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Facile reduction of coordinated α-imino acids to amino acids by dithionite and borohydride

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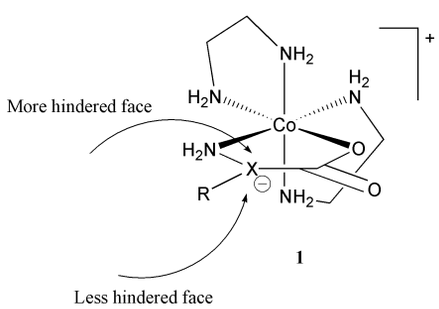
Sodium dithionite was found to reduce a range of α-iminoacidato ligands, chelated to cobalt(III), to the related α-aminoacidato species. For the bis(1,2-ethanediamine) system, the distribution of the products showed a small stereoselectivity towards the Λ*R*,Δ*S* diastereoisomers. The most significant selectivity was exhibited in the preparation of the valinato complex, *via* reduction of the related imine, $[(en)_2Co(Vim)]^{2+}$, where Λ*R*,Δ*S*/Λ*S*,Δ*R* = 4.2. Smaller selectivities (≈1.5) were observed in reductions using borohydride reagents. Reduction of the α-imino acid by sodium dithionite is optimised under acidic conditions (pH 3.5–5.5) in a dinitrogen atmosphere, whilst the corresponding reduction by sodium borohydride is optimised under mildly alkaline conditions (pH 9–10). Reduction conditions are therefore available to suit molecules with either acid or base sensitive functional groups. A mechanism for the reduction of the chelated imino acids by dithionite is proposed.

Introduction

One of the routes available for the synthesis of novel amino acids is that which makes use of the metal ion promoted activation of chelated α-imino acids. The acidity of the protons on the β-carbon atom adjacent to the imine group is enhanced and the imine group is activated towards attack by nucleophiles. This allows the imino acid to be functionalised in a variety of ways,[1] and reduction of the imino acid would then give the related β-functionalised α-aminoacidato complex.

In early work, the availability of cobalt(III) α-iminoacidato complexes was rather limited. These were usually prepared by the base catalysed intramolecular condensation of pentaammine α-keto acid complexes of cobalt(III)[2] or by elimination reactions.[1a] More recently however, oxidative methods for preparing α-iminoacidato complexes using thionyl chloride, $SOCl_2$, and *N*-bromosuccinimide (NBS) have been developed.[3] These methodologies have greatly extended the range of α-iminoacidato complexes that are available for functionalisation because the synthetic routes are compatible with ancilliary ligands other than just ammonia. They have therefore provided a means of synthesising chiral compounds with which the stereoselective control of the subsequent chemistry can be investigated. In this study we set out to establish whether or not the configuration of didentate ligands around the cobalt(III) ion in these complexes could be used to control the stereochemistry of the reduction of the imine group.

The proton on the α-carbon atom of a chelated α-amino acid is relatively acidic, undergoing exchange in basic aqueous solution.[4] Under such conditions an equilibrium mixture of the possible diastereoisomeric complexes is formed. Mutarotation studies on aminoacidatobis(1,2-ethanediamine)cobalt(III) complexes have shown that the preferred orientation for the protonation of the intermediate carbanion, **1**, by water, depends on the nature of the substituent on the α-carbon atom.[4e] However, the magnitude of the selectivity is not large. The ratio of the rate constants for protonation from each face of the carbanion varies between 0.5 and 1.6. Since this reprotonation is formally similar to the addition of dihydrogen to the related imine complex, one might expect to observe preferences in the reduction reactions. The purpose of this study was to examine, therefore, the use of agents such as sodium dithionite and sodium borohydride in the stereoselective reduction of α-iminoacid anions chelated to cobalt(III). Of particular interest in these investigations was an evaluation of the influence of the $Co(en)_2$ chiral auxiliary on the stereochemistry of the resulting amino acid products.



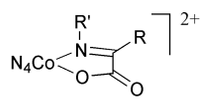
In order to study the stereoselectivity of the reaction, it was first necessary to establish the optimal conditions under which the imino acid, and not the metal centre, was reduced, and the range of imino acids that could be reduced using these reagents. Rationalising the stereochemical results also required an understanding of the mechanism of the reduction reaction, and this led to some investigations which provided information about intermediates along the reaction pathway.

Results and discussion

General comments on syntheses

Complexes of the form $[Co(en)_2(aa)]^{2+}$ were prepared using methods similar to those described in the literature.[1a,3d] In some instances, most notably in the preparation of the phenylglycinato complex, the use of the corresponding dimethylsulfoxide complex $[Co(en)_2(dmso)_2]^{3+}$ as starting material

improved the yield of the desired complex.[5] With the exception of $[Co(en)_2(Gim)]^{2+}$ †, the iminoacidato complexes were prepared by thionyl chloride oxidation of the corresponding α-aminoacidato complexes.[3d] NBS was used as the oxidant for the preparation of $[Co(en)_2(Gim)]^{2+}$, **2a**, from $[Co(en)_2(gly)]^{2+}$.[3f] Tetraammine complexes, $[Co(NH_3)_4(iminoacidato)]^{2+}$, were prepared in the usual way by intramolecular Schiff base condensation between coordinated ammonia and a coordinated α-keto acid anion of the precursor pentaammine complexes.[2b] Fig. 1 illustrates the range of iminoacidato complexes that were



2	R' = H,	R = H	Gim
3	"	R = CH_3	Aim
4	"	R = $CH(CH_3)_2$	Vim
5	"	R = $(CH_2)_2SCH_3$	Mim
6	"	R = $CH_2(4\text{-}C_6H_4OH)$	Yim
7	"	R = $(CH_2)_4NH_2$	Kim
8	"	R = C_6H_5	phenylGim
9	"	R = $(CH_2)_2COOH$	Eim
10	R' = CH_3	R = H	sarim
11	"	R = CH_3	N-MeAim
12	"	R = $CH(CH_3)_2$	N-MeVim
13	R'-R = $(CH_2)_3$		Pim
14	R'-R = $(CH_2)_4$		pipim

a $N_4 = (en)_2$
b $N_4 = (NH_3)_4$
c N_4 = tris (2-aminoethyl)amine = tren
d N_4 = bpy

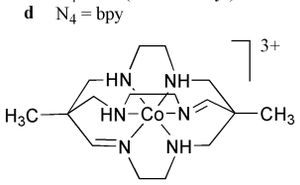


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Fig. 1 Substrates for dithionite reduction: **2a**–**9a**, **3b**–**3d**, **4b**, **8b**–**12b**, **12a**–**14a**, **15**. Substrates for detailed study of borohydride and/or borodeuteride reduction: **2a**–**4a**.

prepared and used in this study. These were used to determine the range of application of dithionite as a reducing agent. The complexes $[Co(en)_2(Aim)]^{2+}$, **3a**, and $[Co(en)_2(Vim)]^{2+}$, **4a**, were used to examine the mechanism of the reduction reaction in more detail and for comparative studies using borohydride reagents.

Reduction of $[Co(en)_2(Aim)]Cl_2\cdot H_2O$ and $[Co(en)_2(Vim)](ClO_4)_2\cdot 1.5H_2O$ by $NaBH_4$ and $Na_2S_2O_4$

Reductions of iminoacidato complexes by sodium borohydride were carried out in buffered, slightly basic aqueous solution (pH 9–10) to limit reduction of the metal centre and subsequent decomposition of the labile cobalt(II) complex. Reduction of the imine proved facile under these conditions. The reactions were complete within 30 seconds (20 °C) and were quenched by rapidly adsorbing the reaction mixture on to an ion exchange column and washing the adsorbed complexes with water. After elution with hydrochloric acid and evaporation to dryness, the residues were analysed by ^{1}H NMR spectrometry. The influence of other, more bulky, alkyl borohydride reagents on the diastereoselectivity of the reduction reaction was also investigated. These reagents included $Na(CH_3CO_2)_3BH$, $Na(ClCH_2CO_2)_3BH$, $Na(C_6H_5CO_2)_3BH$, NH_3BH_3 and $N(CH_2CH_2OH)_3BH_3$. The complex $[Co(en)_2(Aim)]^{2+}$, **3a**, was treated with these reagents by a similar procedure to that used for $NaBH_4$.

Reactions involving sodium dithionite were stirred for up to half an hour before reduction of the imine was complete. During this time the odour of SO_2 was apparent and a faint suspension of colloidal sulfur became noticeable about 15 minutes after the reaction had commenced. The reaction was quenched and the complexes recovered in the manner described above. The outcome of the reaction was influenced by a number of parameters, including the ratio of dithionite to complex, the pH of the solution and the presence/absence of oxygen in the atmosphere above the reaction mixture.

In general, the reduction was performed with a molar ratio of dithionite : complex of 5. If the dithionite : complex ratio was decreased to 3 or less then reduction of the imine was incomplete, probably because of the loss of dithionite ion to competing reactions with oxygen, water and the metal centre.[6] However, if the ratio was increased beyond 5 reduction of the metal centre became a significant problem. Under these latter circumstances little aminoacidato complex was recovered after quenching; instead a black precipitate, presumably CoS or finely divided Co,[7] formed and Co^{2+} was also detected by ion exchange chromatography of the quenched reaction mixture. Conducting the reaction under a nitrogen atmosphere reduced the reaction time from thirty to five minutes, presumably because dithionite was no longer consumed by reaction with O_2. This is probably the best reduction strategy, since it also limits reduction of the metal ion and other side reactions.

The pH of the solution was found to have a significant influence on the reduction reaction. Sodium dithionite and $[Co(en)_2(Vim)]^{2+}$, **4a**, were mixed in buffers having a pH in the range 3.5 to 9.5. The mixtures of products were separated from paramagnetic cobalt(II) ions by ion exchange chromatography and the components identified by ^{1}H NMR spectrometry. At pH > 5.5 there was increased reduction of the metal centre and a corresponding decrease in the total amount of cobalt(III) complexes recovered. Previous studies, involving the reduction of the metal centre of various complexes, have been performed in the pH range 6.3 to 13.0.[8]

Identification of products

The product mixtures were analysed by ^{1}H NMR spectrometry. The equilibrium ratio of the aminoacidato diastereoisomers has previously been found to be $\Lambda R,\Delta S : \Lambda S,\Delta R = 1$ and 2 for the alaninato and valinato complexes respectively.[4c,e,9]

The intensity of the large singlet at 3.39 ppm that appears in spectra from dithionite reductions was identified as 1,2-ethanediamine by NMR methods. This arises presumably from reduction of the Co(III) centre. The amount of 1,2-ethanediamine that was recovered from the column as part of the mixture was rather variable. It could be estimated, on the basis of the masses of the residues following chromatography, and the relative integrals of the resonances in the ^{1}H NMR spectrum, that 5 to 10% of the complex was lost as a result of reduction of Co(III) to Co(II) by dithionite when the reduction was carried out in the presence of air.

Resonances at 1.32 and 1.29 ppm, corresponding to the methyl groups of the $[Co(en)_2(Vim)]^{2+}$ complex, **4a**, indicate that not all of the imine had been reduced. The efficacy of dithionite in reducing the imine was estimated by comparison of the integral of these signals with those of the methyl groups of the valinato complex. In a typical example, 94% of the valinato complexes were produced from the imine complex after a reaction time of 30 minutes.

The diastereoselectivity of the reduction reaction was determined by comparing the integrals of the resonances assigned to each of the diastereoisomers. The methyl group doublets are not particularly useful from this point of view

† The abbreviations for the imino acidate ligands are derived by adding the letters 'im' to the single letter abbreviation for the amino acid to which it is related. For example, glycine, G, gives rise to the related imine ligand abbreviation Gim, likewise alanine, Aim, and valine, Vim.

as they overlap or are too close in both spectra. The methine multiplets, however, only overlap slightly in the spectra of the alaninato complexes and are totally separated in those of the valinato complexes (at the magnetic fields employed for these studies). Treatment of $[Co(en)_2(Vim)]^{2+}$, **4a**, with $S_2O_4^{2-}$ at pH 3.5–6.5 gave a diastereoisomeric ratio, $\Lambda R,\Delta S$: $\Lambda S,\Delta R$, of the product valinato complexes of 4.2. By contrast, the reduction of $[Co(en)_2(Vim)]^{2+}$ by BH_4^- in mildly alkaline conditions (pH 9–10) showed rather less stereoselectivity: $\Lambda R,\Delta S$: $\Lambda S,\Delta R = 1.5$.

Reductions of $[Co(en)_2(Aim)]^{2+}$, **3a**, with both sodium dithionite and sodium borohydride resulted in a $\Lambda R,\Delta S$: $\Lambda S,\Delta R$ ratio of 1.5 for the diastereoisomers of the alaninato complexes $[Co(en)_2(ala)]^{2+}$. Chromatographic separation and analysis of the product complexes formed in such reactions confirmed the ratio of isomers determined by ^1H NMR spectrometry. Increasing the bulk of the reducing agent through the use of the acyl borohydride reagents did not influence the diastereoselectivity of the reaction. However, it is conceivable that the triacyloxyborohydride reagents may react with the solvent to give a smaller reagent, possibly $HB(OH)_3$ (or one of its sodium salts), which is the effective reducing agent. The steric demand of such a compound would not be much different from that of BH_4^- in this situation.

Reduction of other coordinated α-imino acids by sodium dithionite

The facility with which dithionite ion reduces different imino acidato complexes was studied using the complexes depicted in Fig. 1. These include a range of α-imino derivatives of naturally occurring amino acids as well as synthetic iminoacidato complexes. The reduction reactions were carried out under the same conditions as those described previously for reductions of $[Co(en)_2(Vim)]^{2+}$, **4a**, and $[Co(en)_2(Aim)]^{2+}$, **3a**.

In general, sodium dithionite did not reduce N-alkylimino acids chelated to cobalt(III) (**10a**–**14a**). For example, the $[Co(en)_2(Vim)]^{2+}$ complex, **4a**, could be reduced by dithionite but the N-methyl analogue, **12a**, could not. This is in direct contrast to the activity of BH_4^-, which readily reduced all such species. ^1H NMR spectra of the residues recovered after reaction of dithionite with complexes **10a**–**14a** contained no signals due to the corresponding aminoacidato complexes. Instead they contained large signals due to the presence of uncoordinated 1,2-ethanediamine, indicating that reduction of the metal centre and consequent decomposition of the complex had occurred. Variation of the number of molar equivalents of dithionite used in the experiment and removing O_2 from the solution did not alter these results. It is difficult to distinguish between two possible explanations of the results — whether the imine could *not* be reduced under the experimental conditions, or whether it *was* reduced, and the resulting amino acid complex decomposed rapidly *via* reduction of the Co(III) centre.

In order to gain more information on this last issue, the 'cage' complex **15**, which contains the group R–N=C– and in which the Co(III) ion is much more stable towards reduction, was mixed with dithionite and monitored by ^{13}C NMR spectrometry. No signals corresponding to the reduced imine were detected in the resulting spectra. This result implies that the R–N=C– iminoacidato complexes are not reduced to the corresponding aminoacidato complexes before the cobalt centre is reduced.

By contrast, BH_4^- was capable of reducing all iminoacidato complexes used in the study and the cage imines. The diastereoselectivities of the reduction reactions of iminoacidato complexes were determined by a similar process to that described for isomers that resulted from the reduction of $[Co(en)_2(Vim)]^{2+}$ and $[Co(en)_2(Aim)]^{2+}$. Regardless of the nature of the side chain on the imino acid, the isomer distribution, $\Lambda R,\Delta S$: $\Lambda S,\Delta R$, of the resulting amino acids was approximately 1.5.

The $[Co(en)_2(Gim)]^{2+}$ complex, **2a**, proved to be very susceptible to reduction of the metal centre. Under the usual reaction conditions the solution turned from clear orange to brown with a black suspension within seconds of addition of dithionite. However, addition of a molar equivalent of dithionite to a solution of $[Co(en)_2(Gim)]^{2+}$ in a deoxygenated buffer solution at pH 4.1 for 30 seconds resulted in the quantitative isolation of $[Co(en)_2(gly)]^{2+}$ from the quenched reaction mixture. $[Co(en)_2(Gim)]^{2+}$ was also reduced by BH_4^-, using the established conditions for this reducing agent.

A group of complexes, $[Co(N_4)(Aim)]^{2+}$ ($N_4 = (en)_2$, $(NH_3)_4$, tren, $(bpy)_2$, (**3a**–**d**)) were used to gain a measure of the utility of sodium borohydride and sodium dithionite as reducing agents for the iminoacid anion in different tetraamine complexes. Sodium borohydride has been used previously to reduce tetraammine complexes of α-imino acids and proved capable of reducing iminoacidato ligands of **3a**–**d** with only minor reduction of the metal centre.[2] Dithionite ion did not prove to be a useful reagent for reducing the imine of $[Co(bpy)_2(Aim)]^{2+}$ (**3d**) because the complex was not stable enough to reduction of the metal ion. Some cobalt(II) production also occurred during reduction of $[Co(NH_3)_4(Aim)]^{2+}$, **3b**, while only traces of cobalt(II) were produced during reductions of the imine in the $[Co(en)_2(Aim)]^{2+}$, **3a**, and $[Co(tren)(Aim)]^{2+}$, **3c**, ions. These results crudely correlate with the cobalt(III) electrochemical reduction potentials of the complexes. The bpy and ammonia complexes have more positive potentials than the en and tren complexes.

Deuterium labelling experiments

Reduction of $[Co(en)_2(Aim)]^{2+}$, **3a**, by $NaBD_4$ under the same conditions as $NaBH_4$ resulted in the introduction of a deuteron at the α-carbon of the amino acid. The reaction was carried out in a buffered H_2O solution, so the deuteron came from the borodeuteride ion, not the solvent. The deuteron must also remain in this position during recovery of the product by ion-exchange chromatography since there were no methine signals in ^1H NMR spectra of the resulting residues. The implication is that there is very little or no exchange of protons or deuterons at the α-carbon and therefore no mutarotation of the chelated amino acid after reduction and during workup of the reaction mixture. When a mixture of the ΛR and ΔS isomers of the alaninato complex was resubmitted to the reaction conditions, no ΛS or ΔR isomers could be detected in the product. Therefore there was no significant proton exchange on the α-carbon under the reaction conditions.

It was anticipated that the dithionite reduction reaction in D_2O would result in an amino acid complex bearing a deuterium label on the α-carbon, since the proton that is added to the α-carbon in the reduction of $[Co(en)_2(Aim)]^{2+}$, **3a**, by dithionite ion must come from water. However, the material recovered from the reduction of $[Co(en)_2(Aim)]^{2+}$ or $[Co(en)_2(Vim)]^{2+}$, **4a**, by dithionite in D_2O contained no labelled amino acid. This is in spite of the fact that products isolated in the same manner from previous reductions by $NaBD_4$ retained the deuteron on the α-carbon atom. This observation eliminates the possibility that the label could have been exchanged during the isolation procedures.

In a new experiment, $[Co(en)_2(Vim)]^{2+}$, **4a**, was reduced by dithionite in D_2O and the resulting complexes were isolated by precipitating them from solution with excess ethanol. The pale orange solid was dissolved in a minimum quantity of 6 M DCl, thereby generating a distinctive odour of SO_2, and then diluted with H_2O and treated by ion-exchange chromatography in the usual way. This procedure yielded the deuterated valinato complex and the same technique was used to isolate the corresponding deuterated alaninato complex. The solid intermediate isolated from these reactions was not stable enough to recrystallise but was analysed by a number of techniques, including

elemental microanalysis, IR spectrometry and electro-spray mass spectrometry. It was difficult to identify the species conclusively, mostly because of the presence of other sulfur containing species such as $S_2O_4^{2-}$ and $S_2O_3^{2-}$ in the anionic component of the compound but adducts of the imine can be inferred.

Mechanism of the reduction of $[Co(N_4)(\alpha\text{-iminoacidato})]^{2+}$ by $Na_2S_2O_4$ and $NaBH_4$

The lack of incorporation of the D label when the dithionite experiments were conducted in D_2O (unless DCl was added subsequently), implies the presence of an acid sensitive, sulfur containing intermediate formed during the reduction process. In the acidic conditions under which the chromatographic isolations were conducted, or upon dissolving the precipitated products in acid solution (H^+/H_2O or D^+/D_2O), a proton or deuteron is inserted. Once installed, the H or D label remains intact in neutral to acidic media.

A few studies of reductions by dithionite ion have reported isolating sulfinate adducts of some organic compounds.[10] For example, nicotinamides form sulfinate adducts, which are stable at high pH but which convert to dihydronicotinamides in acid conditions.[10b] By analogy, the acid sensitive intermediates isolated in these experiments would have the form of **16**. It follows that the mechanism of the reduction may be ascribed to the nucleophilic sulfur atom of dithionite ion initially adding to the α-carbon of the imino acid, **17**. Upon rearrangement and loss of SO_2 from this unstable adduct, the complex which remains is the intermediate, sulfinate adduct, **16**. Under acidic conditions, the sulfinate moiety is protonated and eventual loss of neutral SO_2 and capture of H or D generates the amino-acidato complex, **18**, as shown in Fig. 2.

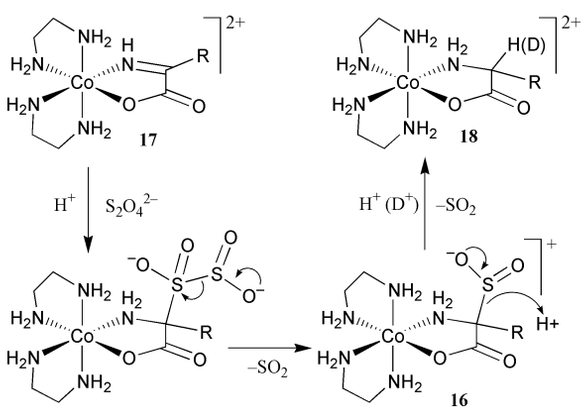

Fig. 2 Proposed mechanism for the dithionite reduction.

It is not clear why the *N*-methyliminoacidato ligands are not reduced by dithionite, but one possible rationalisation would be that the substitution of an electron withdrawing proton on the imine nitrogen atom by an electron donating alkyl group may make the imine group less electrophilic and therefore less vulnerable to nucleophilic attack by dithionite. Electron transfer to the cobalt(III) ion and subsequent ligand exchange reactions on cobalt(II) could therefore dominate over imine reduction. Borohydride ion is a better nucleophile and reducing agent and may be less influenced by the alkylation.

The mechanism of the reduction of $[Co(N_4)(\alpha\text{-im})]^{2+}$ by BH_4^- is distinctly different to that by dithionite. Reduction of $[Co(en)_2(Aim)]^{2+}$ by BD_4^- in H_2O resulted directly in the deuterated amino acidato complex, $[Co(en)_2(NH_2C\mathbf{D}(CH_3)COO)]^{2+}$. The implication here is that the deuterium (or protium) comes from the borodeuteride (borohydride) species itself and that there must be an activated complex, in which the D–αC bond is formed through nucleophilic addition to the imine carbon atom. This mechanism is in general agreement with that proposed for reduction of species such as carbonyls by BH_4^-.[11]

In all instances of reduction involving bis(1,2-ethanediamine) complexes, the formation of the Λ*R*,Δ*S* diastereoisomers were favoured over the Λ*S*,Δ*R* diastereoisomers. This preference contrasts with that observed in the mutarotation and reprotonation studies, where the relative carbanion protonation rates for attack on each face are observed to depend on the nature of the carbon substituent.[4e]

Reduction of the imine occurred predominantly by attack of the reducing agent at the more hindered face of the imino-acidato ligand, giving rise to an excess of the Λ*R*,Δ*S* diastereo-isomers and, with the exception of the glycine imine and valine imine complexes, the size of the side chain had little effect on the isomer distribution of the resulting amino acid complexes; Λ*R*,Δ*S* : Λ*S*,Δ*R* = 1.5.

When $[Co(en)_2(Gim)]^{2+}$ was reduced by BD_4^- or by $S_2O_4^{2-}$/D_2O/DCl, the resulting isomer distribution was Λ*R*,Δ*S* : Λ*S*,Δ*R* = 1. This might be expected on steric grounds since the 'side chain' of glycine is a proton and would not therefore effect the selectivity of the addition significantly.

By far the most substantial result arose when $[Co(en)_2(Vim)]^{2+}$ was reduced by $S_2O_4^{2-}$, where the isomer distribution was Λ*R*,Δ*S* : Λ*S*,Δ*R* = 4.2. It is reasonable to argue in this instance that the bulk of the 2-propyl group of the imino acid imposes a greater steric constraint on the reaction than the methylene β-carbon of the side chains of most of the other imino acid complexes. By implication, if the steric bulk of the imino acid substituent were increased, the selectivity should be even greater.

Conclusion

It has proven possible to reduce iminoacidato ligands chelated to cobalt(III) relatively rapidly using either sodium borohydride or sodium dithionite especially in the absence of dioxygen. The former reagent may be used more generally, while the latter is unreactive towards N-alkyl imine complexes. On the other hand, greater stereoselectivities have been observed in reductions using dithionite ion than were seen when borohydride reagents were used.

An important difference between the two reagents also is that one is optimal under acidic conditions while the other is optimal under mildly basic conditions. This dichotomy could be useful in situations where the reactant or product have acid or base sensitive functional groups. These reagents and the incorporation of a chiral auxiliary enhance the armoury that is available for synthesis of amino acids using a simple inorganic system instead of the usual array of organic protecting and deprotecting reagents.

Experimental

Nuclear magnetic resonance spectra of the complexes dissolved in D_2O or 0.1 M DCl were acquired using a Jeol JNM-FX 200 Fourier transform NMR spectrometer or a Varian Instruments Gemini 300 NMR spectrometer. Chemical shifts in ^1H NMR spectra are reported relative to sodium trimethylsilylpropane sulfonate (NaTPS), 0.00 ppm. Chemical shifts in ^{13}C NMR spectra are reported relative to dioxane, 67.4 ppm (relative to TMS). Multiplicities of signals in the ^1H NMR spectra are indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br). Visible spectra were measured using a Hewlett-Packard HP 8450A spectrophotometer; 0.1 M HCl was used as the solvent unless otherwise specified. IR spectra were obtained using KBr discs in a Perkin-Elmer PE 1800 FTIR spectrometer.

Most solvents and chemicals used for syntheses were analytical reagent grade. Commercial MMM CF_3SO_3H was distilled before use. dmf was dried over $CaSO_4$ before use. Ion

exchange chromatography was performed with analytical grade Dowex 50WX2 (H^+ or Na^+ form, 200–400 mesh, Bio-Rad) or SP Sephadex C-25 (Na^+ form, Pharmacia). The dimensions of resin columns are given as diameter × length in cm. Complexes present in the collected eluents were recovered by evaporation (≈20 Torr in a Büchi rotary evaporator, with a water bath temperature of less than 40 °C.

The complexes $[Co(en)_2(Gim)](ClO_4)_2$,[3f] $[Co(en)_2(Aim)]Cl_2$, $[Co(en)_2(Vim)]Cl_2$, $[Co(en)_2(Vim)](ClO_4)_2$, $[Co(en)_2(Mim)]Cl_2$, $[Co(en)_2(Yim)]Cl_2$, $[Co(en)_2(Kim)]Cl_2$, $Co(en)_2(Pim)]Cl_2$, $Co(en)_2(pipim)]Cl_2$,[3d] $[Co(en)_2(phenylGim)](ClO_4)_2$, $[Co(bipy)_2(Aim)]Cl_2$,[5] $[Co(tren)(A)]Cl_2$, $[Co(tren)(Aim)]Cl_2$,[1a] $[Co(en)_2(N\text{-}MeAim)]Cl_2$, $[Co(en)_2(N–MeVim)]Cl_2$,[2a] $[Co(NH_3)_4(Aim)]Cl_2$, $[Co(NH_3)_4(sarim)]Cl_2$, $[Co(NH_3)_4(Eim)]Cl_2$, $[Co(NH_3)_4(phenylGim)](ClO_4)_2$,[2b] and $[Co((CH_3)_2$ − diimino-sar)]$ZnCl_4$·Cl[12] were prepared using literature procedures.

Separation of the complex diastereoisomers in aqueous conditions, was achieved by adsorbing a small quantity of the complex (*ca.* 80 mg) on a Sephadex column (4.0 × 45.0 cm) and eluting the isomers with 0.05 M sodium citrate. The complexes were desalted by adsorption on small beds of Dowex, washing with water and then eluting with HCl prior to analysis of the residue by ^{1}H NMR spectrometry following evaporation.

The reducing agents $Na(CH_3CO_2)_3BH$, $Na(ClCH_2CO_2)_3BH$ and $Na(C_6H_5CO_2)_3BH$ were prepared according to the method of Marchini *et al.*[13] NH_3BH_3 was prepared according to the method of Hu *et al.*[14] The triethanolamine–borohydride adduct $N(CH_2CH_2OH)_3BH_3$ was isolated as a white precipitate which resulted from addition of $NaBH_4$ (3.1 g) to a solution of triethanolamine (1 L) in acetonitrile (50 mL).

Reduction of $[Co(en)_2(Aim)]^{2+}$ and $[Co(en)_2(Vim)]^{2+}$ with $NaBH_4$

These experiments were performed under similar conditions to those used in previously published work.[2a] In a typical experiment, $[Co(en)_2(Aim)]Cl_2$·H_2O (3 g) was dissolved in H_2O (200 mL). $NaHCO_3$ (0.1 g) and Na_2CO_3 (0.1 g) were added to ensure that the solution was slightly basic (pH 9.5). $NaBH_4$ (1 g) was added and the solution stirred for 30 seconds before being rapidly adsorbed, under suction, on a column of Dowex resin (4 × 10, Na^+ form). The column was washed with H_2O (1 L), under suction, and then with more H_2O (1 L) without suction. Residual Co^{2+} and remaining Na^+ ions were removed with 1M HCl (1 L) and the mixture of $[Co(en)_2(ala)]^{2+}$ diastereoisomers eluted with 2 M HCl and the solvent removed by rotary evaporation.

The product of these reduction reactions was a mixture of diastereoisomers of $[Co(en)_2(ala)]^{2+}$, or $[Co(en)_2(val)]^{2+}$. The signals in the ^{1}H NMR spectra of these complexes have been assigned previously to the Λ*R*,Δ*S* and Λ*S*,Δ*R* diastereoisomers.[9] For $[Co(en)_2(ala)]Cl_2$: δ_H (300 MHz; solvent 0.1 M HCl; standard NaTPS) 3.78 (1H, q, Λ*R*,Δ*S*-α-CH), 3.69 (1H, q, Λ*S*,Δ*R*-α-CH), 1.47 (3H, d, Λ*R*,Δ*S*-β-CH_3), 1.48 (3H, d, Λ*S*,Δ*R*-β-CH_3). For $[Co(en)_2(val)]Cl_2$: 3.73 (1H, d, Λ*R*,Δ*S*-α-CH), 3.57 (1H, d, Λ*S*,Δ*R*-α-CH), 2.35 (1H, m, Λ*R*,Δ*S*-β-CH), 2.28 (1H, m, Λ*S*,Δ*R*-β-CH), 1.14, 0.94 (6H, 2d, Λ*R*,Δ*S*-γ-methyls), 1.14, 0.99, (6H, 2d, Λ*S*,Δ*R*-γ-methyls). Integration of the methine proton quartets in the ^{1}H NMR spectra of residues obtained following reduction of both $[Co(en)_2(Aim)]^{2+}$ and $[Co(en)_2(Vim)]^{2+}$ revealed a Λ*R*,Δ*S* : Λ*S*,Δ*R* ratio of approximately 1.5.

In order to verify this measurement of selectivity, the diastereoisomers were separated.[15] The residue was dissolved in 1 M NaCl (500 cm^3) and adsorbed on a column of Dowex AG50W-X8 resin (2 × 75 cm) that had previously been equilibrated with 1 M NaCl solution. Elution with 1 M NaCl resulted in an almost total separation of the diastereoisomers. Fractions (250 mL) were desalted by HCl elution on columns of Dowex resin (4 × 6, H^+ form) and the eluate taken to dryness by rotary evaporation. Fractions 15–18 of the eluate proved, by ^{1}H NMR spectrometry of the resulting residues, to be mixtures of the diastereoisomers. These fractions were combined, and gave a 1 : 1 ratio of diastereoisomers by integration of the relevant peaks in the ^{1}H NMR spectrum. Comparison of the combined weight of residues from fractions 1–14 with the weight of residues from fractions 19 and higher revealed a Λ*R*,Δ*S* : Λ*S*,Δ*R* ratio of 58 : 42. Resubmission of the Λ*R*,Δ*S* mixture to the reaction conditions did not result in the production of any Λ*S*,Δ*R* alaninato complex. A similar reduction experiment was performed on $[Co(en)_2(Vim)]^{2+}$. Integration of the α-methine ^{1}H NMR signals of the resulting valinato diastereoisomers gave a Λ*R*,Δ*S* : Λ*S*,Δ*R* ratio of approximately 1.5.

Reduction of $[Co(en)_2(Aim)]^{2+}$ by acylborohydride reagents

The following reducing agents were used: $Na(CH_3CO_2)_3BH$ $Na(ClCH_2CO_2)_3BH$, $Na(C_6H_5CO_2)_3BH$, NH_3BH_3 and $N(CH_2CH_2OH)_3BH_3$. Reduction reactions were performed under similar conditions to those described above for sodium borohydride. In most cases there was still a significant amount of unreacted starting material, and in all cases the observed Λ*R*,Δ*S* : Λ*S*,Δ*R* ratio was approximately 1.5.

Reduction of $[Co(en)_2(Aim)]^{2+}$ by $NaBD_4$

$[Co(en)_2(Aim)]Cl_2$·H_2O (0.3 g) was dissolved in H_2O (30 mL) and small amounts of $NaHCO_3$ and Na_2CO_3 were added to the reaction mixture in order to ensure that the solution was slightly basic (pH 9.5). $NaBD_4$ (0.1 g) was added and the solution stirred for 30 seconds before being rapidly adsorbed, under suction, on a column of Dowex resin (4 × 6, Na^+ form). After thoroughly washing the adsorbed complexes with H_2O (1 L) and 1 M HCl (500 mL), they were eluted with 2 M HCl and the eluate taken to dryness by rotary evaporation. The ^{1}H NMR spectrum of this residue showed no sign of the α-methine quartet and the methyl group resonances were broadened into a single signal.

Reductions of $[Co(N_4)(iminoacidato)]^{2+}$ by $Na_2S_2O_4$

$[Co(en)_2(Vim)](ClO_4)_2$ (0.100 g, 2.03×10^{-4} mol) was dissolved in acetate buffer solution (pH 4.10, 5 M, 10 mL) at 25 °C. Sodium dithionite (0.195 g, 1.02×10^{-3} mol) was added to the stirred solution. After 30 minutes the reaction was quenched by diluting it with water (≈90 mL) and passing the solution down a column of Dowex ion exchange resin (2.5 × 7.0 cm) with the aid of suction provided by a water pump. The adsorbed material was washed with water and with 0.5 M HCl (to remove traces of Co^{2+}) before being eluted from the column as a single fraction with 3 or 4 M HCl. The solvent was removed by rotary evaporation and the residue analysed by ^{1}H NMR spectrometry.

Variations to this procedure included: different substrate concentrations, temperature (0 °C, 25 °C), pH (3.0, 4.1 (acetate); 5.5, 6.5, 7.5 (Bis Tris); 8.5 (HEPES); 9.5 (carbonate)) and use of an air or N_2 atmosphere. If the reaction was performed in D_2O the solution was unbuffered; the pH of the unbuffered solution was approximately 4.5. The related complexes $[Co(NH_3)_4(Vim)]Cl_2$ and $[Co(en)_2(N–MeVim)]Cl_2$ were reacted with dithionite at pH 4.1 using the procedure described above.

$[Co(en)_2(Gim)](CF_3SO_3)_2$, (0.10 g, 1.8×10^{-4} mol) was dissolved in acetate buffer (0.5 M, pH 4.1, 10 mL). Nitrogen was bubbled through the solution for 20 minutes before sodium dithionite (0.035 g, 1.8×10^{-4} mol) was added, with vigorous stirring. After 30 seconds the reaction was quenched as described for reduction of $[Co(en)_2(Aim)]Cl_2$. ^{1}H NMR (D_2O): δ 4.2–5.8 (br, en–NH_2), 3.75 (s, 2H, NH_2–CH_2), 2.6–3.0 (br, 8 H, en–CH_2).

The complexes $[Co(NH_3)_4(Aim)]Cl_2$, $[Co(NH_3)_4(N–MeAim)]Cl_2$, $[Co(bipy)_2(Aim)]Cl_2$, *p*-$[Co(tren)(Aim)]Cl_2$, $[Co(en)_2(Yim)]Cl_2$, $[Co(en)_2(Kim)]Cl_2$, $[Co(en)_2(phenylGim)]$-

$(ClO_4)_2$, $[Co(en)_2(Mim)]Cl_2$, $[Co(en)_2(Pim)]Cl_2$, $[Co(en)_2$-$(pipim)]Cl_2$, $[Co(NH_3)_4(sarim)]Cl_2$, $[Co(NH_3)_4(Eim)]Cl_2$, and $[Co(NH_3)_4(phenylGim)](ClO_4)_2$ were reacted with dithionite in the manner described for the reduction of $[Co(en)_2(Aim)]Cl_2$. $[Co((CH_3)_2$-diimino-sar$)]ZnCl_4 \cdot Cl$ (0.050 g) was dissolved in a mixture of D_2O (0.70 mL) and acetate buffer (0.5 M, pH 4.1, 0.4 mL). Portions of sodium dithionite (4 × 0.040 g, were added to this solution every 2 hours. The reaction was monitored by ^{13}C NMR spectrometry. No conversion of imine to amine was detected.

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