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Chromium(V) Peptide Complexes: Synthesis and Spectroscopic Characterization

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A series of stable Cr(V) model complexes that mimic the binding of Cr(V) to peptide backbones at the C-terminus of proteins have been prepared for *N*,*N*-dimethylurea derivatives of the tripeptides Aib_3-DMF, AibLAlaAib-DMF, and AibDAlaAib-DMF (Aib = 2-amino-2-methylpropanoic acid, DMF = *N*,*N*-dimethylformamide). The Cr(II) precursor complexes were synthesized by the initial deprotonation of the amide and acid groups of the peptide ligands in DMF with potassium *tert*-butoxide in the presence of $CrCl_2$. The Cr(II) intermediates thus formed were then immediately oxidized to Cr(V) using *tert*-butyl hydroperoxide. Spectroscopic and mass-spectrometric analyses of the Cr(V) complexes showed that a new metal-directed organic transformation of the ligand had occurred. This involved a DMF solvent molecule becoming covalently bound to the amine group of the peptide ligand, yielding a urea group, and a third coordinated deprotonated urea nitrogen donor. A metal-directed oxidative coupling has been proposed as a possible mechanism for the organic transformation. The Cr(V/IV) reduction potential was determined for the three Cr(V) complexes using cyclic voltammetry, and in all cases it was quasi-reversible. These are the first isolated and fully characterized Cr(V) complexes with non-sulfur-containing peptide ligands.

Introduction

While Cr(VI) is a well-recognized human carcinogen,[1] numerous in vitro studies have shown that Cr(VI) does not interact with, or damage, isolated DNA.[2,3] However, in the presence of reductants, Cr(VI) causes a wide range of DNA damage similar to that observed in vivo, including DNA−Cr(III) adducts, DNA−DNA cross-links, and DNA−protein cross-links.[2−5] The uptake−reduction model has been proposed to account for the effects of Cr(VI) in vivo and in vitro.[5,6] In this model, tetrahedral $[CrO_4]^{2-}$ enters the cell readily via general anion transport channels. Once inside the cell, Cr(VI) is reduced by cellular components producing reactive intermediates, including Cr(VI) esters, Cr(V) and Cr(IV) complexes, free radicals, and reactive oxygen species (ROS), with the Cr ultimately reduced to Cr(III).

Among the most important cellular molecules that are thought to be involved in the reduction of Cr(VI) to Cr(III) are the following: glutathione (γ-glutamylcysteinylglycine, GSH); cysteine; ascorbate; catecholamines such as DOPA.[4,5] Formation of relatively stable Cr(V) intermediates is commonly detected by EPR spectroscopy during the uptake of Cr(VI) by cells or tissues,[5,7,8] as well as during the in vitro reduction of Cr(VI) with GSH,[9] ascorbate[10] and DOPA.[11] Several researchers have shown that a direct correlation exists between the concentration of such intermediates and the level of DNA damage in vitro.[2,3,11,12] The importance of Cr(V) as a reactive intermediate in Cr(VI)-induced genotoxicity is further increased in vivo by the formation of relatively stable Cr(V) complexes with carbohydrates and glycoproteins within the cells and on the cell surface.[4,5,13]

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(1) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; International Agency for Research on Cancer: Lyon, France, 1990.
(2) Kortenkamp, A.; Casadevall, M.; Da Cruz Fresco, P.; Shayer, R. O. J. *NATO ASI Ser., Ser. 2* **1997**, *26*, 15−34.
(3) Stearns, D. M.; Wetterhahn, K. E. *NATO ASI Ser., Ser. 2* **1997**, *26*, 55−72.
(4) Codd, R.; Dillon, C. T.; Levina, A.; Lay, P. A. *Coord. Chem. Rev.* **2001**, *216−217*, 533−577.
(5) Levina, A.; Codd, R.; Dillon, C. T.; Lay, P. A. *Prog. Inorg. Chem.* **2003**, *51*, 145−250.
(6) Connett, P. H.; Wetterhahn, K. E. *Struct. Bond.* **1983**, *54*, 93−124.
(7) Wetterhahn Jennette, K. *J. Am. Chem. Soc.* **1982**, *104*, 874−875.
(8) Liu, K. J.; Shi, X.; Jiang, J.; Goda, F.; Dalal, N.; Swartz, H. M. *Ann. Clin. Lab. Sci.* **1996**, *26*, 176−184.
(9) Levina, A.; Zhang, L.; Lay, P. A. *Inorg. Chem.* **2003**, *42*, 767−784.
(10) Zhang, L.; Lay, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12624−12637.
(11) Pattison, D. I.; Davies, M. J.; Levina, A.; Dixon, N. E.; Lay, P. A. *Chem. Res. Toxicol.* **2001**, *14*, 500−510.
(12) Casadevall, M.; Kortenkamp, A. *Carcinogenesis* **1995**, *16*, 805−809.

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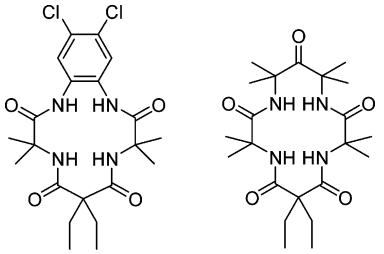
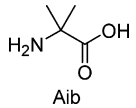
Proteins and peptides are the most abundant cellular constituents and are also one of the most important classes of biomolecules known to bind metal ions naturally.[14] DNA−Cr(III)−protein cross-links are among the most common DNA lesions detected following the in vivo exposure to Cr(VI).[2−5] This observation implicates the involvement of Cr−peptide intermediates in the Cr(VI)-induced DNA damage. Despite this implication, relatively few studies have been conducted on the coordination chemistry of Cr, particularly in its higher oxidation states (IV−VI), with peptides (except for GSH).[9,15]

Ligands with deprotonated amide nitrogen donors are known to stabilize high oxidation states of metal ions.[16] For instance, stable Cr(V) complexes of two related macrocyclic tetraamide ligands, H_4mampa and H_4mac, have been characterized by X-ray crystallography.[17] These square-pyramidal oxo complexes ($[Cr^V(O)L]^-$, where L = mampa or mac) were synthesized by the reactions of the deprotonated ligands with $CrCl_2$, followed by oxidation of the Cr(II) intermediates with an organic peroxide.[17]



In addition, Cr(V) complexes of N-deprotonated glycine or alanine peptides (tri-, tetra-, or pentamers) have been generated via the reductions of Cr(VI) in methanol solutions of the ligands[18] or via the oxidations of the corresponding Cr(III)−peptide complexes with Pb(IV) or I(VII) in aqueous solutions.[19] Six-coordinate octahedral structures ($[Cr^V(O)L(OR)]^{2-}$, where L is a tetradentate ligand, coordinated in the square plane, and R is H or Me) were assigned to these complexes, on the basis of EPR spectroscopic and mass-spectrometric studies.[18,19]

There has been a considerable work on the stabilization of higher oxidation states of other metal ions, usually Cu(III) or Ni(III), by deprotonated peptides or related ligands.[16,20−25] Certain Ni(II) and Cu(II) complexes of such ligands react spontaneously with atmospheric oxygen in solutions to produce M(III) complexes.[20−22] This reaction is immediately followed by ligand oxidation, leading to divalent metal complexes of oxidized ligands.[20−22] Substitution of methylene hydrogens in the peptide ligands increases the stability of the Cu(III) and Ni(III) oxidation states.[16,23] Consequently, the ligand Aib_3 (where Aib is a synthetic amino acid, 2-amino-2-methylpropanoic or α-aminoisobutyric acid), in which all the methylene hydrogens were substituted for methyl groups, was used to prepare relatively stable Cu(III)− and Ni(III)−peptide complexes,[23] and the crystal structure of one such complex, $[Cu^{III}(H_{-2}Aib_3)]\cdot 2H_2O\cdot 0.5NaClO_4$, has been solved.[24]



In this work, we report the syntheses and definitive structural characterizations of Cr(V) complexes with tripeptide ligands, Aib_3, AibLAlaAib, and AibDAlaAib, as well as unusual covalent transformations of the ligands during such syntheses.

Experimental Section

Caution! *Chromium(V) compounds are mutagenic and possibly carcinogenic;[4,5] contact with skin and inhalation should be avoided.*

Chemicals. The following chemicals of analytical or higher purity grade were used as received: benzyl alcohol; HCl (37% w/w in H_2O), H_2SO_4 (98% w/w in H_2O), and $CaCl_2$ from Ajax; L-alanine, D-alanine, 2-amino-2-methylpropanoic acid (α-aminoisobutyric acid), benzylchloroformate, *tert*-butyl hydroperoxide (70% w/v in H_2O), L-cysteine, *N*,*N*′-dicyclohexylcarbodiimide, potassium *tert*-butoxide, 4-toluenesulfonic acid, CH_3COOH, HBr in CH_3COOH (30% w/w), $NaCNBH_3$, NaH, $NaClO_4$, and NaOH (all Aldrich); L-ascorbic acid, ferrocene, $CrCl_2$ (anhydrous), KBr, NaCl, Na_2SO_4, $NaHCO_3$ Na_2CO_3, $NaH_2PO_4\cdot 2H_2O$, and Pd/C (10% w/w) (all Merck); *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid) (HEPES, Research Organics); glutathione (reduced form, Sigma). Tetra-*n*-butylammonium tetrafluoroborate (Fluka) was recrystallized from ethyl acetate. Triethylamine (Aldrich) was freshly distilled off CaH_2 before use. The following solvents (HPLC grade, Aldrich) were used as received: acetonitrile; acetone; benzene; *N*,*N*-dimethylformamide (DMF); dimethyl sulfoxide (DMSO); hexamethylphosphoramide; sulfolane; ethanol; and methanol. Other solvents, including diethyl ether, hexane, dichloromethane, and ethyl acetate, were distilled off CaH_2 before use. Water was purified by the Milli-Q technique. Stock solutions used in the preparation of the buffers (1.0 M of NaH_2PO_4 or HEPES) were treated with Chelex-100 ion-exchange resin (BioRad) to remove traces of heavy metal ions, and the pH values of the buffers were adjusted using ultrapure NaOH (99.99%, Aldrich).

Syntheses. The tripeptide α-aminoisobutyryl-α-aminoisobutyryl-α-aminoisobutyric acid (Aib_3) was prepared using the previously published method.[23] Similar methods were used for the syntheses of two new tripeptides, α-aminoisobutyryl-L-alanine-α-aminoisobutyric acid (AibLAlaAib) and α-aminoisobutyryl-D-alanine-α-aminoisobutyric acid (AibDAlaAib). These are outlined as follows.

(13) Codd, R.; Irwin, J. A.; Lay, P. A. *Curr. Opinion Chem. Biol.* **2003**, *7*, 213-219.
(14) Hay, R. W. *Bio-Inorganic Chemistry*; Ellis Horwood: Chichester, U.K., 1984.
(15) Levina, A.; Lay, P. A. *Inorg. Chem.* **2004**, *43*, 324−335.
(16) Margerum, D. W. *Pure Appl. Chem.* **1983**, *55*, 23−34.
(17) Collins, T. J.; Slebodnick, C.; Uffelman, E. S. *Inorg. Chem.* **1990**, *29*, 3433−3436.
(18) Headlam, H. A.; Lay, P. A. *Inorg. Chem.* **2001**, *40*, 78−86.
(19) Headlam, H. A. Ph.D. Thesis, The University of Sydney, 1998.
(20) Paniago, E. B.; Wetherburn, D. C.; Margerum, D. W. *Chem. Commun.* **1971**, 1427−1428.
(21) Bossu, F. P.; Paniago, E. B.; Margerum, D. W.; Kirksey, S. T.; Kurtz, J. L. *Inorg. Chem.* **1978**, *17*, 1034−1042.
(22) Weeks, C. L.; Turner, P.; Fenton, R. R.; Lay, P. A. *J. Chem. Soc., Dalton Trans.* **2002**, 931−940.
(23) Kirksey, S. T.; Neubecker, T. A.; Margerum, D. W. *J. Am. Chem. Soc.* **1979**, *101*, 1631−1633.
(24) Diaddario, L. L.; Robinson, W. R.; Margerum, D. W. *Inorg. Chem.* **1983**, *22*, 1021−1025.

The benzylcarbonyl (Cbz) and benzyl ester (OBz) protected peptide CbzAlaAibOBz was synthesized from CbzAla and AibOBz by the dicyclohexylcarbodiimide (DCC) method of amino acid coupling.[26] The Cbz protecting group was removed from the dipeptide by treating with HBr (30% w/w) in glacial CH_3COOH.[27] The ammonium nitrogen of the formed AlaAibOBz was then deprotonated using a saturated aqueous solution of Na_2CO_3 followed by extraction of the dipeptide into dichloromethane. The dipeptide was then coupled to CbzAib by the DCC method, leading to CbzAibAlaAibOBz. The protecting groups were then removed by catalytic hydrogenation on Pt/C,[26] leading to free tripeptides, AibAlaAib. Details of the syntheses and characterizations of the tripeptides are given in the Supporting Information, including full assignments of the ^1H and ^{13}C NMR signals (Figure S1 and Tables S1 and S2) and full IR spectra (Figure S2).

Na[Cr^V(O)(H$_{-3}$Aib$_3$-DMF)]·4.5H$_2$O (1). In a N_2-atmosphere box, anhydrous $CrCl_2$ (0.42 g, 3.1 mmol) was added to a deoxygenated stirred solution of Aib_3 (1.0 g, 3.1 mmol) in DMF (50 mL). A purple precipitate rapidly formed, and potassium *tert*-butoxide (1.1 g, 9.3 mmol) was added after 5 min, resulting in a color change to gray/black with simultaneous dissolution of the purple precipitate. At 15 min after dissolution of the precipitate, aqueous *tert*-butyl hydroperoxide (70% w/v, 10 mL) was added, and the mixture was stirred for 12 h at 22 °C. Further manipulations were performed under ambient atmosphere. The reaction solution was diluted to 100 mL with water, filtered through a Celite pad, and loaded onto a Sephadex (DEAE A-25, Amersham) column (2.5 × 20 cm). The column was flushed with water (500 mL) to elute any nonanionic species and DMF. An aqueous solution of NaCl (0.20 M) was then used to elute a deep orange band, leaving a bright yellow band on the column. The latter was eluted with 2.0 M NaCl and contained $[CrO_4]^{2-}$ (λ_{max} = 372 nm, no EPR signals).[28] The orange fraction was taken to dryness under reduced pressure, and the solid was extracted with acetonitrile. The acetonitrile solution was filtered and again taken to dryness, and the acetonitrile extraction process was repeated. Yield of deep brown solid: 0.50 g (31.5%). ESMS (−ve ion; MeCN): *m*/*z* 408.4. UV−vis (MeCN) [λ/nm (ϵ/dm^3 mol^{-1} cm^{-1})]: 300 (5500), 420 (1900). IR (solid mixture with KBr; ν_{max}/cm^{-1}): 3400 w (broad), 2977 w, 2931 w, 2872 w, 1686 s, 1622 s, 1531 m, 1454 m, 1373 s, 1308 m, 1195 m, 1022 w, 972 m, 655 w, 479 m. Anal. Calcd for $C_{15}H_{33}N_4CrNaO_{10.5}$: C, 35.16; H, 6.49; N, 10.93. Found: C, 34.93; H, 6.57; N, 10.36.

Na[Cr^V(O)(H$_{-3}$Aib$_L$AlaAib-DMF)]·3.5H$_2$O (2). This compound was prepared using the same procedure as for **1**, except that Aib$_L$AlaAib (1.0 g, 3.86 mmol) was used in the place of Aib_3. A longer time (12 h) was required for the reaction of $CrCl_2$ with the ligand in deoxygenated DMF as, unlike Aib_3, this ligand was only sparingly soluble. Yield of brown-black solid: 0.42 g (22.6%). ESMS (−ve ion; MeCN): *m*/*z* 394.5. UV−vis (MeCN) [λ/nm (ϵ/dm^3 mol^{-1} cm^{-1})]: 289 (5.9 × 10^3), 398 (2.7 × 10^3). CD (MeCN) [λ/nm ($\Delta\epsilon$/cm^2 mmol^{-1})]: 237 (15.4), 274 (−7.4), 307 (1.1), 340 (2.4), 398 (−6.0), 488 (3.2). IR (solid mixture with KBr; ν_{max}/cm^{-1}): 3360 w (broad), 2976 w, 2934 w, 2873 w, 1681 s, 1620 s, 1456 m, 1372 s, 1344 m, 1311 m, 1204 w, 970 w, 900 w, 470 w. Anal. Calcd for $C_{14}H_{29}N_4CrNaO_{9.5}$: C, 35.00; H, 6.08; N, 11.66. Found: C, 34.95; H, 5.63; N, 11.18.

Na[Cr^V(O)(H$_{-3}$Aib$_D$AlaAib-DMF)]·H$_2$O (3). This compound was prepared using the same procedure as for **2**, except that Aib$_D$AlaAib·H_2O (1.0 g, 3.61 mmol) was used in the place of Aib$_L$AlaAib. Yield of brown-black solid: 0.43 g (27.2%). ESMS (−ve ion; MeCN): *m*/*z* 394.5. UV−vis: identical with that of **2**. CD: identical with that of **2** but with opposite signs for the $\Delta\epsilon$ values. IR: identical with that of **2**. Anal. Calcd for $C_{14}H_{24}N_4CrNaO_7$: C, 38.62; H, 5.56; N, 12.87. Found: C, 38.17; H, 5.40; N, 12.48.

Mass-Spectrometric Characterization of the Ligand from the Complex 1. A stock solution of **1** in H_2O (∼1.0 mM) was freshly prepared. This solution (0.50 mL) was mixed with aqueous solutions of ascorbic acid (AsA, 0.50 mL, 40 mM) or L-cysteine (Cys, 0.50 mL, 40 mM). Complete reduction of Cr(V) to Cr(III) by AsA or Cys (judged from the disappearance of the brown color of Cr(V)) was achieved in ∼1 min or ∼5 h, respectively (22 °C). Mass spectra of the reaction mixtures were taken at ∼5 h after mixing. The pH values of the reaction mixtures (measured at 5 h after the mixing) were as follows: 6.6 for the aqueous solution; 4.5 for the solution containing AsA; 6.2 for the solution containing Cys.

Analytical Techniques. Elemental analyses (C, H, N) were performed by the Microanalytical Laboratory at the Australian National University, Canberra.

EPR-spectroscopic measurements were carried out on a Bruker EMX X-band spectrometer equipped with an ER 041XG microwave bridge, an EMX 148T microwave bridge controller, an EMX 035M NMR gaussmeter, an EMX 032T field controller, and an EMX 120 modulation amplifier. Spectra were recorded at 22 °C from solutions contained in a quartz flat cell. EPR spectrometer operating parameters were as follows: operating frequency, ∼9.7 GHz; microwave power, 6.36 mW; modulation frequency, 100 kHz; modulation amplitude, 0.5 G; center field, 3480 G; sweep width, 100 G; time constant, 40.96 ms; conversion time, 20.48 ms; receiver gain, 1 × 10^4; number of scans, 10. Simulation of the EPR spectra were performed using the program WinSim, and second-order corrections were applied in the simulations.[29]

Electrospray mass spectrometry (ESMS) was performed using a Finnigan LCQ mass spectrometer; experimental settings were as follows: sheath gas (N_2) pressure, 60 psi; spray voltage, 5.0 kV; capillary temperature, 200 °C; capillary voltage, 19 V; tube lens offset, 25 V; *m*/*z* range, 100−2000 (both in positive- and negative-ion modes). Analyzed solutions were injected into a flow of H_2O/MeOH (1:1 v/v, flow rate 0.20 mL min^{-1}). Acquired spectra were the averages of 10 scans (scan time 10 ms). Simulations of the mass spectra were performed using IsoPro software.[30]

Vibrational spectra (IR) were recorded by the diffuse reflectance technique on a Bio Rad FTS-40 spectrometer with KBr as the matrix (ν = 400−4000 cm^{-1}). Electronic absorption (UV−vis) spectra were recorded on a Varian Cary 5 spectrophotometer using a 1-cm path length quartz cell (λ = 200−800 nm). Circular dichroism (CD) spectra were acquired on a JASCO 710 spectropolarimeter. The instrument settings were as follows: wavelength range, 200−700 nm; scan rate, 100 nm min^{-1}; response time, 0.25 s. The reported CD spectra were the averages of five scans.

Cyclic voltammetry (CV) was performed using a BAS 100B electrochemical analyzer. The complexes **1**−**3** were dissolved in DMF with (^{n}Bu$_4$N)BF_4 (0.10 M) as the supporting electrolyte. A three-electrode system with a glassy-carbon working electrode (3.0-mm diameter), an aqueous Ag/AgCl reference electrode, and a Pt

(25) Hamburg, A.; Chih, H.; Getek, T. A. *Inorg. Chem.* **1985**, *24*, 2593−2594.
(26) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 2.
(27) Ben-Ishai, D.; Berger, A. *J. Org. Chem.* **1952**, *17*, 1564−1570.
(28) Brasch, N. E.; Buckingham, D. A.; Evans, A. B.; Clark, C. R. *J. Am. Chem. Soc.* **1996**, *118*, 7969−7980.
(29) Duling, D. R. *J. Magn. Reson.* **1994**, *B104*, 105−110. The software is available via the Internet at http://EPR.niehs.nih.gov/.
(30) Senko, M. *IsoPro 3.0*; Sunnyvale, CA, 1998.

wire counter electrode were used. Full *iR* compensation was applied for all scans. The ferrocenium/ferrocene ($Fc^{+/0}$) couple was used as an internal redox potential standard in all experiments.

Results

Syntheses of the Ligands. Although several modifications were reported in the literature for the synthesis of the tripeptide Aib_3,[25,31] the original procedure of Margerum and co-workers[23] was mainly followed in this work. Important variations, which improved the yields by ~20%, were a short (15 min) reaction time for the removal of the Cbz group from CbzAibAibOBz and deprotonation of the resultant AibAibOBz·HBr prior to its use in the coupling reaction with CbzAib (see the Supporting Information for details). The same method was successfully used for the syntheses of new tripeptides AibLAlaAib and AibDAlaAib.

Syntheses of the Cr(V) Complexes. The synthetic procedure used in the preparation of the Cr(V) complexes **1**−**3** was based on the techniques used by Collins and co-workers for the syntheses of Cr(V) complexes with tetra-amide macrocyclic ligands H_4mampa and H_4mac.[17] The use of more polar peptide ligands in this work required the use of a different solvent/base combination to form a Cr(II)−ligand complex (DMF/KOBu^t instead of tetrahydrofuran/^{t}BuLi).[17] Upon addition of $CrCl_2$ to a DMF solution of the tripeptide ligand, a purple-colored precipitate formed, suggesting the formation of a Cr(II)−peptide intermediate (possibly involving coordination through the carboxylate and amine groups). A rapid color change was observed from purple to deep gray-brown with simultaneous dissolution of the purple precipitate when KOBu^t was added to the mixture, indicating the formation of a deprotonated peptide complex of Cr(II). Oxidation of the Cr(II) complex was performed using an aqueous solution of ^{t}BuOOH, which resulted in a rapid color change from dark gray to deep orange-brown. The Cr(V) complexes formed in this way were anionic and were purified by anion-exchange chromatography.

Structures and Spectroscopy of the Cr(V) Complexes. In the previously studied Cu(III) and Ni(III) complexes of Aib_3,[16,23,24] the ligand was coordinated via the amine nitrogen, two deprotonated amide nitrogens, and an oxygen of the carboxylate group in a square-planar geometry. It was anticipated that the Cr(V)−peptide complexes would display the same ligand coordination with an oxo group in an axial position.[4,17,18] However, the data obtained from elemental analyses for **1**−**3** were inconsistent with this expected structure. Much better agreement between the observed and calculated elemental compositions (C, H, N) was obtained if a DMF molecule was incorporated into the chemical formula, suggesting that a DMF molecule was coordinated to the Cr center or had become covalently bound to the peptide ligand or was a solvate of crystallization. It is improbable that DMF would remain coordinated to Cr(V) if the final complex was six-coordinate, since water was used during its synthesis and chromatographic purification (see Experimental Section) and would be expected to have displaced the labile DMF ligand, which would be weakly bound through the carbonyl oxygen.[32] For similar reasons, it is unlikely that DMF would be present as a solvent of crystallization. Since the results of the elemental analyses were inconsistent with the coordination of a simple deprotonated Aib_3 ligand, a detailed spectroscopic analysis was required to characterize the Cr(V) complexes.

The IR spectra of **1**−**3** (shown in Figure S2, Supporting Information, in comparison with those of the corresponding ligands) were consistent with the formation of pure Cr complexes (since the spectra were similar for all of the complexes, Figure S2). The spectra of the complexes were distinguished from those of the ligands by the absence of broad signals at $\nu = 2500-3500$ cm^{-1} (due to the N−H stretching vibrations of the amide and amine groups) and the presence of sharp signals at ~970 cm^{-1}, characteristic for Cr≡O bonds (Figure S2).[17,33] The latter feature supports the formation of a Cr(V) oxo complex.

The negative-ion ESMS spectra of **1**−**3** displayed essentially only one ion in each case, located at $m/z = -408.4$ for **1** and −394.5 for **2** and **3**; the isotope distribution patterns were consistent with the formulations $[C_{15}H_{24}N_4O_6Cr]^-$ for **1** and $[C_{14}H_{22}N_4O_6Cr]^-$ for **2** and **3** (Figure 1). No significant signals were observed at $-m/z > 1000$ or in the positive-ion mode ($m/z = 100-2000$). The ESMS data (Figure 1) are consistent with the observations from ion-exchange chromatography (Experimental Section), showing that the isolated Cr(V) complexes are monoanionic species, as well as with the results of elemental analyses, showing the binding of a DMF molecule to each of the complexes **1**−**3**. The formulations of the complexes, $[Cr^V(O)(H_{-7}L\text{-DMF})]^-$ (where L = Aib_3 or AibAlaAib), deduced from the ESMS data, point to covalent binding of a DMF molecule to the ligand (see Discussion), since the charge balance is inconsistent with DMF binding as a neutral ligand to the Cr(V) center.

Typical EPR spectra of **1**−**3** (in MeCN, 22 °C) are shown in Figure 2, a list of EPR spectral parameters (determined by simulations of the spectra)[29] is given in Table 1, and typical simulation results are shown in Figure S3, Supporting Information. The main feature in the EPR spectra of all three compounds (Figure 2) was a seven-line signal centered at $g_{iso} \sim 1.98$; this multiplet pattern is consistent with the coupling from three equivalent ^{14}N nuclei ($I = 1$). There were also weak satellite peaks due to hyperfine splitting of ^{53}Cr (9.55% abundance, $I = 3/2$). The magnitudes of the splitting (the $A_{iso}(^{53}Cr)$ values in Table 1) were consistent with a metal-centered radical, confirming that the Cr atom is d^1 and in the formal oxidation state of Cr(V).[33] The ^{14}N superhyperfine coupling constants obtained from the seven-line signals (the $a_{iso}(^{14}N)$ values in Table 1) observed here are consistent with those expected from three spectroscopically equivalent amide ^{14}N atoms.[17,18,33] Superhyperfine

(31) Lockwood, M. A.; Blubaugh, T. J.; Collier, A. M.; Lovell, S.; Mayer, J. M. *Angew. Chem., Int. Ed.* **1999**, *38*, 225−227.

(32) Dossing, A.; Engberg, P.; Hazell, R. *Acta Chem. Scand.* **1997**, *51*, 849−854.

(33) (a) Farrell, R. P.; Lay, P. A. *Comments Inorg. Chem.* **1992**, *13*, 133−175. (b) Barr-David, G.; Charara, M.; Codd, R.; Farrell, R. P.; Irwin, J. A.; Lay, P. A.; Bramley, R.; Brumby, S.; Ji, J.-Y.; Hanson, G. R. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1207−1216.

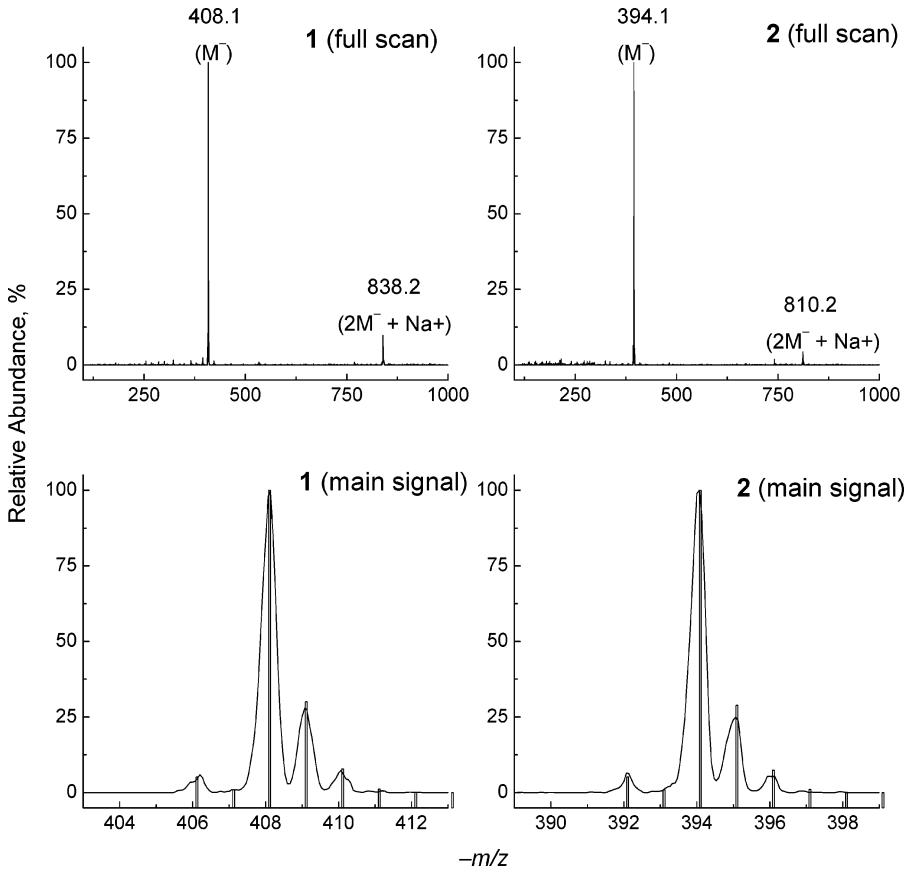


Figure 1. Typical negative-ion ESMS data for **1** and **2** (∼1 mM in MeCN, ∼5 min after dissolution, 22 °C). The spectra for **3** were not significantly different from those of **2**. Experimental (lines) and simulated (columns, $[C_{15}H_{24}N_4O_6Cr]^-$ for **1** and $[C_{14}H_{22}N_4O_6Cr]^-$ for **2**) isotopic distributions of the main signals are also shown.

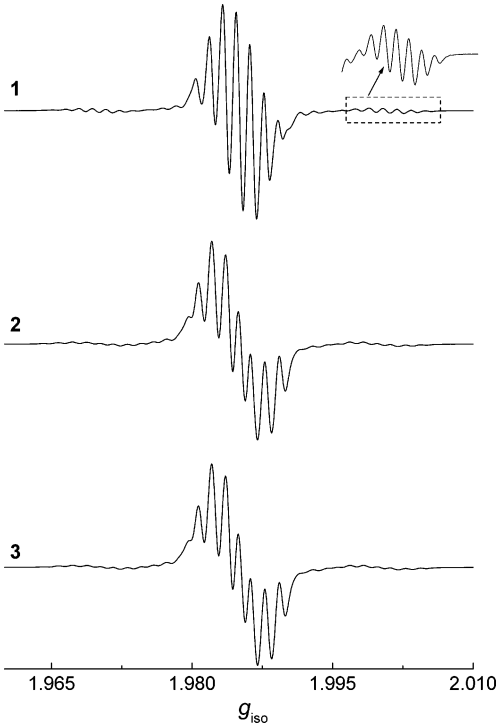


Figure 2. Typical EPR spectra of freshly prepared (within ∼5−10 min) solutions of **1** (17.2 mM), **2** (15.6 mM), and **3** (17.5 mM) in MeCN at 22 °C. The inset shows an expanded view of one of the signals due to ^{53}Cr hyperfine splitting. Spectral parameters are listed in Table 1.

Table 1. EPR Spectroscopic Parameters of **1**−**3** (MeCN solutions, 22 °C, Figure 2)

param	complex **1**	complex **2**	complex **3**
	Species 1		
abundance, %	100	54.0	58.6
g_{iso}	1.9817	1.9822	1.9822
line width, 10^4 cm^{-1}	1.2	1.1	1.2
$A_{iso}(^{53}Cr)$, 10^4 cm^{-1}	16.9	14.8	14.8
$a_{iso}(^{14}N)$, 10^4 cm^{-1}	2.36	2.36	2.36
$a_{iso}(^{1}H)$, 10^4 cm^{-1}		0.65	0.66
	Species 2		
abundance, %		46.0	41.4
g_{iso}		1.9831	1.9830
line width, 10^4 cm^{-1}		1.3	1.3
$A_{iso}(^{53}Cr)$, 10^4 cm^{-1}		16.7	16.7
$a_{iso}(^{14}N)$, 10^4 cm^{-1}		2.67	2.69
$a_{iso}(^{1}H)$, 10^4 cm^{-1}		0.72	0.74

couplings from amine nitrogens coordinated to Cr(V) are often not observed and, when they are resolved, the magnitude of the couplings are different from those due to amide nitrogens.[18,34] These results confirm that the coordination of the tripeptides to the Cr(V) center was different from that of the previously reported Cu(III/II) and Ni(III/II) complexes of Aib_3.[16,20,21,23,24]

An excellent simulation ($R^2 = 0.997$) was obtained for the EPR spectrum of **1** assuming coupling from three equivalent amide nitrogens (^{14}N) coordinated to a single Cr

(34) Weeks, C. L.; Levina, A.; Dillon, C. T.; Turner, P.; Fenton, R. R.; Lay, P. A. *Inorg. Chem.* **2004**, *43*, 7844−7856.

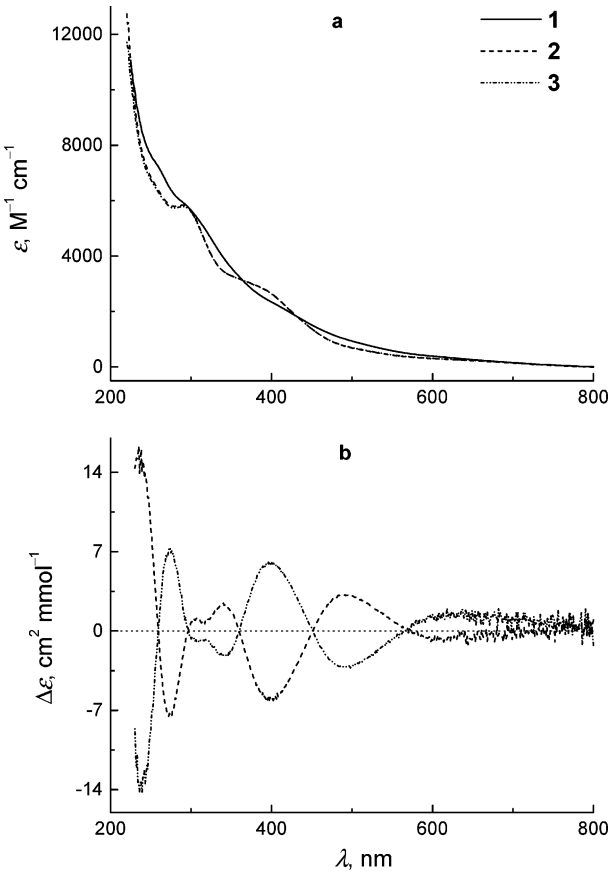


Figure 3. UV−vis (a) and CD (b) spectra of freshly prepared (within 5−10 min) solutions of **1**−**3** in MeCN ([Cr] = 0.35 mM, 22 °C). No significant CD signals were observed for **1**.

center (Figure S3). Three equivalent ^{14}N nuclei were also used in the EPR simulations for **2** and **3**, taking into account the additional coupling from the α-hydrogen of the Ala. The ^{1}H superhyperfine coupling constants for **2** and **3** (Table 1) were close to the values observed for Cr(V) diolato complexes.[33] Good fits (R^2 = 0.993 or 0.994, respectively) were obtained only when two separate species were used to simulate the observed spectra for **2** or **3** (Table 1 and Figure S3), while significantly lower R^2 values (0.941 or 0.952, respectively) were obtained when only one species was used (Figure S3). The optimized EPR parameters for the two species were consistent between the spectra of **2** and **3** (Table 1), which suggests that the improvement of the fit for the two-species model was not caused simply by an increase in the number of varied parameters. By contrast, including a second species into the simulations for **1** did not result in a significant improvement of the fit (R^2 = 0.998, Figure S3).

The electronic absorption (UV−vis) spectrum for **1** in MeCN (Figure 3a) was characterized by a relatively featureless absorption at 400−800 nm, and two poorly resolved shoulders were evident at ∼420 nm and ∼300 nm. Identical electronic absorption spectra were obtained for complexes **2** and **3** (Figure 3a), and these were similar to the spectrum obtained for **1**, although the resolved bands were more prominent with a distinct maximum at 289 nm due to a ligand-to-metal charge transfer (LMCT) transition and a shoulder at 398 nm (d−d transitions) and a weak band at

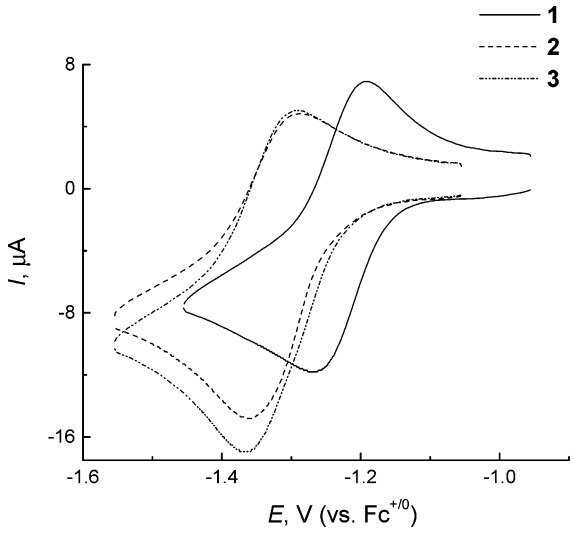


Figure 4. Cyclic voltammograms of **1**−**3** ([Cr] = 1.0 mM, 22 °C) in DMF with (^{n}Bu$_4$N)BF$_4$ (0.10 M) as the supporting electrolyte at a glassy carbon electrode.

650 nm. The different electronic transitions for **2** and **3** were more easily observed with the aid of CD spectra. The CD spectra obtained for **2** and **3** (Figure 3b) were mirror images, showing that the chirality of the ligands was retained in the Cr(V) complexes and that **2** and **3** are enantiomeric. The values of molar extinction coefficients in UV−vis and CD spectra of **1**−**3** are listed in the Experimental Section.

Cyclic voltammetry of **1**−**3** in DMF solutions (in the presence of (^{n}Bu$_4$N)BF$_4$ as a supporting electrolyte) showed quasi-reversible Cr(V/IV) redox couples for all of the complexes (Figure 4); the $E_{1/2}$ values (vs Fc$^{+/0}$) were −1.23 V for **1**, −1.33 V for **2**, and −1.34 V for **3**, and the respective peak-to-peak potential separations (ΔE_p) were 67, 58, and 57 mV. While the ΔE_p values for **2** and **3** corresponded to those of reversible redox reactions,[35] the values of cathodic and anodic peak currents for these two compounds were unequal ($i_{pc} > i_{pa}$, Figure 4), which indicates that the Cr(IV) counterparts of **2** and **3** are unstable and some decomposition or reorganization of the Cr(IV) species occurs on the time scale of the experiments. Under similar conditions (DMF solutions, 0.10 M (Bu$_4$N)BF$_4$, 22 °C), **1**−**3** are significantly weaker oxidants ($E_{1/2}$ = −1.23 to −1.34 V vs Fc$^{+/0}$) than the Cr(V) complexes with 2-hydroxycarboxylato or macrocyclic amido ligands, which can also be reduced quasi-reversibly to Cr(IV) ($E_{1/2} \sim -0.9$ V vs Fc$^{+/0}$).[36−38]

Stability of the Cr(V) Peptide Complexes. The complexes **1**−**3** are hygroscopic in the solid state, but samples stored in a desiccator at 22 °C (protected from light) showed no signs of decomposition (by UV−vis and EPR spectroscopies and ESMS) for at least 3 years. Solutions of **1**−**3** in DMF or DMSO ([Cr] = 10 mM) or in MeCN ([Cr] = 1.0 mM) were stable for at least 1 month at 22 °C. Despite the high stability of **1**−**3** in nonaqueous media, repeated

(35) Willard, H. H.; Merritt, L. L.; Dean, J. A.; Settel, F. A. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth: Belmont, CA, 1988.
(36) Judd, R. J.; Hambley, T. W.; Lay, P. A. *J. Chem. Soc., Dalton Trans.* **1989**, 2205−2210.
(37) Dillon, C. T. Ph.D. Thesis, The University of Sydney, 1995.
(38) Levina, A. Unpublished results, 2003.

Table 2. Typical Results of Stability Studies for the Cr(V)−Peptide Complexes

complex[a]	buffer	pH	addition	% Cr(V) remaining[b,c]	% Cr(V) decomposd via eq 2[c]
1	phosphate	7.0		23	96
1	phosphate	7.0	GSH (5.0 mM)	0	100
1	phosphate	7.0	H_2O_2 (5.0 mM)	22	60
1	phosphate	4.0		32	100
1	HEPES	7.4		18	95
2	phosphate	7.0		68	82
3	phosphate	7.0		71	87

[a] In all cases, [Cr] = 0.30 mM. Stock solutions of **1**−**3** in DMF ([Cr] = 30 mM, prepared on the day of experiments) were used for the preparation of the aqueous solutions of the complexes. [b] Reaction time 7 d at 22 °C. [c] Determined from the changes in UV−vis spectra of the solutions. The amounts of remaining Cr(V) species and the Cr(VI) decomposition species were determined from their characteristic absorbances at 534 nm and 350−372 nm, respectively (Figure S5).[28,40,41] The proportion of Cr(V) decomposed by disproportionation (eq 1) was calculated from the amount of Cr(VI) generated.

attempts to grow X-ray-quality crystals of these compounds have been unsuccessful, as yet.

The characteristic seven-line EPR signals of **1**−**3** (Figure 2 and Table 1) persisted for at least 1 week in aqueous phosphate buffers (0.10 M, pH 7.0, [Cr] = 0.30 mM) at 22 °C. A comparison of the EPR spectra for fresh and 1-week-old aqueous solutions (Figure S4 in Supporting Information) showed a significant decomposition of **1** during that time, as evident from a decrease in signal-to-noise ratio and the appearance of a new EPR signal (broad singlet, g_{iso} = 1.9660). The absence of the ^{14}N coupling for the latter signal suggests that it is due to a hydrolysis product, where the N-donors were fully or partially replaced with O-donors.[33] The decomposition of **2** or **3** under the same conditions was much less obvious (Figure S4).

The lower stability of **1** compared with **2** or **3** was confirmed by UV−vis spectroscopy (Table 2; typical spectra are shown in Figure S5, Supporting Information). Decomposition in aqueous solutions for most of the known Cr(V) complexes occurs via parallel disproportionation (eq 1) and ligand/solvent oxidation (eq 2) routes, with the former reaction typically predominant in basic media and the latter process becoming more important with the decrease in the pH value.[9,34,39,40]

$$3\mathrm{Cr(V)} \rightarrow 2\mathrm{Cr(VI)} + \mathrm{Cr(III)} \quad (1)$$

$$\mathrm{Cr^{V}{=}O} + \mathrm{S} \rightarrow \mathrm{Cr(III)} + \mathrm{S{=}O} \quad (\mathrm{S} = \text{organic substrate}) \quad (2)$$

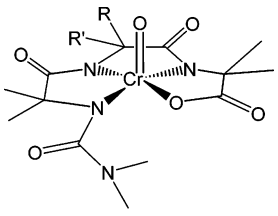
The UV−vis spectrum of the decomposed solution of **1** in aqueous phosphate buffer (0.10 M, pH 7.0, [Cr] = 0.30 mM) after 7 days at 22 °C shows a general decrease in absorbance at 300−800 nm compared with that of a fresh solution (Figure S5), which is indicative of the predominant formation of Cr(III) under these conditions.[4,41] The data contained in Table 2 suggest that complexes **1**−**3** decompose mainly (>80%) via eq 2 (where the ligand acts as a reductant) at the physiological range of pH values (4.0−7.4)[41] in the absence of added oxidants or reductants.

Addition of H_2O_2 (5.0 mM) to a solution of **1** (0.30 mM) at pH 7.0 (0.10 M phosphate buffer, reaction time 7 days at 22 °C) leads to a partial oxidation of the Cr(V) complex with the formation of $[CrO_4]^{2-}$, detected from its characteristic absorbance at λ_{max} = 372 nm[28] (Table 2 and Figure S5). Reactions of **1** with the most common biological reductants (Red), glutathione (GSH), L-cysteine (Cys), or ascorbic acid (AsA), in phosphate buffer (0.10 M, pH 7.0, [Red] = 5.0 mM, [Cr] = 0.30 mM, 22 °C) led to a complete reduction of Cr(V) to Cr(III) within ∼5 min (for AsA), ∼10 h (for Cys), or ∼30 h (for GSH), as determined from the disappearance of the Cr(V) EPR signals and the changes in UV−vis spectra (an example for the reaction with GSH is shown in Figure S5).

Reduction of **1** by AsA or Cys in aqueous solutions leads to the release of the free ligand, LH_4 = Aib_3-DMF, as shown by ESMS of the reduction products (m/z = −343.2 for LH_3^-, +345.1 for $LH_4{\cdot}H^+$, and +367.2 for $LH_4{\cdot}Na^+$, Figure S6 and Table S3 in Supporting Information). No signals due to the parent ligand (Aib_3, fw = 273.2) were observed (Figure S6). Thus, ESMS data for the decomposed solutions of **1** strongly support the formation of a covalent bond between the peptide ligand and a molecule of solvent (DMF) during the synthesis of the Cr(V) complex.

Discussion

On the basis of the results of spectroscopic and analytical studies, a molecular structure was assigned for the anionic complexes in **1**−**3**, in which a DMF solvent molecule has become covalently bound to the amine group of the ligand, to form a substituted urea group. The amine nitrogen of a tripeptide is thus converted to a deprotonated amido nitrogen that is coordinated to the Cr(V) center.



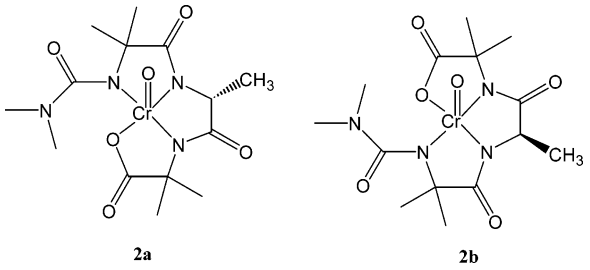
1: R = R' = Me

2,**3**: R = H, R' = Me

The EPR spectra for solutions of **2** or **3** in MeCN (Figure 2 and Table 1) that show the presence of at least two species can be explained by the existence of two stereoisomers of **2** or **3**, in which the methyl group of the Ala residue is located

(39) Krumpolc, M.; Roček, J. *Inorg. Chem.* **1985**, *24*, 617−621.
(40) Levina, A.; Lay, P. A.; Dixon, N. E. *Inorg. Chem.* **2000**, *39*, 385−395.
(41) Lay, P. A.; Levina, A. *J. Am. Chem. Soc.* **1998**, *120*, 6704−6714.

either above or below the equatorial plane with respect to the oxo ligand (**2a**,**b** or mirror images of these structures for **3**).



2a **2b**

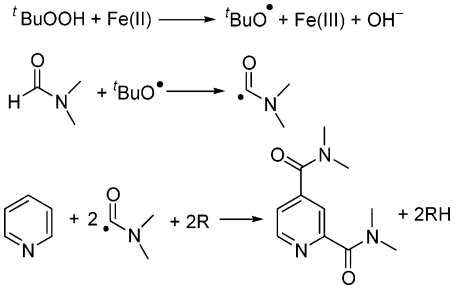
A slight preference for one of the stereoisomers in solutions, as detected by EPR spectroscopy (~55% vs ~45%, Table 1), may be due to unfavorable steric interactions in **2b**, where the oxo group and the methyl group are on the same side of the ligand plane.

The stability of **1**−**3** in neutral aqueous solutions (Table 2) is comparable with that of Cr(V) complexes with macrocyclic tetraamide ligands[37,42] and is much higher than that of well-studied Cr(V) 2-hydroxycarboxylato complexes (which persist for a few minutes at pH 7.0 and 22 °C).[39,40] This high stability of **1**−**3** is in agreement with the observation that they are weak oxidants in nonaqueous media, as shown by electrochemical studies (Figure 4). The higher stability of **2** and **3**, compared with **1**, in aqueous media (Table 2), is consistent with their lower reduction potentials in DMF solutions (Figure 4). These observations contradict the suggestion[16,23] that the presence of methylene hydrogen atoms in peptide ligands (such as AibAlaAib) will make them more susceptible to the reactions with oxidizing metal centers (such as Cr(V)), compared with the complexes of Aib_3, where such hydrogen atoms are absent. The stability of **1** may be decreased due to the steric clashes of eight methyl groups in the ligand (compared with seven such groups in **2** and **3**).

To our knowledge, the conversion of the peptide ligands in **1**−**3** to substituted urea derivatives represents a novel type of organic transformation. Probably the closest analogy for such a reaction is the oxidative coupling of amides (such as DMF) with heteroaromatic compounds (such as pyridine) in the presence of organic peroxides and Fe(II) compounds, which is thought to proceed through the formation of free carbamoyl radicals (Scheme 1).[43]

Rapid formation of Cr(II) complexes during the reactions of the tripeptide ligands with $CrCl_2$ and the relatively high yields of **1**−**3** (higher than those expected for a radical chain reaction, similar to that in Scheme 1)[43] suggest that the oxidative coupling of DMF to the tripeptides is more likely to occur via a Cr-directed process without the release of free radical species into the reaction medium. Schemes 2 and 3 illustrate two alternative schemes that are plausible to explain the formation of the Cr(V) product with the urea/peptide ligands. Reduction of **1** with L-cysteine or ascorbate, which

Scheme 1. Proposed Mechanism for the Oxidative Coupling Reaction of DMF and Pyridine[43]



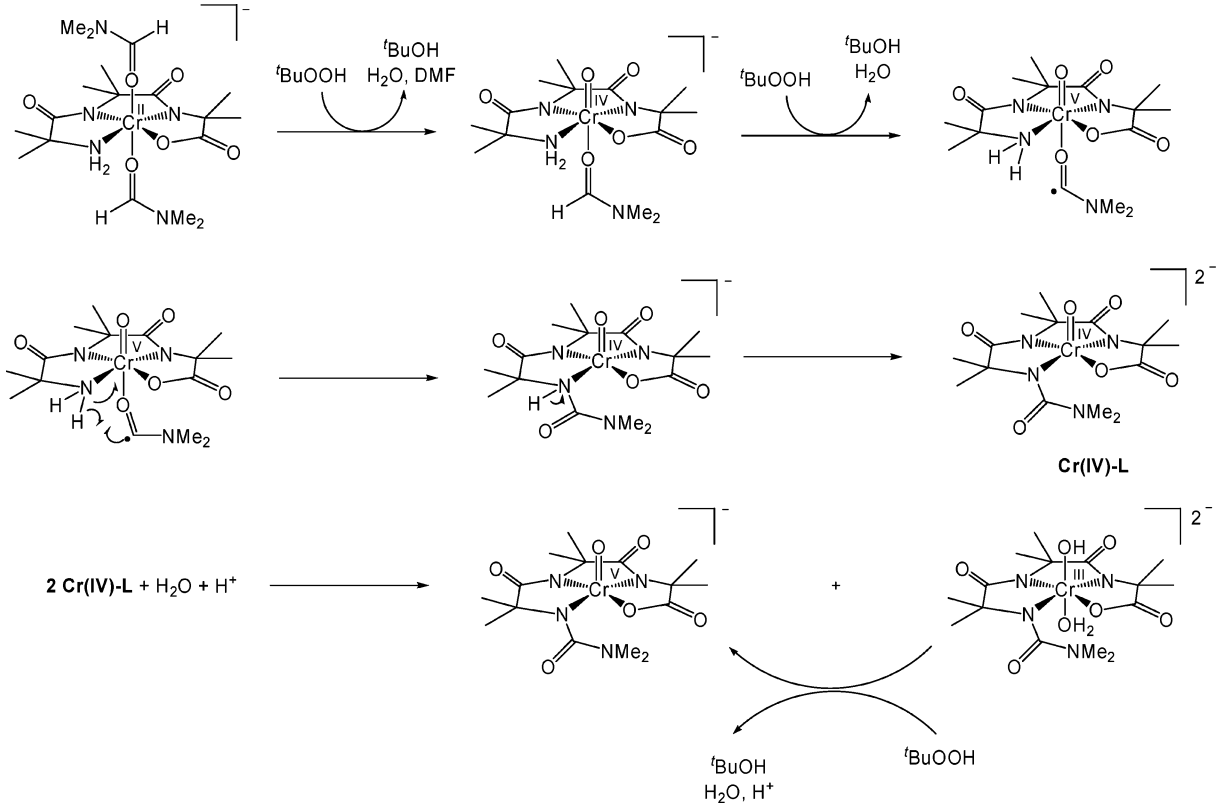
also act as ligands for the formed Cr(III) ions,[41] leads to the release of the modified peptide ligand, Aib_3-DMF, which is stable for at least several hours in aqueous solutions at pH 4−6 and 22 °C (as shown by ESMS of the reduction products of **1**, Figure S6 and Table S3).

Unsuccessful attempts to generate Cr(V) complexes of Aib_3 and related ligands by oxidation of the corresponding Cr(II) complexes in polar organic solvents other than DMF (e.g., DMSO, sulfolane, or hexamethylphosphoramide)[44] show that the replacement of the amine group in the peptide ligands with an amido donor is crucial for the stabilization of Cr(V). Previously, reduction of Cr(VI) in the presence of peptide ligands (Gly_n or Ala_n, $n = 3-5$) or oxidation of the corresponding Cr(III)−peptide complexes led to the formation of Cr(V)−peptide complexes, which were detectable by EPR spectroscopy but were too unstable to isolate.[18,19] Similarly, no stable Cr(V) complexes could be isolated for a modified tripeptide ligand, *N*,*N*-dimethyl-AibAibAib, where the site for the formation of a DMF adduct was blocked (synthesis and characterization of this ligand are described in the Supporting Information).[44] It is likely that the coordination of Cr(V) to the unprotected amino groups of the peptide ligands was the main reason for the instability of these complexes.[18,19,44]

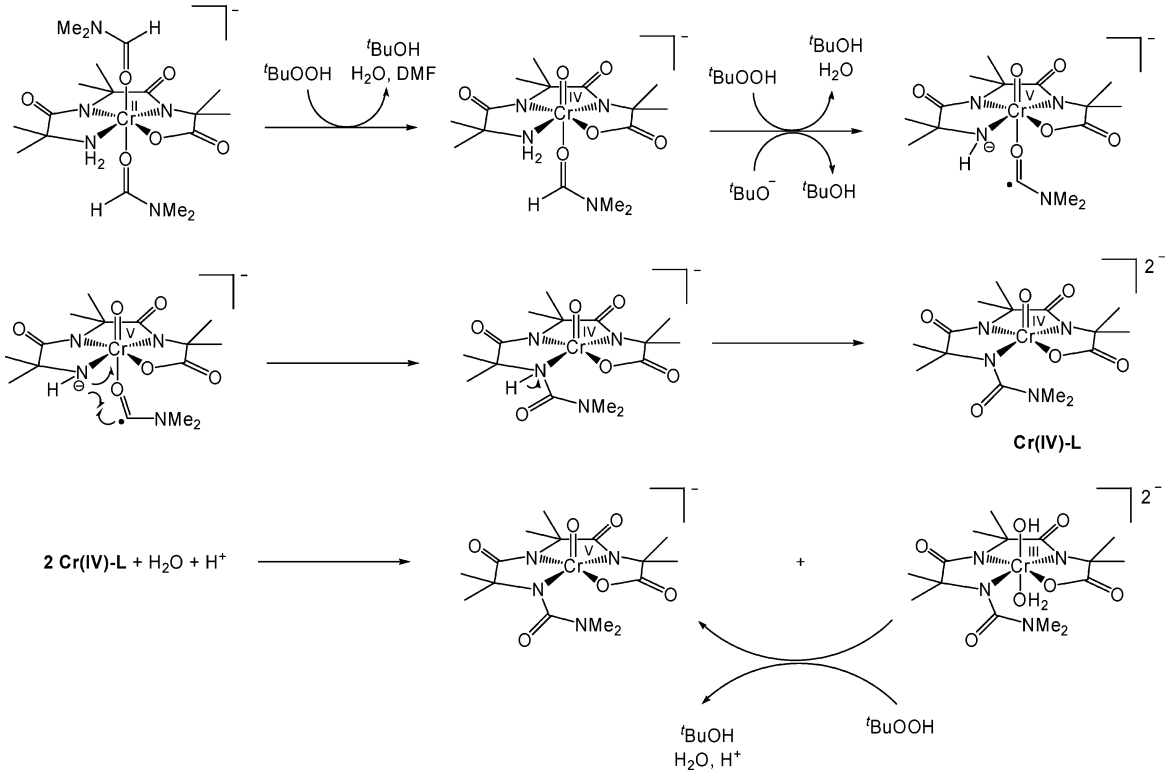
Although the syntheses of **1**−**3**, involving the use of Cr(II) and organic solvents (see Experimental Section), are not compatible with biological conditions, the formation of stable Cr(V) complexes with amido and carboxylato donors (such as **1**−**3**) is relevant to the proposed role of Cr(V) as a major reactive species in Cr(VI)-induced genotoxicity.[4,5,45] Reactions of Cr(VI) with intracellular reductants are likely to lead to the formation of stable Cr(III) complexes with proteins and peptides,[5] which can be subsequently reoxidized to Cr(V/VI) species by cellular oxidants,[46] including H_2O_2-producing enzymatic systems,[47] and peroxides of proteins or lipids (which are similar to ^{t}BuOOH in chemical properties).[48] Such reoxidations are likely to be responsible for the long-term toxicity caused by the exposure to Cr(VI), which leads to accumulation of Cr(III) in cells.[5,45,46] The possibility

(42) Dillon, C. T.; Lay, P. A.; Bonin, A. M.; Dixon, N. E.; Collins, T. J.; Kostka, K. L. *Carcinogenesis* **1993**, *14*, 1875−1880.
(43) Arnone, A.; Cecere, M.; Galli, R.; Minisci, F.; Perchinunno, M.; Porta, O.; Gardini, G. *Gazz. Chim. Ital.* **1973**, *103*, 13−29.
(44) Barnard, P. J. Ph.D. Thesis, University of Sydney, 2002.
(45) Levina, A. Lay, P. A. *Coord. Chem. Rev.* **2005**, *249*, 281−298.
(46) Dillon, C. T.; Lay, P. A.; Bonin, A. M.; Cholewa, M.; Legge, G. J. F. *Chem. Res. Toxicol.* **2000**, *13*, 742−748.
(47) Mulyani, I.; Levina, A.; Lay, P. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 4504−4507.
(48) Knott, H. M.; Baoutina, A.; Davies, M. J.; Dean, R. T. *Arch. Biochem. Biophys.* **2002**, *400*, 223−232.

Scheme 2. Proposed Mechanism for the Oxidative Coupling of DMF with a Tripeptide Ligand during the Syntheses of **1**−**3** in Which the Coordinated DMF Radical Reacts with the Amine of the Peptide Ligand



Scheme 3. Proposed Mechanism for the Oxidative Coupling of DMF with a Tripeptide Ligand during the Syntheses of **1**−**3** in Which the Coordinated DMF Radical Reacts with the Deprotonated Amine of the Peptide Ligand



of formation of stable (and potentially DNA-damaging)[4,5] Cr(V)−peptide complexes as a result of cellular reoxidation of Cr(III)−peptide complexes is supported by the results of this work. Synthesis and characterization of the Cr(V)−peptide complexes **1**−**3** (which are the first isolated Cr(V) complexes with non-sulfur-containing peptide ligands)[9,49] will

facilitate the studies of biological activities of such compounds.

Acknowledgment. The Australian Research Council (ARC) is gratefully acknowledged for the funding for an ARC Professorial Fellowship to P.A.L., for Large and Discovery grants, and for RIEF and LIEF equipment grants for the NMR, EPR, and ESI-MS instrumentation.

(49) Lay, P. A.; Levina, A. In *Comprehensive Coordination Chemistry II*; McCleverty, J. A., Meyer, T. J., Eds.; Elsevier: Oxford, U.K., 2004; Vol. 4, pp 313−413.

Supporting Information Available: Details of syntheses and characterizations of the ligands Aib_3, AibLAlaAib, AibDAlaAib, and *N*,*N*-dimethyl-AibAibAib, IR spectra of **1**−**3** and the corresponding ligands, typical results of simulations of EPR spectra for **1**−**3**, typical results of stability studies of **1**−**3** in aqueous solutions (EPR and UV−vis spectra), and ESMS data for the reduction products of **1** in aqueous solutions (typical spectra and assignment of the signals). This material is available free of charge via the Internet at http://pubs.acs.org.

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