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Biomimetic Ligands for Transition Metals: Catechol-Containing Peptides

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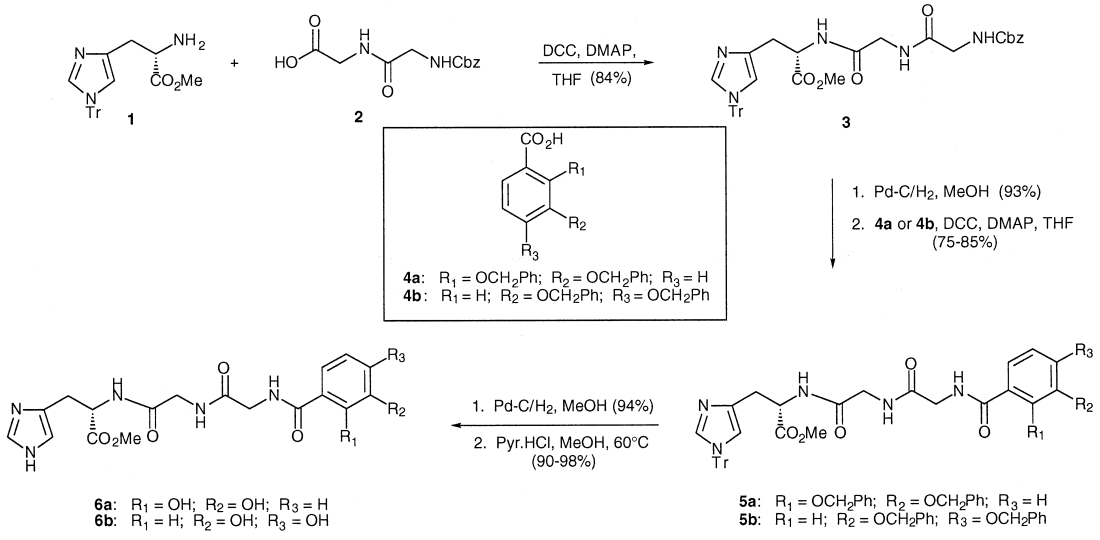
Abstract—The tetrapeptide ligands **6a** and **6b** containing a catechol moiety have been synthesised and their metal binding with Fe(III), Mn(III) and Cu(II) has been studied using fluorescence spectroscopy.

One of the major challenges in current organic chemistry is to find efficient catalysts for the chemo- and stereoselective insertion of one oxygen atom into various organic molecules under mild conditions. Transition metal-containing enzymes capable of achieving such transformations are well known[1,2] but the main efforts in this area have followed a non-biomimetic course. However, there are limited reports illustrating the effectiveness of peptides[3–5] and α-amino acids[6] as ligands of metallic species in asymmetric reactions. In addition, simple peptides such as the physiologically active tripeptide, Gly–His–Lys have been shown to exhibit strong chelating ability to metal ions.[7] Such peptides all contain a histidyl residue and chelation of metal ions has been shown to involve the terminal amino nitrogen and histidine imidazole nitrogen. This chelating domain is also a feature of serum albumins (His–Gly–Gly).[8] These systems have been shown to produce oxidising species upon complexation.[9] The aim of this project was to design and synthesize small peptides containing the



Scheme 1.

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key His–Gly–Gly structural motif along with a metal-coordinating group at the N-terminus. In addition to being a good ligand for redox-active transition metals, the N-terminus group should also act as a fluorsecent probe such that metal binding could be followed by observing fluorescence quenching. In this way, potential new oxidation catalysts could be readily identified. We now wish to report on the synthesis and metal-binding of two such peptide-based ligands.

The N-terminus group chosen was the 1,2-dihydroxybenzene (catechol) group. This group is found in siderophores,[10] the natural iron chelators and it also possesses suitable fluorescent properties to act as a probe for binding.[11] The regioisomeric ligands **6a** and **6b**, differing only in the positioning of the 1,2-diol functionality with respect to the attaching chain were synthesised as outlined in Scheme 1. Tritylation of the imidazole nitrogen of histidine methyl ester was accomplished in 75% yield

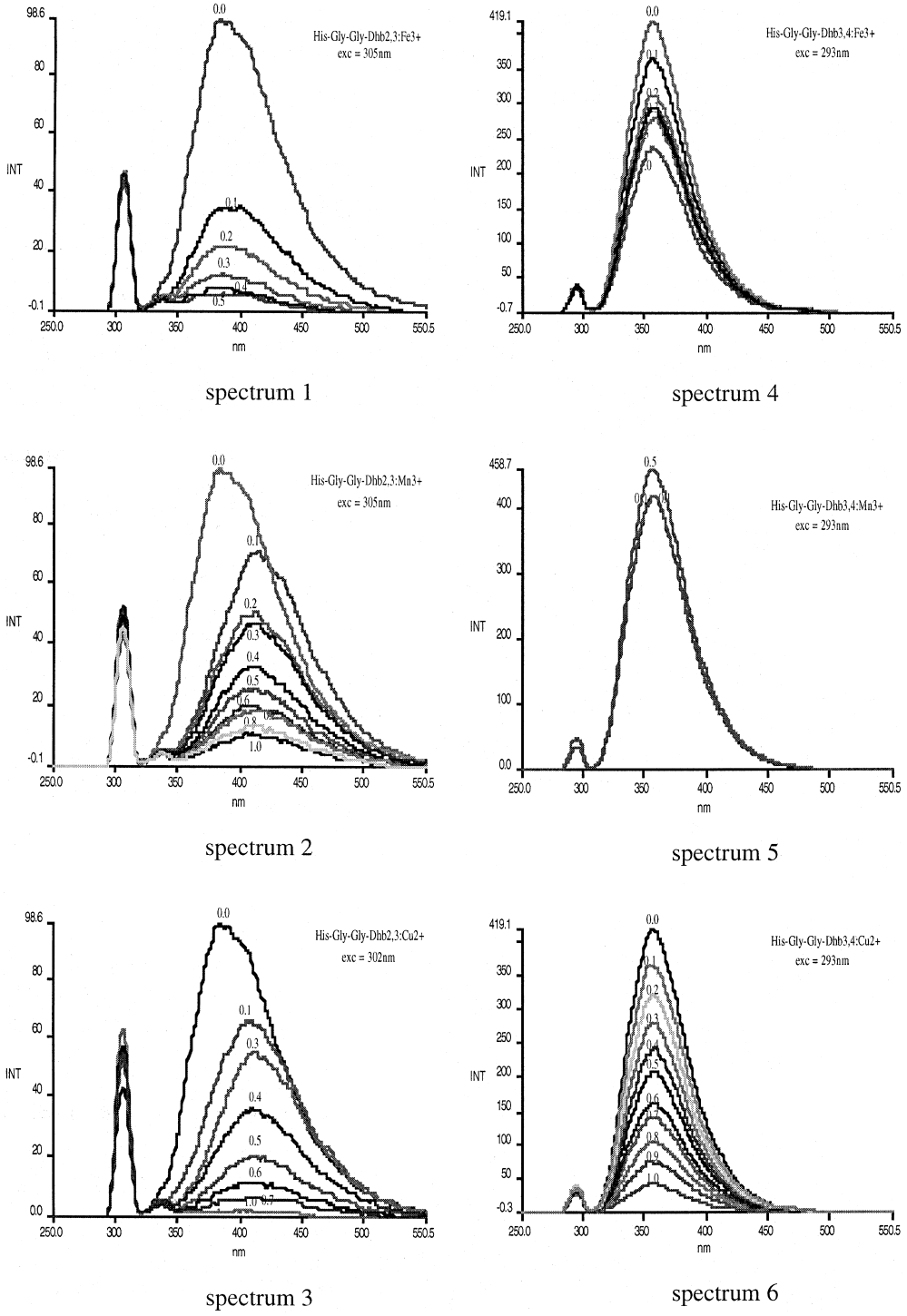

Figure 1. Uncorrected fluorescence emission of **6a** and **6b** in response to the addition of Fe(III), Mn(III) and Cu(II) metal cations in methanol: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 molar equivalents. Exicitation was carried out at 305nm for (**6a**) and 293nm for (**6b**).

under standard conditions. *N*-Trityl hisitidine methyl ester **1** was then condensed with *N*-Cbz protected diglycine **2** to give the protected tripeptide **3** in 84% yield. Considerable experimentation was carried out to find the most suitable protecting groups for the phenolic hydroxyl groups in the dihydroxybenzoate moiety. The conditions for the removal of ketal-based protecting groups proved to be too severe for the system and finally, benzyl ether protection was chosen. Removal of the Cbz group from tripeptide **3** followed by condensation separately with the protected 2,3-dihdroxybenzoic acid **4a** and the protected 3,4-dihydroxybenzoic acid **4b**, gave the pseudotetrapeptides **5a** and **5b** in yields consistently between 75% and 85%. Catalytic hydrogenation removed the benzyl ethers in high yield and finally treatment with pyridinium hydrochloride in methanol removed the trityl group to give **6a** and **6b** in overall yields of some 90% from **5a** and **5b**.

Although fluorescence has been widely used in peptide chemistry, it has usually been employed as a means of detection and quantification of small amounts of peptides either through the fluorscence properties of tryptophan, derivatisation with the dansyl[12] group or incorporation of non-natural amino acids[13] with specific fluorescent properties. The use of fluorescence quenching to explore metal binding has previously been accomplished by Imperiali who derivatised the peptides studied using a dansyl group.[12c,d] In our case, the dihydroxybenzoate group, in addition to metal chelation shows excellent fluorescence properties with excitation of **6a** at 305 nm leading to strong emission at 385 nm and excitation of **6b** at 293 nm leading to strong emission at 360 nm. The results of metal binding studies with Fe(III) chloride, Mn(III) acetate and Cu(II) acetate are shown in Figure 1. Spectrum 1 shows that a progressive quenching of fluorescence occurs up to 0.5 molar equivalents of Fe(III) to **6a**. Spectrum 2 shows a similar progressive quenching of fluorescence emission of **6a** on addition of Mn(III). However, in this case complete quenching requires 1 molar equivalent of Mn(III). Spectrum 3 shows the results obtained for **6a** and Cu(II). This latter combination closely mirrors the results for **6a** and Mn(III). From this series of results, we conclude that Fe(III) forms a preferential 2:1 complex with **6a** involving 2 molecules of **6a** to each Fe(III) ion whilst Mn(III) and Cu(II) form 1:1 complexes. The fluorescence quenching provides evidence for binding of metal ion to the catechol unit but we have no direct evidence of the site(s) of further co-ordination. Clearly, the histidine imidazole would be the most favoured option for the next co-ordination site in **6a**. This would then produce a 3-point attachment of **6a** to metal ions. The 1:1 complexes formed with Mn(III) and Cu(II) could also involve metal binding via the amide groups.

The behaviour of peptide **6b** under similar conditions with Fe(III), Mn(III) and Cu(II) is shown in spectra 4, 5 and 6 respectively. In spectrum 4, fluorescence quenching with Fe(III) is again observed. However, this time it is much less complete than in the case of **6a** and Fe(III) as judged by the degree of quenching (c.f. spectrum 1). Additionally, it does not plateau out at 0.5 molar equivalents of metal to ligand. On titration with Mn(III) (spectrum 5), virtually no quenching is observed in marked contrast to the corresponding case with **6a** (spectrum 2). Finally, titration with Cu(II) (spectrum 6) shows a similar result to that obtained with ligand **6a** (spectrum 3), namely progressive quenching but at a notably slower 'rate' and leaving a small but noticeable residual fluorescence. We interpret these results as showing that with Fe(III) and particularly Mn(III), peptide **6b** shows little or no co-ordination via the catechol unit. This is explicable in terms of the twist required in the system to incorporate binding to both the imidazole and the catechol in **6b** owing to the disposition of the diol functionality in this ligand. However, it appears that **6b** can form a complex with Cu(II) involving the catechol group although a comparison of the quenching with that observed for **6a** with Cu(II) would indicate a more weakly bound complex.

In summary, we have prepared two peptide-based catechol-containing ligands and have explored their metal binding using the fluorescent properties of the catechol unit. These studies have shown that the 2,3-dihydroxybenzamide unit in **6a** is a better system for metal chelation than the 3,4-dihydroxybenzamide unit in **6b**. With a rapid screening method available and reliable chemistry which can be transfered to the solid phase, we are now in a position to investigate related ligands and explore their chemical reactivity.

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