_1 symmetrical tilting (Cp′-Ti-Cp′) of 255 ± 3 cm^{-1} is indicative of a bent Cp'_2Ti^{2+} metallocene unit and can be used for simple evaluation, whether this unit has remained unchanged or otherwise. All modes related to the metallocene unit did not significantly differ from those of DMTDC and main indicative bands are summarized in Table 2.

NMR spectroscopy

The ^{1}H NMR spectra of compounds **1–5** show typical triplet-like signals in the aromatic region of methylcylopentadienyl rings and a downfield shift of ligand's α-protons, owing to the electronic changes in this centre as a consequence of α-amino acid co-ordination again. Owing to the fast chemical exchange, the NH_3^+ protons could not be observed. The ^{13}C NMR spectra show an up-field shift of carboxylic group ^{13}C resonances and up-field shift of α-carbons' signals opposite to ^{1}H NMR, in comparison with uncoordinated α-amino acids; $\delta_{(COO)}$ ppm, amino acid: 171.44 (Gly), 181.90 (2-Me-Ala), 175.51 (*N*-Me-Gly), 179.85 (L-Ala), 179.84 ppm (D-Ala); $\delta_{(COO)}$ ppm, complex: 175.20 (**1**), 181.58 **2**, 174.31 **3**, 178.41 **4**; 178.39 **5**). The ^{14}N NMR shifts clearly correspond to ammonium group chemical shifts; $\delta_{(complex)}$ ppm: −353.43 **1**, −329.68 **2**, −335.68 **3**, −340.37 **4**, −340.84 **5**; $\delta_{(aminoacid)}$ ppm: −354.10 (Gly), −328.75 (2-Me-Ala), −350.20 (*N*-Me-Gly), −339.74 (L-Ala), −340.03 (D-Ala).[11,23]

X-ray crystallography

The crystal structure of complex **3**, which has been reported in preliminary form,[14] along with the key structural parameters, is discussed and shown in Fig 1 and Table 3. The unit

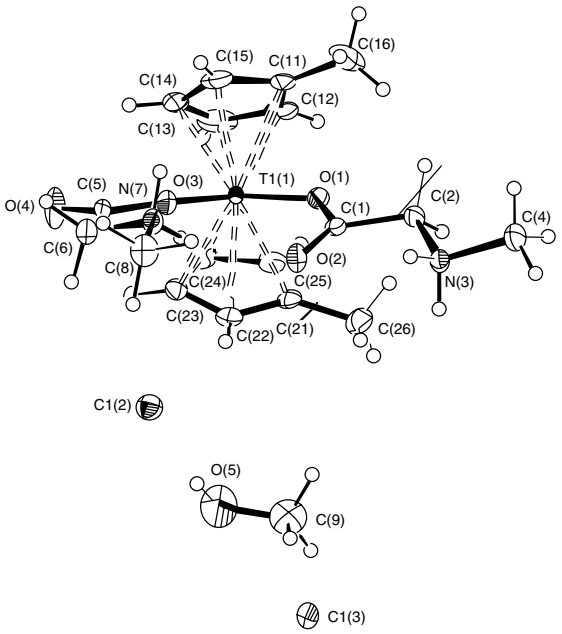

Figure 1. Cationic unit of compound **3**, ORTEP presentation — formula $C_{18}H_{28}N_2O_4Ti \cdot 2(Cl) \cdot CH_4O$; thermal ellipsoids with 40% probability.

Table 2. Vibrational modes of $Cp_2'Ti^{2+}$ — fragment in complexes **1–5**

Vibrational mode	Compound										DMTDC	
	1		2		3		4		5			
	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR	Raman
ν(C-H), Cp′	3112s, b	3115s	3115s, b	3115s	3125s, b	3124s	3125s, b	3117s	3125s, b	3121s	3110s, b	3114s
	3001w,	2993w,	3002w,	3000w,	2998m,	2990w,	2998w,	2978m,	2998w,	2983w,	2984w,	2898m,
	2960w	2958m	2977w	2975w	2968w	2964w	2968w	2966w	2968w	2974w	2965w	2964w
$\nu(CH_3)$	2936s	2936s	2922s	2940s	2931s	2931s	2931s	2931s	2941s	2931s	2925s	2924vs
ν(C-CH_3)	1505vs	1507s	1505s	1505s	1502vs	1507m-s	1502vs	1502m-s	1504s	1506s	1500s	1504s
ν(C-C), Cp′	1259m	1258m	1086m	1077m	1082m	1082w-m	1082m	1079m	1080w-m	1083m	1262m	1255s
δ(C-H), Cp′	1055m-s	1056s	1054s	1061s	1056ws	1059s	1056s	1057m-s	1056s	1056s	1051s	1052vs
γ(C-H), Cp′	860m,	851m,	852s	855s	858m,	853s,	858m,	845m,	858w-m,	845s	870m,	860m,
	853s	849m			850s	848sh	850s	832m	851s		823m	822w
a_1-tilting (Cp′-Ti-Cp′)		254m		258s		254m		252m-s		253m	251m	250m

cell is built up from discrete cationic units, containing co-crystallized solvent molecules that are connected through H···Cl and H···O bonds, which bonding situation is presented in Fig. 1. One can see some degree of distortion of one α-amino acid ligand from the L-Ti-L plain (where L = *N*-CH_3-Gly), arising from steric demands of ring's methyl groups and *N*-methyl groups of α-amino acid ligands (Fig. 1).[14] The C(1)–O(2) and C(5)–O(4) bond lengths compare nicely with the double-bond distances in R_2CO (R = H, CH_3) but the O(1)–C(1) (1.290 Å) and/or O(3)–C(5) (1.293 Å) bonds are significantly shorter than the C–O single-bond distances in alcohols [d (C–O) = 1.42 Å], thus the coordinated carboxyl group resembles C–O bond lengths found typically in esters.[24,25] The titanocene core bond angles of **3** (α = Cg(1)-Ti-Cg(2) and β = L-Ti-L; Cg = ring centroid) are only slightly affected by ligand exchange comparing to the mother metallocene: $\alpha = 133.06°$, $\beta = 95.16°$ (**3**); $\alpha = 130.2°$, $\beta = 93.15°$ (DMTDC).[26] The intra-molecular H(71)–O(2) bond length of 2.01 Å is substantially shorter than the rest of H-bonds observed for **3**. This connection between two α-amino acid ligands represents an unusual example of an intra-molecular H-bond among the known structures of such type of compounds.[5,7,14,23]

Hydrolytic stability

All compounds undergo partial decomposition to species I (Fig. 4) in D_2O even at low pD = 2.90 ± 0.05 (pD values of D_2O-solutions after dissolution of complexes **1–5**), which is clearly seen in the formation of new signals in ^{1}H NMR, in contrast to findings of Mokdsi and Harding,[6] who did not report any changes of DMTDC–Gly complex at pD = 2. New signals arising during 45–60 min in the aromatic region of ^{1}H NMR spectra (2.08ppm, s; two broad singlets at 6.45 and 6.55ppm), were assigned to fragment **I**, i.e. hydrated species $[Cp'_2Ti(D_2O)_2]^{2+}$. The ^{1}H and ^{13}C NMR spectra of species **I** are depicted in Fig. 2.

Additional signals arising in the α-proton region of the ligands as well as in the aromatic region, were assigned to the protonated form of α-amino acid, i.e $RCHNH_3^+COOH$ (regarding results of Jardetzky and Roberts[27]) and to the partially hydrolysed complex $[Cp'_2Ti(A)(D_2O)]^{2+}$, respectively. The whole process reached equilibrium 60–90 min after dissolution and no further changes were observed after 5, 10 and 24 h. Comparing the integral intensities of original and new signals we estimated the original complexes to species **I** ratios after 24 h as 0.33 for all compounds **1–5**. Electronic changes evoked by ligand exchange, appear clearly in the Cp′-rings' region and ring-methyl groups' region of ^{1}H NMR spectra of compounds **1–5**, where the sets of discrete signals are found, whilst due to very slight differences in chemical shifts between α-protons of the mother complexes ($[Cp'_2Ti(A)_2]^{2+}$) and of their hydrolytic products, individual signals cannot be distinguished. This fact was manifested only by appearance of broad signals or not clearly resolved multiplets observed about 0.2 ppm downfield from the ^{1}H-signal of α-protons of protonated α-amino

Table 3. X-ray data of compound **3** with selected bond lengths (Å) and bond angles (deg) (Cg = ring centroid)

Formula	$C_{18}H_{28}N_2O_4Ti \cdot 2(Cl) \cdot CH_4O$	Formula weight	487.27		
Crystal system	centric, orthorombic	Crystal size (mm)	0.15 × 0.20 × 0.35, red		
Space group	*Pbca* no. 61	*a*	9.5310(3) Å		
b	18.2980(5) Å	*c*	26.6350(5) Å		
Z	8	*V* (Å^3)	4645.1(2)		
μ(MoKa) (mm^{-1})	0.630	Diffractometer	Nonius Kappa CCD		
F (000)	2048.0	θ_{max}	27.5°		
D_{calc} (g/cm^3)	1.3934	*T* (K)	150		
Reflections measured (total)	36780	Reflections unique, R_{int}	5307; 0.024		
Reflections with $I > 2\sigma(I)$	4323	*R* (all data)	0.0353		
ωR (all data)	0.08	ρ_{max} (e Å^{-3})	0.37		
Weighting scheme	$\omega = 1/[\s^2^(Fo^2^) + (0.0392P)^2^ + 2.4268P]$ [where $P = (Fo^2^ + 2Fc^2^)/3$]				
Programs used	audit creation method: SHELXL-97, PLATON for Windows and ORTEP III				
Selected bond lengths [Å] and bond angles (deg)					
Ti–O1	1.9792	C11–C12	1.405	Cg(1)–Ti–Cg(2)	133.06
Ti–O3	1.9431	C11–C15	1.387	Cg(1)–Ti–O(1)	103.46
O1–C1	1.2901	C12–C13	1.374	Cg(1)–Ti–O(3)	106.47
C1–O2	1.222	C13–C14	1.364	Cg(2)–Ti–O(1)	106.65
O3–C5	1.293	C14–C15	1.412	Cg(2)–Ti–O(3)	105.75
C5–O4	1.216	C–C(mean)	1.3907	O1–Ti–O3	95.15
C2–N3	1.480	C11–C16	1.495	Ti–O1–C1	137.22
C4–N3	1.489	C21–C25	1.415	Ti–O3–C5	147.29
N7–C8	1.483	C21–C22	1.402	O2–C1–O1	109.37
N7–C6	1.481	C22–C23	1.411	O3–C5–O4	113.86
Ti–Cp1	2.0564	C23–C24	1.389	C2–N2–C4	125.78
Ti–Cp2	2.0544	C24–C25	1.410	C6–N7–C8	126.83
Ti–Cg(1)	2.0537	C–C(mean)	1.4054		
Ti–Cg(2)	2.0534	C21–C26	1.495		
Hydrogen bonds					
H31...C13	2.220	H71...O2	2.01	H5...C12	2.40
H32...C13	2.344	H72...C13	2.20		

acids. A similar situation was also observed for other ^{1}H-signals of the ligands. The ^{13}C signals of appropriate carbons and their changes in chemical shift are in opposite, up-field directions, which is in accord with literature data.[28] Typical spectra, with explanation of signals, are shown in Figs 3 and Fig. 4.

Further increase of the solution pD above the value of pD = 4.9(pH = 4.5) resulted in very fast formation of insoluble yellowish precipitates and decomposition of the complexes **1–5**. The first evidence of the forming precipitate was observed at pD = 4.00(pH = 3.6). No signals of Cp′-rings, only signals due to protonated ligands, were observed in the ^{1}H NMR spectra on increasing pD up to 6.5–7.0. In order to characterize the precipitate formed, we have prepared the decomposition products of compound **1** and DMTDC for comparison under the same conditions (see Experimental). The same type of product was obtained starting from DMTDC and/or complex **1** and it was characterized by elemental analysis and IR and Raman spectroscopy (Fig. 5).

Regarding the microanalytical results which meet the formula $Ti[\eta^5\text{-}C_5H_4(CH_3)]_{0.65}O_{0.3}(OH)_{1.9}$, and IR and Raman spectra [broad IR signals at 3419[ν(OH)], 1630, strong broad bands at 800, 625 ± 5 cm^{-1}[ν(Ti-O)],[29–31] missing Raman signals at 254 cm^{-1} (a_1-tilting)] and 'fine structure' of the bands at 800 and 625 cm^{-1}, which points out the presence of oligomeric and/or polymeric units, this compound was attributed to posses an oligomeric and/or polymeric structure, e.g. $[Ti(Cp')_x(O)_y(OH)_z]_n$, containing only one η^5-bonded ring. The proposed structure corresponds well with results published by Toney and Marks[13] concerning behaviour of bent metallocenes of Ti, Zr, V and Mo in aqueous media and Carraher *et al.*,[32] reporting synthesis of TDC-polymers with α-amino acids and/or di-peptides in DMSO. Mokdsi and Harding[6] also observed similar features, e.g. formation of

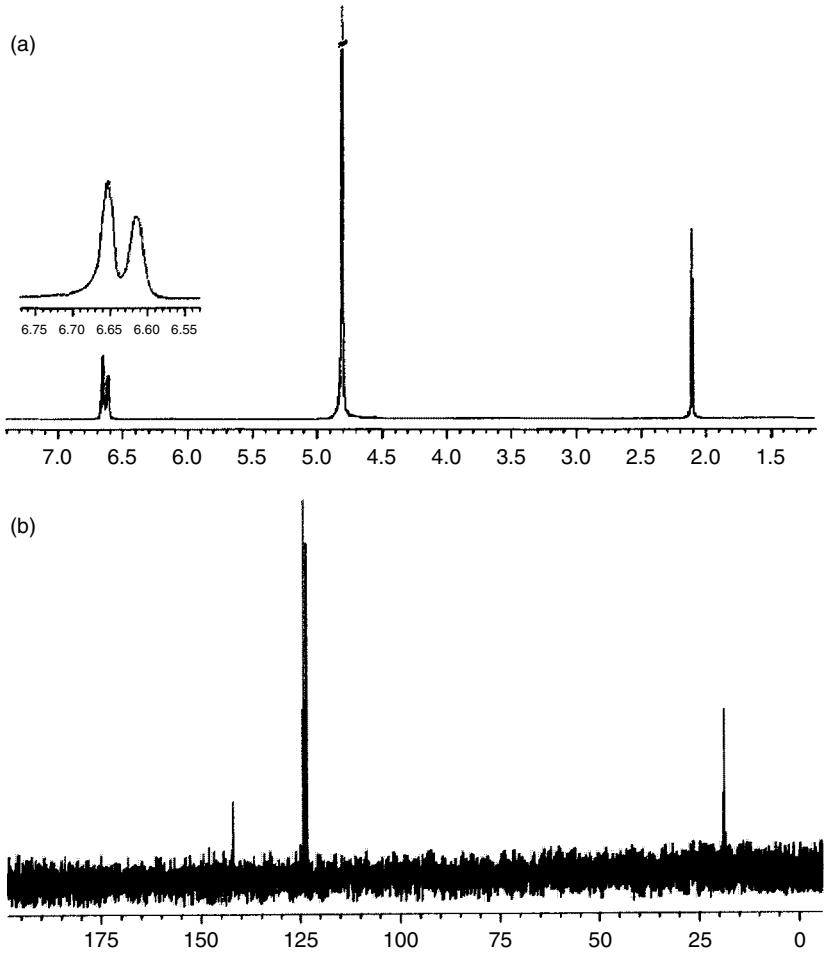

Figure 2. The ^{1}H (a) and ^{13}C (b) NMR spectra of $Cp'_2Ti(D_2O)_2^{2+}$ at pD = 2.91 at 293 K; 100 mg of DMTDC in 600 µl of D_2O. ^{1}H 2.10 (CH_3), 6.61 (s, broad, α-H = H_2, H_5, 4H), 6.65 ppm (s, broad, β-H = H_3, H_4, 4H); ^{13}C 18.67 (CH_3), 123.42 (C_2, C_5), 124.01 (C_3, C_4), 142.02 ppm (C_1).

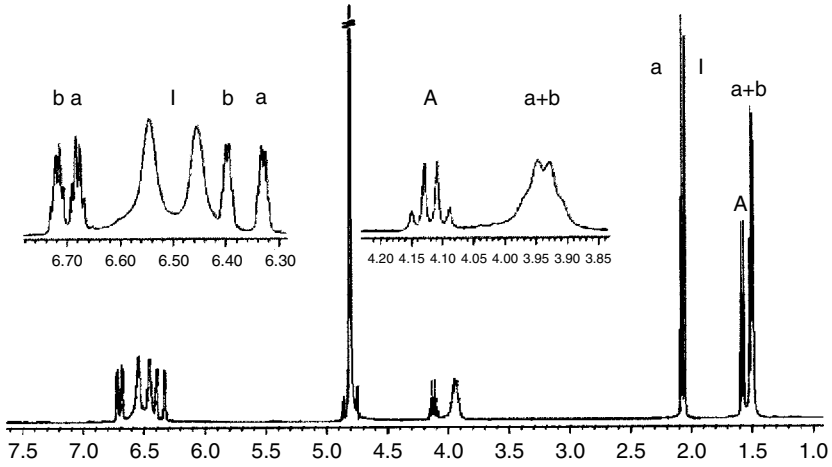

Figure 3. The ^{1}H NMR spectrum of complex **4** after 24 h in D_2O at pD = 2.91, 100 mg/600 µl D_2O; **a**, mother compound $[Cp'_2Ti(L\text{-}Ala)_2]^{2+}$: 1.50, 2.05, 3.94, 6.33 and 6.68 ppm; **b**, hydrated compound $Cp'_2Ti(L\text{-}Ala)(D_2O)^{2+}$: 1.50, 3.94, 6.39 and 6.72 ppm; species **I**, $Cp'_2Ti(D_2O)_2^{2+}$: 2.08 ppm and two broad singlets at 6.45 and 6.55ppm; **A**, free L-Ala: 1.58 (CH_3), 4.12 ($C_\alpha H$) ppm.

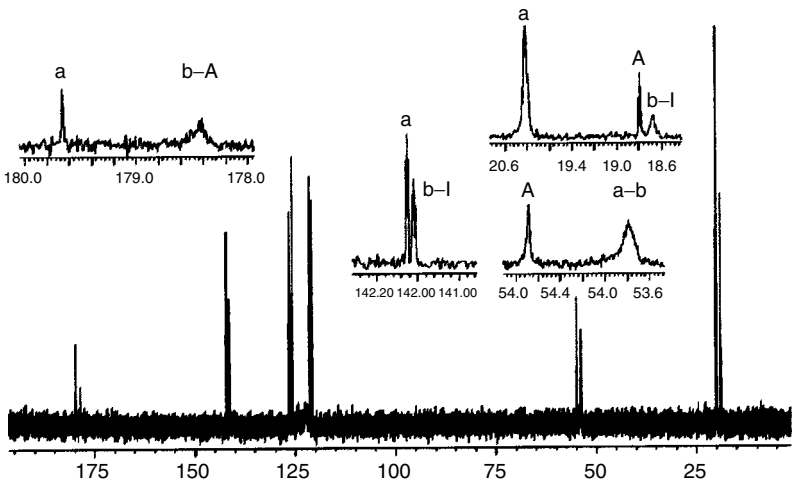

Figure 4. The ^{13}C NMR spectrum of complex 4 after 24 h in D_2O at pD = 2.91, 100 mg/600 µl D_2O; **a**, mother compound $[Cp'_2Ti(L\text{-}Ala)_2]^{2-}$; **b**, hydrated compound $Cp'_2Ti(L\text{-}Ala)(D_2O)^{2+}$; species **I**, $Cp'_2Ti(D_2O)_2^{2+}$; **A**, free L-Ala.

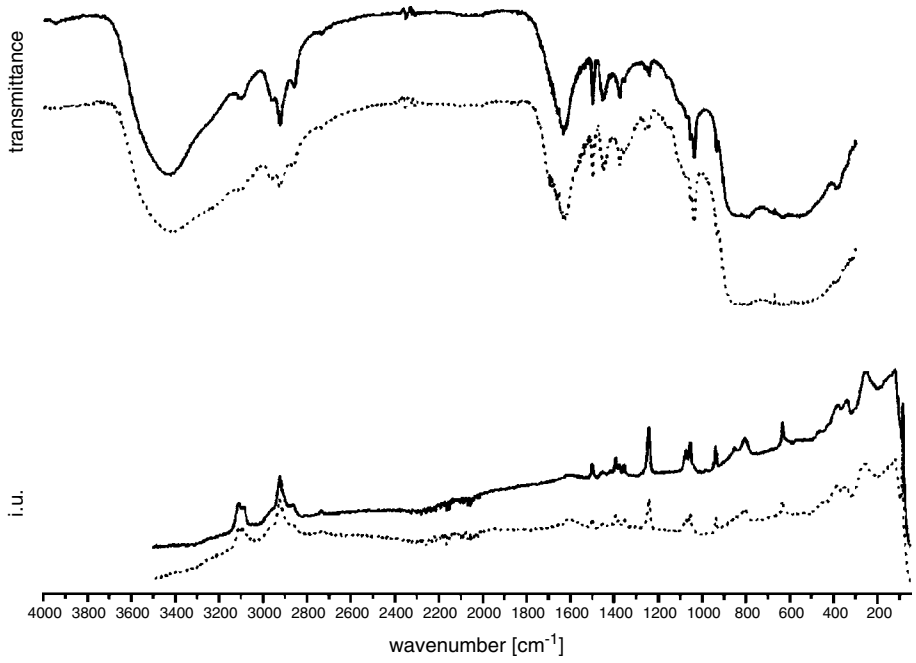

Figure 5. IR (4000–300 cm^{-1}) and Raman (3500–50 cm^{-1}) spectra of the decomposition product of DMTDC (solid line) and complex **1** (dotted line).

yellow precipitate, but did not characterize the solid formed or calculate the degree of aromatic rings hydrolysis at elevated pD. Over 21 and 40% of the DMTDC–Gly complex precipitated after 15 min, and after 24 h it was over 56 and 88% at pD = 5.7–6.0 and 7.5–7.8, respectively.[6] The percentage of ring hydrolysis at physiological pD was estimated from the ratio of Cp′ protons to glycine CH_2 protons to <5%, despite more then 90% of the original complex precipitating. Composition of aqueous solutions and no ^1H NMR informative spectra were given and/or studied.[8]

Moksdi and Harding did not observe any decomposition and/or considerable ring hydrolysis during 24 h (less then 2–5%, based on ring signals, were estimated). In our experience decomposition of DMTDC and DMTDC–Gly complex (**1**) starts rapidly at pD ≈ 4.0, and at pD approaching 7.0 both are completely transformed into insoluble yellowish species. Thus any ^1H NMR-based estimation of percentage of ring hydrolysis failed (pD ≈ 4.5, very small signal of Cp′-rings was found which could not be accurately integrated; pD ≈ 7.0, no signal of Cp′-rings was found).

CONCLUSIONS

A series of five DMTDC α-amino acid complexes was synthesized at high yield (80–95%) and the structural situation in the solid state elucidated by means of several methods. The general bonding pattern for **1–5** was clearly and unambiguously stated from IR, Raman and X-ray crystallography results, in contrast to the situation in water solution of **1–5**. Hydrolytic stability of **1–5** in D_2O was studied by ^1H NMR spectroscopy and results obtained were compared with earlier work.[6] All compounds lost α-amino acid ligands during 60 min at pD 2.9 to form a mixture of the appropriate complex $[Cp'_2Ti(A)_2]^{2+}$ the partially hydrated species $[Cp'_2Ti(A)(D_2O)]^{2+}$ the ionized α-amino acid and hydrated metallocene $[Cp'_2Ti(D_2O)_2]^{2+}$. Rapid decomposition of the bent metallocene unit of **1–5** occurred at pD > 4.9(pH > 4.5) accompanied by complete destruction of their structures to yield yellowish insoluble solids possessing oligomer and/or polymer structures, which was even more evident at pD 7.0, when nearly 100% of starting material precipitated. The mother DMTDC complex followed a similar hydrolytic pattern as compounds **1–5**, yielding the same (or a very similar) product, as in the case of complex **1**, which was demonstrated in separate experiments [compounds match the formula $Ti[\eta^5\text{-}C_5H_4(CH_3)]_{0.65}O_{0.3}(OH)_{1.9}$]. Thus, the Cp′-rings stay metal bound predominantly at low pDs from 2.9 to ca. 4.0 (pH 2.5–3.5). In the light of results presented, it is obvious that increased hydrolytic stability of DMTDC under physiological conditions, and also its α-amino acid complexes, is an issue, that is at least very optimistic and/or the results published by Mokdski and Harding[6] were not accurately interpreted.

Supplementary material

Crystallographic data of compound **3** has been deposited with the Cambridge Crystallographic Data Centre: CCDC 205 636. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk or www:www.ccdc.cam.ac.uk).

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