Covalent and Selective Labeling of Proteins with Heavy Metals. Synthesis, X-ray Structure, and Reactivity Studies of *N*-Succinimidyl and *N*-Sulfosuccinimidyl Ester Organotungsten Complexes

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New functionally substituted η^5-cyclopentadienyl and 2-oxaallyl (η^1-enolate) tungsten complexes bearing an *N*-succinimidyl or an *N*-sulfosuccinimidyl ester have been prepared and fully characterized. The molecular structures of [η^5-((succinimidooxy)carbonyl)cyclopentadienyl]methyltricarbonyltungsten(II) (**2**) and [η^5-((succinimidooxy)carbonyl)cyclopentadienyl]iodotricarbonyltungsten(II) (**5**) were solved by X-ray crystallography. The reactivity of these activated esters toward a range of amines and amino acids has been studied. While the *N*-succinimidyl ester enolate was found to be unreactive, *N*-succinimidyl-substituted cyclopentadienyl complexes were quite reactive, leading to the expected stable organometallic amides. Bovine serum albumin (BSA), a 66 kDa molecular mass globular protein, could be labeled with fair yields, and conjugates were characterized by IR spectroscopy of the CO ligands. Organotungsten *N*-succinimidyl esters thus appear as promising reagents for the labeling of proteins with heavy metals.

Introduction

The labeling of biological systems with heavy metals is a key step in the exploration and resolution of structures by both transmission electron microscopy and X-ray diffraction analysis of crystals.[1] In this area, we have been interested in exploring new tools based on the organometallic chemistry of heavy transition metals for the resolution of three-dimensional crystal structures of proteins. The X-ray analysis of such complex systems is greatly limited by several problems, among them the difficulty of producing crystals of adequate quality[2] as well as the necessity of preparing heavy-metal protein derivatives that are isomorphous with the native protein.[3] With the exception of mercury reagents that react covalently with thiol groups[4] and trimethyllead acetate, which reacts with carboxylic groups,[5] the typical reagents used are inorganic salts that react rather nonspecifically in a noncovalent way with charged side chains of proteins inside the crystal lattice.[3]

We have therefore undertaken the design of organometallic complexes that would be able to react covalently with targeted side chains of proteins, more precisely with primary amino groups of lysine residues. These amino acid residues are generally abundant and are usually located at the surface of proteins, because of their hydrophilic character.[6] We had previously prepared dicobalt hexacarbonyl[7,8] and cyclopentadienylrhenium tricarbonyl *N*-succinimidyl esters[9] and tested their reactivity toward proteins. Starting from this work, we describe here the synthesis of *N*-succinimidyl and *N*-sulfosuccinimidyl esters of tungsten(II), the crystal structures of [η^5-((succinimidooxy)carbonyl)cyclopentadienyl]methyltricarbonyltungsten(II) and [η^5-((succinimidooxy)carbonyl)cyclopentadienyl]iodotricarbonyltungsten(II) and their reactivity with amines, amino acids, and a model protein. Some of the results reported in this paper have been communicated in preliminary form.[10]

Results

Synthesis of Organotungsten Carboxylic Acids. The preparation of substituted-cyclopentadienyl half-sandwich complexes of group 6 can be achieved in two main ways: (1) electrophilic substitution of sodium cyclopentadienide followed by complexation of the corresponding metal carbonyl[11] or (2) metalation with alkyllithium as described for the preparation of iodocy-

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(1) Blundell, T. L.; Johnson, L. N. *Protein Crystallography*; Academic Press: New York, 1976.
(2) North, A. C. T.; Phillips, D. C. *Prog. Biophys. Mol. Biol.* **1969**, *19*, 1.
(3) Blake, C. C. F. *Adv. Protein Chem.* **1968**, *23*, 59.
(4) Petsko, G. A. *Methods Enzymol.* **1985**, *114*, 147.
(5) Holden, H. M.; Rayment, I. *Arch. Biochem. Biophys.* **1991**, *291*, 187.
(6) Metzler, D. E. *Biochemistry*; Academic Press: New York, 1977.
(7) Salmain, M.; Vessières, A.; Butler, I. S.; Jaouen, G. *Bioconjugate Chem.* **1991**, *2*, 13.
(8) Varenne, A.; Salmain, M.; Brisson, C.; Jaouen, G. *Bioconjugate Chem.* **1992**, *3*, 471.
(9) Salmain, M.; Gunn, M.; Gorfti, A.; Top, S.; Jaouen, G. *Bioconjugate Chem.* **1993**, *4*, 425.
(10) Gorfti, A.; Salmain, M.; Jaouen, G. *J. Chem. Soc., Chem. Commun.* **1994**, 433.
(11) Hart, W. P.; Macomber, D. W.; Rausch, M. D. *J. Am. Chem. Soc.* **1980**, *102*, 1196.

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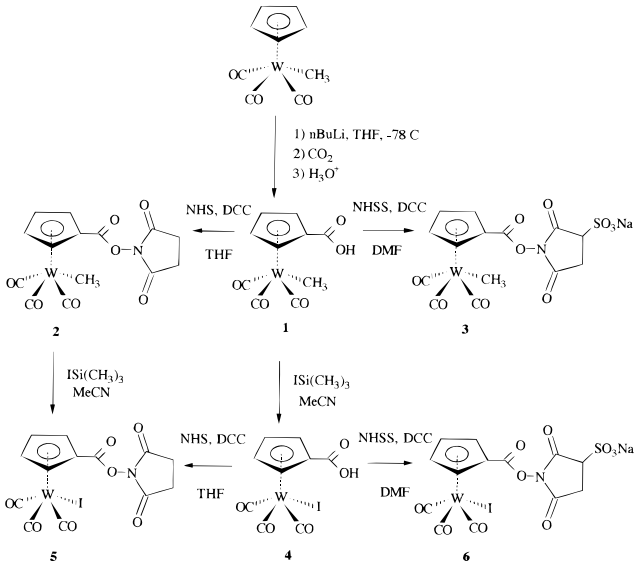
Scheme 1. Synthetic Routes to [(Succinimidooxy)carbonyl)cyclopentadienyl]- and [((Sulfosuccinimido)oxy)carbonyl)cyclopentadienyl]tungsten Complexes[a]



[a] Legend: DCC, *N*,*N*′-dicyclohexylcarbodiimide; NHS, *N*-hydroxysuccinimide; NHSS, *N*-hydroxysulfosuccinimide.

clopentadienyl complexes.[12] The synthesis of [η^5-carboxycyclopentadienyl]methyltricarbonyltungsten(II) (**1**) had been previously carried out by the first method: that is, preparation and subsequent saponification of the methyl ester.[13] We employed a strategy, based on the second method, involving lithiation of (η^5-Cp)W(CO)$_3$-CH$_3$ followed by addition of CO_2, as shown in Scheme 1; subsequent hydrolysis led directly to the expected carboxylic acid **1** with a yield of 71%.

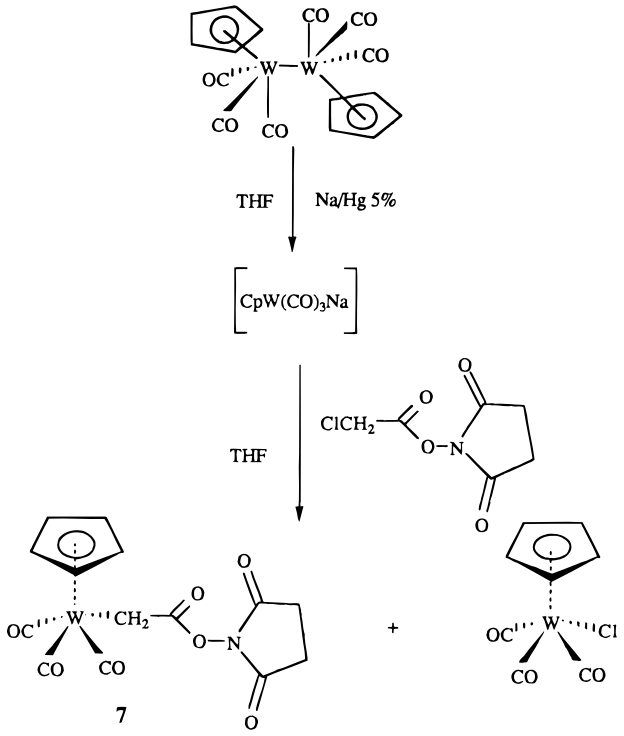
The action of iodotrimethylsilane on **1** gave almost quantitatively the acid **4**, which bears two heavy atoms linked together. This reaction involving substitution of the methyl ligand of **1** is similar to that previously observed.[13]

The ^{1}H NMR data for the acids **1** and **4** show different resonances for the cyclopentadienyl protons. Lower field resonances are observed for the iodo derivative, perhaps resulting from the electronegativity of the iodo ligand. For the same reason, IR ν_{CO} bands show a 30 cm^{-1} shift to high wavenumbers on going from **1** to **4**.

Synthesis of Organotungsten *N*-Succinimidyl Esters. Compounds **2** and **5** were prepared by following the Anderson method of synthesis of "activated" esters from the corresponding acids **1** and **4**, respectively.[14] Alternatively, **5** was synthesized from ester **2** by the action of iodotrimethylsilane at room temperature. Again, the cyclopentadienyl protons of **2** and **5** exhibit different resonances in the ^{1}H NMR but do not differ from those of the corresponding acids. Conversely, a significant shift of the ν_{CO} bands toward high wavenumbers is observed for esters **2** and **5** relative to acids **1** and **4**, presumably as a result of the electronegativity of the *N*-succinimidyl ester group.

In the η^1-enolate series, an *N*-succinimidyl ester function was attached α to the position of tungsten by

Scheme 2. Synthesis of (η^5-Cyclopentadienyl[((succinimidooxy)carbonyl)-methyl]carboxymethyl)tricarbonyltungsten (7)



a method modified from the literature, as depicted in Scheme 2.[15] The cyclopentadienyltricarbonyltungsten anion was generated by treating [CpW(CO)$_3$]$_2$ with sodium amalgam. Addition of *N*-succinimidyl chloroacetate led to **7**, but as the minor compound (yield 15%). The major orange complex separated by TLC was identified as CpW(CO)$_3$Cl by ^{1}H NMR and IR spectroscopy.[16]

A second experiment using *N*-succinimidyl iodoacetate led only to CpW(CO)$_3$I. This last result, that is the predominance of metal−halogen exchange over nucleophilic substitution, is quite similar to what had been previously observed during the reaction of the metal anion with esters and α-bromo ketones and secondary α-chloro esters.[17] Wilkinson et al. have suggested several mechanisms to explain this side reaction.[18] One such mechanism involves the attack of the organometallic anion on the halogen. In our case, the high electron-withdrawing ability of the *N*-succinimidyl ester moiety should favor this mechanism by developing an electrophilic character for the chloride in *N*-succinimidyl chloroacetate. The reactivity of the cyclopentadienyltungsten anion toward N-succinimidyl chloroacetate differs from that of the molybdenum analogue, which was recently reported in the literature.[19] In this latter case, another mechanism also suggested by Wilkinson et al. is involved.

Synthesis of *N*-Sulfosuccinimidyl Esters 3 and 6. Sodium *N*-sulfosuccinimidyl esters were developed

(12) Lo Sterzo, C.; Miller, M. M.; Stille, J. K. *Organometallics* **1989**, *8*, 2331.
(13) Macomber, D. w.; Rausch, M. D. *J. Organomet. Chem.* **1983**, *258*, 331.
(14) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
(15) (a) Hillis, J.; Ishaq, M.; Gorewith, B.; Tsustui, M. *J. Organomet. Chem.* **1976**, *116*, 91. (b) Doney, J. J.; Bergman, R. G.; Heathcock, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 3724.
(16) King, R. B.; Stone, F. G. A. *Inorg. Synth.* **1963**, *7*, 107.
(17) Burkhardt, E. R.; Doney, J. J.; Bergman, R. G.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 2022.
(18) Poli, R.; Wilkinson, G.; Motevalli, M.; Hursthouse, M. B. *J. Chem. Soc., Dalton Trans.* **1985**, 931.
(19) El Mouatassim, B.; El Amouri, H.; Vaissermann, J.; Jaouen, G. *Organometallics* **1995**, *14*, 3296.

Table 1. Summary of Crystallographic Data for 2 and 5

	2	5
chem formula	$WC_{14}H_{11}O_7N$	$WIC_{13}H_8O_7N$
fw	489.07	598.95
cryst syst	triclinic ($P\bar{1}$)	triclinic ($P\bar{1}$)
Z	2	2
a, Å	8.6563(7)	11.549(6)
b, Å	12.746(2)	12.421(2)
c, Å	16.038(2)	12.763(9)
α, deg	111.363(9)	96.47(4)
β, deg	93.147(9)	111.10(6)
γ, deg	108.509(8)	105.99(2)
V, Å^3	1533.2	1595.4
F(000)	928	1096.00
ρ(calcd), g cm^{-3}	2.12	2.5
μ(Mo Kα), cm^{-1}	77.298	93.699
cryst size, mm	0.20 × 0.30 × 0.30	0.30 × 0.35 × 0.60
diffractometer	Enraf-Nonius CAD4	Enraf-Nonius CAD4
monochromator	graphite	no
radiation; λ, Å	Mo Kα; 0.710 73	Mo Kα; 0.710 73
temp, °C	20	20
scan type	$\omega-2\theta$	$\omega-2\theta$
scan range θ, deg	0.53 + 0.60 tan θ	0.57 + 0.73 tan θ
2θ range, deg	$2 \le 2\theta \le 53$	$2 \le 2\theta \le 53$
no. of rflns collcd	6641	6780
no. of rflns used ($I > 3\sigma(I)$)	4885	4039
R	0.024	0.036
R_w[a]	0.032	0.050
transmissn factor (max, min, av)	1.1388, 0.8549, 1.6017	1.2769, 0.8139, 1.0022
secondary extinction	2.2426×10^{-7}	1.3053×10^{-7}
weighting scheme	non-Poisson contribution	non-Poisson contribution
GOF[b]	0.987	1.305
no. of least-squares params	482	416
residual electron density, e Å^{-3}	1.028	0.962

[a] $R_w = [\sum_i W_i(|F_o| - |F_c|)^2/\sum_i |F_o|^2]^{1/2}$. [b] GOF $= [\sum_i W_i(|F_o| - |F_c|)^2/(N_o - N_v)]^{1/2}$: N_o, number of observations; N_v, number of variables.

as an alternative to *N*-succinimidyl esters because of their aqueous solubility and their enhanced hydrophilicity.[20] Hence, *N*-sulfosuccinimidyl esters are useful reagents for the conjugation of proteins with labels in an aqueous medium. Their mode of preparation is similar to that of *N*-succinimidyl esters, with the exception that DMF is used as the solvent for solubility reasons. *N*-Sulfosuccinimidyl esters of tungsten (**3** and **6**) were prepared accordingly. As expected, these complexes are water soluble but are often isolated as mixtures with some of the starting materials (see below).

Their ^{1}H NMR spectra show a complex pattern in the 3−4.4 ppm region corresponding to the protons of the *N*-sulfosuccinimidyl ring. The two protons of the CH_2 group are inequivalent because of the neighboring asymmetrical carbon and give rise to two well-resolved doublets of doublets centered at 3.25 and 3.05 ppm. $CH(SO_3^-)$ appears in the form of a doublet of doublets centered at 4.25 ppm. The spectra also show that the final crude product contains unreacted *N*,*N*′-dicyclohexylcarbodiimide and *N*-hydroxysulfosuccinimide. In order to avoid hydrolysis, we did not try to purify these compounds any further.

X-ray Structures of 2 and 5. Complex **2** was crystallized by slow diffusion of a CH_2Cl_2 solution into hexane at room temperature to give yellow single crystals belonging to the triclinic space group $P\bar{1}$, with a = 8.6563(7) Å, b = 12.746(2) Å, c = 16.038(2) Å, α = 111.363(9)°, β = 93.147(9)°, γ = 108.509(8)°, and ρ(calc) = 2.12 g cm^{-3} for Z = 2. Crystal data collection parameters are listed in Table 1. Bond lengths and selected bond angles are listed in Table 2.

Complex **5** was crystallized by evaporation of a CH_2Cl_2 solution containing 10−15% of diethyl ether at room temperature to give red single crystals belonging to the triclinic space group $P\bar{1}$, with a = 11.549(6) Å, b = 12.421(2) Å, c = 12.763(9) Å, α = 96.47(4)°, β = 111.10(7)°, γ = 105.99(2)°, and ρ(calc) = 2.5 g cm^{-3} for Z = 2. Crystal data collection parameters are listed in Table 1. Bond lengths and selected bond angles are listed in Table 3.

Complexes **2** and **5** have the normal "four-legged piano stool" geometry common to this family of complexes.[21] Each asymmetric unit is composed of two molecules, labeled 1 and 2. For complex **5**, the iodine ligand is in the syn position with respect to the ipso carbon of the Cp ring in molecule 1 (I1−W2−C5 = 96.4(3)° and W1−I1 = 2.826 Å) and in the anti position in molecule 2 (I2−W2−C18 = 135.8(2)° and W2−I2 = 2.841 Å) (Figure 1). In contrast, in complex **2**, the methyl group is in the syn position in each molecule (Figure 2).

For both complexes, the substituted cyclopentadienyl carbon atoms (C5 and C18) are in maximally staggered positions. The pattern of W−C(η^5) bond distances is as follows: two long (C6, C7 in molecule 1 and C20, C22 in molecule 2), two medium (C8, C9 in molecule 1 and C20, C21 in molecule 2), and one short (C5 in molecule 1 and C18 in molecule 2). The W1−C5 and W2−C18 distances are significantly shorter than the remaining W−C(η^5) bonds and are in agreement with the values reported for the complex [η^5-C_5H_4COMe]W(CO)$_3$Me.[20a]

(20) Staros, J. V. *Biochemistry* **1982**, *21*, 3950.

(21) (a) Rogers, R. D.; Atwood, J. L.; Rausch, M. D.; Macomber, D. W. *J. Crystallogr. Spectrosc. Res.* **1990**, *20*, 555. (b) Bueno, C.; Churchill, M. R. *Inorg. Chem.* **1981**, *20*, 2197. (c) Doney, J. J.; Bergman, R. G.; Heathcock, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 3724. (d) Töfke, S.; Berhens, U. *J. Organomet. Chem.* **1987**, *331*, 229.

Table 2. Interatomic Distances (Å) and Selected Bond Angles (deg) for 2

W1−C27	2.284(6)	W2−C28	2.306(6)
W1−C1	1.964(5)	W2−C15	1.985(5)
W1−C2	1.988(5)	W2−C16	1.980(4)
W1−C3	1.971(4)	W2−C14	2.014(6)
W1−C5	2.315(4)	W2−C18	2.331(4)
W1−C6	2.303(4)	W2−C19	2.327(5)
W1−C7	2.331(5)	W2−C20	2.325(5)
W1−C8	2.379(6)	W2−C21	2.355(5)
W1−C9	2.373(5)	W2−C22	2.363(5)
O1−C1	1.162(8)	O9−C15	1.138(6)
O2−C2	1.140(7)	O10−C16	1.146(5)
O3−C3	1.150(5)	O8−C14	1.116(7)
O4−C4	1.191(6)	O11−C17	1.183(6)
C10−C11	1.501(5)	C25−C26	1.477(5)
C11−C12	1.544(9)	C24−C25	1.494(9)
C12−C13	1.483(7)	C23−C24	1.509(7)
O5−N1	1.374(4)	O12−N2	1.387(4)
O5−C4	1.387(6)	O12−C17	1.381(6)
O6−C10	1.202(6)	O14−C26	1.204(6)
O7−C13	1.191(8)	O13−C23	1.172(8)
N1−C10	1.370(8)	N2−C26	1.366(7)
N1−C13	1.386(6)	N2−C23	1.386(6)
C4−C5	1.469(5)	C17−C18	1.474(5)
C5−C6	1.414(6)	C18−C19	1.421(7)
C5−C9	1.410(6)	C18−C22	1.424(7)
C6−C7	1.423(6)	C19−C20	1.413(6)
C7−C8	1.405(7)	C20−C21	1.402(9)
C8−C9	1.408(5)	C21−C22	1.402(6)
C27−W1−C1	73.2(2)	C28−W2−C15	73.8(2)
C27−W1−C3	74.5(2)	C28−W2−C16	73.4(2)
C1−W1−C2	79.1(2)	C15−W2−C14	77.6(2)
C2−W1−C3	78.7(2)	C16−W2−C14	78.5(2)
C1−W1−C3	106.7(2)	C15−W2−C16	101.3(2)
C27−W1−C2	133.4(2)	C28−W2−C14	134.6(2)
C5−W1−C6	35.7(2)	C18−W2−C19	35.5(2)
C6−W1−C7	35.8(1)	C19−W2−C20	35.4(1)
C7−W1−C8	34.7(2)	C20−W2−C21	34.9(2)
C8−W1−C9	34.5(1)	C21−W2−C22	34.6(1)
C9−W1−C5	35.0(2)	C22−W2−C18	35.3(2)
O4−C4−C5	127.0(4)	O11−C17−C18	128.2(4)
O4−C4−O5	122.9(4)	O11−C17−O12	122.9(3)
O5−C4−C5	110.0(4)	O12−C17−C18	108.9(4)
C4−O5−N1	111.1(3)	C17−O12−N2	111.9(4)
O5−N1−C13	120.4(4)	O12−N2−C23	121.6(4)
O5−N1−C10	122.5(4)	O12−N2−C26	121.0(4)

Table 3. Interatomic Distances (Å) and Selected Bond Angles (deg) for 5

W1−I1	2.826(1)	W2−I2	2.841(1)
W1−C1	2.00(1)	W2−C15	2.00(1)
W1−C2	1.99(1)	W2−C16	1.97(1)
W1−C3	1.95(1)	W2−C14	1.99(1)
W1−C5	2.29(1)	W2−C18	2.26(1)
W1−C6	2.38(1)	W2−C19	2.29(1)
W1−C7	2.38(1)	W2−C20	2.37(1)
W1−C8	2.31(1)	W2−C21	2.40(1)
W1−C9	2.32(1)	W2−C22	2.33(1)
O1−C1	1.14(2)	O9−C15	1.14(2)
O2−C2	1.14(1)	O10−C16	1.17(2)
O3−C3	1.16(2)	O8−C14	1.12(1)
O4−C4	1.16(2)	O11−C17	1.21(1)
C10−C11	1.52(2)	C25−C26	1.45(2)
C11−C12	1.49(2)	C24−C25	1.53(2)
C12−C13	1.50(2)	C23−C24	1.50(2)
O5−N1	1.38(1)	O12−N2	1.34(1)
O5−C4	1.42(2)	O12−C17	1.39(1)
O6−C10	1.17(2)	O14−C26	1.22(2)
O7−C13	1.23(2)	O13−C23	1.17(2)
N1−C10	1.36(2)	N2−C26	1.38(2)
N1−C13	1.34(2)	N2−C23	1.38(2)
C4−C5	1.48(2)	C17−C18	1.47(2)
C5−C6	1.40(2)	C18−C19	1.45(2)
C5−C9	1.46(2)	C18−C22	1.41(2)
C6−C7	1.36(2)	C19−C20	1.37(2)
C7−C8	1.41(2)	C20−C21	1.41(2)
C8−C9	1.44(2)	C21−C22	1.38(2)
I1−W1−C1	76.9(4)	I2−W2−C15	74.8(4)
I1−W1−C2	76.2(4)	I2−W2−C16	75.7(4)
C1−W1−C3	74.5(6)	C15−W2−C14	78.7(6)
C2−W1−C3	77.4(6)	C16−W2−C14	77.0(6)
C1−W1−C2	105.9(6)	C15−W2−C16	109.1(6)
I1−W1−C3	133.5(4)	I2−W2−C14	132.7(4)
C5−W1−C6	34.7(5)	C18−W2−C19	37.2(4)
C6−W1−C7	33.3(4)	C19−W2−C20	34.3(4)
C7−W1−C8	34.8(5)	C20−W2−C21	34.5(5)
C8−W1−C9	36.3(5)	C21−W2−C22	33.8(4)
C9−W1−C5	36.9(5)	C22−W2−C18	35.8(4)
O4−C4−C5	129(1)	O11−C17−C18	126(1)
O4−C4−O5	121(1)	O11−C17−O12	124(1)
O5−C4−C5	110(1)	O12−C17−C18	111(1)
C4−O5−N1	110(1)	C17−O12−N2	111.4(9)
O5−N1−C13	122(1)	O12−N2−C23	122(1)
O5−N1−C10	120(1)	O12−N2−C26	120(1)

The mean planes formed by the Cp and the *N*-succinimidyl rings are almost perpendicular (complex **5**, angle of 89° for molecule 1 and 103° for molecule 2; complex **2**, 82° for molecule 1 and 105° for molecule 2).

The X-ray crystal structure of **5** establishes clearly the asymmetric nature of the molecule. The orientation of the ester functionality in the plane of the cyclopentadienyl ring places the *N*-succinimidyl ring in a chiral environment. (For the moment, we ignore the extra complication of $W(CO)_3I$ rotation.) In principle, the four methylene protons of the *N*-succinimidyl ring are rendered nonequivalent in the ^1H NMR spectrum; however, one would expect rapid rotation about the oxygen−nitrogen bond, thus generating local C_2 symmetry within the heterocycle. This low barrier toward rotation of the *N*-succinimidyl moiety can equilibrate two pairs of protons (H_A with $H_{A'}$; H_B with $H_{B'}$), but the protons within each methylene group should remain inequivalent (see Figure 3 for denomination).

We must next consider the barrier to rotation of the ester linkage with respect to the cyclopentadienyl ring. If such a process becomes rapid on the NMR time scale, the effective molecular symmetry becomes C_s, whereby H_A and H_B (and also $H_{A'}$ and $H_{B'}$) are related by a mirror plane and so all four methylene protons in the *N*-succinimidyl unit become chemical shift equivalent. Experimentally, the 500 MHz ^1H NMR spectrum of **5** appears as a sharp singlet at room temperature. However, as depicted in Figure 3, when the sample is cooled, the nonequivalence of the methylene protons becomes evident.

These data yield a barrier of approximately 14 kcal mol^{-1} which, as discussed previously, we can attribute to slowed rotation about the Cp ester linkage. In the ^{13}C NMR spectrum, the methylene carbons appear as a singlet at all accessible temperatures; this observation is consistent with rapid rotation of the succinimidyl ring with respect to the oxygen−nitrogen bond.

The rotational barriers of π-bonded ML_n units relative to arene or cyclopentadienyl rings have been reviewed.[22] In the absence of steric hindrance, the barriers are normally low and are controlled primarily by the interactions of the frontier orbitals of the organometallic fragment with the organic π-manifold. In the case at

(22) McGlinchey, M. J. *Adv. Organomet. Chem.* **1992**, *34*, 285.

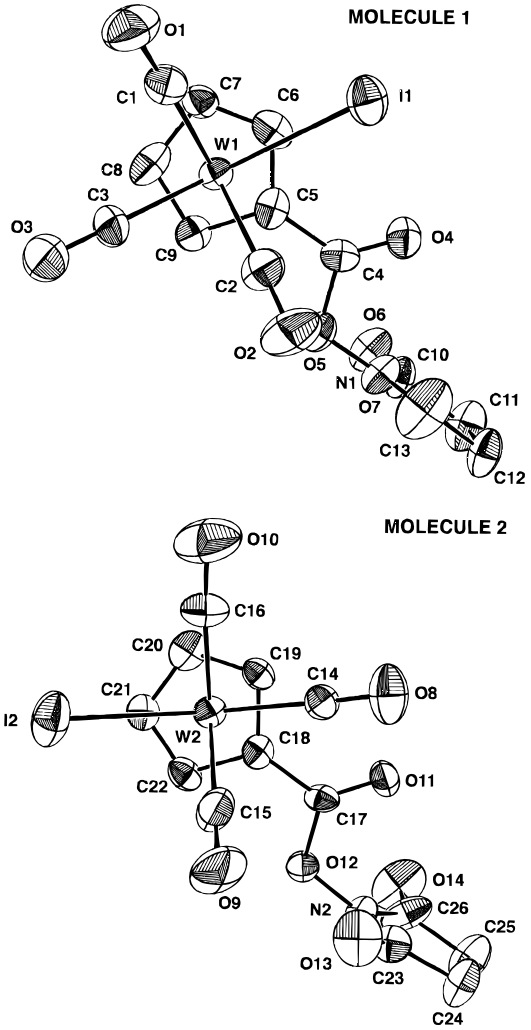


Figure 1. ORTEP drawing of **5** (molecules 1 and 2): projection on the cyclopentadienyl plane.

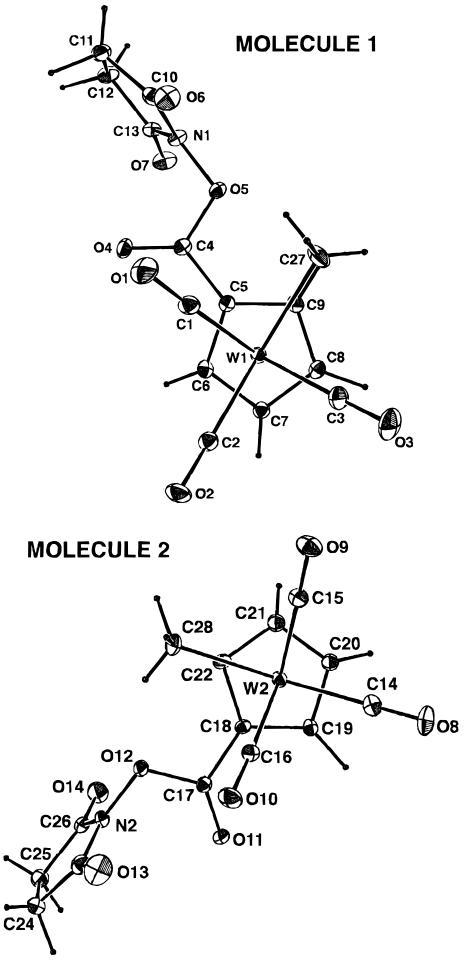


Figure 2. ORTEP drawing of **2** (molecules 1 and 2): projection on the cyclopentadienyl plane.

hand, the crystallographically determined structure reveals that the $W(CO)_3I$ fragment is not aligned such that the W−I bond is parallel to the potential mirror plane of the molecule but rather at an angle of 64° (see Figure 1). Likewise, in all the other cases which we describe here, the ML_4 unit adopts a diagonal orientation relative to the $C_5H_4CO_2R$ ring. In the case where $ML_4 = W(CO)_3X$ (X is iodide or methyl), such a rotamer should render all four CH units in the cyclopentadienyl ring different in both the ^1H and ^{13}C regimes. However, it is only at −100 °C that the cyclopentadienyl protons lose their triplet character and broaden markedly; at this temperature, the *N*-succinimidyl methylene protons are still well-resolved. In contrast, the Cp ring carbons show broadening by −70 °C, and by −100 °C they have almost disappeared into the base line; it is not possible to obtain a limiting spectrum at accessible temperatures. Nevertheless, assuming reasonable chemical shift separations, the barrier to tetrapodal rotation must be low, probably less than 8 kcal mol^{-1}. Clearly, the very large difference between this value and the barrier toward ester rotation demonstrate that these two processes are not correlated. Indeed, such behavior is reminiscent of numerous other cases, e.g. $(C_6Et_6)Cr(CO)_3$[23,24] and $(C_5Ph_5)Fe(CO)(HC{=}O)PMe_3$,[25] where spinning of the tripod is facile and is independent of the rotation of the peripheral groups relative to the central ring to which the metal is attached.

In an attempt to find an electronic rationale for the favored orientation of the $W(CO)_3X$ moiety relative to the cyclopentadienyl ring, we carried out a series of molecular orbital calculations at the extended Hückel level. For simplicity, the system examined initially involved the interaction of a $[W(CO)_4]^{2+}$ fragment with a $[C_5H_4CO_2H]^-$ ligand. As shown in Figure 4, the vacant frontier orbitals of the $[W(CO)_4]^{2+}$ unit have primarily d_{xz} and d_{yz} character (e in C_{4v}) and are ideally suited for overlap with the highest occupied orbitals (π_2 and π_3) of the carboxycyclopentadienide ligand.

The overlap of the tungsten d_{z^2} orbital with the π_1 combination of the organic ligand is rather poor, and the interaction between the metal and the five-membered ring essentially involves only two sets of frontier orbitals. The barrier to rotation of the tetrapod is calculated to be approximately 5 kcal mol^{-1}, somewhat less than the barrier estimated from the NMR data. It is apparent from inspection of π_2 of the $[C_5H_4CO_2H]^-$ ring that there exists a substantial π-bonding interaction with the ester linkage; this overlap is lost upon rotating the side chain and imposes the barrier to ester

(23) Iverson, D. J.; Hunter, G.; Blount, J. F.; Damewood, J. R., Jr.; Mislow, K. *J. Am. Chem. Soc.* **1981**, *103*, 5942.

(24) Mailvaganam, B.; Frampton, C. S.; Top, S.; Sayer, B. G.; McGlinchey, M. J. *J. Am. Chem. Soc.* **1991**, *113*, 1177.

(25) Li, L.; Decken, A.; Sayer, B. G.; McGlinchey, M. J.; Brégaint, P.; Thépot, J.-Y.; Toupet, L.; Hamon, J.-R.; Lapinte, C. *Organometallics* **1994**, *13*, 682.

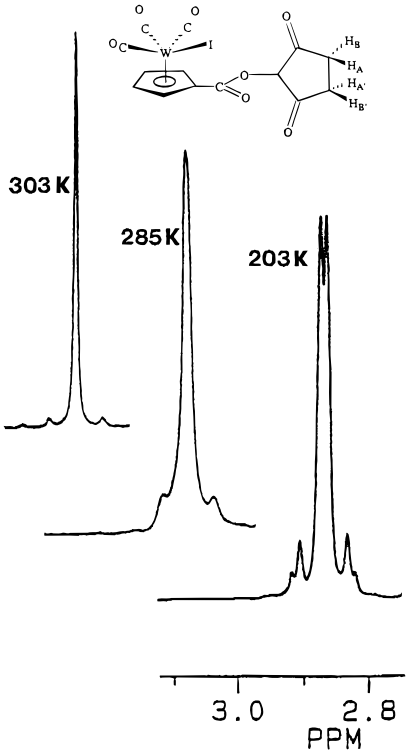


Figure 3. Sections of the 500 MHz NMR spectrum of **5** showing the methylene protons of the *N*-succinimidyl ring at different temperatures (solvent dichloromethane-d_2).

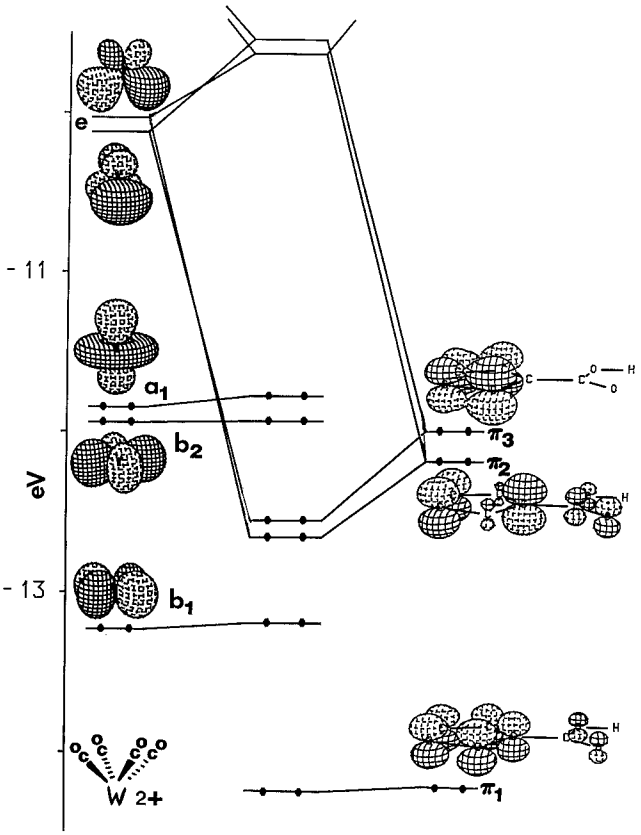


Figure 4. Frontier orbital interactions between $[W(CO)_4]^{2+}$ and $[C_5H_4CO_2H]^-$.

rotation which is detectable by the *N*-succinimidyl methylene protons in **5**.

In fact, the d_{xz} (or d_{yz}) frontier orbitals of the $[W(CO)_4]^{2+}$ fragment are also involved in back-donation to the π^* manifold of the four carbonyl ligands. Substitution of an iodide ligand for a CO does not markedly change the frontier orbitals of the $[W(CO)_3I]^+$ moiety relative to those of the $[W(CO)_4]^{2+}$ fragment (see Figure 5), and the diagonal orientation of the $W(CO)_3I$ tetrapod is predicted by the EHMO calculations and found experimentally by X-ray crystallography.

Reactivity of *N*-Succinimidyl Esters and *N*-Sulfosuccinimidyl Esters with Benzylamine and *β*-Alanine. To study the reactivity of our *N*-succinimidyl organotungsten reagents, we proceeded step by step. The first set of experiments consisted of testing the reactivity of esters **2**, **5**, and **7** with an amine soluble in an organic solvent, namely benzylamine in THF. Whereas no reaction occurred with **7** after 48 h, high yields of amides **8** and **9** were obtained at room temperature (Scheme 3). Their spectral features are given in Table 4.

Interestingly, the reaction process as observed by TLC showed a marked difference in the kinetics for esters **2** and **5**, the latter giving the faster reaction. Thus, the iodo ligand apparently has a remarkable effect on the reactivity of the *N*-succinimidyl ester toward the amine. In contrast, an organometallic moiety in the α-position "deactivates" the *N*-succinimidyl ester, which becomes unreactive toward amines. The pK_a values of the corresponding acids are collected in Table 5.

The differences in reactivity among the three *N*-succinimidyl esters toward amines seem to be directly correlated with the pK_a values of the corresponding acids, the strongest acid **4** reacting the most rapidly. On the other hand, ester **7**, which is derived from a very weak acid, does not react with amines at all.

The second set of experiments involved the water-soluble amino acid *β*-alanine, with which the reaction was performed in a 1/1 THF/water mixture (v/v). In this case, a mixture of two compounds resulting from two competitive reactions was simultaneously precipitated by addition of ethyl ether. Indeed, in aqueous media, competitive hydrolysis of *N*-succinimidyl esters leading to their carboxylic acid precursors is generally encountered, thus reducing, sometimes severely, coupling yields.[26] Hydrolysis levels are dependent on several factors, the nature of the reagent being one of them.[26c] In our case, for complex **2**, the ratio of amide **9** to acid **1** was 90/10. On the other hand, for complex **5**, the ratio of amide **10** to acid **4** was 60/40 (proportions indicated were calculated from the ^{1}H NMR signals of Cp rings for both species). Hence, the hydrolysis reaction becomes important in the case of the iodo derivative and leads to a decrease in the final yield of the amide.

The last set of experiments consisted of coupling *β*-alanine with equimolar quantities of the *N*-sulfosuccinimidyl esters **3** and **6** in aqueous solution. As observed before, mixtures of the corresponding amide and acid were obtained in the ratio 55/45 from **3** and 70/30 from **6**.

Coupling of Bovine Serum Albumin (BSA) with 2, 3, 5, and 6. Bovine serum albumin is a transport protein of molecular mass 66 000 Da and has an isoelectric point of 4.8.[27] It is readily available and inexpensive, thus making it an interesting model pro-

(26) (a) Cuatrecasas, P.; Parikh, I. *Biochemistry* **1972**, *11*, 2291. (b) Carlsson, J.; Drevin, H.; Axén, R. *Biochem. J.* **1978**, *173*, 723. (c) Vaidyanathan, G.; Zalutsky, M. R. *Bioconjugate Chem.* **1990**, *1*, 269.
(27) Peters, T., Jr. *Adv. Protein Chem.* **1985**, *37*, 1.

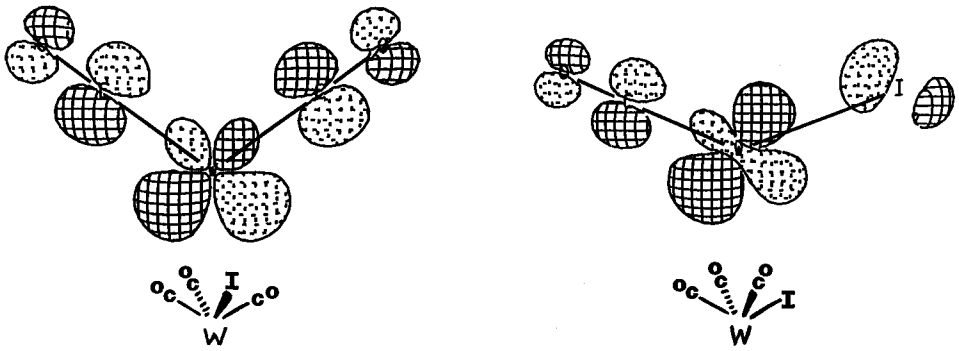


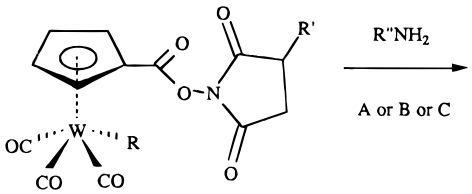
Figure 5. Vacant frontier orbitals of the $[WCO)_3I]^+$ fragment.

Table 4. Condensation of Benzylamine and β-Alanine with Esters 2, 3, 5, and 6: Spectroscopic Data

reagent	amide	yield (%)	IR ν (cm^{-1})[a]	^1H NMR ∂ (ppm)	anal.
2	**8**	60	2006 (s), 1920 (s) (ν_{CO}); 1641 (m), 1554 (m) (ν_{CONH})	7.34 (m, 5H, H benzyl), 5.90 (s, 1H, NH), 5.72 (t, 2H, J = 2.3 Hz, Cp H(2,5)), 5.44 (t, 2H, J = 2.3 Hz, Cp H(3,4)), 4.55 (d, 2H, J = 5.7 Hz, CH_2), 0.51 (s, 3H, Me)[b]	calcd for $C_{17}H_{15}O_4NW$: C, 42.42; H, 3.12; N, 2.91 found: C, 42.46; H, 3.20; N, 2.96
5	**9**	79	2047 (m), 2028 (m), 1976 (s), 1947 (s) (ν_{CO}); 1642 (m), 1556 (m) (ν_{CONH})	7.36 (m, 5H, H benzyl), 6.41 (s, 1H, NH), 6.04 (t, 2H, J = 2.3 Hz, Cp H(2,5)), 5.67 (t, 2H, J = 2.3 Hz, Cp H(3,4)), 4.58 (d, 2H, J = 5.7 Hz, CH_2)[b]	calcd for $C_{16}H_{12}O_4NIW$: C, 32.39; H, 2.02; N, 2.40 found: C, 33.24; H, 2.29; N, 2.40
2/3	**10**	53/26	2023 (s), 1955 (s), 1909 (s) (ν_{CO}); 1710 (s), 1619 (m), 1557 (m)) ν_{CONH})	7.60 (s, 1H, NH), 6.06 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 5.71 (t, 2H, J = 2.4 Hz, Cp H(3,4)), .51 (td, 2H, $J1$ = 2.8 Hz, $J2$ = 6.8 Hz, CH_2NH), 2.57 (t, J = 6.8 Hz, CH_2COO), 0.44 (s, 3H, Me)[c]	calcd for $C_{13}H_{13}O_6NW$: C, 33.69, H, 2.81; N, 3.02 found: C, 33.78; H, 2.87; N, 3.06
5/6	**11**	30/30	2036 (s), 1971 (s), 1934 (s) (ν_{CO}); 1711 (s), 1628 (m), 1558 (m) (ν_{CONH})	7.75 (s, 1H, NH), 6.38 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 6.10 (t, 2H, J = 2.4 Hz, Cp H(3,4)), 3.55 (q, 2H, J = 6.8 Hz, CH_2NH), 2.57 (t, 2H, J = 6.8 Hz, CH_2COO)[c]	calcd for $C_{12}H_{10}O_6NIW$: C, 25.04, H, 1.74; N, 2.73 found: C, 25.60; H, 1.88; N, 2.50

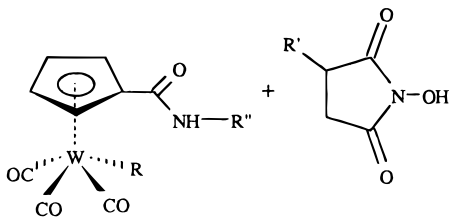
[a] KBr pellet. [b] In $CDCl_3$. [c] In acetone-d_6.

Scheme 3. Reaction of [(Succinimidcyclopentadienyl]- and [((Sulfosuccinimido)oxy)carbonyl)cyclopentadienyl]tungsten Complexes with Primary Amines



R = CH_3 or I

R' = H (solvent A = THF) and R" = $C_6H_5CH_2$
or R' = H (solvent B = THF/water pH 9.6 1/1) and R" = HOOC-CH_2-CH_2
or R' = SO_3Na (solvent C = water pH 9.6) and R" = HOOC-CH_2-CH_2



8 (R = CH_3, R" = $C_6H_5CH_2$)
9 (R = I, R" = $C_6H_5CH_2$)
10 (R = CH_3, R" = HOOC-CH_2-CH_2)
11 (R = I, R" = HOOC-CH_2-CH_2)

tein for reactivity studies of the *N*-succinimidyl esters. Moreover, the X-ray structure of its human homolog has been reported recently.[28] Numerous examples of covalent coupling of haptens have been described in the literature with the intent of producing anti-hapten antibodies.[29] Its primary structure contains 59 lysine residues: i.e., 60 potential sites of conjugation. We carried out a series of coupling experiments with the four reagents **2**, **3**, **5**, and **6** under conditions similar to those previously optimized with the Re(I) derivative.[9] Briefly, the protein was incubated in a basic buffer in the presence of 60 equiv of organometallic reagent in DMF (10% of the total volume of incubation) or buffer in the case of *N*-sulfosuccinimidyl esters. After 24 h, gel filtration chromatography of the mixture was performed, allowing any noncovalently reacted labeling agent to be removed. The conjugates were then characterized by their average coupling ratio (CR = [M]/[P]). The coupling yield CY was also measured. [M] and [P] were evaluated by two analytical methods—IR quantitative analysis of the ν_{CO} bands on nitrocellulose membranes for the metal−carbonyl label and the Bradford method for the protein.[30]

As reported in Table 6, the coupling ratios are markedly dependent on the nature of the ester and the ligands coordinated to the metal.

Table 5. pK_a Values of Organotungsten Carboxylic Acids

compd	pK_a	ref
1	4.4 ± 0.1	13[a]
4	4.3 ± 0.05	this work[a]
[(η^5-Cp)(η^1-CH_2COOH)$(CO)_3$W]	8.35 ± 0.1	36[b]

[a] In ethanol/water, 1/1. [b] In dioxane/water, 1/1.

(28) Min He, X.; Carter, D. C. *Nature* **1992**, *358*, 209.
(29) Erlanger, B. F. *Methods Enzymol.* **1980**, *70*, 85.
(30) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

Table 6. Conjugation of BSA with Activated Esters 2, 3, 5, and 6

reagent	[M] (M)	[P] (M)	CR[a]	CY[b] (%)
2[c]	2.85×10^{-4}	1.1×10^{-5}	27.4	46
3[c]	1.41×10^{-4}	1.2×10^{-5}	12.1	20
5[c]	1.48×10^{-4}	0.9×10^{-5}	16.4	27
6[d]	1.11×10^{-4}	2.0×10^{-5}	5.4	9

[a] CR = coupling ratio = [M]/[P]. [b] CY = coupling yield. [c] $[P]_{init} = 1.5 \times 10^{-5}$ M; 60 equiv of reagent. [d] $[P]_{init} = 3.0 \times 10^{-4}$ M; 60 equiv of reagent.

Discussion

The usefulness of heavy-metal derivatives of crystallized proteins is often hampered by the lack of isomorphism of these derivatives compared to that of the native protein crystals. Classical preparations consist of passive diffusion of heavy-metal salts across the crystal lattice, taking advantage of the high solvent content of protein crystals. Those salts then react nonspecifically with oppositely charged side chains of amino acids.

Targeted reagents that react covalently with specific amino acids would thus improve the preparation of isomorphous derivatives. Among all the potential targets, we chose the ϵ-amino groups of lysine residues because their hydrophilic character implies that they are usually located at the surface of globular proteins.

Two series of cyclopentadienyltungsten reagents were prepared that possess either an *N*-succinimidyl or an *N*-sulfosuccinimidyl ester function. The first series requires the presence of a minimum amount (10%) of organic solvent to help solubilization; however, the second series has no such requirement and could therefore be used for the labeling of proteins in their crystalline state.

In preliminary experiments, their reactivity toward model amines and amino acids was tested. From these observations, the following conclusions were drawn. First, the η^1-enolate derivative is totally unreactive toward amines. Second, the iodo reagent gives better yields in organic solvent than in aqueous medium because the hydrolysis side reaction becomes important. Thus, whereas in a pure organic solvent the reaction of aminolysis is clean and leads only to the expected compounds, a more or less important side reaction of hydrolysis of the *N*-succinimidyl ester to the acid occurs in aqueous solution. Third, *N*-sulfosuccinimidyl reagents give lower yields than do their *N*-succinimidyl counterparts, probably because they are less pure and tend to be more hydrolyzed.

One model protein (BSA) was then successfully coupled to cyclopentadienyltungsten carbonyl moieties by simple incubation of the reagents in basic pH followed by purification by either gel filtration or dialysis and provided conjugates with coupling ratios ranging from 5 to 27, depending on the reagent.

Coupling yields are always lower for iodo esters compared to methyl esters, whereas yields of the hydrolysis compound increase. Both electronic and steric factors could be invoked here. At the extended Hückel level of approximation, the formal charge at the ester carbon (site of reaction with the O- and N-nucleophiles) is unchanged (+1.22 in both cases). The only significant difference is found for the tungsten atom, which bears a charge of +1.12 in the methyl complex but +1.19 in the iodo system. Moreover, differences in pK_a values for both related acids are small.

Finally, coupling yields with BSA are lower than those with β-alanine. It is especially remarkable in the case of the two *N*-sulfosuccinimidyl esters, for which yields of 26−30% were measured with β-alanine compared to only 9−20% with the protein. This result seems to reflect the fact that for proteins all the potential reactive sites are not equally accessible to the reagents, depending on their relative position in the tertiary structure, although lysine side chains are mostly directed to the outer part of globular proteins.

Conclusion

New heavy-metal reagents were successfully designed for the site-specific chemical modification of proteins. These reagents take advantage of the selective reactivity of *N*-succinimidyl and *N*-sulfosuccinimidyl esters toward amino groups and the high stability of $CpW(CO)_3R$ moieties. Their reactivity has been successively tested on amines, amino acids, and model proteins. We have thus demonstrated that all of the reagents, except the η^1-enolate derivative, couple with these compounds. The organotungsten conjugates have been characterized by IR spectroscopy by using the ν_{CO} vibrations. These heavy-transition-metal reagents appear to be promising agents for the labeling of biological systems under nondenaturating conditions, which is a prerequisite to any structural study of proteins by X-ray crystallographic methods.

Experimental Section

General Comments. All organometallic reactions were performed under a dry argon atmosphere by using standard Schlenk techniques. [η^5-Cyclopentadienyl]tungsten tricarbonyl methyl was prepared from $W(CO)_3(CH_3CN)_3$[31] and CpNa in THF at reflux followed by addition of CH_3I.[32] *N*-Succinimidyl chloroacetate was synthesized according to the method of Anderson et al.[14] from chloroacetic acid. *N*-Hydroxysulfosuccinimide was prepared according to the literature.[19] Cyclopentadienyltungsten tricarbonyl dimer was purchased from Strem Chemicals. Other chemicals were obtained from Aldrich or Fluka. THF was distilled from Na−benzophenone, and DMF was distilled under CaH_2. The other solvents were used without any purification.

Bovine serum albumin (grade V) was purchased from Sigma. Phosphate-buffered saline (PBS; 0.1 M, pH 7.1) and carbonate/bicarbonate (0.1 M, pH 9.6) buffer were prepared from demineralized water. Exclusion gel chromatography was performed with prepacked Econopac desalting columns (Bio-Rad) with PBS as the eluent.

IR quantitative analysis was performed with a MB-100 FT spectrometer (Bomem) equipped with an InSb detector. Briefly, 10 μL samples are deposited on 9 mm diameter nitrocellulose membranes and subsequently air-dried. The resulting spectra were compared with those of standard solutions of metal carbonyl complexes. Mass spectra were obtained at the ENSCP, Paris. Elemental analysis was performed at the Université Pierre et Marie Curie, Paris.

For the crystal structure analysis, intensity data were collected on an Enraf-Nonius CAD4 diffractometer using Mo Kα radiation. Cell dimensions and orientation matrices were obtained from the least-squares refinement of 25 reflections for $\theta \leq 12°$. Intensities of three standard reflections were

(31) Tate, D. P.; Knipple, W. R.; Angl, J. M. *Inorg. Chem.* **1962**, *1*, 433.

(32) Piper, T. S.; Wilkinson, G. *J. Inorg. Nucl. Chem.* **1956**, *3*, 104.

monitored every 1 h. These showed no appreciable changes during data collection. Absorption corrections were made with the Difabs program.[33] Computations were performed on a μ-VAX 3100/40 by using programs from Enraf-Nonius SDP described by Frenz.[34] Atomic scattering factors for neutral atoms were taken from ref 35. The structure was solved by direct methods using MULTAN software.[36] The *E*-map with the highest combined figures of merit yielded the positions of the W and I atoms, and the remaining non-hydrogen atoms were found from subsequent difference maps. Hydrogen atoms of the two molecules were calculated by assuming a trigonal orientation and a 0.95 Å bond length.

Cyclopentadienyl proton NMR resonances were assigned by comparison with data reported in the literature.[11,13] Variable-temperature ^{1}H and ^{13}C NMR experiments were performed on a Bruker AM 500 spectrometer operating at 500 MHz for protons and 125.72 MHz for ^{13}C. Molecular orbital calculations were carried out by using the program CACAO.[37]

(η^5-Carboxycyclopentadienyl)methyltricarbonyltungsten (1). To a solution of $CpW(CO)_3CH_3$ (0.5 g; 1.44 mmol) in 20 mL of THF was added 1.3 mL of *n*-BuLi in hexane (2.1 mmol) at −78 °C. After 45 min of stirring, 2 g of CO_2 solid was added while the temperature was kept at −78 °C for 10 min. The temperature was raised to 20 °C, and the mixture was hydrolyzed with 20% HCl, followed by extraction with dichloromethane. The organic layer was extracted with a 20% aqueous Na_2CO_3 solution. The new aqueous phase was treated with 20% HCl until total precipitation. The precipitate was dissolved in dichloromethane, the solution dried, and the solvent removed under vacuum **1** (0.4 g, 71%) as a yellow powder was obtained; mp 176 °C (lit.[13] 187 °C).

^{1}H NMR ($CDCl_3$, 200 MHz): ∂ 5.81 (t, 2H, J = 2.4 Hz, Cp H(2,5)) 5.51 (t, 2H, J = 2.4 Hz, Cp H(3,4)), 0.52 (s, 3H, Me). IR (KBr): ν_{CO} at 2018 (s), 1917 (s) cm^{-1}; ν_{COO} at 1683 cm^{-1}. MS (EI): *m/z* 392 ([M]$^+$), 336 ([M − 2CO]$^+$), 321 ([M − 2CO − Me]$^+$), 308 ([M − 3CO]$^+$).

[η^5-((Succinimidooxy)carbonyl)cyclopentadienyl]methyltricarbonyltungsten (2). **1** was activated in 20 mL of THF with 1.1 equiv of *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide at room temperature for 6 h. After filtration, **2** was purified by preparative TLC (eluent toluene/acetone, 10/3) and recrystallized in CH_2Cl_2/pentane: yield 70%; mp 136 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): ∂ 5.89 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 5.59 (t, 2H, J = 2.4 Hz, Cp H(3,4)), 2.89 (s, 4H, CH_2-CH_2), 0.59 (s, 3H, CH_3). IR (KBr): ν_{CO} at 2025 (s), 1948 (s), 1925 (s) cm^{-1}; ν_{COO} at 1806 (w), 1782 (m), 1742 (s) cm^{-1}. Anal. Calcd for $C_{14}H_{11}O_7NW$: C, 34.36; H, 2.25; N, 2.86. Found: C, 34.47; H, 2.30; N, 2.83.

Sodium [η^5-(((3-Sulfosuccinimido)oxy)carbonyl)cyclopentadienyl]methyltricarbonyltungstate (3). **1** was activated with 1.1 equiv of *N*-hydroxysulfosuccinimide and *N,N'*-dicyclohexylcarbodiimide in DMF at room temperature for 24 h. After filtration, the solvent was removed under vacuum to yield a yellow oil which, triturated in ethyl ether, led to a yellow-gray powder.

^{1}H NMR (D_2O, 250 MHz): ∂ 5.93 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 5.67 (t, 2H, J = 2.4 Hz, Cp H(3,4)), 4.34 (dd, 1H, J = 8.2 Hz, C(H)SO_3^-), 3.25 (dd, 1H, J = 8.2 Hz, J = 18.9 Hz, CH^a_2), 3.05 (dd, 1H, J = 8.2 Hz, J = 18.9 Hz, CH^b_2), 0.38 (s, 3H, CH_3). IR (KBr): ν_{CO} at 2024 (m), 1931 (s) cm^{-1}; ν_{COO} at 1803 (w), 1778 (m), 1741 (s) cm^{-1}; $\nu_{SO_3^-}$ at 1220 (s), 1045 (s) cm^{-1}.

[η^5-Carboxycyclopentadienyl]iodotricarbonyltungsten (4). To a solution of **1** (0.58 mmol, 0.23 g) in 20 mL of acetonitrile was added 0.164 mL (1.15 mmol) of $ISi(CH_3)_3$. After 1 h, water was added to the mixture to precipitate the acid **4**, which was extracted with ethyl ether. The organic phase was decanted, dried, and evaporated off. **4** (0.22 g, 76%) was obtained as a red powder: mp 210 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): ∂ 6.08 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 5.85 (t, 2H, J = 2.4 Hz, Cp H(3,4)). IR (KBr): ν_{CO} at 2034 (s), 1965 (s), 1920 (s) cm^{-1}; ν_{COO} at 1691 cm^{-1}. Anal. Calcd for $C_9H_5O_5IW$: C, 21.45; H, 1.00. Found: C, 21.68; H, 0.87.

[η^5-(Succinimidooxy)carbonyl)cyclopentadienyl]iodotricarbonyltungsten (5). **2** was activated within 2 h by the same procedure. Purification and recrystallization were performed as for **2**: yield 98%; mp 154 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): ∂ 6.18 (t, 2H, J = 2.4 Hz, Cp H(2,5)), 6.00 (t, 2H, J = 2.4 Hz, Cp H(3,4), 2.90 (s, 4H, CH_2-CH_2). IR (KBr): ν_{CO} at 2042 (m), 1954 (s) cm^{-1}; ν_{COO} at 1806 (w), 1776 (m), 1710 (s) cm^{-1}. Anal. Calcd for $C_{13}H_8O_7NIW$: C, 25.98; H, 1.34; N, 2.33. Found: C, 26.89; H, 1.38; N, 2.39.

Sodium [η^5-(((3-Sulfosuccinimido)oxy)carbonyl)cyclopentadienyl]iodotricarbonyltungstate (6). **4** was activated to its *N*-sulfosuccinimidyl ester in the same manner as **1**, leading to the production of a red-gray powder.

^{1}H NMR (D_2O, 250 MHz): ∂ 6.28 (s, Cp H(2,5)), 5.93 (s, Cp H(3,4)), 4.35 (m, C(H)SO_3^-), 3.25 (m, CH^a_2, 3.03 (m, CH^b_2). IR (KBr): ν_{CO} at 2041 (s), 1955 (s) cm^{-1}; ν_{COO} at 1807 (w), 1780 (m), 1741 (s) cm^{-1}; $\nu_{SO_3^-}$ at 1234 (s), 1045 (s) cm^{-1}.

(η^5-Cyclopentadienyl)[((succinimidooxy)carbonyl)methyl]tricarbonyltungsten (7). To a solution of $[CpW(CO)_3]_2$ (0.6 mmol, 0.4 g) in 20 mL of THF was added a 5% Na/Hg amalgam (50 mg of Na in 1 g of Hg). The mixture was stirred at room temperature until a yellow color developed. The solution was then transferred to a equimolar solution of *N*-succinimidyl 1-chloroacetate in 30 mL of THF at −20 °C and allowed to react for 16 h. Compound **7** was then purified by preparative TLC (eluent ethyl ether/pentane, 1/2). **7** (0.1 g, 15%) was obtained as a yellow powder: mp 170 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): ∂ 5.65 (s, 5H, Cp H), 2.84 (s, 4H, CH_2CH_2), 2.23 (s, 2H, CH_2). IR (KBr): ν_{CO} at 2037 (s), 1957 (s), 1903 (s) cm^{-1}; ν_{COO} at 1794 (w), 1733 (s) cm^{-1}. Anal. Calcd for $C_{14}H_{11}O_7NW$: C, 34.36; H, 2.25; N, 2.86. Found: C, 34.36; H, 2.35; N, 2.90.

Reaction of 2 with Benzylamine. A 0.31 mmol amount of **2** in THF was coupled with 0.31 mmol (0.034 mL) of benzylamine at room temperature. After 6 h, the solvent was removed under vacuum and compound **8** was purified by preparative TLC (eluent toluene/acetone, 10/3) and recrystallized in dichloromethane/pentane at −20 °C: mp 182 °C. Spectroscopic data are reported in Table 4.

Reaction of 5 with Benzylamine. The same procedure was applied, except that the reaction time was 2 h. Compound **9** was crystallized from dichloromethane/pentane: mp 178 °C dec.

Reaction of 2 with β-Alanine. β-Alanine (15 mg, 0.17 mmol) was dissolved in 8 mL of carbonate buffer, and 1 equiv of **2** in the same volume of THF was added. After 18 h at room temperature, the mixture was acidified with concentrated HCl and extracted with dichloromethane. The solvent was removed under vacuum, and compound **10** containing 10% of **1** was precipitated in CH_2Cl_2/pentane. Compound **10** was further purified by washing of the raw powder with chloroform.

Reaction of 5 with β-Alanine. The same procedure was applied. A red powder containing 61% of **11** and 39% of **4** was obtained (percentages evaluated by ^{1}H NMR). Compound **11** was further purified by washing of the raw powder with chloroform.

(33) Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 159.
(34) Frenz, B. A. In *Computing in Crystallography*; Schenk, H., Olthof-Hazikamp, R., Eds.; Delft University Press: Delft, The Netherlands, 1978; p 64.
(35) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, U.K., 1974; Vol. 4.
(36) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declerq, J. P.; Woolfson, M. M. Multan 80, a System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data; Universities of York (York, England) and Louvain (Louvain, Belgium).
(37) Mealli, C.; Proserpio, D. M. *J. Chem. Educ.* **1990**, *67*, 399.
(38) Ariyaratne, J. P. K.; Bierrum, A. M.; Green, M. L. H.; Ishaq, M.; Prout, C. K.; Swainwick, M. G. *J. Chem. Soc. A* **1969**, 1309.

Reaction of 3 with β-Alanine. β-Alanine and **3** were allowed to react in equimolar quantities in carbonate buffer for 18 h at room temperature. The mixture was acidified with concentrated HCl and extracted with dichloromethane. The solvent was removed under vacuum, and a mixture of amide **10** (56%) and acid **1** (44%) was precipitated in CH_2Cl_2/pentane.

Reaction of 6 with β-Alanine. β-Alanine and **6** were allowed to react in carbonate buffer as described above. A mixture of amide **11** (68%) and acid **4** (32%) was obtained after crystallization in CH_2Cl_2/pentane.

Labeling of BSA. To 1.8 mL of a 27.8 μM BSA solution in carbonate buffer was added 0.2 mL of a 1.5×10^{-3} M solution (corresponding to 60 equiv) of organotungsten reagent (in DMF for **2** and **5**; in carbonate buffer for **3** and **6**). The mixture was incubated for 24 h at room temperature, brought to 3 mL with buffer, filtered on a 0.45 μm porosity filter (Millex GV), and chromatographed by gel filtration to remove any unreacted labeling reagent. The first four elution fractions were pooled and analyzed as previously described.[9]

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Supporting Information Available: Tables of fractional coordinates, bond angles, least-squares planes, and anisotropic thermal factors for compounds **2** and **5** (27 pages). Ordering information is given on any current masthead page.

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