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Synthesis and Reactivity of a Transition Metal-Carbonyl Imidoester Designed for the Selective and Covalent Labelling of Biological Macromolecules.

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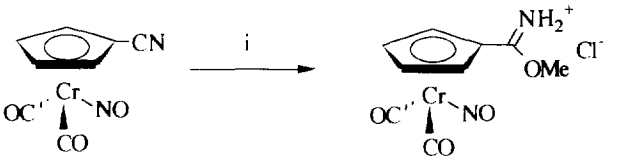
Abstract *:* The first transition metal-carbonyl water-soluble imidoester has been prepared in several steps and characterised by classical spectroscopic methods. It is aimed at the selective conjugation of amine-containing biomolecules such as proteins in aqueous medium, leading to conjugates that retain their net charge. A preliminary reactivity study with bovine serum albumin (BSA) is reported. Copyright © 1996 Published by Elsevier Science Ltd

In the emerging field of bio-organometallic chemistry, one of the most promising developments lies in the utilisation of metal complexes as probes for the monitoring of biochemical processes.[1] They indeed possess unique features which allow them to be detected at very low concentrations in complex biological media.[2] In this area, we took interest into a special class of organometallic complexes, i.e. metal-carbonyl complexes, which have a specific infrared spectrum in the 1800-2150 cm^{-1} region, thanks to the stretching modes of the carbonyl ligands. These non-isotopic probes were successfully used in a new immunoassay method named Carbonyl-MetalloImmunoAssay (CMIA).[3] One of the major stages for the development of a new test is to prepare biomolecules labelled with these probes. For amine-containing biomolecules (like proteins), two classes of selective metal-carbonyl reagents have been described to date. The first one chronologically is composed of esters of N-hydroxysuccinimide which react with amines to give amides.[4] The second class consists of pyrylium salts which react with amines to yield pyridinium salts.[5] While both classes display a high reactivity towards proteins, they both have different drawbacks. N-succinimidyl esters and pyrylium salts are not water-soluble, which necessitates addition of an organic cosolvent that could be detrimental to protein stability. Water-soluble N-sulfosuccinimidyl esters were synthesized but proved less reactive because of their increased tendency to hydrolyse.[6] Furthermore, N-succinimidyl esters induce a change in the protein net charge by converting ammonium groups into amides. From thiol-containing biomolecules, a metal carbonyl N-maleimide complex has been recently described.[7]

Imidoesters have long been utilised for the selective chemical modification of protein amino groups.[8] They are water-soluble and form amidines which are positively charged at physiological pH. Here, we report the preparation of the first example of a water soluble organometallic imidoester.

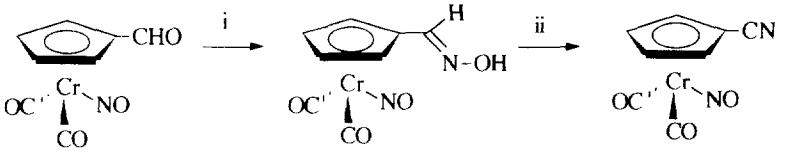
Imidoester **4** was prepared in 60 % yield by the base-catalysed addition of methanol to nitrile **3**[9] as preconized by Moeller et al.[10], followed by protonation with gaseous HCl. Unsurprisingly, the classical acid-catalysed Pinner synthesis failed in our case because of the electron-attracting character of the organometallic group in the α-position of the nitrile.[11]



3 **4**

i : K_2CO_3 cat. in MeOH then HCl_g in Et_2O

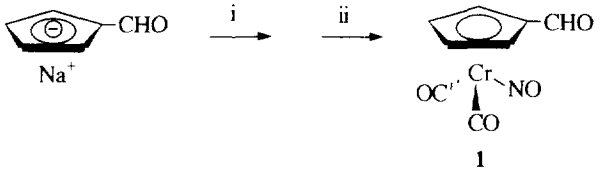
(η^5-Cyanocyclopentadienyl)(dicarbonyl)(η^3-nitroso)chromium **3** was prepared by a new route within two steps, starting from (η^5-formylcyclopentadienyl)(dicarbonyl)(η^3-nitroso)chromium **2**.[12] The oxime intermediate **2** was dehydrated under mild conditions in the presence of 1.2 eq. of dicyclohexylcarbodiimide (DCC) and a catalytic amount of $CuSO_4$.[13] This nitrile had been previously prepared in four steps from the methylic ester analogue with a similar yield.[14]



1 **2** **3**

i : $HONH_3Cl$, pyridine in EtOH; ii : DCC, NEt_3, $CuSO_4$ cat. in CH_2Cl_2

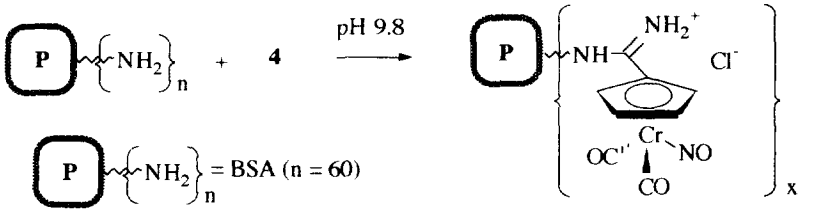
Aldehyde **1** was prepared by complexation of formylcyclopentadienyl sodium with (tricarbonyl)(triammine)chromium[15] instead of $Cr(CO)_6$.[16] This complex, which is used for the complexation of arenes, proved a very advantageous alternative to $Cr(CO)_6$ because it is non volatile, easy to prepare[17] and handle and reacts stoechiometrically and at lower temperatures than $Cr(CO)_6$ (THF reflux versus DMF reflux).



1

i : $Cr(CO)_3(NH_3)_3$, THF; ii : CH_3COOH then diazald

A preliminary labelling experiment of a model protein, namely bovine serum albumin (BSA), was carried out with **4**. The primary structure of BSA is comprised of 59 lysines and one N-terminal residue, i.e. 60 theoretical sites of coupling. BSA and 60 molar eq. of **4** were dissolved in carbonate buffer pH 9.8 and incubated at room temperature (25°C) for 24 h. The resulting conjugate was purified by gel filtration chromatography using phosphate buffer pH 7.4 as the eluent. The fractions containing the protein were pooled and concentrated by centrifugal ultra-filtration.



An actual coupling of 1.3 ± 0.1 chromium carbonyl entities per protein molecule was measured by IR spectroscopy on 3mm-diameter KBr micropellets as previously described,[18] taking advantage of the presence of the two ν_{CO} detected at 2032 and 1964 cm^{-1}. This relatively weak coupling seems to be related to the slow decomplexation of the organochromium reagent in aqueous medium monitored by IR spectroscopy of the carbonyl ligands. However, this preliminary result is encouraging and now requires optimisation of the coupling reaction conditions.

In summary, the first transition metal carbonyl imidoester has been prepared, characterised, and its coupling ability with a model protein demonstrated.

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9. **3** (0.17 g, 0.45 mmol) and K_2CO_3 (0.013 g) were stirred in methanol 24 h at room temp. The raw product was flash-chromatographed on silica gel (eluent: chloroform / hexane 2/1), dissolved in ethylic ether and saturated with gaseous HCl. A beige precipitate (0.08 g; 60 %) was obtained. ^1H NMR (D_2O, 200 MHz) ∂ in ppm 4.1 (3H, s, CH_3O-), 5.5 (2H, t, J= 2.5 Hz, Cp), 6.1 (2H, t, J= 2.5 Hz, Cp). IR (KBr) ν in cm^{-1} 2031, 1958 ν_{CO}; 1706 ν_{NO}; 1636 $\nu_{C=N}$. Elem. anal. Calc for $CrC_9H_9N_2O_4Cl$: C, 36.44; N, 9.44; H, 3.06. Found: C, 37.93; N, 9.10; H, 3.44.
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12. **1** (0.4 g), hydroxylamine hydrochloride (0.5 g) and pyridine (0.2 ml) were refluxed in ethanol for 90 min. After elimination of the solvent under reduced pressure, the brown residue was extracted with ethylic ether and washed with water. 0.373 g of **2** as a yellow-red oil were obtained. ^{1}H NMR ($CDCl_3$, 200 MHz) δ in ppm 5.4 (2H, broad s, Cp), 5.7 (2H, broad s, Cp) 7.0 (1H, s, N-OH) 7.7 (1H, s, CH=N). IR ($CHCl_3$) ν in cm^{-1} 2026, 1957 ν_{CO}; 1706 ν_{NO}.To a deep blue solution of $CuSO_4$ (0.05 g) and pyridine (0.125 ml) in H_2O was added **2** (1.5 mmol), triethylamine (0.44 ml) and DCC (0.37 g, 1.8 mmol) in dichloromethane. The mixture was stirred 1 h then the excess of DCC was destroyed by addition of formic acid and the solvent evaporated. The residue was suspended in dichloromethane, the urea filtered. The organic phase was washed with water, filtered on silica gel. 0.25 g (73 %) of **3** were obtained as a red oil. IR (CH_2Cl_2) ν in cm^{-1} 2238 ν_{CN}; 2036, 1969 ν_{CO}; 1716 ν_{NO}.
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15. Formylcyclopentadienyl sodium (30 mmol) prepared as in ref. 16 and (tricarbonyl)(triammine)chromium (12 mmol) prepared as is ref. 17 were refluxed in THF for 150 min and allowed to cool down to room temp. Acetic acid (1.4 ml) was slowly added, followed by 3.4 g of Diazald after 1 h. After 75 min, the mixture was extracted with ether/pentane, washed with water and chromatographed on silica gel (eluent: ether / hexane 1 /10). **1** (0.94 g, 34%) was obtained as an orange-red powder. IR (THF) ν in cm^{-1} 2028, 1962, ν_{CO}; 1712, ν_{NO}; 1693 ν_{CO}.
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