

A "Second-Generation" Transition Metallo-Carbonyl Reagent for Protein Labelling Based on the $(\eta^5\text{-cyclopentadienyl})\text{Fe}(\text{CO})_2(\eta^1\text{-N-imidato})$ System

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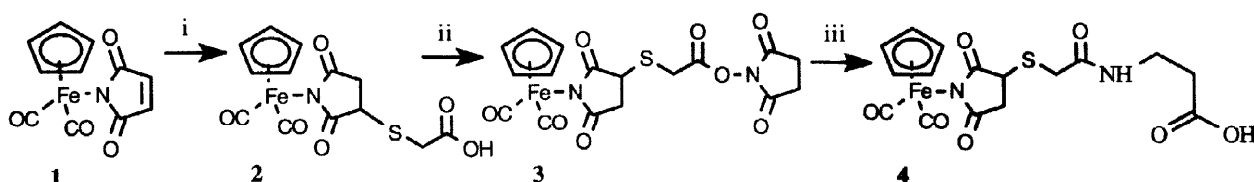
Abstract : Complex $(\eta^5\text{-Cp})\text{Fe}(\text{CO})_2(\eta^1\text{-N-imidato})$ is shown to be the basis of a new family of IR-detectable reagents for the controlled selective labelling of biomolecules. One example of an N-succinimidyl ester is reported here together with its reactivity with a model protein. © 1998 Elsevier Science Ltd. All rights reserved.

Several families of transition metallo-carbonyl complexes have been recently used as IR-detectable reagents for the labelling of drugs¹, proteins² and oligonucleotides³. These complexes and their bioconjugates display intense, sharp absorption bands owing to the stretching vibrations of metal-coordinated CO ligands in the 1850–2150 cm^{-1} region. This region is devoid of any absorption bands from biomolecules and biological matrices are virtually transparent, allowing their detection down to the picomole level.⁴

Ideally, the labelling agents should be stable in the biological medium (non oxidisable), soluble to some extent in water or water-organic solvent mixtures and contain a functional group capable of interacting with the desired target function borne by the biomolecule. As the organometallic systems fulfilling all these conditions are still rare, it is interesting to envisage the transformation of already known labelling reagents into new, "second-generation" markers selective of other functional groups.

We have recently introduced complex $(\eta^5\text{-cyclopentadienyl})\text{Fe}(\text{CO})_2(\eta^1\text{-N-imidato})$ **1** as a marker for thiol functions of biomolecules.⁵ Subsequent studies showed that **1** also readily alkylated the imidazole ring of histidine at neutral pH and the amino function of β -alanine at basic pH.⁶ Reaction of **1** with a model protein, namely bovine serum albumin (BSA) and subsequent analysis of the protein conjugates revealed that **1** was able to alkylate cysteine, histidine and/or lysine residues, according to the applied reaction conditions.⁷

Here we report that **1** can be easily transformed into a "second-generation" metallo-carbonyl marker **3** containing an N-succinimidyl (NS) activated ester function by a two-step procedure.



i : thioglycolic acid ; ii : NHS and DCC ; iii : β -alanine in THF/water pH 9

Addition of thioglycolic acid to **1** at pH 9–10 resulted in a 48 % yield of **2** after work-up. This complex was then reacted with N-hydroxysuccinimide (NHS) and N,N'-dicyclohexylcarbodiimide (DCC) in dichloromethane to afford **3** with 77 % yield.⁸ Complex **3** was able to acylate β -alanine in a THF-water

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medium at pH 9 overnight to yield complex **4** with 79 % yield.⁹

Three BSA-labelling experiments were carried out using a 60-fold molar excess of complex **3** over protein, that is in stoichiometric amounts relative to the number of lysine residues, at pH 7.0 (phosphate buffer), 8.0 and 9.0 (borate buffers). Bioconjugates were purified by gel filtration chromatography and an average number of CpFe(CO)₂ entities bound per protein molecule equal to 7, 11 and 12 was calculated from IR measurements performed on the 2053 cm⁻¹ band, respectively.¹⁰

We have thus demonstrated that complex (η⁵-cyclopentadienyl)Fe(CO)₂(η¹-N-imidato) could be considered as a synthon for the preparation of new IR-detectable labelling reagents. Complex **3** which possesses new labelling capabilities is the first example of this. Work is now in progress to extend this concept to the synthesis of other families of reagents, in particular iodoacetamido derivatives.

References and notes

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8. A suspension of **1** and thioglycolic acid in water pH 9 was stirred 2 d at r.t. After washing with chloroform, the aqueous phase was acidified to pH 2 and extracted with chloroform. Crystallisation from CH₂Cl₂ / ether / hexanes afforded **2** as a yellow solid. ¹H NMR (δ, CDCl₃): 5.07 (s, 5H, Cp), 3.9 (m partly overlapped, H-3), 3.84 and 3.35 (d, 1H, S-CH₂), 3.09 and 2.45 (dd, 1H, succinimide CH₂). IR (CHCl₃, ν cm⁻¹): 2055, 2000 (Fe-CO), 1725 (CO acid), 1645, 1630 (CO imide). Elem anal: Calcd (found) for C₁₃H₁₁FeNO₆S: C 42.76 (42.85), H 3.04 (3.00), N 3.84 (3.87), S 8.78 (8.41). Compound **2** was activated into **3** by action of NHS and DCC in CH₂Cl₂. ¹H NMR (δ, CDCl₃): 5.05 (s, 5H, Cp), 4.31 and 3.64 (d, 1H, S-CH₂), 3.96 (dd, 1H, H-3), 3.07 and 2.44 (dd, 1H, H-4), 2.86 (s, 4H, succinimide). IR (CHCl₃, ν cm⁻¹): 2055, 1995 (Fe-CO), 1810, 1785, 1740 (CO ester), 1645 (CO imide). Elem anal: Calcd (found) for C₁₇H₁₄FeN₂O₈S: C 44.18 (43.81), H 3.05 (3.06), N 6.06 (6.06), S 6.94 (6.87).
9. A stoichiometric mixture of **3** and β-alanine in water pH 9 / THF was stirred 2 h at r.t. After washing with chloroform, the aqueous phase was acidified to pH 2 and extracted with chloroform. Evaporation of the solvent yielded **4** as a yellow oil. ¹H NMR (δ, CDCl₃): 7.7 (br s, 1H, COOH), 5.07 (s, 5H, Cp), 4.2 (very br, 1H, NH), 3.73 (m partly overlapped, 1H, H-3), 3.67 and 3.35 (d, 1H, S-CH₂), 3.60 (m, 2H, N-CH₂), 3.07 and 2.47 (dd, 1H, H-4), 2.65 (br t, 2H, CH₂-COO). IR (CDCl₃, ν cm⁻¹): 2055, 2050 (Fe-CO), 1740 (CO acid), 1685 (amide I), 1655 (imide), 1515 (amide II).
10. To a 1 ml of a 50 μM BSA solution at pH 7, 8 or 9 was added 0.1 ml of a 0.03 M DMF solution of **3**. After 1 h at r.t., protein conjugates were purified by gel filtration chromatography and analysed by IR spectroscopy by deposition of 10 μl of conjugate solution on nitrocellulose and air drying.