Metal Complexes of Biologically Important Ligands, 105[◇]

Catalytic Formation of Oligopeptides from α-Amino Acid Esters with (*p*-Cymene)ruthenium(II) Complexes☆

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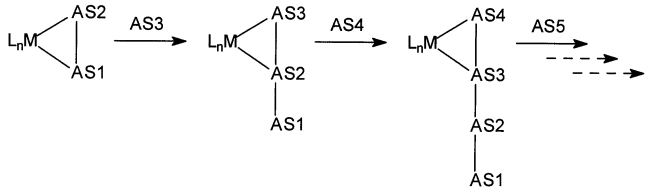
Consecutive addition of one-equivalent portions of glycine ethyl ester to [(*p*-cymene)Ru(GGGOMe−H^+)Cl] leads to considerable amounts of (tetra- to nonapeptide)ruthenium complexes in a one-pot reaction, in which the (*p*-cymene)RuCl fragment acts as a catalyst. The analogous reaction with alanine methyl ester affords AGGG and AAGGG complexes as the main products. The course of these metal-catalyzed peptide oligomerizations has been followed by mass spectrometry. The synthesis and characterization of the pentapeptide complex [(C_6Me_6)Ru(GGGGGOMe−H^+)] is reported.

Introduction

There have been several reports on the formation of peptides from α-amino acid esters[2] or α-amino acids[3] in the presence of metal ions or metal complexes. Recently, our group reported a metal-promoted peptide synthesis in which non-activated α-amino acid esters are assembled in the coordination spheres of half-sandwich complexes of ruthenium, rhodium, and iridium.[4] This method permits the elongation of a coordinated peptide ester at its *N*-terminus with a defined amino acid sequence. In previous studies[4] we focused on stoichiometric reactions between peptide complexes and amino acid esters. However, according to the proposed mechanism, it should be possible to produce polypeptides using only catalytic amounts of these organometallic complexes (Scheme 1).

Scheme 1



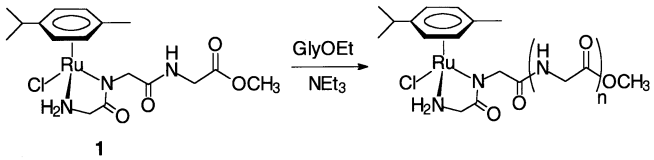
Therefore, we became interested in ascertaining whether longer peptide chains could be obtained by repetitive additions of α-amino acid esters to half-sandwich (peptide)metal complexes. In this paper, we report on the formation of tetra- to nonapeptides by means of a one-pot reaction of [(*p*-cymene)Ru(GGGOMe−H^+)Cl] (**1**) with glycine or alanine esters according to Scheme 1.

[◇] Part 104: Ref.[1].

Results and Discussion

Repetitive additions (every 24 h) of one equivalent of glycine ethyl ester (GlyOEt) to [(*p*-cymene)Ru(GGGOMe−H^+)Cl] (**1**) in the presence of triethylamine gave ruthenium complexes with increased chain length of the peptide (Scheme 2). The reaction resembles living polymerization processes.[5] A large excess of α-amino acid ester was avoided in order to reduce autocondensation reactions.

Scheme 2



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The course of the metal-catalyzed peptide oligomerization was followed by FAB mass spectrometry. This analytical tool allows the simultaneous determination of the various intact (peptide)Ru^{II} complexes that are present in the reaction mixture.[6] The relative amount of each peptide complex was estimated by comparing the appropriate peak heights. Table 1 and Figure 1A show how the distribution of the higher peptides changes as the complex is "fed" with successive one-equivalent portions of glycine ester. Significant amounts of hepta- to nonapeptides are obtained after the addition of 8 equivalents of GlyOEt. The time course of product formation reflects the statistical growth of the peptide chain. The limiting factor of this reaction was the increased viscosity of the solution after the addition of several portions of the amino acid. It should be emphasized, however, that the presence of the lipophilic (*p*-cymene)Ru^{II} fragment strongly increases the solubility of the otherwise

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notoriously insoluble polyglycine peptides in organic solvents.

Figure 1. Catalytic formation of polypeptide complexes by consecutive addition (every 24 h) of glycine ethyl ester (A) or alanine methyl ester (B)

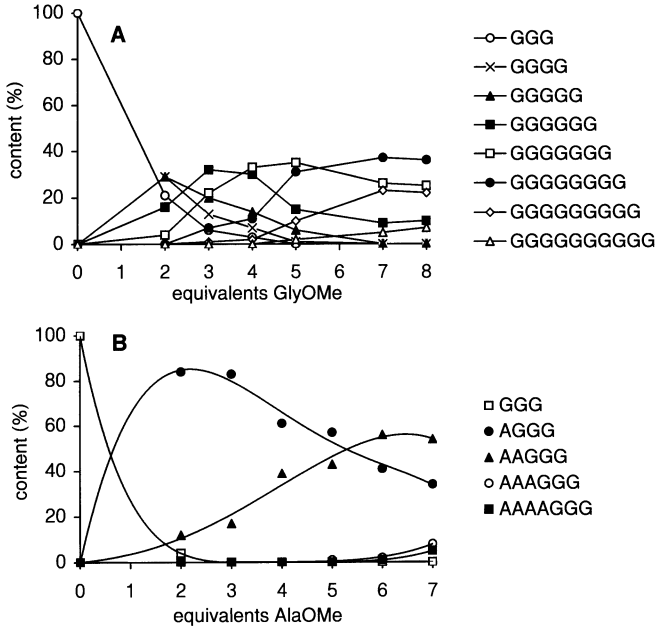
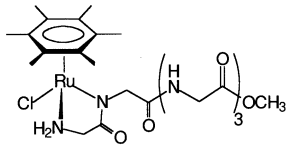


Table 1. Formation of (oligoglycine)ruthenium complexes following successive additions of one-equivalent portions of glycine ethyl ester

Peptide	Start	Portion no. 2	3	4	5	7	8
triglycine	100	21	6	3	0	0	0
tetraglycine	0	29	13	7	1	0	0
pentaglycine	0	29	20	14	6	0	0
hexaglycine	0	17	32	30	15	9	10
heptaglycine	0	4	22	33	35	26	25
octaglycine	0	0	7	11	31	37	26
nonaglycine	0	0	1	2	10	23	22
decaglycine	0	0	0	0	2	5	7

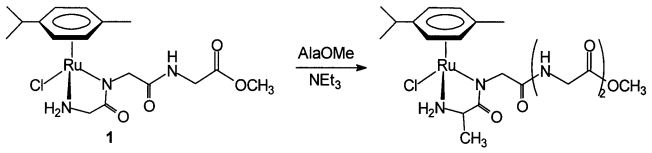
For comparison, a (pentaglycine methyl ester)ruthenium complex **2** was prepared directly from $[(C_6Me_6)RuCl_2]_2$ and GGGGGOMe. *N*,*N*-coordination in **2** is proven by the IR absorptions at 1660 and 1579 cm^{-1}.[4]



2

While with glycine ethyl ester and **1** the relative amount of any single product does not exceed 40% because of similar reactivities of all the products, with alanine methyl ester (AlaOMe) and **1** a different situation is encountered. Scheme 3 illustrates the reaction of **1** with one equivalent of AlaOMe. The larger steric bulk of this amino acid seems to prevent it from undergoing successive condensation reactions. Therefore, a substantial build-up of the AGGG complex (up to 84%) and the AAGGG complex (up to 56%) is observed (Figure 1B). Only minor amounts of peptide complexes with more than two alanine residues are produced, even after the addition of 7 equivalents of AlaOMe.

Scheme 3



The experiments described herein demonstrate that (*p*-cymene)Ru^{II} complexes are able to catalyze the formation of oligopeptides from simple amino acid esters. We are currently investigating whether this strategy can be applied to generate oligopeptides with a different amino acid content and/or a longer chain length. In certain cases, this new synthetic approach might be an interesting alternative to conventional methods of peptide synthesis.

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Experimental Section

Reactions were carried out in Schlenk tubes under N_2 or Ar. Methanol (puriss., < 0.05% H_2O) was saturated with N_2 and stored over molecular sieves (3 Å). $[(p\text{-cymene})RuCl_2]_2$ and $[(C_6Me_6)RuCl_2]_2$ were prepared according to literature procedures.[7] The free amino acid esters were prepared from the hydrochlorides by treatment with NEt_3 in dichloromethane. NEt_3·HCl precipitated upon addition of pentane. − IR: Perkin-Elmer 841, Nicolet 520; PE = polyethylene. − NMR: JEOL EX 400. − MS: Finnigan MAT 90 mass spectrometer.

[(p-Cymene)Ru(GGGOMe−H⁺)Cl] (**1**): A solution of 288 mg (1.2 mmol) of GGGOMe·HCl and 1.2 mmol of NaOMe in 10 ml of methanol was added to a suspension of 367 mg (0.6 mmol) of $[(p\text{-cymene})RuCl_2]_2$ in 20 ml of methanol. After stirring for 2 h, the mixture was concentrated in vacuo to a volume of 2 ml. NaCl precipitated upon the addition of 10 ml of dichloromethane and 3 ml of pentane and was separated by centrifugation. The solvent was then removed in vacuo. After the addition of pentane, an oily red compound was obtained. The product was purified by recrystallization from methanol/diethyl ether. Yellow powder. Yield 543 mg (94%). − M.p. 203−206°C (decomposition). − IR (KBr): $\tilde{\nu}$ = 3333 cm^{-1} (m), 3275 (m, sh), 3128 (m, NH), 1748 (s, C=O, ester), 1665 (s, amide I, uncoord.), 1583 (vs, amide I, coord.), 1535 (m, amide II). − IR (PE): $\tilde{\nu}$ = 286 cm^{-1} (w), 253 (m, RuCl). − ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ = 1.27 [d, ^3J = 7 Hz, 6 H, $CH(CH_3)_2$], 2.21 (s, 3 H, $ArCH_3$), 2.83 [sept, ^3J = 7 Hz, 1 H, $CH(CH_3)_2$], 3.15 (m, 2 H, NH_2CH_2), 3.76 (s, 3 H, OCH_3), 3.94 (m, 2 H, CH_2CO_2), 4.11 (d, ^2J = 17 Hz, 1 H, NCH_2CO), 4.78 (d, ^2J = 16 Hz, 1 H, NCH_2CO), 5.36−5.58 (m, 4 H, C_6H_4). − ^{13}C NMR (68 MHz, $CDCl_3/CD_3OD$): δ = 18.63 ($ArCH_3$), 22.00, 23.10 [$CH(CH_3)_2$], 31.13 [$CH(CH_3)_2$], 40.94 (CH_2CO_2), 46.76 (NH_2CH_2), 52.54 (OCH_3), 54.74 (NCH_2CONH), 80.83, 81.95, 82.10, 82.47, 96.46, 101.76 (C_6H_4), 170.71 (CO_2Me), 174.45, 180.36 (CON). − $C_{17}H_{26}ClN_3O_4Ru \cdot 1/2\ H_2O$ (481.9): calcd. C 42.37, H 5.65, N 8.72; found C 42.56, H 5.87, N 8.55.

[(C$_6$Me$_6$)Ru(GGGGGOMe−H$^+$)Cl] (**2**): 311 µl (0.19 mmol) of NaOMe/MeOH (1.19 M) was added dropwise to a suspension of 65 mg (0.19 mmol) of GGGGGOMe·HCl and 62 mg (0.09 mmol) of $[(C_6Me_6)RuCl_2]_2$ in 15 ml of methanol. After 1 h, a small amount of a precipitate was separated by centrifugation and the solvent was removed in vacuo. The residue was treated with 5 ml of dichloromethane, 1 ml of methanol, and 2.5 ml of pentane and NaCl was removed by centrifugation. The product precipitated upon addition of 30 ml of pentane and was purified by recrystallization from methanol/dichloromethane (2:1)/diethyl ether. Orange crystals. Yield 40 mg (35%). − M.p. 198°C (decomposition). − IR (KBr): $\tilde{\nu}$ = 3289 cm^{-1} (s, NH), 3243 (sh, NH), 1758 (m, C=O, ester), 1743 (m, C=O, ester), 1691 (sh, amide I, uncoord.), 1660 (vs, amide I, uncoord.), 1579 (vs, amide I, coord.). − ^{1}H NMR (400 MHz, CD_3OD): δ = 2.13 (s, 18 H, $ArCH_3$), 3.14 (s, 2 H, NH_2CH_2), 3.31 (CH_2 together with CD_2HOD), 3.37 (s, 2 H, CH_2), 3.73 (s, 3 H, OCH_3), 3.89 (br. s, 2 H, CH_2), 3.95 (s, 2 H, CH_2). − $C_{23}H_{36}ClN_5O_6Ru\cdot H_2O$ (633.1): calcd. C 43.63, H 6.05, N 11.06; found C 44.01, H 6.24, N 11.02.

Peptide Synthesis: 213 mg (0.45 mmol) of $[(p\text{-cymene})RuCl_2]_2$ was dissolved in 20 ml of methanol and 347 µl (2.5 mmol) of NEt_3 was added as co-catalyst. Once per day one portion of 57 µl (0.55 mmol) of GlyOEt was added to the solution. Just before adding the third portion, a sample of 1.9 ml (first data point) was removed from the reaction vessel. Peptide complexes were isolated after precipitation with 30 ml of diethyl ether and were analyzed by mass spectrometry. The reaction volume was kept constant by adding 2 ml of methanol. Subsequent data points were obtained similarly. As the amount of complex in the solution decreased, the amount of GlyOEt added was decreased accordingly. After addition of the seventh portion, the viscosity increased and magnetic stirring became problematic. The solution showed thixotropic properties. − Peptide synthesis with AlaOMe was performed analogously using 152 mg (0.32 mmol) of $[(p\text{-cymene})RuCl_2]_2$ and 250 µl of NEt_3 dissolved in 20 ml of methanol.

MS Analysis: FAB-MS measurements were performed using *m*-NBA as the matrix. For each peptide complex present, three to four well-separated peak groups can be identified, corresponding to [M − Cl$^-$], [M − Cl$^-$ + H$^+$ + Na$^+$], [M + H$^+$], and [M + Na$^+$]. Ionization occurs without decomposition of the complex. Semi-quantitative analyses were performed by comparing the sums of the relevant peak heights.

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