An Organometallic Substitute for the Radioactive Bolton–Hunter Reagent

Monique Savignac,[a] André Sasaki,[b] Pierre Potier[b] and Gérard Jaouen*[a]

[a] *Ecole Nationale Supérieure de Chimie de Paris, CNRS U.R.A. 403, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05 France*

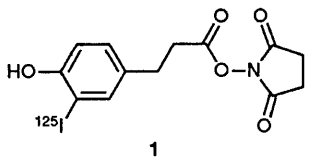
[b] *Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif-sur-Yvette, France*

Novel organometallic reagents, such as the stable cobalt and molybdenum carbonyl complexes of hex-4-ynoic acid *N*-hydroxysuccinimide ester which are detectable at very low concentrations by Fourier transform infrared spectroscopy, couple with amine residues of peptides under mild conditions and provide a non-radio isotopic alternative for the widely used radioactive Bolton–Hunter tracer.

The widespread dissemination since the 1960s of extremely sensitive and specific radioisotopic bioassays revolutionized many aspects of biology and clinical chemistry.[1] Among the techniques of conjugation labelling to introduce a highly radioactive ^{125}I atom, on peptides and proteins, the Bolton–Hunter strategy has proved to be particularly useful.[2] The monoiodinated acylating agent **1** reacts with the terminal or side-chain amino groups present in most proteins and peptides to form amide bonds.[3] However, the consequent need for radiological protection procedures, the generation of radioactive waste as well as other limitations such as the short lifetime of some labelled reagents helped promote the search for alternative bioassays: for example with labelling substances such as enzymes, fluorescent or luminescent compounds.[4]

Since the above substitutes for radiological assays possess their relative merits and demerits the search for new ligand-binder assays is still very active.[4] In this context, organometallic ligand-binder assays seem an attractive method to explore.

Many analytical techniques are available for detecting and quantitating metals and metallic compounds *e.g.*, colorimetric assays,[5] electron microscopy,[6] atomic absorption spectrometry,[7] neutron activation analysis, luminescence,[8]



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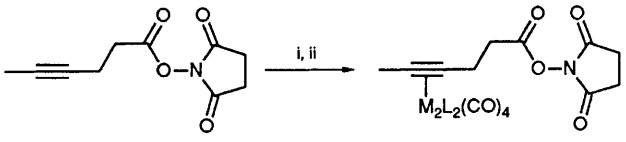
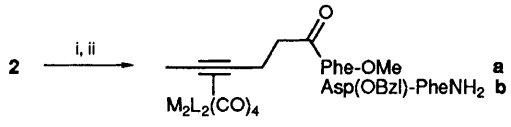


4 2 a M : Mo, L = cp (50%)
b M : Co, L = CO (53%)

Scheme 1 *Reagents*: i, $Co_2(CO)_8$ or $Mo_2L_2(CO)_4$; ii, THF



5 a_1 M : Mo, L = cp (45%)
a_2 M : Co, L = CO (53%)
b M : Mo, L = cp (30%)

Bzl = benzyl

Scheme 2 *Reagents*: i, H-Phe-OMe or Asp (OBzl)-$PheNH_2$; ii, THF

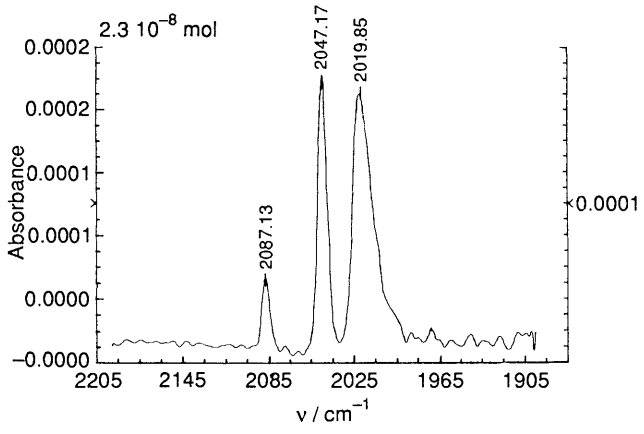


Fig. 1 IR spectrum of a 2.3 × 10^{-8} mol l^{-1} CCl_4 solution of **5a₂** in the ν CO region, with the use of an ultra-low-volume (16 μl) gold light-pipe cell and recorded on a Bomem Milkelson 100 spectrometer equipped with a liquid-nitrogen cooled, indium-antimonide (In Sb) detector and a beam condensor (to produce a 1.0 mm IR microbeam).[16]

fluorescence[9] and electrochemistry.[10] In addition, organometallic chemistry has now reached a level of development compatible with potentially easy tailor-made labelling of ligands and binders.

An obvious strategy for the metallic labelling of peptides and proteins consists of the adaptation of recognized methods in radioassays. We now report as an example of this approach an organometallic substitute[11] for the Bolton–Hunter procedure.

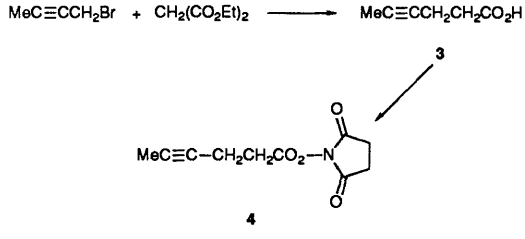
The Bolton–Hunter acylating agent **1** usually produces conjugates which retain the specific properties of the label as well as the molecular recognition (binding) properties of the labelled peptides and proteins. This species has been particularly useful when direct labelling of tyrosine can lead to loss of immuno or biological activity or when the substrate lacks the appropriate tyroxyl residue for iodination. In addition, direct introduction of ^{125}I can also cause severe damage to the biological species.

With the exception of the nature of the markers, there is a clear analogy between compounds **1** and **2**. The most direct way to mark a six-electron arene ligand as shown in **1** would be to introduce a twelve electron moiety such as a $Cr(CO)_3$ unit in order to adjust the similarity with **1**. Unfortunately, the presence of a phenolic group in the precursor of **1** makes the complex too unstable for further biological use. Since small alkyne clusters have proven to be particularly stable under biological conditions,[12] we have developed a mode of access to **2**, shown its coupling capabilities with peptides, and analysed its low concentration possibilities of detection by Fourier transform infrared (FTIR) spectroscopy.

Compounds **2a** and **2b** were easily prepared from **3** *via* **4** as indicated below† (Scheme 1). These compounds **2a** and **2b** show a long term stability (several years) when kept at 4 °C protected from sunlight. It is worth noticing that the solubility of such species in biological solvents [H_2O or H_2O plus dimethylformamide (DMF)] can be considerably increased, if needed, by introduction of a sulphonyl group on the succinimidyl unit.

Scheme 2 depicts the preparation‡ of conjugates between organometallic activated esters **2** and the free amino groups of peptides. The next step consists of finding a suitably sensitive analytical technique with which to monitor very low concentrations of metallic markers. *A priori*, each of the above reported techniques[5–10] may be used for this purpose. However, we now show that another type of analysis can be performed. Metal-carbonyl moieties exhibit extremely intense, characteristic absorptions, in their IR spectra at about ν CO 2000 cm^{-1}. These absorptions of coordinated carbonyls

† Compound **3** was prepared according to the literature method: *conditions*, NaOH, MeOH, then KOH, EtOH, followed by heating in refluxing dioxane.[13] Total yield for three steps: 73%. Treatment of **3** with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide gave **4**.



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A 0.5 × 10^{-3} mol dm^{-3} solution of **4** in anhydrous tetrahydrofuran (THF) was added to a solution of $M_2L_2(CO)_4$; compounds **2a** and **2b** were obtained after stirring for 3 h at room temperature. Following purification by TLC (silica gel) these compounds were isolated in about 50–55% yield and characterized.

Selected data: **3**: m.p. 100 °C;[14] ^1H NMR (200 MHz, $CDCl_3$) δ: 1.77 (t, *J* 3 Hz, 3H); 2.50 (m, 2H); 2.56 (m, 2H); 9.65 (s, 1H). **4**: m.p. 65 °C; yield 95%; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.53 (m, 2H); 2.80 (m, 2H); 2.83 (m, 2H). **2a**: ^1H NMR (250 MHz, CD_2Cl_2) δ: 2.49 (t, 2H); 2.71 (s, 3H); 2.81 (s, 4H); 3.07 (t, 2H); 5.28 (s, 10H). IR (KBr) ν̄ CO: 1976.3, 1897.3, 1826 (sh), 1819, 1782 (w), 1734 cm^{-1}. Mass spectrometry (desorption chemical ionisation with NH_3) *m/z*: 659 ($M+NH_4^+$), 644 ($M+H^+$), 435 [$cp_2Mo_2(CO)_4$].

‡ After deprotection, a solution of H-Phe-OMe or Asp(OBzl)-$PheNH_2$ was added to a THF solution of **2a** and **2b**. The mixture was stirred at room temperature for 4 h, the solvent evaporated, and the product purified by TLC (silica gel; eluent: ethyl acetate).

Selected data: **5a₁**: ^1H NMR (250 MHz, CD_2Cl_2) δ: 2.02 (m, 2H); 2.66 (s, 3H); 2.94 (m, 2H); 3.11 (m, 2H); 3.71 (s, 3H); 4.81 (m, 1H); 5.23 (s, 5H); 5.24 (s, 5H); 5.87 (d, 1H); 7.11–7.29 (m, 5H). IR (KBr) ν̄ CO: 1973, 1895, 1823, 1730 cm^{-1}. Mass spectrometry (DCI-NH_3) *m/z*: 708 (M + H^+), 652 (M − 2CO).

fall into a window between the absorption of most organic molecules including those of peptides and proteins.[15] This fact associated with the advent of sensitive, multiscanning and relatively cheap FTIR spectrometers provides a new way of assaying bioligands, as shown in Fig. 1. This example depicts the IR spectrum (10 scans; 4 cm^{-1}) of a 2.3×10^{-8} mol dm^{-3} solution of $\mathbf{5a_2}$ in CCl_4 using a benchtop Bomem Michelson 100 spectrometer.

To reach such a level of detection (about a picomole of marker in the measurement cell) we have designed a gold light-pipe cell for the IR analysis of ultra-low volumes of extremely dilute organic solutions.[16]

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References

1 J. P. Gosling, *Clin. Chem.*, 1990, **36**, 1408.
2 A. E. Bolton and M. Hunter, *Biochem. J.*, 1973, **133**, 529.
3 J. J. Langone, *Methods in Enzymology*, eds. J. J. Langone and H. Van Vunakis, Academic Press, New York, 1980, **70**, p. 221.
4 L. J. Kricka, *Ligand-Binder Assays: Labels and Analytical Strategies*, Marcel Dekker, New York and Basel, 1985.
5 C. L. Baigent and G. Muller, *Experientia*, 1980, **36**, 472; J. H. W. Leuvering, P. J. H. Thal, M. Van der Waart and A. H. W. M. Schuurs, *J. Immunoassay*, 1980, **1**, 77.
6 W. P. Faulk and G. M. Taylor, *Immunochemistry*, 1971, **8**, 1021; J. F. Hainfield, *Science*, 1987, **236**, 450.
7 D. A. Skoog and D. M. West, *Analytical Chemistry*, Holt, Rinthart and Wilson, New York, 1979, pp. 482–496; M. Cais, *Methods in Enzymology*, eds. J. J. Langone and H. Kunakis, Academic Press, New York, 1983, **92**, 445.
8 T. Hara, M. Toriyama and K. Tsukagoshi, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2267; P. S. Forgione and W. A. Henderson, 1983, US Patent 4 375, 972; R. A. Stott, G. H. G. Thorpe, L. J. Kricka and T. P. Whitehead, in *Analytical Applications of Bioluminescence and Chemiluminescence*, eds. L. J. Kricka, P. E. Stanley, G. H. G. Thorpe and T. P. Whitehead, Academic Press, London, 1984, pp. 233–238.
9 T. D. Barela and A. D. Sherry, *Anal. Biochem.*, 1976, **71**, 351; N. J. Marshall, S. Dakubu, T. Jackson and R. P. Ekins in *Monoclonal Antibodies and Developments in Immunoassay*, eds. A. Albertini and R. Ekins, Elsevier, Amsterdam, 1981, pp. 101–108; P. Halonen, O. Meurman, T. Lovgren, I. Hemmila and E. Soini, *Curr. Topics Microbiol. Immunol.*, 1983, **104**, 133.
10 W. R. Heineman, C. W. Anderson and H. B. Halsall, *Science*, 1979, **204**, 865; D. A. Skoog and D. M. West, *Fundamentals of Analytical Chemistry*, Saunders College Publishing, Philadelphia, 4th edn., 1982, pp. 459–471; M. J. Doyle, H. B. Halsall and W. R. Heineman, *Trends Anal. Chem. 2*; 1983, XI–XII; K. R. Wehmeyer, M. J. Doyle, H. B. Halsall and W. R. Heinemann, *Methods in Enzymology*, eds. J. J. Langone and H. Van Kunakis, Academic Press, New York, 1983, 92, 432; I. A. Alam and G. D. Christian, *Anal. Lett.*, 1982, **15B**, 1449.
11 N. A. Sasaki, P. Potier, M. Savignac and G. Jaouen, *Tetrahedron Lett.*, 1988, **29**, 5759.
12 G. Jaouen, A. Vessieres, S. Top, M. Savignac, A. A. Ismail and I. S. Butler, *Organometallics*, 1987, **5**, 1985.
13 N. A. Sasaki, J. L. Morgat and P. Potier, *Int. J. Peptides Protein Res.*, 1986, **27**, 360.
14 E. R. H. Jones, G. H. Whitham and M. C. Whiting, *J. Chem. Soc.*, 1954, 3201.
15 I. S. Butler, A. Vessieres and G. Jaouen, *Comments Inorg. Chem.*, 1989, **8**, 269.
16 A. Vessieres, G. Jaouen, M. Salmain and I. S. Butler, *Appl. Spectrosc.*, 1990, **44**, 1092.