Novel *N*-Succinimidyl and *N*-Sulfosuccinimidyl Organotungsten Reagents for the Labelling of Biological Systems

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New organotungsten reagents bearing a *N*-succinimidyl or *N*-sulfosuccinimidyl ester function have been prepared, specifically coupled with amines, amino acids and proteins and provide a promising basis for the preparation of heavy metal labelling agents designed for X-ray structural analysis of biological systems.

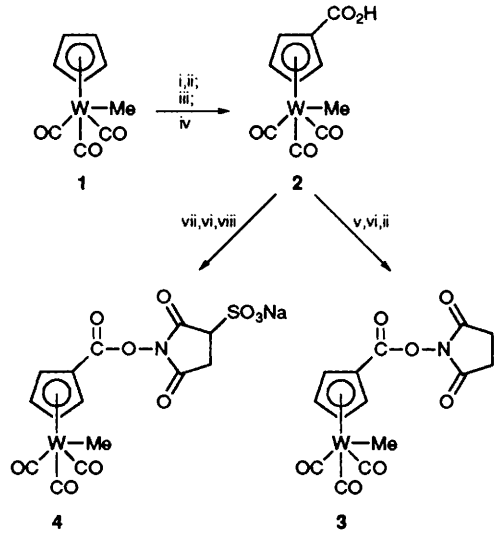
We are currently investigating methods of introduction of transition metal organocomplexes into molecules of biological interest.[1] For example, several applications in the field of immunoassays of low molecular mass molecules have been reported where organometallic probes are quantified by either spectroscopic methods (FT-IR of metal–carbonyl complexes,[2] AAS of metals[3]) or electrochemical methods[4] particular to this kind of complexes.

At a more fundamental level, introduction of heavy metals into protein crystals is a key step for the resolution of their three-dimensional structure by X-ray crystallography.[5] Until now, the widespread 'isomorphous replacement method' merely used inorganic salts as labelling agents which react rather non-specifically with charged side chains of the proteins inside the crystal lattice. Still, only a few covalently reacting agents are used.[6] Starting from our previous experience with transition metal complexes of cobalt(0)[7] bearing a *N*-succinimidyl ester function, new *N*-succinimidyl (NS) and *N*-sulfosuccinimidyl (NSS) reagents of tungsten(II) have been designed and their reactivity tested with simple amines, amino acids and proteins.

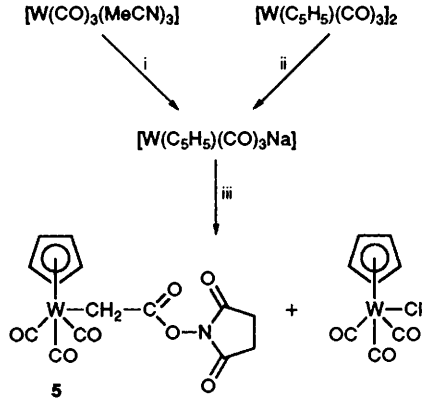
Two series of complexes were prepared† in which the NS or NSS function is attached either to the C_5H_5 ligand (compounds **3** and **4**, Scheme 1) or more directly to the metal (compound **5**, Scheme 2). The acid **2** was synthesized and isolated intermediately by a novel lithiation/carbonatation method. In the second case, ester **5** was obtained with a yield of 10–20% by electrophilic addition of *N*-succinimidyl 2-chloroacetate on $[W(C_5H_5)(CO)_3Na]$ generated either from $[W(CO)_3(MeCN)_3]$ and $Na(C_5H_5)$ or from $[W_2(C_5H_5)_2(CO)_6]$ and a 5% Na–Hg amalgam. The major compound of the rection was indeed identified as $[W(C_5H_5)(CO)_3Cl]$. Attempts to improve the yield in ester **5** by using *N*-succinimidyl 2-iodoacetate were unsuccessful as $[W(C_5H_5)(CO)_3I]$ was only obtained this time, as similarly noticed before.[8] Interestingly, we notice that compound **4** is insoluble in most organic solvents (except DMF) but highly soluble in water.

In a second step, esters **3**, **4** and **5** were allowed to react with benzylamine and β-alanine in totally organic, partially aqueous and totally aqueous media (Scheme 3). In the first case, the expected amide **6** was obtained in a pure form after TLC purification.‡ In the second and third cases, β-alanine which is only water-soluble was coupled to either ester **3** or **4** and led to the expected amide **7**§ as deduced from the NMR data along with formation of acid **2** (8 and 40%, respectively) resulting from the competitive hydrolysis in both aqueous media. No further purification was attempted.

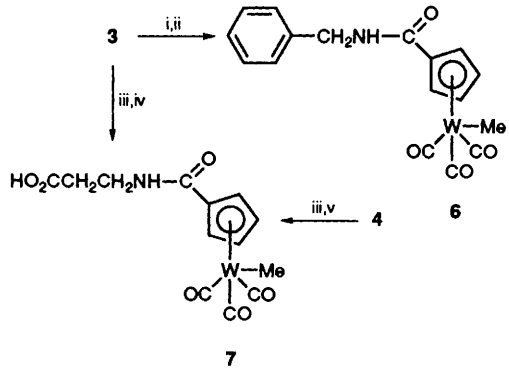
On the other hand, the reactivity of ester **4** was tested with benzylamine and β-alanine ethyl ester in THF with periodical TLC analysis. No reaction occurred after 48 h in both cases. A tentative explanation of this result is that the electron-withdrawing effect of the *N*-succinimidyl group on the carbonyl is counterbalanced by the electron-donating effect of the $W(C_5H_5)CH_2$ moiety which results in the inactivation of the *N*-succinimidyl ester toward nucleophiles.



Scheme 1 *Reagents*: i, Bu^nLi; ii, THF; iii, CO_2; iv, H_3O^+; v, NHS; vi, DCC; vii, NHSS; viii, DMF



Scheme 2 *Reagents*: i, $(C_5H_5)Na$; ii, Na–Hg 5%; iii, *N*-succinimidyl 2-chloroacetate



Scheme 3 *Reagents*: i, benzylamine; ii, THF; iii, β-alanine; iv, THF–carbonate buffer 1 : 1; v, carbonate buffer

Preliminary protein labelling studies were performed with ester **3** on bovine serum albumin (BSA) and lysozyme. These proteins possess 60 and 7 potential labelling sites, respectively. Reaction was achieved in the previously described conditions[7] and resulted in the covalent introduction of 17 and 1–2 organotungsten moieties on BSA and lysozyme, respectively.

Tungsten(II) carbonyl entities can thus be covalently linked to amines, amino acid and proteins in a fast and efficient manner. In this purpose, ester **4** could reveal to be very useful because no organic cosolvent which could be harmful to proteins and induce denaturation is needed.

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Footnotes

† Compound **2** was prepared by lithiation of **1** (0.3 g; 1.44 mmol) with Bu^nLi (1.3 ml, 2.1 mmol, 1.6 mol dm^{-3} in hexane) in THF at −78 °C for 30 min, followed by the addition of 2 g of CO_2. The mixture was allowed to warm up, acidified with conc. HCl and extracted with CH_2Cl_2, 0.4 g (71%) of a yellow powder was obtained. Treatment of **2** (0.4 g; 1 mmol) with stoichiometric amounts of *N*-hydroxysuccinimide (NHS) and *N,N'*-dicyclohexylcarbodiimide (DCC) in THF led to compound **3** (0.34 g; 70%). Alternatively, treatment of **2** (0.2 g; 0.5 mmol) with stochiometric amounts of *N*-hydroxysulfosuccinimide (NHSS) and DCC in DMF led to compound **4** (0.15 g; 51%) obtained as a yellow powder (in mixture with **2**) by rapid stirring of the oily residue in ethyl ether. $[W(C_5H_5)(CO)_3]Na$ was generated *in situ* by rection of $W(CO)_3(MeCN)_3$ (1 g; 2.6 mmol) with an excess of $Na(C_5H_5)$ in THF. The addition of *N*-succinimidyl 2-chloroacetate (0.5 g, 2.6 mmol) led to compound **5** (0.2 g; 15%) which was purified by TLC (silica gel; eluent: diethyl ether–pentane 1:2).

Selected spectroscopic data: for **2**: mp 176 °C decomp. (lit. 187 °C);[9] ^1H NMR (200 MHz, $CDCl_3$): δ 5.81 [t, 2H, *J* 2.4 Hz, Cp H (3,4)]; 5.51 [t, 2H, *J* 2.4 Hz, Cp H (2,5)]; 0.52 s, 3H, W–Me). IR (KBr) ν/cm^{-1}: 2018, 1917 ν WCO, 1683 ν COO. MS (EI) *m/z* 392 (M^+), 336 ([M − 2CO]$^+$); 321 ([M − 2CO − Me]$^+$), 308 ([M − 3CO]$^+$). **3**; mp 136 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 5.89 [t, 2H, *J* 2.4 Hz, Cp H(3,4)]; 5.59 [t, 2H, *J* 2.4 Hz, Cp H(2,5)]; 2.89 (s, 4H, COONS); 0.59 (s, 3H, W-Me). IR (KBr) ν/cm^{-1}: 2025, 1948, 1925 ν WCO, 1806w, 1781m, 1736s ν COONS. MS (EI) *m/z* 489 (M^+), 405 ([M − 3CO]$^+$). **4**: ^1H NMR (200 MHz, D_2O) 5.93 [t, 2H, *J* 2.4 Hz, Cp H(3,4)]; 5.67 [t, 2H, *J* 2.4 Hz, Cp H(2,5)]; 4.34 [dd, 1H, *J* 8.2 Hz, C(H)SO_3^-]; 3.25 (dd, 1H, J_1 8.2, J_2 18.9 Hz, CH_{2a}); 3.05 (dd, 1H, J_1 3.3, J_2, 18.9 Hz, CH_{2b}); 0.38 (s, 3H, W-Me). IR (KBr) ν/cm^{-1} 2024s, 1931s ν WCO, 1803w, 1778m, 1741s ν COONSS, 1220s, 1045s ν SO_3^-. **5**: mp 170 °C. ^1H NMR (250 MHz, $CDCl_3$): 5.65 (s, 5H, Cp), 2.84 (d, 4H, NS), 2.23 (s, 2H, CH_2). IR (KBr) ν/cm^{-1} 2037s, 1957s, 1903s ν WCO, 1794w, 1747sh, 1733s ν COONS. Satisfactory elemental analyses were obtained.

‡ Benzylamine (34 μl, 0.31 mmol) was coupled to compound **3** (0.15 g; 0.31 mmol) in THF at room temp. for 18 h. The resulting amide **6** was purified by TLC (silica gel; eluent: toluene–acetone 10:3) and crystallised as a yellow powder (0.09 g; 60%) from a CH_2Cl_2–pentane mixture at −20 °C.

Selected spectroscopic data: for **6**: mp 182 °C. ^1H NMR (200 MHz, $CDCl_3$) 7.34 (m, 5H, H benzyl), 5.90 (s, 1H, NH), 5.72 [t, 2H, *J* 2.3 Hz, Cp H(3,4)], 5.44 [t, 2H, *J* 2.3 Hz, Cp H(2,5)], 4.55 (d, 2H, *J* 5.7 Hz, CH_2) 0.51 (s, 3H, W–Me). IR (KBr) ν/cm^{-1} 2006s, 1920s ν WCO, 1641m ν amide I, 1554m, ν amide II. MS (EI) *m/z* 481 (M^+); satisfactory elemental analyses were obtained.

§ β-Alanine (0.027 g; 0.3 mmol) was coupled to a stoichiometric amount of compound **3** in a carbonate buffer (0.1 mol dm^{-3}, pH = 9.6)–THF 1:1 mxiture. The resulting amide **7** (0.08 g; 58%, containing 8% of **2**) was obtained by extraction of the acidified aqueous phase by CH_2Cl_2, followed by evaporation and crystallisation from a CH_2Cl_2–pentane mixture at −20 °C. In a similar manner, β-alanine (0.036 g; 0.4 mmol) was coupled to compound **4** (0.120 g; 0.025 mmol) in carbonate buffer. The resulting amide **7** (0.05 g containing 40% of **2**) was extracted and crystallised as above.

Selected spectroscopic data for **7**: ^1H NMR (250 MHz, CD_3COCD_3). δ 7.6 (s, 1H, NH), 6.06 [t, 2H, *J* 2.4 Hz, Cp H(3,4)], 5.71 [t, 2H, *J* 2.4 Hz, Cp H(2,5)], 3.51 (td, 2H, J_1 2.8, J_2 6.8 Hz, CH_2NH), 2.57 (t, 2H, *J* 6.8 Hz, CH_2COO), 0.44 (s, 3H, W–Me). IR (KBr) ν/cm^{-1} 2023s, 1955s, 1909s ν WCO, 1710s ν COOH, 1619m ν amide I, 1557m ν amide II. MS (DCI NH_3): *m/z* 464 (MH^+), 448 (M − Me)$^+$.

References

1 A. D. Ryabov, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 931; G. Jaouen, A. Vessières and I. S. Butler, *Acc. Chem. Res.*, 1993, **76**, 361.
2 M. Salmain, A. Vessières, P. Brossier, I. S. Butler and G. Jaouen, *J. Immunol. Methods*, 1992, **148**, 65.
3 M. Cais, E. Slovin and L. Snarsky, *J. Organomet. Chem.*, 1978, **160**, 223; P. Chéret and P. Brossier, *Res. Comm. Chem. Pathol. Pharmacol.*, 1986, **54**, 237; F. Mariet and P. Brossier, *Res. Comm. Chem. Pathol. Pharmacol.*, 1990, **68**, 251.
4 K. Digleria, H. Allen, O. Hill, J. McNeill and M. J. Green, *Anal. Chem.*, 1986, **58**, 1203; B. Limoges, C. Degrand, P. Brossier and R. L. Blankespoor, *Anal. Chem.*, 1993, **65**, 1054.
5 T. L. Blundell and L. N. Johnson, *Protein Crystallography*, Academic Press, New York, 1976.
6 H. M. Holden and I. Rayment, *Arch. Biochem. Biophys.*, 1991, **291**, 187.
7 A. Varenne, M. Salmain, C. Brisson and G. Jaouen, *Bioconjugate Chem.*, 1992, **3**, 471.
8 E. R. Burkhardt, J. J. Doney, R. G. Bergman and C. H. Heathcock, *J. Am. Chem. Soc.*, 1987, **109**, 2022.
9 D. M. Macomber and M. D. Rausch, *J. Organomet. Chem.*, 1983, **258**, 331.