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Transition metal-carbonyl labeling reagent containing iodoacetamido function: $CpFe(CO)_2$ [η^1-*N*(1)-4-iodoacetamidophthalimidato]-synthesis and reaction with the phenytoin and ethosuximide anions

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Abstract—The first transition metal-carbonyl complex containing iodoacetamido function, $CpFe(CO)_2$[η^1-*N*(1)-4-iodoacetamidophthalimidato] was synthesized and used for labeling of two drugs: phenytoin and ethosuximide. © 1998 Published by Elsevier Science Ltd. All rights reserved

Keywords: metal-carbonyl; iodoacetamido; phenytoin anion; ethosuximide anion

Transition metal carbonyl complexes having substituents able to react selectively with functional groups present in molecules of biological and pharmacological interest, currently attract considerable interest as IR-detectable labeling reagents [1]. The detection of the bioconjugates is feasible in a pico- or subpicomole scale by virtue of very strong absorption bands corresponding to the ν(CO) modes, appearing at 2100–1850 cm^{-1} i.e. in the region which is generally free from other interferring absorptions, even in aqueous solutions and biological systems [2,3]. This approach was successfully applied for the labeling of haptens in immunoassays (CMIA-carbonylmetalloimmunoassay) [2], for labeling of steroid hormones for the study of hormone-receptor interaction [4], and labeling of oligonucleotides [5,6]. Most of the labeling procedures described up to now is based on organometallic complexes containing active *N*-hydroxysuccinimide ester function which reacts with the amino group present in the biomolecule and forming the amido bond. Another approach uses organometallic complexes having pyrylium system [7], or iminoester [8], as a reactive function (target function-NH_2).

We have recently reported the synthesis of $CpFe(CO)_2$(η^1-N-maleimidato), which contains a maleimide system reactive towards thiol groups and can be used for labeling of e.g. cysteine-containing peptides or proteins [9]. However, we have demonstrated that it can also label histidine and (in basic medium) lysine residues [10]. Another approach developed recently in our laboratory in collaboration with the Prof. Jaouen's group is the use of the $CpFe(CO)_2$– complexes of phthalimide containing in the 3- or 4-position isothiocyanato functions reactive towards amino groups [11].

In this communication we report the synthesis of the first metallocarbonyl marker containing the $CpFe(CO)_2$(η^1-N-phthalimidato) system and iodoacetamido group as the reactive function. This function is believed to be specific for thiols (cysteines), although it can also react with methionines (MeS-), histidines (imidazole) and lysines (NH_2) [12,13]. However, prompted by recent interest in the development of organometallic tracers for immunoassays of antiepileptic and sedative drugs [2,14,15], we decided to study the reactivity of our reagent toward anions of two important representants of this group of drugs: phenytoin (5,5-diphenylhydantoin) and ethosuximide (2-methyl-2-ethylsuccinimide).

We synthesized the bioconjugates of these products which are of interest as potential markers in carbonylmetalloimmunoassays (CMIA).

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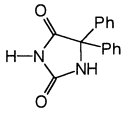
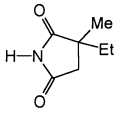


phenytoin



ethosuximide

Experimental

All reactions were carried out under argon. Dichloromethane was distilled from CaH_2. $CpFe(CO)_2[\eta^1$-*N*(1)-4-aminophthalimidato] (**1**) was prepared according to the earlier published procedure [11]. All reagents and other solvents (reagent grade) were used as received from Aldrich or Fluka. Column chromatographies were carried out using Kieselgel 60 (230–400 mesh ASTM) purchased by Merck. Instrumentation: IR-Specord 75 IR; ^{1}H NMR: Varian Gemini 200BB (200 MHz); FAB MS: Finnigan MAT 95. IR spectra were recorded in $CHCl_3$, ^{1}H-NMR in $CDCl_3$ and referenced to internal TMS, FAB spectra in m-nitrobenzyl alcohol matrices.

$CpFe(CO)_2(\eta^1$-*N*(1)-4-*iodoacetamidophthalimidato*) (**2**)

To a solution of **1** (310 mg, 0.92 mmol) in CH_2Cl_2 (5 ml) DCC (206 mg, 1.0 mmol) and iodoacetic acid (186 mg, 1.0 mmol) were added. After 1 h stirring at r.t. the solid formed was filtered off and the filtrate evaporated to dryness. Column chromatography (eluent $CHCl_3$) and crystallization (CH_2Cl_2-ether) gave **2** as yellow solid. Yield 386 mg (83%). IR (cm^{-1}): 2050 and 1995 cm^{-1} (Fe–CO), 1690 cm^{-1} (CO–amide), 1655 (CO–phthalimide), 1535 (amide-II). ^{1}H NMR ($CHCl_3$): 8.10, b, 1H, NH; 7.76, d ($J = 8.0$ Hz), 1H and 7.58, d ($J = 8.0$ Hz), 1H, H-5 and H-6; 7.69, s, 1H, H-3; 5.12, s, 5H, Cp; 3.90, s, 2H, CH_2. Residual ether (0.2 molecule) shows signals at 3.49, q and 1.22, t.

Analysis: Found: C, 40.61, H, 2.37; N, 5.83, I, 24.75. Calc. for $C_{17}H_{11}N_2O_5IFe \times 0.2\ C_4H_{10}O$: C, 40.35; H, 2.19; N, 5.54; I, 25.08.

Reaction of **2** *with Na-phenytoin*

A solution of **2** (102 mg, 0.20 mmol) and phenytoin (51 mg, 0.20 mmol) in 0.05 M NaOMe in MeOH (4 ml) was incubated at 50°C for 24 h, cooled to r.t. and poured to water (20 ml). The yellow precipitate was filtered off, dried under vacuum and recrystallized from CH_2Cl_2- pentane. Yield 107 mg (85%). IR (cm^{-1}): 2045, 1990 (Fe–CO); 1780, 1725 (phenytoin and amide CO); 1645 (phthalimide CO); ^{1}H-NMR: 9.11, bs, 1H (NH); 7.3–7.8, m, 13H (aromatic Hs); 5.07, s, 5H (Cp); 4.34, s, 2H (CH_2); FAB MS [positive ions, m/e (intensity)]: 631 (24%), M + H; 575 (56%), M + H-2CO. Elemental analysis: Found: C 61.08; H 3.72; N 8.82. Calc. for $C_{32}H_{22}N_4O_7Fe$: C 60.97; H 3.52; N 8.89.

Reaction of **2** *with Na-ethosuximide*

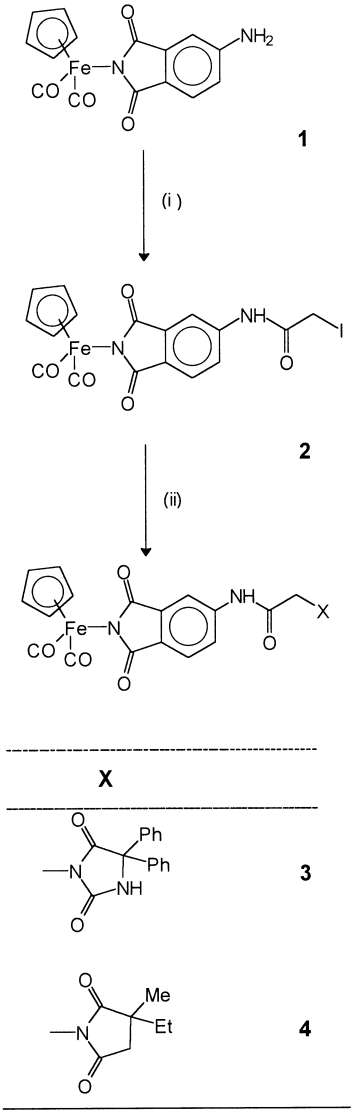
A solution of **2** (102 mg, 0.20 mmol) and ethosuximide (30 mg, 0.21 mmol) in 0.05 M NaOMe in MeOH (4 ml) was incubated at 50°C for 2 d, cooled to r.t., poured to water (20 ml) and extracted with dichloromethane (3×5 ml). The combined extracts were dried with sodium sulphate and evaporated to dryness to give yellow oil. The oil was dissolved in ether ($\sim$20 ml), diluted with hexanes and slowly concentrated *in vacuo* to give an yellow solid (53 mg, 51%). IR (cm^{-1}): 2045, 1990, (Fe–CO); 1775, 1720 (amide and succinimide CO); 1645 (phthalimide CO); ^{1}H-NMR: 8.32 bs, 1H, NH; 7.72, d ($J = 8$ Hz), 1H and 7.54, d ($J = 8$ Hz), 1H, H-5 and H-6; 7.67, s, 1H, H-3; 5.12, s, 5H, Cp; 4.38, s, 2H, $COCH_2$; 2.77, d ($J = 18$ Hz) 1H and 2.54, d ($J = 18$ Hz), 1H, succinimide ring CH_2, 1.82 m, 2H CH_2 (in Et); 1.38, s, 3H, Me; 0.94, t ($J = 7$ Hz), 3H, CH_3 (in Et); FAB MS [positive ions, m/e (intensity)]: 520 (26%), M + H; 464 (51%), M + H-2CO. Elemental analysis: Found: C 55.68; H 4.12; N, 8.32. Calc. for $C_{24}H_{21}N_3O_7Fe$: C 55.51; H 4.08; N, 8.09.

RESULTS AND DISCUSSION

Our results are summarized in Scheme 1.

We have found that readily accessible 4-aminophthalimidato complex **1** [11] readily reacts with iodoacetic acid and *N*,*N*′-dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperature to give the 4-iodoacetamido derivative **2** in high yield. Its structure was confirmed by spectroscopic as well as elemental analyses data. The amino group in **1** can be therefore acylated under mild conditions and **1** is a potential labeling reagent for biomolecules containing carboxylic functions. This possibility, however, will be a subject of further studies.

The iodoacetamido complex **2** readily reacts with sodium salts of phenytoin and ethosuximide (prepared by dissolving of the corresponding NH acids in methanol containing the equimolar amount of sodium methoxide) to afford conjugates **3** and **4,** respectively. The structures of these complexes were confirmed by spectroscopic and elemental analysis data. Their FAB mass spectra (*meta*-nitrobenzyl alcohol as a matrix) display, in the positive ions mode, peaks corresponding to M + H and M + H-2CO ions and in the negative ions mode to M-H and M-H-2CO ions. ^{1}H NMR spectra of conjugates **3** and **4** show characteristic singlet of —$COCH_2N$— protons (4.44 ppm for **3** and 4.38 ppm for **4**), shifted downfield in comparison to the signal of the —$COCH_2I$ protons in **2** (3.90 ppm). The complexes **3** and **4** are yellow air-stable microcrystalline solids, soluble in polar organic



Scheme 1. (i) ICH_2COOH—DCC; (ii) phenytoin (ethosuximide)-CH_3ONa-CH_3OH.

solvents (ethanol, dimethylsulfoxide, dimethylformamide), in chloroform, and practically insoluble in water. Their IR spectra show very strong absorption bands at around 2050 cm^{-1} and 1990 cm^{-1} i.e. in the region where the detection is particularly sensitive [2,3].

Compound **2** as containing the iodoacetamido function is also a potential label for biomolecules containing HS-groups [12,13]. Feasibility of such labeling is currently under study in our laboratory.

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