Synthesis and Structural Characterization of η^6-Arene–Ruthenium(II) Complexes of Alanine and Guanine Derivatives

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Abstract

The η^6-arene–ruthenium(II) complexes of L-alanine (L-alaH) and L-alanine methyl ester (L-alaMe), $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}ala)Cl]$ (**1**) and $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}alaMe)Cl_2]$ (**2**) have been prepared and their structures established by X-ray structural analysis. The crystal lattice of **1** contains two diastereomers with opposite chiralities at the metal centre. Epimerization of these species is relatively slow in aqueous solution. Reaction of **1** with 9-ethylguanine (9Etgua) yields $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}ala)(9\text{-}Etgua)]Cl$ (**3**) for which two diastereomers are observed in solution and in the solid state. In contrast L-alanine methyl ester in **2** may be replaced by 9-ethylguanine leading to the formation of $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)Cl_2]$ (**4**). N7 coordination of the nucleobase is exhibited by both **3** and **4**.

Introduction

Interest in the antitumour properties of ruthenium(II) complexes has concentrated mainly on *cis*-$[RuCl_2(DMSO)_4]$ [1]. Upon application of equitoxic dosages, this compound displays an activity towards Lewis lung carcinoma similar to that of cisplatin *cis*-$[PtCl_2(NH_3)_2]$, both towards primary tumor growth and metastases formation [2]. In the case of two further solid mouse tumours, B16 melanoma and MCa mammary carcinoma, the ruthenium(II) derivative exhibits an activity greater than cisplatin. Furthermore *cis*-$[RuCl_2(DMSO)_4]$ clearly displays a better therapeutic index in comparison to cisplatin for these tumours [3, 4]. It has been suggested by Clarke [5] that active ammine–ruthenium(III) complexes such as *fac*-$[RuCl_3(NH_3)]_3$ [5] or ImH *trans*-$[RuCl_4Im)_2]$ [6] will be reduced to more labile ruthenium(II) derivatives before interacting with cellular components, so that the former may be regarded as prodrugs. On the basis of this reasoning, selective attack on solid hypoxic tumours could be feasible, as ruthenium(III) would be more readily reduced inside the tumour than in normal tissues.

In view of the documented activity of *cis*-$[RuCl_2(DMSO)_4]$ it appeared to be of interest to study the antitumour properties of other ruthenium(II) complexes. Studies of the oncological properties of organoruthenium(II) derivatives are effectively restricted to ruthenocene [5], for which the stability of the organometallic bonds prevents metal binding to bioligands. In contrast, complexes of the type $[(\eta^6\text{-}C_6H_6)Ru(aa)Cl]$ (aa = amino acidate ligand), which have previously been prepared [7] for glycine and D,L-alanine by their reaction with dimeric $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$, contain a labile Ru–Cl bond and should be capable of binding DNA. In the present paper we report the preparation and structural characterization of $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}ala)Cl]$ (**1**) and $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}alaMe)Cl_2]$ (**2**) (L-alaMe = L-alanine methyl ester). As a model investigation for their interaction with nucleic acids, we have studied their reaction with 9-ethylguanine (9Etgua). Whereas the mixed amino acid/nucleobase complex $[(\eta^6\text{-}C_6H_6)Ru(L\text{-}ala)(9Etgua)]Cl$ (**3**) may be synthesized from **1**, reaction of **2** with 9-ethylguanine leads to replacement of L-alanine methyl ester by the nucleobase and the formation of $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)Cl_2]$ (**4**). This complex may also be prepared by direct reaction of $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ with 9-ethylguanine. X-ray structural analyses are presented for **1**–**4**.

Experimental

IR spectra were recorded as 1% KBr discs on a Perkin-Elmer 297 spectrometer. ^1H NMR spectra were recorded on a Bruker AM400 spectrometer in D_2O with $(CH_3)_3SiCD_2CD_2COONa$ as internal reference. Elemental analyses were performed with a Perkin-Elmer 240 instrument. L-Alanine (alaH), L-alanine methyl ester hydrochloride (alaMe·HCl) and 9-ethylguanine (9Etgua) were purchased from Sigma Chemie GmbH and used as received; $RuCl_3 \cdot 3H_2O$ was a gift from Degussa AG. $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ was prepared as described in the literature [8].

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Preparation of Complexes 1–4

[(η^6-C_6H_6)Ru(L-ala)Cl] (1)

One hundred mg (0.2 mmol) of $[(\eta^6\text{-}C_6H_6RuCl_2]_2$ were dissolved with heating in 20 ml of water and the solution filtered. Thirty-six mg (0.4 mmol) of L-alanine were added to the solution, which was reduced in volume to 10 ml and cooled to 6 °C to yield orange crystals of **1** (yield 86%). *Anal.* Found: C, 35.5; H, 3.99; N, 4.60. Calc. for $C_9H_{12}NO_2ClRu$ (M = 302.7): C, 35.71; H, 3.99; N, 4.63%. IR: 3290, 3240 $\nu(NH_2)$, 1625 $\nu(CO)$ cm^{-1}. ^1H NMR (D_2O): 1.17, 1.26, 1.27, 1.31, 1.34, 1.36 (6d, 3H, ala CH_3), 2.86, 3.16, 3.18, 3.45, 3.52, 3.53 (6q, 1H, ala CH), 5.79, 5.82, 5.90, 5.92 (4s, 6H, C_6H_6).

[(η^6-C_6H_6)Ru(L-alaMe)Cl_2] (2)

A total of 0.4 ml 1 M NaOMe solution and 100 mg (0.2 mmol) of $[(\eta^6\text{-}C_6H_6)RuCl_2]_2$ were added to a solution of 56 mg (0.4 mmol) of L-alanine methyl ester hydrochloride. After stirring for 12 h at room temperature (r.t.) the orange precipitate was filtered off and recrystallized from a methanol/water solution (**2**, yield 91%). *Anal.* Found: C, 33.9; H, 4.34; N, 3.90. Calc. for $C_{10}N_{15}NO_2Cl_2Ru$ (M = 353.2): C, 34.01; H, 4.28; N, 3.97%. IR: 3280, 3200 $\nu(NH_2)$, 1725 $\nu(CO)$, 1585 $\delta(NH_2)$ cm^{-1}. ^1H NMR (D_2O): ester hydrolysis occurs leading to the formation of **1** and methanol.

[(η^6-C_6H_6)Ru(L-ala)(9Etgua)]Cl·$2H_2O$ (3)

Fifty-six mg of 9-ethylguanine were added to a solution of 100 mg of **1** (0.3 mmol) in 10 ml H_2O. After stirring for 1 h at r.t. the solution was reduced in volume to 2 ml at 60 °C. Yellow crystals of **3** were obtained upon cooling to r.t. (yield 66%). *Anal.* Found: C, 37.6; H, 4.46; N, 16.3. Calc. for $C_{16}H_{21}N_6O_3ClRu\cdot 2H_2O$ (M = 517.9): C, 37.11; H, 4.87; N, 16.23%. ^1H NMR (D_2O): diastereomer **3a**, 1.29 (d, 3H, ala CH_3), 1.44 (t, 3H, 9Etgua CH_3), 2.45 (q, 1H, ala CH), 4.16 (q, 2H, 9Etgua CH_2), 5.86 (s, 6H, C_6H_6), 7.99 (s, 1H, 9Etgua H8); diastereomer **3b**, 0.82, 0.82 (2d, 3H, ala CH_3), 1.45 (t, 3H, 9Etgua CH_3), 3.56, 3.57 (2q, 1H, ala CH), 4.17 (q, 2H, 9Etgua CH_2), 5.85 (s, 6H, C_6H_6), 8.00 (s, 1H, 9Etgua H8).

[(η^6-C_6H_6)Ru(9Etgua)Cl_2] (4)

Method 1: 36 mg (0.2 mmol) of 9-ethylguanine were added to an aqueous solution (20 ml) of 71 mg (0.2 mmol) of **2** and stirred at r.t. for 30 min. The orange solution was reduced in volume to 3 ml and cooled to 0 °C to precipitate orange crystals of **4** (yield 69%).

Method 2: 100 mg (0.2 mmol) of $[(\eta^6\text{-}C_6H_6)\text{-}RuCl_2]_2$ were dissolved with heating in 20 ml of water and the solution filtered. Seventy two mg (0.4 mmol) of 9-ethylguanine were added and the volume reduced to 1 ml. After addition of 5 ml of methanol, the solution was cooled to 0 °C to precipitate orange crystals of **4** (yield 64%). *Anal.* Found: C, 36.2; H, 3.56; N, 16.1. Calc. for $C_{13}H_{15}N_5OCl_2Ru$ (M = 429.3): C, 36.38; H, 3.52; N, 16.31%. IR: 3380, 1700, 1620 cm^{-1}. ^1H NMR (D_2O): 1.46 (3t, 3H, 9Etgua CH_3), 4.17 (3q, 2H, 9Etgua CH_2), 5.94, 6.05, 6.10 (s, C_6H_6), 8.10, 8.20, 8.38 (s, 9Etgua H8).

X-ray Structural Analyses of 1–4

Suitable crystals of **1** and **3** were obtained from aqueous solution; for **2** and **4** methanol/water was used. The crystal lattice of **3** contains two water molecules for each diastereomer, that of **4** three water molecules in the asymmetric unit. Whereas these solvent molecules are retained by **3** upon drying, they are lost by **4**, as demonstrated by the elemental analysis. Crystal and refinement data for **1**–**4** are summarized in Table 1. Unit cell constants were obtained from a least-squares fit to the settings of 25 reflections recorded on an Enraf-Nonius CAD4 diffractometer. Intensities were collected on the diffractometer at varied scan rates in the ω– or θ–2θ mode with graphite-monochromated Cu Kα or Mo Kα radiation. The choice of wavelength was influenced by the crystal size. Three monitor reflections were controlled at regular intervals during data collection; no significant alterations in intensity were recorded. Empirical absorption corrections were performed for all data sets. The structures were solved by Patterson and difference syntheses and refined by full-matrix least-squares. The asymmetric units of **1** and **3** each contain two diastereomers with respectively R and S chiralities at the ruthenium atoms. Two independent molecules with different conformations at the amino acidate C–N bond are found in the unit cell of **2**. For **3**, which crystallizes in the triclinic space group $P\bar{1}$, the ruthenium, benzene and 9-ethylguanine atoms of the two diastereomers are related by a pseudo-centre of symmetry, which leads to extremely strong correlations of their positional and thermal parameters. It was, therefore, necessary to restrain their refinement by including bond length parameters (±0.01 Å) for equivalent distances in the two diastereomers. Under these conditions, with anisotropic temperature factors for the Ru, Cl, O and N atoms and hydrogen atoms at geometrically calculated positions, R converged to 0.038 for **3**. Anisotropic thermal parameters were introduced for all non-hydrogen atoms in **2** and **4** and for the Ru, Cl and O atoms in **1**. Hydrogen atoms were introduced, where possible, at calculated positions with d(C–H) = 1.08 Å. Terminal reliability indices are listed in Table 1 where $R_w = [\Sigma w(F_o - F_c)^2/\Sigma wF_o^2]^{1/2}$ with weights given by $w = (\sigma^2(F_o) + p^2F_o^2)^{-1}$. Final difference syntheses were effectively contourless. Calculations were performed with the SDP program suite (Enraf-Nonius)

TABLE 1. Crystal and refinement data for **1**–**4**

	Compound			
	1	**2**	**3**	**4**[a]
Space group	$P2_1$	$C2$	$P1$	$P\bar{1}$
a (Å)	11.833(2)	36.313(3)	11.184(2)	10.358(3)
b (Å)	7.945(1)	6.422(1)	11.964(3)	11.767(3)
c (Å)	10.914(1)	12.069(2)	8.337(2)	7.927(2)
α (°)	90	90	110.71(3)	101.28(2)
β (°)	93.58(2)	106.11(1)	92.93(3)	90.30(3)
γ (°)	90	90	93.90(3)	110.06(2)
V (Å^3)	1024.0(5)	2704(1)	1038(1)	887(1)
Z	4	8	2	2
D_c (g cm^{-3})	1.96	1.74	1.54	1.81
Radiation	Cu Kα	Cu Kα	Mo Kα	Mo Kα
μ (cm^{-1})	149.6	132.6	9.0	12.0
Scan method	ω	ω	ω	$\theta-2\theta$
$2\theta_{max}$ (°)	130	140	50	45
Reflections measured	1797	2764	3900	2315
Reflections observed	1550	2694	3695	2085
Rejection criterion	$F_o^2 < 2\sigma(F_o^2)$	$F_o^2 < 2\sigma(F_o^2)$	$F_o^2 < 2\sigma(F_o^2)$	$F_o^2 < 2\sigma(F_o^2)$
R	0.080	0.054	0.038	0.034
R_w	0.082	0.057	0.038	0.034
P	0.007	0.010	0.007	0.010

[a]The asymmetric unit of **4** contains three water molecules of crystallization.

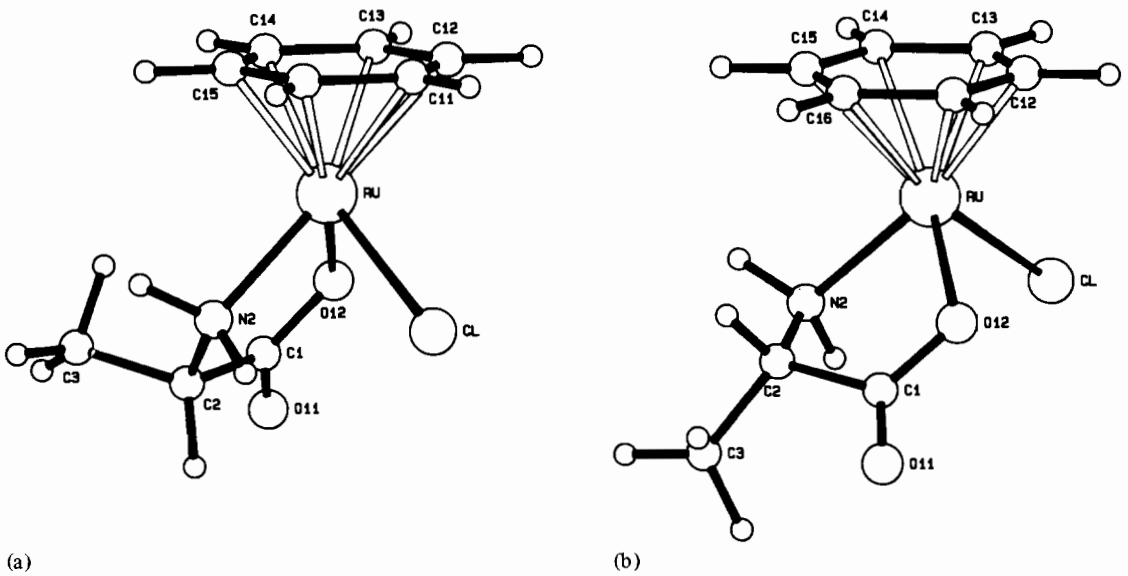

Fig. 1. The two diastereomers of **1**.

with SHELX-76 [9] and with local programs. Atomic coordinates with equivalent isotropic temperature factors are given in Table 2; bond lengths to the ruthenium atoms are given in Table 3.

Discussion

As depicted in Fig. 1, two diastereomers with opposite chiralities at the metal centre are found in the unit cell of **1**. Diastereomer **1a** displays the S_C, R_{Ru}, diastereomer **1b** the S_C, S_{Ru} configuration. In general, a relative stabilization of one diastereomer may be achieved through preferential intramolecular hydrogen bonding, by interaction with solvent molecules or by a reduction in intramolecular steric contacts. A weak intramolecular N–H···Cl hydrogen bond is exhibited by diastereomer **1a** with N...Cl and H···Cl distances of 2.84 and 2.42 Å respectively and

TABLE 2. Atom coordinates with equivalent isotropic temperature factors ($Å^2 \times 10^3$)

Atom	x/a	y/b	z/c	U_{eq}
1 Molecule a				
Ru	0.1293(2)	0.0	0.7775(1)	33(1)
Cl	0.0917(8)	0.2915(9)	0.8119(6)	54(4)
O11	−0.1131(13)	0.0393(22)	0.4906(12)	29(9)
O12	−0.0222(10)	−0.0060(37)	0.6704(11)	43(10)
N2	0.1724(14)	0.1176(23)	0.6091(11)	26(4)
C1	−0.0236(17)	0.0323(41)	0.5552(16)	48(7)
C2	0.0852(15)	0.1027(25)	0.5065(15)	27(5)
C3	0.1261(27)	−0.0314(37)	0.4201(27)	69(9)
C11	0.2105(16)	−0.0118(28)	0.9697(16)	38(5)
C12	0.1083(16)	−0.0993(28)	0.9690(16)	30(5)
C13	0.0831(16)	−0.2221(28)	0.8801(16)	43(7)
C14	0.1601(16)	−0.2573(28)	0.7919(16)	70(10)
C15	0.2623(16)	−0.1698(28)	0.7925(16)	67(9)
C16	0.2876(16)	−0.0470(28)	0.8814(16)	56(9)
1 Molecule b				
Ru	0.6346(2)	0.0025(3)	0.7788(2)	33(1)
Cl	0.5924(8)	−0.2842(9)	0.8310(6)	57(5)
O11	0.3936(15)	0.0276(30)	0.4965(16)	56(13)
O12	0.4787(11)	0.0089(33)	0.6824(12)	46(10)
N2	0.6784(17)	−0.0709(26)	0.6002(12)	39(6)
C1	0.4803(15)	0.0053(47)	0.5646(15)	40(6)
C2	0.5980(15)	0.0163(30)	0.5135(14)	29(4)
C3	0.6065(20)	−0.0532(29)	0.3846(17)	34(5)
C11	0.5914(13)	0.2413(24)	0.8562(16)	40(7)
C12	0.6149(13)	0.1187(24)	0.9456(16)	69(9)
C13	0.7183(13)	0.0341(24)	0.9507(16)	60(8)
C14	0.7984(13)	0.0722(24)	0.8662(16)	53(8)
C15	0.7749(13)	0.1948(24)	0.7768(16)	41(6)
C16	0.6714(13)	0.2794(24)	0.7718(16)	34(6)
2 Molecule a				
Ru	0.6080(1)	0.0	0.0666(1)	36(1)
Cl1	0.6114(1)	−0.2656(6)	0.2100(2)	50(2)
Cl2	0.6508(1)	−0.2064(7)	−0.0086(3)	61(2)
O11	0.7325(3)	−0.0694(19)	0.2419(8)	66(6)
O12	0.7440(3)	0.1137(19)	0.0966(8)	64(5)
N2	0.6597(2)	0.0919(19)	0.1919(7)	41(5)
C1	0.7239(3)	0.0660(23)	0.1716(9)	46(6)
C2	0.6901(3)	0.2101(24)	0.1551(11)	49(6)
C3	0.7022(4)	0.4005(26)	0.2224(15)	67(8)
C4	0.7782(4)	−0.0094(37)	0.1054(14)	74(9)
C11	0.5502(2)	0.0673(15)	0.0706(7)	61(8)
C12	0.5720(2)	0.2491(15)	0.0947(7)	50(6)
C13	0.5922(2)	0.3180(15)	0.0188(7)	61(8)
C14	0.5906(2)	0.2052(15)	−0.0812(7)	64(8)
C15	0.5688(2)	0.0235(15)	−0.1053(7)	76(9)
C16	0.5486(2)	−0.0455(15)	−0.0294(7)	78(9)
2 Molecule b				
Ru	0.6408(1)	0.0159(2)	0.5502(1)	41(1)
Cl1	0.6011(1)	0.2272(7)	0.6347(3)	63(2)
Cl2	0.6326(1)	0.2841(6)	0.4058(2)	55(2)
O11	0.5178(3)	−0.1879(22)	0.2791(9)	70(6)
O12	0.5260(3)	−0.5183(22)	0.3385(10)	78(7)
N2	0.5874(2)	−0.0790(18)	0.4335(7)	41(4)
C1	0.5344(3)	−0.3174(27)	0.3455(11)	52(7)
C2	0.5686(3)	−0.2777(24)	0.4494(9)	46(6)
C3	0.5544(4)	−0.2733(30)	0.5548(11)	62(8)
C4	0.4926(5)	−0.5873(41)	0.2508(15)	95(12)
C11	0.6589(2)	−0.2997(19)	0.6027(8)	58(7)
C12	0.6761(2)	−0.2345(19)	0.5187(8)	63(8)
C13	0.6975(2)	−0.0509(19)	0.5344(8)	73(10)
C14	0.7016(2)	0.0674(19)	0.6341(8)	103(11)
C15	0.6844(2)	0.0022(19)	0.7180(8)	87(10)
C16	0.6630(2)	−0.1814(19)	0.7023(8)	69(9)
3 Molecule a				
Ru	0.8981(1)	0.2946(1)	0.1828(1)	30(1)
Cl	0.8285(4)	−0.2633(4)	−0.5457(7)	66(2)
Ow1	0.6112(11)	0.5406(14)	0.3987(25)	97(7)
Ow2	0.3772(16)	0.5231(16)	0.3195(29)	105(9)
O6	0.8680(8)	−0.0063(11)	−0.1103(16)	56(6)
O11	0.7341(9)	0.2648(11)	0.5904(14)	50(5)
O12	0.8224(9)	0.3348(9)	0.4170(10)	42(5)
N1	0.6903(9)	−0.0821(11)	−0.2775(16)	37(6)
N2	0.5231(10)	−0.1810(11)	−0.4456(16)	46(5)
N3	0.5036(9)	0.0048(9)	−0.2272(17)	44(6)
N7	0.7251(6)	0.2088(8)	0.0790(16)	32(6)
N9	0.5320(9)	0.1946(10)	0.0072(18)	50(7)
N21	0.9234(9)	0.1400(9)	0.2451(14)	36(5)
C1	0.7977(13)	0.2496(8)	0.4695(16)	44(4)
C2	0.5708(10)	−0.0782(12)	−0.3179(18)	43(4)
C4	0.5736(10)	0.0926(12)	−0.1048(19)	42(4)
C5	0.6922(9)	0.0972(9)	−0.0487(15)	34(4)
C6	0.7627(9)	0.0083(10)	−0.1470(16)	38(4)
C8	0.6236(9)	0.2574(12)	0.1210(18)	41(4)
C91	0.4077(9)	0.2298(11)	0.0214(16)	43(3)
C92	0.3830(10)	0.2879(10)	−0.1070(14)	59(3)
C11	1.0597(5)	0.4194(5)	0.2577(6)	29(2)
C12	1.0857(5)	0.3132(5)	0.1309(6)	33(2)
C13	1.0147(5)	0.2670(5)	−0.0246(6)	37(2)
C14	0.9176(5)	0.3270(5)	−0.0532(6)	42(2)
C15	0.8916(5)	0.4331(5)	0.0736(6)	53(3)
C16	0.9626(5)	0.4793(5)	0.2291(6)	39(2)
C21	0.8313(8)	0.1272(7)	0.3581(12)	44(2)
C31	0.8644(10)	0.0528(9)	0.4651(14)	64(3)
3 Molecule b				
Ru	0.1019(1)	0.7054(1)	0.8172(1)	32(1)
Cl	0.1601(4)	1.2565(4)	1.5450(6)	53(2)
Ow1	0.3735(12)	0.4390(15)	0.6044(23)	86(7)
Ow2	0.6322(11)	0.4906(13)	0.6885(23)	72(6)
O6	0.1356(8)	1.0130(11)	1.1112(17)	53(6)
O11	0.2610(11)	0.7346(14)	0.4034(13)	66(7)
O12	0.1788(9)	0.6577(8)	0.5857(10)	35(5)
N1	0.3094(10)	1.0823(11)	1.2729(18)	44(7)
N2	0.4902(13)	1.1745(12)	1.4507(20)	68(7)
N3	0.4925(9)	0.9967(11)	1.2280(16)	43(7)
N7	0.2738(7)	0.7966(10)	0.9119(14)	39(6)
N9	0.4694(8)	0.8057(10)	0.9872(15)	34(6)

(continued)

(continued)

TABLE 2. *(continued)*

Atom	*x/a*	*y/b*	*z/c*	U_{eq}
N21	0.0668(9)	0.8489(9)	0.7376(13)	37(5)
C1	0.1989(11)	0.7405(8)	0.5251(14)	34(3)
C2	0.4307(10)	1.0860(12)	1.3122(18)	42(4)
C4	0.4272(9)	0.9074(11)	1.0989(17)	32(4)
C5	0.3057(8)	0.8965(9)	1.0585(14)	31(3)
C6	0.2421(9)	0.9979(10)	1.1339(16)	37(4)
C8	0.3723(9)	0.7376(12)	0.8880(17)	38(4)
C91	0.5907(10)	0.7673(12)	1.0075(18)	51(4)
C92	0.5884(12)	0.6660(12)	1.0743(18)	78(4)
C11	−0.0801(5)	0.6239(8)	0.7879(10)	65(3)
C12	−0.0602(5)	0.7165(8)	0.9478(10)	68(3)
C13	0.0359(5)	0.7169(8)	1.0616(10)	50(2)
C14	0.1122(5)	0.6249(8)	1.0154(10)	52(2)
C15	0.0923(5)	0.5323(8)	0.8555(10)	43(2)
C16	−0.0038(5)	0.5318(8)	0.7418(10)	53(3)
C21	0.1269(8)	0.8486(6)	0.5852(13)	46(2)
C31	0.2028(10)	0.9647(9)	0.6129(17)	74(3)
4				
Ru	0.7310(1)	0.7187(1)	0.2840(1)	25(1)
Cl1	0.7229(2)	0.7104(1)	−0.0230(2)	35(1)
Cl2	0.9643(2)	0.8630(1)	0.2946(2)	38(1)
Ow1	0.0626(4)	0.6748(4)	0.0375(6)	56(2)
Ow2	0.0136(4)	0.8878(4)	0.8475(5)	50(2)
Ow3	0.1298(5)	0.5054(5)	0.2149(7)	83(3)
O6	0.3928(4)	0.6821(3)	0.1079(5)	43(2)
N1	0.3364(5)	0.8506(4)	0.1059(5)	30(2)
N2	0.2624(5)	1.0123(4)	0.0856(6)	43(2)
N3	0.4700(5)	1.0570(4)	0.2408(6)	33(2)
N7	0.6684(4)	0.8727(4)	0.3062(5)	29(2)
N9	0.6803(5)	1.0678(4)	0.3848(6)	32(2)
C2	0.3603(6)	0.9756(5)	0.1461(7)	34(3)
C4	0.5586(5)	1.0052(5)	0.2830(6)	29(3)
C5	0.5501(5)	0.8837(5)	0.2374(6)	28(2)
C6	0.4278(6)	0.7950(5)	0.1472(7)	31(3)
C8	0.7421(6)	0.9849(5)	0.3943(7)	31(3)
C11	0.5800(5)	0.5338(4)	0.2493(5)	57(4)
C12	0.7116(5)	0.5264(4)	0.2490(5)	57(4)
C13	0.8100(5)	0.5963(4)	0.3851(5)	56(4)
C14	0.7769(5)	0.6735(4)	0.5215(5)	56(4)
C15	0.6453(5)	0.6809(4)	0.5218(5)	61(4)
C16	0.5469(5)	0.6110(4)	0.3857(5)	58(4)
C91	0.7315(6)	1.1977(5)	0.4721(7)	38(3)
C92	0.7944(9)	1.2810(6)	0.3552(9)	63(4)

an N–H...Cl angle of 101°. This leads to the adoption of a relatively planar conformation for the chelate ring as evidenced by the observed absolute ring torsion angles of ≤10.7°. The ammine nitrogen N2 is displaced by 0.20 Å from the best least-squares plane through the remaining four ring atoms. The closest intramolecular contact between the amino acidate methyl group and the benzene ligand is observed between H33(CH_3) and H14 (C_6H_6), which display a distance of 3.11 Å. Formation of an N–H···Cl intramolecular hydrogen bond is no longer favourable for diastereomer **1b**, as evidenced by the N···Cl and

TABLE 3. Bond lengths (Å) to the ruthenium atoms

1a		**1b**	
Ru–Cl	2.392(7)	Ru–Cl	2.408(7)
Ru–O12	2.079(11)	Ru–O12	2.066(12)
Ru–N2	2.152(11)	Ru–N2	2.130(12)
Ru–C11	2.25(2)	Ru–C11	2.15(2)
Ru–C12	2.26(2)	Ru–C12	2.07(2)
Ru–C13	2.18(2)	Ru–C13	2.08(2)
Ru–C14	2.08(2)	Ru–C14	2.18(2)
Ru–C15	2.07(2)	Ru–C15	2.26(2)
Ru–C16	2.16(2)	Ru–C16	2.25(2)
2a		**2b**	
Ru–Cl1	2.409(3)	Ru–Cl1	2.409(3)
Ru–Cl2	2.404(3)	Ru–Cl2	2.404(4)
Ru–N2	2.140(8)	Ru–N2	2.146(8)
Ru–C11	2.157(6)	Ru–C11	2.170(11)
Ru–C12	2.152(9)	Ru–C12	2.154(11)
Ru–C13	2.156(9)	Ru–C13	2.161(8)
Ru–C14	2.166(8)	Ru–C14	2.184(8)
Ru–C15	2.171(8)	Ru–C15	2.199(10)
Ru–C16	2.166(7)	Ru–C16	2.192(9)
3a		**3b**	
Ru–N7	2.115(6)	Ru–N7	2.112(7)
Ru–O12	2.080(7)	Ru–O12	2.062(6)
Ru–N21	2.120(7)	Ru–N21	2.098(7)
Ru–C11	2.177(5)	Ru–C11	2.159(7)
Ru–C12	2.176(6)	Ru–C12	2.150(8)
Ru–C13	2.165(6)	Ru–C13	2.165(8)
Ru–C14	2.154(5)	Ru–C14	2.188(7)
Ru–C15	2.155(6)	Ru–C15	2.197(8)
Ru–C16	2.166(6)	Ru–C16	2.182(8)
4			
Ru–Cl1	2.416(1)	Ru–C12	2.420(1)
Ru–N7	2.101(4)	Ru–C11	2.164(3)
Ru–C12	2.163(4)	Ru–C13	2.154(4)
Ru–C14	2.147(4)	Ru–C15	2.148(5)
Ru–C16	2.157(4)		

closest H...Cl distances of 3.24 and 2.90 Å. As furthermore, the alaninate methyl group is now positioned on the opposite side of the chelate ring to the benzene moiety, so that close intramolecular contacts between the ligands are no longer possible, the energetically favourable puckered conformation is adopted by the five-membered ring. N2 and C2 are displaced by respectively −0.27 and +0.27 Å from the best least-squares plane through the chelate ring. The following ring torsion angles are observed (beginning with Ru–O12, ending with Ru–N2): −6.0, −14.6, 33.5, −41.8, 31.1°. Intermolecular N–H···Cl or N–H···O hydrogen bonds are not exhibited by the crystal lattice, so that it may reasonably be assumed that the cocrystallization of diastereomers **1a** and **1b** is a result of their relatively small energy difference.

For complex **2**, two independent molecules with dramatically different conformations at the C2–N2 bond are found in the asymmetric unit. Respective values of the torsion angle Ru–N2–C2–C3 in the two molecules are 128.3 and 68.7°. The conformation of **2a** is stabilized by an N2–H···O11 intramolecular hydrogen bond with distances N···O and H···O of 2.74 and 2.14 Å and an N–H···O angle of 113°. This ammine hydrogen atom is positioned at 2.59 Å from Cl1. A similar N–H···O stabilization is not observed for molecule **2b**; the apposite H···O distances are 2.66 and 2.65 Å (Fig. 2).

Reaction of **1** with 9-ethylguanine leads to displacement of the chlorine atom and formation of the mixed amino acidate/nucleobase complex **3**, in which the imidazole nitrogen N7 of the purine base is coordinated. As for **1**, two diastereomers (cations) with differing chiralities at the metal centre are observed in the unit cell of **3** (Fig. 3). On account of the lower priority of N7 of the purine base in comparison to Cl, the chiralities of the ruthenium atoms in **3a** and **3b** are now reversed with respect to the analogous diastereomers **1a** and **1b**. Diastereomic cation **3a** displays the S_C, S_{Ru}, diastereomeric cation **3b** the S_C, R_{Ru} configuration. An intramolecular N–H···O6 hydrogen bond with N···O and H···O distances of 2.89 and 1.92 Å and an N–H···O angle of 148° is exhibited by **3a**. The chelate ring is puckered with N21 and C21 displaced respectively 0.22 and −0.20 Å from the best least-squares plane through the five ring atoms. The N–H···O6 interaction in **3b** is markedly weaker than in **3a**, as reflected by the N···O and H···O distances of 3.09 and 2.13 Å for an N–H···O angle of 145°. In addition, the five-membered ring is markedly less puckered with a maximal deviation of −0.13 Å from the best least-squares plane at the carboxyl oxygen O12. The ^{1}H NMR spectrum, obtained upon dissolving crystals of **3** (i.e. a 50:50 ratio for the diastereomers **3a**:**3b**) in D_2O indicates a *c.* 65:35 equilibrium

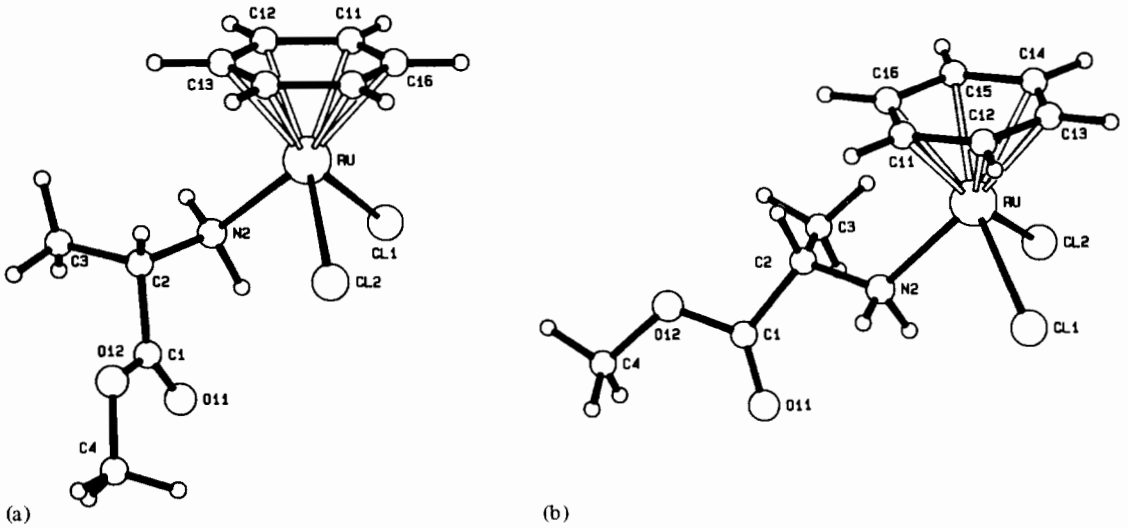

Fig. 2. The two independent molecules of **2**.

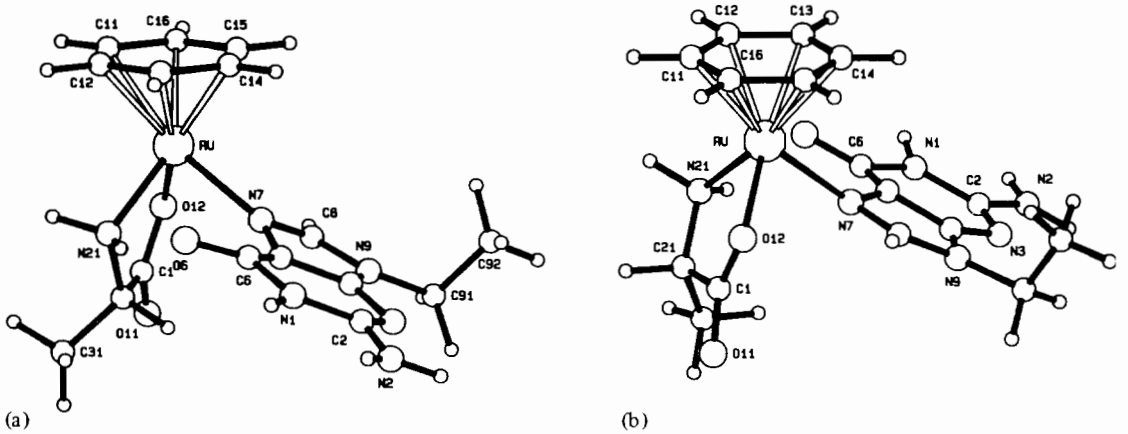

Fig. 3. The two diastereomer cations of **3**.

ratio for **3a**:**3b** in aqueous solution. A preference for diastereomer **3a** would be predicted on the basis of the X-ray structural analysis, as the S_{Ru} configuration at the ruthenium atom allows the formation of a significantly stronger N–H···O6 intramolecular hydrogen bond with retention of a less strained puckered ring conformation. This hydrogen bond should restrain the alaninate proton and methyl substituents on the α-carbon atom C21 to respectively axial and equatorial positions relative to the chelate ring, so that an exchange of these positions will be energetically unfavourable. As a result, the chemical shift for H21 (axial) in **3a** (2.45 ppm) is observed at markedly higher field than in **3b** (3.56, 3.57 ppm); the methyl proton resonances in **3a** occur at lower field (1.29 ppm) than in **3b** (0.82, 0.82 ppm). The presence of two conformations for diastereomer **3b** is indicated by the occurrence of two resonances at closely similar positions for the alaninate protons. A possible explanation is provided by inspection of the cation structure in the solid state (Fig. 3), which suggests that a weak N–H···O hydrogen bond may also be formed for a second orientation of the guanine plane relative to Ru–N7.

Only one signal is observed for the benzene protons in both diastereomers **3a** and **3b**. In contrast, the ^{1}H NMR spectrum of **1** in D_2O indicates that the coordinated chloride is readily substituted by water, as had previously been reported by Dersnah and Baird for $[(\eta^6\text{-}C_6H_6)Ru(\text{D,L-ala})Cl]$ [7]. For a solution concentration of 10 g l^{-1}, **1** displays benzene resonances at respectively 5.79, 5.82, 5.90 and 5.92 ppm. Upon addition of a fivefold excess of LiCl, the lowfield resonances disappear leaving two singlets at 5.83 ppm, which may be assigned to the two diastereomers of $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})Cl]$ in a ratio of *c.* 62:38. Resonances are observed for the alaninate C2 and methyl protons at respectively 3.18 and 1.34 ppm for the preferred diastereomer, and at 3.55 and 1.27 ppm for the second diastereomer. As concluded by Dersnah and Baird [7], the lowfield resonances at 5.92/5.90 ppm may reasonably be attributed to a diastereomeric cation pair $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})(D_2O)]^+$. Their alaninate proton resonances occur at similar positions to those of the diastereomeric pair $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})Cl]$. As ring inversion will be fast on the NMR timescale for such species, an assignment of the ruthenium chiralities is not immediately possible. However, it is interesting to note that the methyl proton resonances for the preferred diastereomers of $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})Cl]$ and $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})(D_2O)]^+$ both occur at lower field. The ^{1}H NMR spectrum indicates that epimerization at ruthenium is a relatively slow process. The observation of six resonances for the alanine methyne and methyl protons as compared to four resonances for the benzene protons suggests the presence of species with differing ring conformations.

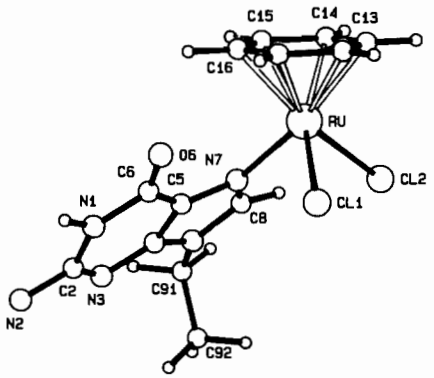

Fig. 4. The molecular structure of **4**.

The ^{1}H NMR spectrum of **2** in D_2O displays resonances at identical positions to those of **1**. The observation of a methanol signal indicates that ester hydrolysis has occurred. As would be expected, the relative intensity of the diastereomeric pair $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})Cl]$ is greater in this case than for the original spectrum of **1**, owing to the increased chloride concentration. Surprisingly reaction of **2** with 9-ethylguanine in water leads to replacement of the alanine methyl ester ligand and formation of $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)Cl_2]$ (**4**), the molecular structure of which is depicted in Fig. 4. It may be assumed that this reaction is more rapid than hydrolysis of **2** in aqueous solution. Arene proton resonances at 5.94, 6.05 and 6.10 ppm, which occur in a ratio 62:14:24, may be attributed to the respective species $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)Cl_2]$, $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)(D_2O)Cl]^+$ and $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)(D_2O)_2]^{2+}$. N7 is once again the coordination site of the guanine derivative.

Our present investigation demonstrates that η^6-arene–ruthenium(II) complexes of amino acids can coordinate N7 of guanine derivatives, i.e. that they should be capable of coordinating this base in DNAs. Initial *in vivo* studies on a complex of this type, namely $[(\eta^6\text{-}C_6H_6)Ru(\text{pro})Cl]$, have established a significant antitumour activity towards P388 leukemia [10]. The observed replacement of alanine methyl ester in $[(\eta^6\text{-}C_6H_6)Ru(\text{alaMe})Cl_2]$ **2** through 9-ethylguanine in $[(\eta^6\text{-}C_6H_6)Ru(9Etgua)Cl_2]$ (**4**) indicates a preference for ruthenium(II) coordination of aromatic nitrogen atoms in such η^6-arene complexes. However participation in a five-membered chelate ring as in $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})Cl]$ (**2**) allows retention of the ammine binding site upon coordination of guanine N7 in $[(\eta^6\text{-}C_6H_6)Ru(\text{L-ala})(9Etgua)]Cl$ (**3**).

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