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A simple regiospecific strategy for labelling hydrogen atoms in α-amino acids

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Simple methods for the regioselective introduction of deuterium labels at the α- and β-carbon atoms of leucine using a Co(III) imino acid complex are described which have a general applicability to the synthesis of a range of labelled amino acids.

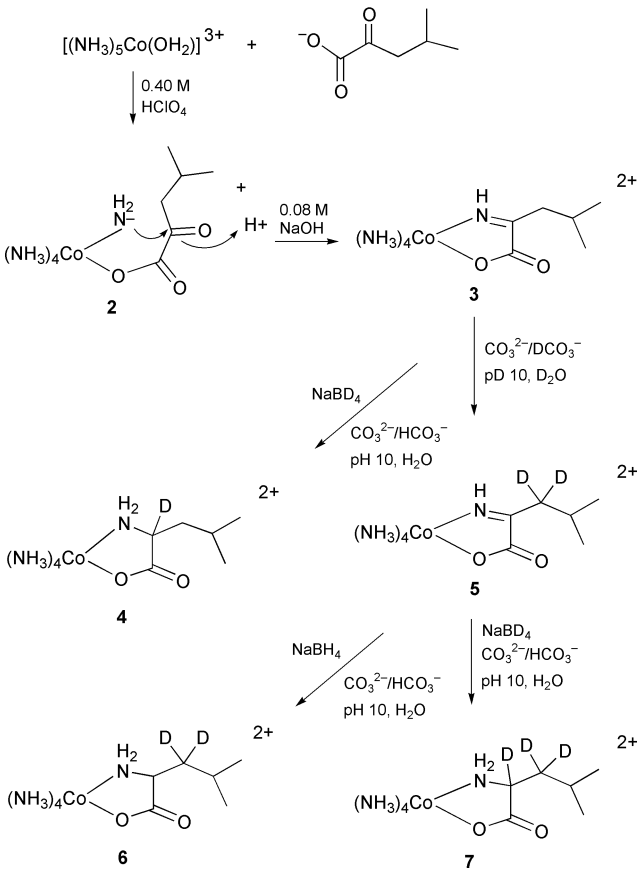
Deuterium labelled amino acids are useful probes of protein structure[1] and biosynthetic pathways for interesting, potentially useful, metabolites.[2] As such they are significant synthetic targets and a substantial catalogue of synthetic[3] and biosynthetic[4] strategies for the preparation of labelled α-amino acids now exists in the literature. Many of these strategies involve complex, multi-step syntheses and their application as universal methods for the preparation of families of deuterium labelled amino acids is limited by relatively low yields or relatively high substrate specificity. Given these restrictions, a simple and general synthetic strategy has been developed by which the metal ion activation and protection of sites in α-imino acids coordinated to Co(III) may be harnessed to incorporate, regioselectively, deuterium or tritium labels on the α- and β-carbon atoms of various α-amino acids.

Syntheses of α-amino acid complexes of Co(III) are readily achieved by intramolecular condensation of coordinated amido ion (NH_2^-) with an α-keto acid coordinated *cis* to the amido ion,[5] Scheme 1. These ammine complexes have three useful properties: the complexes are substitutionally inert, the imine is protected from hydrolysis in aqueous solution, and sites, including the imine-N, imine-C and β-carbon atoms are activated for further reaction. For example, the protons on the β-carbon of $[Co(NH_3)_4(2\text{-iminopropanoate})]^{2+}$ readily exchange with deuterium ions in dilute NaOD solution. Then, it is possible to reduce, rapidly, the imino acid ligand to the corresponding amino acid (alanine) with BH_4^- ion in dilute, basic solution without significant reduction of the metal centre.[5] These reactions can be employed to prepare a range of amino acids containing a specific deuterium label at the α-carbon using BD_4^- ion and/or at the β-carbon using basic D_2O and BD_4^- or BH_4^-. To illustrate this strategy, the synthesis and isolation of three deuterium-labelled analogues of leucine are described.

The leucine–iminato complex, **3**†, was prepared by a method analogous to that for the alanine–iminato complex.[5] The 4-methyl-2-oxopentanoate complex, **2**, was treated with aqueous base to induce intramolecular condensation between the coordinated α-keto acid and amido ion, Scheme 1. Treatment of **3** with sodium borodeuteride (at pH 10) for 60 s gave **4**,‡ in which the α-carbon of the coordinated leucine was completely labelled with deuterium. A sample of **3** was dissolved in a carbonate–deuterium carbonate buffer (pD 10) and exchange of the protons on the β-carbon for deuterium was monitored by ^1H NMR spectrometry to completion. The isolated complex, **5**, was then treated with either sodium borohydride or sodium borodeuteride to prepare the corresponding complexes **6** and **7**,‡ in which coordinated leucine was labelled with deuterium at the β-carbon and at the α- and β-carbon atoms, respectively. Direct introduction of the D label from BD_4^- ion at the α-C atom occurs even in H_2O and base catalyzed proton exchange at the β-C atom leads to capture of D^+ from D_2O. Complexes **3**–**7** were purified by cation-exchange chromatography to remove traces of cobalt(II) generated during their preparation.

The deuterium labelled amino acids **8**–**10**§ were isolated following treatment of the corresponding Co(III) complexes (**4**, **6**, **7**) with excess ammonium sulfide and filtration to remove precipitated CoS. They were purified by ion exchange chromatography (dilute aqueous ammonia as eluent)[6] and characterized by ^1H and ^{13}C NMR spectrometry, microanalysis and electrospray mass spectrometry. Examples of ^{13}C NMR spectra of the deuterium labelled leucine are reproduced in Fig. 1. Coupling between the deuterium labels and the α- and β-carbon atoms generates characteristic splitting patterns in the peaks assigned to these atoms.

Analogous chemistry has been used to introduce deuterium labels into other amino acids, including glycine, alanine and valine, details of which will be published elsewhere. A wide range of (α-imino acidato)cobalt(III) complexes is also readily accessible by oxidation of the corresponding (α-amino acidato)cobalt(III) complexes with $SOCl_2$[7] and the chemistry described here whould provide the means to prepare selectively labelled analogues of many amino acids. The methods are



Scheme 1

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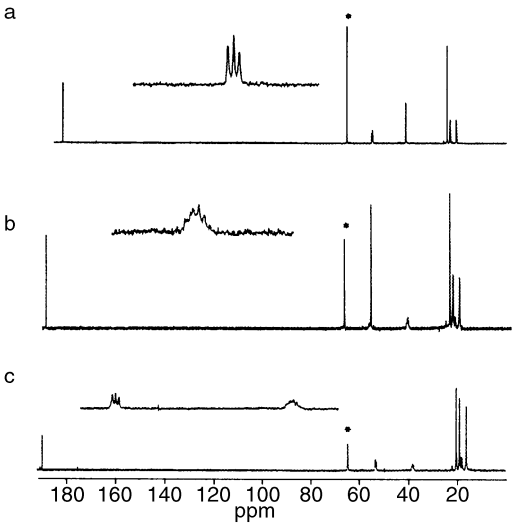

Fig. 1 Comparison of the ^{13}C NMR spectra (D_2O, *1,4-dioxane) of deuteriated-leucines **8**–**10**, a–c respectively from **4**, **6** and **7**. Magnification of spectral peaks due to the α-methine and β-methylene carbon atoms are shown inset, to illustrate splitting of the signals due to the presence of deuterium labels on those atoms.

simple, rt processes and the pure amino acids can be recovered readily.

The stereoselectivity of the chemistry used to prepare the labelled amino acids needs to be addressed. The products isolated from tetraammine complexes are racemic but they can be resolved readily with chiral ion exchange eluents. However, similar chiral cobalt(III) reagents also influence the imino acid[8] reductions stereoselectively and a report on this issue is currently being prepared.

Electrospray mass spectrometric analysis of labelled amino acids by Dr Margaret Shiel and associates of the Department of Chemistry, University of Wollongong, NSW is gratefully acknowledged.

Notes and references

† When necessary, compounds were purified by cation-exchange chromatography (Dowex 50W-X2, 200–400 mesh, BioRad) using HCl solutions as eluents.

For **2**: $[(NH_3)_5Co(OH_2)](ClO_4)_3$[9] (5.00 g, 10 mmol) was added to sodium 4-methyl-2-oxopentanoate (6.1 g, 40 mmol) in $HClO_4$ solution (0.4 M, 4 mmol). The mixture was heated at 45 °C for 3.5 h and the resulting precipitate recrystallized from hot water, producing scarlet crystals of **2** (3.9 g, 76%). δ_H(0.1 M DCl) 4.1 (12 H, br, *cis* 4 × NH_3) 3.5 (3 H, br, *trans* NH_3), 2.73 (2 H, d, β-CH_2), 2.19 (1 H, m, γ-CH), 0.99, 0.98 (6 H, d, 2 × CH_3); δ_C(0.1 M DCl) 188.2 (α-C=O) 174.0 (β-C=O), 44.7 (γ-CH_2), 26.5 (δ-CH), 22.7 (2C, s, 2 × CH_3) (Found: C, 15.3; H, 5.3; N, 15.3; Cl, 14.9; Co, 12.6%. $C_6H_{24}N_5O_{11}Cl_2Co$ requires C, 15.26; H, 5.12; N, 14.83; Cl, 15.02, Co, 12.48%).

For **3**: **2** (3.6 g, 7.6 mmol) was dissolved in NaOH (0.08 M, 7.6 mmol) and stirred at 25 °C (30 s) before addition of acetic acid to pH 4.0. The orange product, **3**, was eluted from ion exchange resin (H^+ form), evaporated to dryness and recrystallized from water by the addition of $HClO_4$ (70%, CAUTION) (2.1 g, 59%). δ_H(0.1 M DCl) 4.1 (3 H, br, NH_3) 3.6 (6 H, br, 2 × NH_3), 3.3 (3 H, br, NH_3), 2.95 (2 H, d, β-CH_2), 2.13 (1 H, m, γ-CH), 0.99, 0.98 (6 H, d, 2 × CH_3); δ_C(0.1 M DCl) 187.6 (O–C=O), 173.0 (α-C=N), 42.3 (β-CH_2), 26.2 (γ-CH), 21.5 (2C, s, 2 × CH_3) (Found: C, 15.6; H, 4.9; N, 15.2; Cl, 14.5; Co, 12.7%. $C_6H_{22}N_5O_{10}Cl_2Co$ requires C, 15.87; H, 4.88; N, 15.42; Cl, 15.61; Co, 12.98%).

‡ For **4**: **3** (0.25 g, 0.8 mmol) was dissolved in a carbonate–bicarbonate buffer solution ($[CO_3^{2-}]$ = $[HCO_3^-]$ = 0.5 M) and $NaBD_4$ (0.08 g, 4 mmol) added. Following vigorous mixing (60 s) the product was rapidly trapped on ion exchange resin (Na^+ form) by suction and washed with water and 0.5 M HCl and the orange product eluted with 2 M HCl. Evaporation gave an orange solid, **4**. The labelled amino acid was removed from this complex without further purification.

Complexes **6** and **7** were prepared by analogous treatment of **5** with $NaBH_4$ and $NaBD_4$, respectively. For **5**: **3** (1.5 mmol, 0.50 g), was dissolved in a carbonate–deuteriobicarbonate buffer ($[CO_3^{2-}]$ = $[DCO_3^-]$ = 0.25 M, pD = 10.0). The resulting deep orange solution was stirred at 25 °C for 10 h and the orange product, **5**, was isolated as above.

§ For **8**–**10**: Complexes **4**, **6** and **7** were individually dissolved in water (10 cm^3) and an 8% solution of $(NH_4)_2S$ added dropwise to reduce and precipitate the cobalt. The resulting CoS suspensions were removed by filtration and the solutions desalted on ion exchange resin (H^+ form); the sorbed material was washed with water before eluting with 0.5 M ammonia solution. Fractions containing the amino acids were evaporated and recrystallized twice from H_2O–propan-2-ol to give white powders (yields from **2** to isolation ~50%). **8**: $\delta_H(D_2O)$ 1.70 (1 H, m, γ-CH), 1.75 (2 H, ABXq, β-CH_2), 0.97, 0.98 (6 H, d, 2 × CH_3); δ_C (D_2O) 187.9 (COOH), 56.8 [1C, t, *J*(CD) 24 Hz, α-CD], 42.4 (β-CH_2), 24.9 (γ-CH), 20.9, 21.0 (2 × CH_3); *m/z* (ES-MS) 133.0 (M^+) (Found: C, 54.4; H, 9.13; N, 10.5%. $C_6H_{12}DNO_2$ requires C, 54.52; H, 9.15; N, 10.6%). **9**: $\delta_H(D_2O)$ 3.73 (1 H, s, α-CH) 1.69 (1 H, m, γ-CH), 0.97, 0.98 (6 H, d, 2 × CH_3); $\delta_C(D_2O)$ 187.8 (COOH) 56.5 (α-CH), 42.3 (1C, m, CD_2), 24.5 (γ-CH), 19.9, 20.9 (2 × CH_3); *m/z* (ES-MS) 134.0 (M^+) (Found: C, 53.2; H, 8.5; N, 10.4%. $C_6H_{11}D_2NO_2$ requires C, 54.1; H, 8.32; N, 10.5%). **10**: δ_H (D_2O) 1.70 (1 H, m, γ-CH), 0.97, 0.98 (6 H, d, 2 × CH_3); $\delta_C(D_2O)$ 187.9 (COOH), 56.6 [1C, t, *J* (CD) 24.45 Hz, α-CD], 42.4 (1C, m, β-CD_2), 24.5 (γ-CH), 19.8, 20.9 (2 × CH_3). *m/z* (ES-MS) 135.0 (M^+) (Found: C, 53.9; H, 7.50; N, 10.3%. $C_6H_{10}D_3NO_2$ requires C, 53.7; H, 7.51; N, 10.4%).

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