Bioorganometallic Chemistry: A Future Direction for Transition Metal Organometallic Chemistry?

GÉRARD JAOUEN* AND ANNE VESSIÈRES

Ecole Nationale Supérieure de Chimie de Paris, UA CNRS 403, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

IAN S. BUTLER

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec, Canada H3A 2K6

Received January 3, 1992

Although research in organometallic chemistry has continued to develop at a furious pace over the past 40 years, with an incredible range of structurally exotic and novel compounds being produced, the time is now ripe for new directions to be explored. The sustained interest in the area is due to (1) the application of organometallic compounds in selective organic synthesis, particularly in the development of industrially important, high-performance, homogeneous catalysts,[1] and (2) the enormous potential of organometallics as precursors in the production of new advanced materials such as ceramics (SiC, GaAs, MnTe, etc.)[2] and other types of solids with important, nonlinear optical properties.[3]

Selectivity in a chemical reaction is an example of the much broader field of molecular recognition, which extends into the realm of molecular biology and is the basis of all bioligand–biological macromolecule associations that eventually lead to the construction of supramolecular assemblies.[4] At present, the contribution of transition metal, inorganic and organometallic complexes to this field is confined almost exclusively to studies involving DNA.[5] Proteic systems, in particular, have been little investigated.[6,7] In fact, there are several types of high-affinity, proteic molecular associations such as enzyme–substrate, hormone–receptor, and antigen–antibody which should be amenable to experimental investigation by organometallic chemistry. In these cases, it will be important to know the precise effect of an organometallic label on molecular recognition. The wide range of synthetic procedures already available in organometallic chemistry bodes well for the labeling step. Once this step has been accomplished and the stability of the labeled molecule in the complex biological medium has been verified, then the biological study can be undertaken.

In this Account, we present a summary of our work on the application of bioorganometallic chemistry mainly in some analytical aspects in immunology, and in the analysis and molecular recognition of active sites in hormone receptors. It should be emphasized that the potential of the field is enormous and is certainly not restricted to the few directions considered here.

Carbonylmetalloimmunoassay (CMIA)

An important area of molecular recognition is that based on antigen–antibody interactions, and studies of these high-affinity interactions have led to the continued development of new immunoassay procedures.[8] Until quite recently, however, the majority of the tracers employed have been radioactive. The advantages of these types of tracer are well-known. They permit rapid, sensitive, and reliable analyses to be performed on a large scale. However, the problems associated with radioactive tracers are also well documented and have restricted their use to those centers which can be routinely inspected. The storage and treatment of radioactive waste are also extremely sensitive ecological and environmental protection issues. These negative factors have constituted a major stumbling block to the continued use of radioimmunoassay techniques, and there is now a worldwide search for nonisotopic markers to replace existing radioactive ones.[8]

The possibility of using organometallic markers as tracers in immunoassays was originally suggested by Cais in 1977, who coined the term, *metalloimmunoassay* (MIA), for the procedure.[9] The first such marker was an estradiol derivative modified in the 17-position by a ferrocenyl group. Although atomic absorption spectrometry was proposed as the detection method, apart from the determination of standard curves for the

Gérard Jaouen was born in France. He is Professor of Chemistry at the Université Pierre et Marie Curie (ENSC Paris) and Director of CNRS Unit 403. His research interests include transition metal organometallics and their application in organic chemistry and biochemistry.

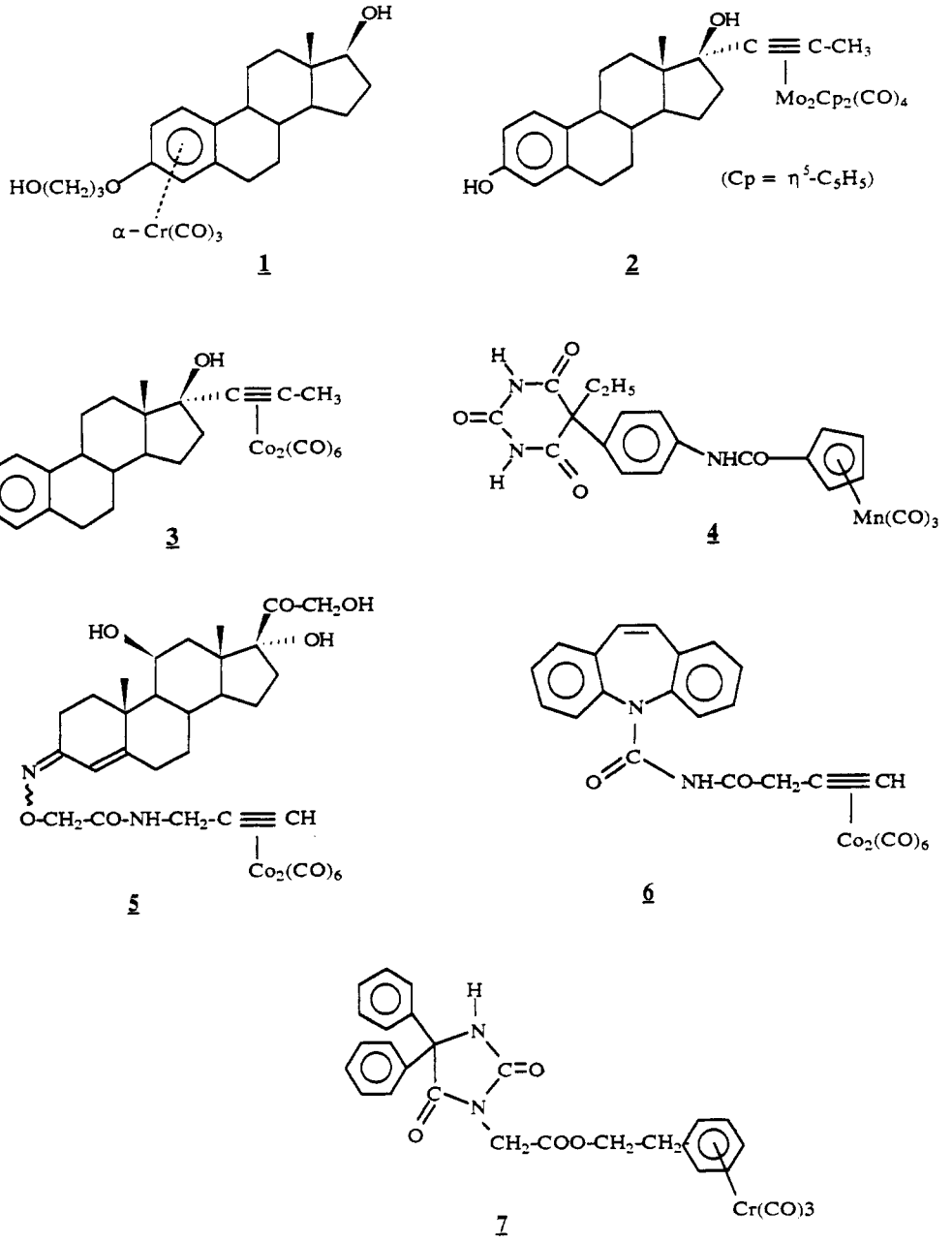
Anne Vessières was born in France and educated at the University of Rennes, where she received Ph.D. degrees in chemistry (1974) and biochemistry (1980). She is currently working as Director of Research for the CNRS in Paris on the use of organometallic complexes in biochemistry, especially in the areas of receptorology and immunology.

Ian Butler was born in England and educated at the University of Bristol (Ph.D., 1964). Following postdoctoral work in the United States, he was appointed to the faculty of McGill University, where he is Professor of Chemistry. He is Senior Editor of the *Canadian Journal of Applied Spectroscopy*, and his research interests include organometallic chemistry, vibrational spectroscopy, and kinetics and mechanisms of inorganic reactions.

(1) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1320–1367.
(2) (a) Laine, R. M., Ed. *Transformation of Organometallics into Common and Exotic Materials: Design and Activation*; Martinus Nijhoff Publishers: Boston, MA, 1988. (b) Harrod, J. F., Laine, R. M., Eds. *Inorganic and Organometallic Oligomers and Polymers*; Kluwer Academic Publishers: 1991.
(3) Wang, Y. *Acc. Chem. Res.* **1991**, *24*, 133.
(4) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304–1319.
(5) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153.
(6) Kostic, N. M. *Comments Inorg. Chem.* **1988**, *8*, 137.
(7) Ryabov, A. D. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 931 and references therein.
(8) Gosling, J. P. *Clin. Chem.* **1990**, *36*, 1408.
(9) Cais, M.; Dani, S.; Eden, Y.; Gandolfi, O.; Horn, M.; Issac, E. E.; Josephy, Y.; Saar, Y.; Slovin, E.; Snarsky, L. *Nature* **1977**, *270*, 534.

0001-4842/93/0126-0361$04.00/0 © 1993 American Chemical Society

Scheme I



marker in solution, there was apparently no attempt to apply the procedure in an actual immunoassay. The concept has recently been reevaluated by Brossier, who has demonstrated the feasibility of such immunoassays for antidepressant and antiepileptic drugs.[10]

Our work has exploited the unique vibrational spectroscopic properties of metal carbonyl markers. These complexes display extremely intense infrared bands in the 2200–1850-cm^{-1} region which are at least 5–10 times more intense than any other bands in the spectra. Also, the wavenumber region of interest affords a spectral window which is devoid of any significant absorptions due to proteins. Because of the high sensitivities of modern Fourier transform infrared (FT-IR) spectrometers, picomole to femtomole quantities of organometallic carbonyl tracers both in the solid state[11] and in chlorinated organic solvents have been successfully detected.[12] These results have led us to develop the *carbonylmetalloimmunoassay* (CMIA) procedure,[13,14] a new type of competitive immunoassay in which the tracer used in an organometallic-labeled molecule and the method of detection is FT-IR spectroscopy. Several methods for incorporating organometallic fragments such as $Cr(CO)_3$, $Mo_2Cp_2(CO)_4$ (Cp = η^5-C_5H_5), $Mn(CO)_3$, and $Co_2(CO)_6$ to molecules of biological interest (e.g., medications and hormones, Scheme I) have been developed. These synthetic methods were modeled on the procedures used in the preparation of immunogens for the fabrication of

(10) Chéret, P.; Brossier, P. *Res. Commun. Chem. Pathol. Pharmacol.* **1986**, *54*, 237.

(11) Butler, I. S.; Vessières, A.; Salmain, M.; Brossier, P.; Jaouen, G. *Appl. Spectrosc.* **1989**, 1497.

(12) Salmain, M.; Vessières, A.; Jaouen, G.; Butler, I. S. *Anal. Chem.* **1991**, *63*, 2323.

(13) Vessières, A.; Salmain, M.; Philomin, V.; Jaouen, G. *Immunoanal. Biol. Spec.* **1992**, *31*, 9.

(14) Salmain, M.; Vessières, A.; Brossier, P.; Butler, I. S.; Jaouen, G. *J. Immunol. Methods* **1992**, *148*, 65.

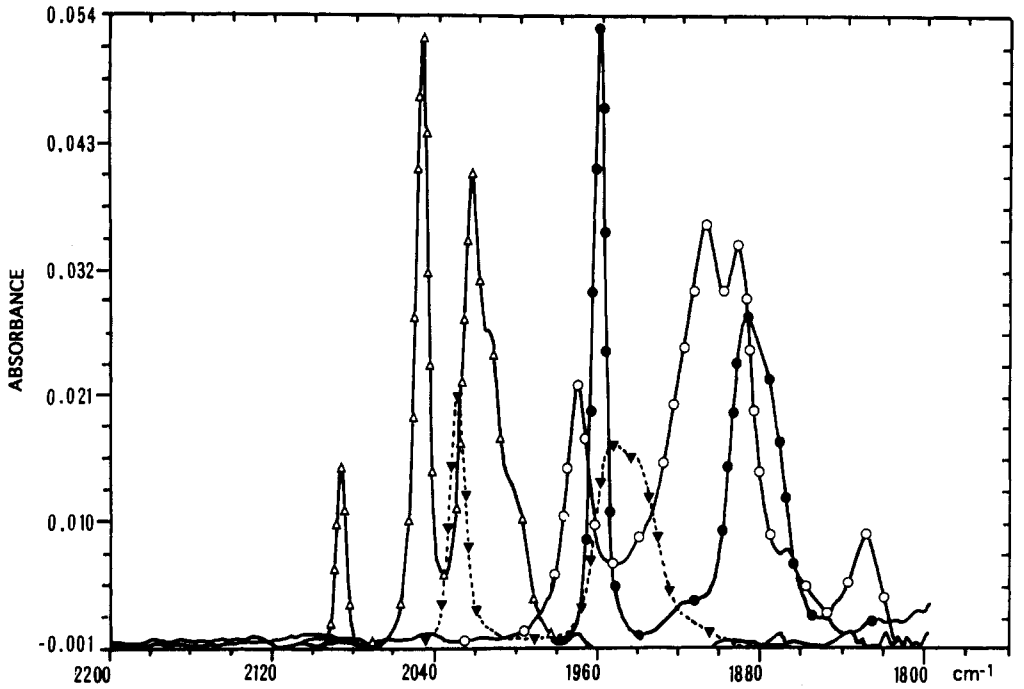


Figure 1. IR spectra of the organometallic complexes 1–4. Expansion of the ν(CO) region. Spectra (64 scans, 4-cm^{-1} resolution) were recorded on a Michelson 100 FT spectrometer equipped with an InSb detector, a 4× beam condenser, and a KBr micro cavity cell (1-mm path length): (●) 1, (○) 2, (▲) 3, (▼) 4.

antibodies and have allowed us to prepare organometallic markers for a variety of molecules which possess specific antibodies. The FT-IR spectra in the ν(CO) region of the organometallic markers 1–4 (Scheme I) are shown in Figure 1.

Since the quantities of tracers used in immunoassays are extremely low, it was essential to know the limit of detection of these markers by FT-IR spectroscopy. There were distinct spectral differences between the various organometallic markers, and they could be split into two groups, depending on the position of their strongest peak: below 2020 cm^{-1}, the Cr and Mo complexes; above 2020 cm^{-1}, the Co and Mn complexes. Because of interference from water vapor bands appearing just below 2020 cm^{-1}, the limit of detection was 10 times lower for the Co and Mn markers than for the Cr and Mo ones. Following optimization of the various factors in the FT-IR analyses (choice of detector, cell, solvent, etc.), the quantitative limit of detection in about 1 min (64 scans, 4-cm^{-1} resolution) was 0.3 pmol for the Co complex and 0.7 pmol for the Mn complex. This limit of detection is fully compatible with the quantities used in most immunoassays, and the feasibility of the CMIA immunoassay procedure has already been demonstrated for two typical hapten assays which are performed routinely in hospitals. The haptens selected were phenobarbital (an anticonvulsive medication) and cortisol (a hormonal steroid). For phenobarbital, the organomanganese marker 4 was used,[15] while for cortisol, the cobalt complex 5 was employed.[16]

For a molecule to be a useful tracer in an immunoassay, it must exhibit good recognition properties for antibodies. We found that the attachment of organometallic fragments in positions near those used in obtaining antibodies gave organometallic tracers that were well recognized by the antibodies. For the cortisol–$Co_2(CO)_6$ complex 5, the level of cross reactivity, determined by using tritiated cortisol as the tracer, varied between 58 and 83%, depending on which particular batch of antibodies and which isomer (5-*Z* or -*E*) were being used.[16] (Note: 100% cross reactivity implies full competition between a compound being assayed and the radioactive tracer.) For the Mn phenobarbital complex, the level of cross reactivity, determined by using [^{14}C]phenobarbital, also varied significantly (between 100 and 200%).[14] We worked routinely with 30-pmol quantities of tracer because this amount produced good IR signal-to-noise ratios and provided excellent conditions for a sensitive immunoassay. Moreover, this quantity is typical of that normally used for radioimmunological assays (RIA) employing [^{14}C]phenobarbital as the tracer, thereby affording us a direct comparison of the CMIA and RIA procedures.

An important parameter in a successful immunoassay is an accurate determination of the antibody titer. This titer value depends on the quantity of tracer and the methods used to separate the free and bound tracer fractions. For the CMIA studies, we opted for solvent extraction by ethyl acetate for phenobarbital and isopropyl ether for cortisol. In both cases, the titers found by charcoal–dextran and organic solvent extraction were identical. Figure 2 illustrates the dilution curves obtained by CMIA for marker 4. The free fractions of tracer which had been solvent extracted were directly proportional to the intensities of the associated ν(CO) peaks located at 2057 (marker 5) and 2032 cm^{-1} (marker 4). The resulting curves were similar to those obtained by RIA, and the titer values increased as the quantity of tracer decreased, as expected. The highest titer value found (1000) in the presence of 30 pmol of tracer was sufficiently high to allow selection of the antibodies for use in the CMIA procedure. It is

(15) Lavastre, I.; Besançon, J.; Brossier, P.; Moise, C. *Appl. Organomet. Chem.* **1990**, *4*, 9.
(16) Philomin, V.; Vessières, A.; Gruselle, M.; Jaouen, G. *Bioconjugate Chem.*, submitted.

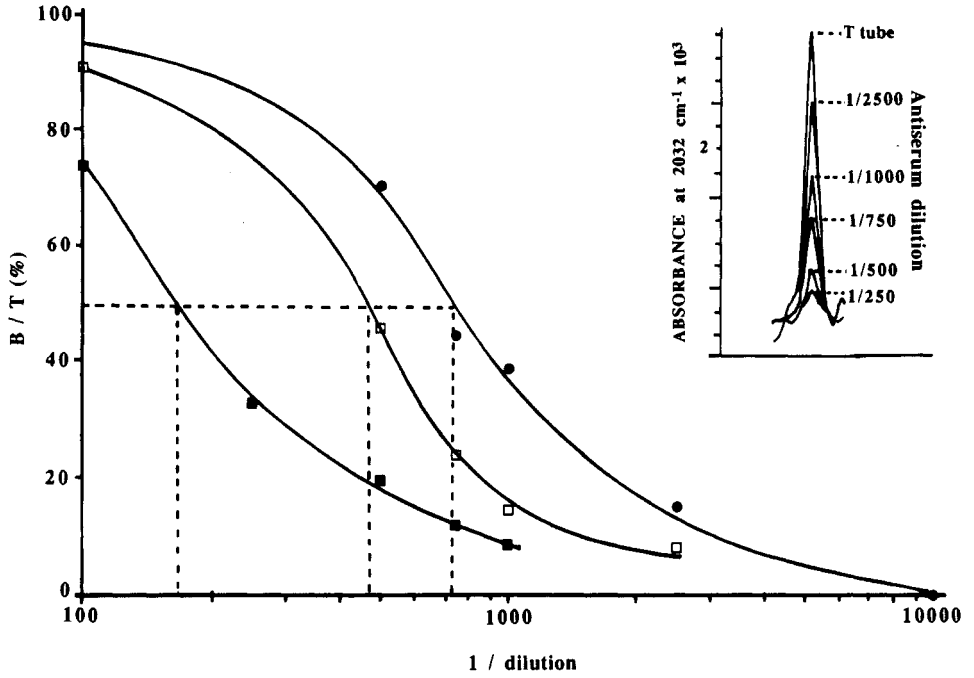


Figure 2. Antiserum dilution curves by CMIA. Variation of the antiserum titer with the quantity of 6 used as tracer: (■) 34 pmol/tube, (□) 129 pmol/tube, (●) 215 pmol/tube. Inset: Variation of the 2032-cm^{-1} ν(C)) peak of 34 pmol of 6 (32 scans, 4 cm^{-1}) following incubation in the presence of various antiserum dilutions and extraction of the free fraction by ethyl acetate.

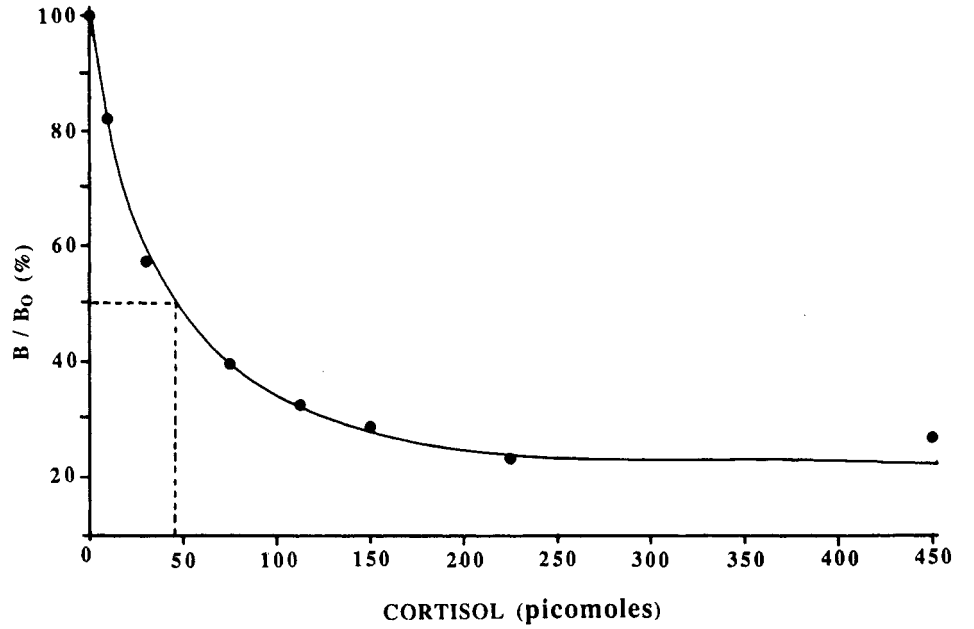


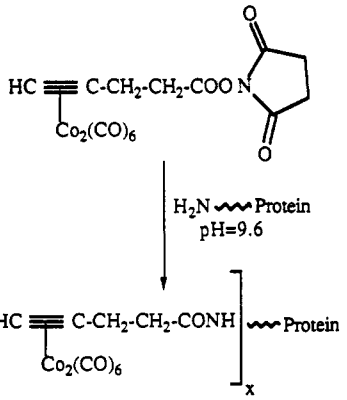
Figure 3. Standard curve by CMIA: 30 pmol of 5 used as tracer; extraction of the free fraction by isopropyl ether.

important to note that the antibody titer obtained by RIA in the presence of the same amount of [^{14}C]-phenobarbital was only 500.[14] Clearly, the titer value depends markedly on the structure of the tracer, with the better results being obtained for the organometallic complex.

Typical standard curves such as that shown in Figure 3 for marker 5 were constructed. The steepest part of the curve is where the greatest sensitivity occurred, and this corresponded to an added quantity of unlabeled cortisol of 25–75 pmol, which is equivalent to ~40–60 μL of serum from a patient suffering from hypercortisolemia. The reproducibility and the coefficient of variation (CV) of the CMIA method were good (standard deviation, 2.1%; CV, 5%).[14] The serum assays showed that routine CMIA procedures may well be possible for 40 μL of sample (CV, 7.5–9%)[13], indicating that the technique is competitive with existing cold immunoassay methods (e.g., fluorescence). In fact, it may be more reliable than some of them (e.g., ELISA), and direct blood assays may even be possible in the future. A distinct advantage of the CMIA method is the absence of overlapping signals due to the parent molecule in the spectral region being used for the assay.

In order for CMIA to be really useful in clinical diagnosis, two questions have to be answered: (1) Can antibodies be labeled directly with sufficient metal carbonyl fragments to increase the sensitivity and ease of the assay? (2) Can simultaneous multiimmunoassays be performed by using a range of different but com-

Scheme II



patible organometallic markers? Concerning the first question, we have already succeeded in labeling anti-human thyroid stimulating hormone (h-TSH) monoclonal antibodies with (*N*-succinimidyl 4-pentynoate)-hexacarbonyldicobalt [$(NS)Co_2(CO)_6$] (see Scheme II).[17] Each monoclonal antibody (named JOSS2-2; a gift from Clonatec, Paris, France) can be labeled with about 30 $Co_2(CO)_6$ fragments at the lysines accessible to the reactant. The quantity of marker present in the protein can in principle be determined directly by FT-IR spectroscopy because the ν(CO) IR absorbances are proportional to the number of $Co_2(CO)_6$ groups directly bound to the protein. The lysine residues implicated in the coupling reaction do not interfere with the association of the hormone h-TSH and its specific antibody JOSS 2-2, indicating that the metal carbonyl labeling does not alter the specific antigen–antibody association. The ratio of the marker/protein coupling (31/1) is also important in increasing the sensitivity of the detection by FT-IR spectroscopy. For heterogeneous assays, the use of antibody/antigen coated on solid phases can considerably simplify the procedure by avoiding the separation of the free and bound fractions of tracer. Certain polymers, such as polystyrene, are highly absorbing in the IR, but there is a useful spectral window between 2200 and 2000 cm^{-1}. In addition, alkyne–$Co_2(CO)_6$ fragments on a solid support can be detected by IR spectroscopy because the three ν(CO) bands normally observed appear at positions above 2000 cm^{-1}. A typical spectrum of the conjugate depicted on a microtitration well is shown in Figure 4. The absorbance of the 2051-cm^{-1} band exhibits good linearity with changes in concentration, while the 2095-cm^{-1} band is too weak for quantitative work, and that at 2023 cm^{-1} is too close to the cutoff of the polystyrene vibrations. Solid-state immunoassays are certainly now feasible because a mounting stage for the microtitration plates has recently become available.

Also, since each metal carbonyl marker exhibits its own characteristic ν(CO) peaks, the possibility of performing several different immunoassays at the same time is conceivable; multianalyte testing is a long-cherished goal of the analytical chemist.[18] A multilabel immunoassay using FT-IR detection and organometallic complexes as markers can now be envisaged. To illustrate the feasibility of this approach, we have attached different organometallic markers on three important antiepileptic drugs, phenobarbital, carbamazepine, and phenytoin (**4**, **6**, and **7**, Scheme I), and we have demonstrated that it is possible to analyze simultaneously and quantitatively a mixture of two or three organometallic complexes, present in amounts of the order of 10–100 pmol.[19] The FT-IR spectra of the complexes are ideal for multicomponent IR analysis, and we can determine their concentrations in a mixture with a quantitative precision of ~3–5%. The drugs are normally administered simultaneously to patients, and since their therapeutic effect is related to their concentrations in the serum of patients, assays are performed extensively in hospitals. To the best of our knowledge, there are no immunoassays (isotopic or nonisotopic) routinely available which permit the simultaneous determination of several drugs. The development of such a multicomponent analytical method would be readily extended to other biological systems, e.g., labeled nucleotides.

Organometallic Binding-Site-Directed Reagents in the Study of Proteins

Estrogen receptors are members of a large family of nuclear proteins which include certain hormonal steroid receptors.[20] The intimate association of a hormone with its receptor is the first step in triggering a hormonal response, one of the regulatory phenomena in organisms. This hormonal response initiates a succession of events, including receptor phosphorylation, release of a protective protein (heat shock protein, HSP 90), receptor dimerization, and subsequent association of this dimer with DNA. It is difficult to propose a mechanism for several reasons, which include the lower stability of these receptor proteins, their low concentration (approximately 10 000 molecules per whole cell), and the existence of a reversible equilibrium between the hormone and its receptor despite a high affinity (K_D = 10^{-9}–10^{-11} M). The structure of a hormone–receptor complex cannot yet be established by X-ray diffraction. However, certain receptors have recently been cloned and sequenced,[21] thus opening up routes to purified receptors.[22] In the interim, alternate avenues have to be explored, and we present here a novel approach based on organometallic chemistry to obtain structural and mechanistic information.

The association site of the estradiol receptor has an acid character[23] and probably contains a sulfhydryl group.[24] Also, the hydroxyl groups located at positions C3 and C17 on estradiol are essential in maintaining a high recognition level, and the 17α-position can apparently tolerate a relatively bulky group without any appreciable reduction in recognition level.[25] Conse-

(17) (a) Varenne, A.; Salmain, M.; Brisson, C.; Jaouen, G. *Bioconjugate Chem.* **1992**, *3*, 471. (b) For previous labeling of peptides, see: Sasaki, N. A.; Potier, P.; Savignac, M.; Jaouen, G. *Tetrahedron Lett.* **1988**, *29*, 5759–5762. Le Borgne, F.; Beaucourt, J. P. *Tetrahedron Lett.* **1988**, *29*, 5649–5652.

(18) Kricka, L. J. *J. Clin. Chem.* **1992**, *38*, 327.

(19) Salmain, M.; Vessières, A.; Brossier, P.; Jaouen, G. *Anal. Biochem.* **1993**, *208*, 117.

(20) (a) Carson-Jurica, M. A.; Schrader, W. T.; O'Malley, B. W. *Endocr. Rev.* **1990**, *11*, 201. (b) O'Malley, B. W. *Mol. Endo.*, **1990**, *4*, 363.

(21) Ojasoo, T.; Raynaud, J. P.; Mornon, J. P. In *Comprehensive Medicinal Chemistry*, Vol. 3, *Membranes and Receptors*; Emmet, J. C., Ed.; Pergamon Press: New York, 1990, p 1175.

(22) Wooge, C. H.; Nilsson, G. N.; Heierson, A.; McDonnell, D. P.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1992**, *6*, 861.

(23) Katzenellenbogen, J. A.; Carlson, K. E.; Heiman, D. F.; Robertson, D. W.; Wei, L. L.; Katzenellenbogen, B. S. *J. Biol. Chem.* **1983**, *258*, 3487.

(24) Jensen, E. V.; Hurst, D. J.; De Sombre, E. R.; Jungblut, P. W. *Science (Washington, D.C.)* **1967**, *158*, 385.

(25) Jaouen, G.; Vessières, A. *Pure Appl. Chem.* **1989**, *61*, 565.

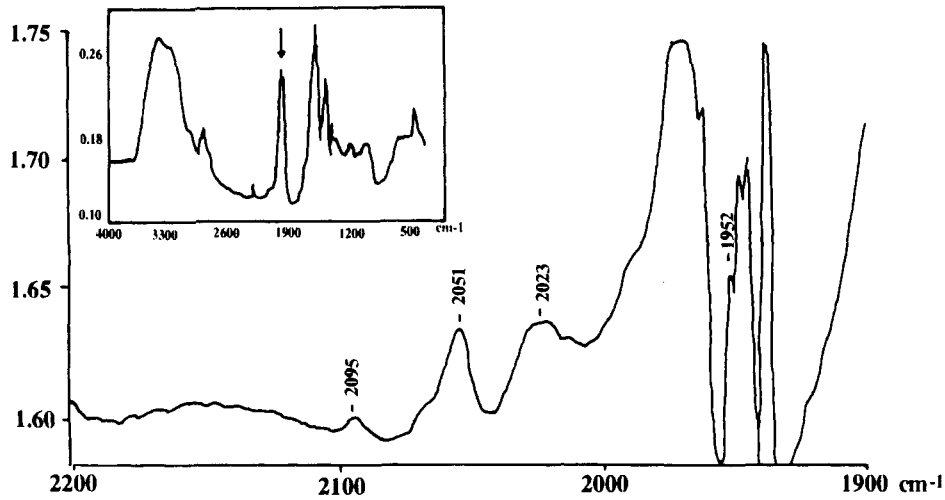
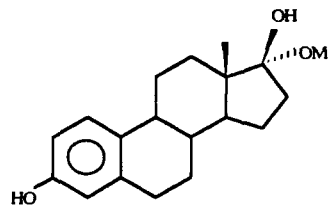


Figure 4. IR spectrum of a $Co_2(CO)_6$–protein complex (protein: antibody JOSS 2-2) on a microtiter well in the ν(CO) region. Inset: IR spectrum of a $Co_2(CO)_6$–protein complex in KBr (whole range, 13-mm KBr pellet). The ν(CO) bonds are indicated with an arrow.

Scheme III



OM = an organometallic unit such as :	Relative Binding Affinities RBA (%) for the nuclear estradiol receptor
2 -C ≡ C-$CH_3/Mo_2Cp_2(CO)_4$	33
3 -C ≡ C-$CH_3/Co_2(CO)_6$	18
8 (η-C_5H_4)Fe(η-C_5H_5)	15
9 -C ≡ C-$CH/Ru_3(CO)_{10}$	8
10 -C ≡ C-C/$[Ru_3(CO)_9]H$	2.6
11 -C ≡ C-$CH/Os_3(CO)_{10}$	2.5

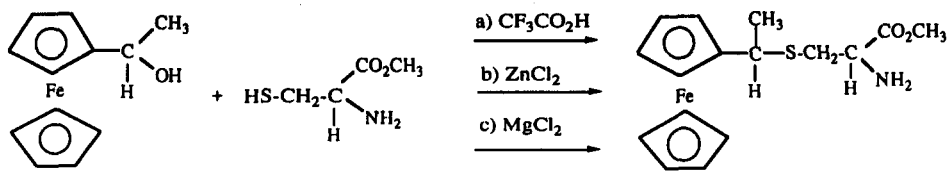
quently, we synthesized several estradiol derivatives which had been labeled with organometallic fragments in strategic positions on the steroidal skeleton (Scheme III). We initially labeled at the 17α-position and showed that these complexes recognize (RBA values) the estradiol receptor. This study led to the discovery of a new family of affinity markers which were produced by direct covalent bond formation.[26,27]

Three groups of organometallic-labeled estradiol derivatives were investigated: (1) Co and ferrocene derivatives (**3**, **8**), which produced extensive inactivation (~80%) of the estradiol receptor, (2) trinuclear Os and Ru complexes (**9**, **10**, **11**), which caused moderate receptor inactivation (~65%); and (3) a Mo cluster (**2**), which only led to a weak inactivation of the receptor (22%). The differences in receptor inactivation can perhaps be attributed to the stabilities of the associated carbenium ions [e.g., pK_{R^+}[HC≡$CCH_2OH/Mo_2Cp_2(CO)_4$] = 3; pK_{R^+}[HC≡$CCH_2OH/Co_2(CO)_6$] = −5.5].[28,29] Moreover, the data suggested that the 17β-OH function of the organometallic-labeled hormone could be selectively activated in the region of the association site of the estrogen receptor. Possibly, there is a reaction with an acidic group to form a carbenium species and, if a nucleophile is located near this transitory alkylating ion, irreversible covalent bond formation can take place to produce the affinity markers. Since hormone **3** could be obtained in two radioactive forms (tritiated at positions 2,4 or with a ^{14}CO label on the cluster), we were able to establish definitively that the organometallic hormone was covalently bound to estradiol receptor. The ^{14}C measurements also indicated that the organometallic hormone did not decompose during the course of the incubation experiments.

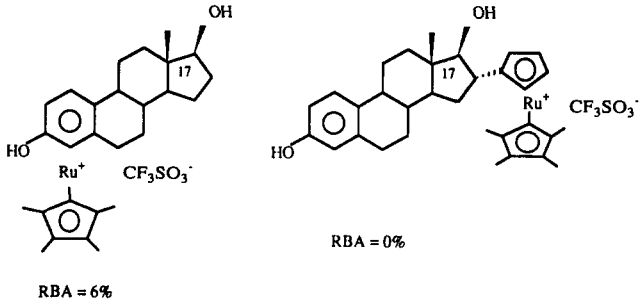
The possibility of a cysteine being located near the association site of the receptor has received some support from inactivation studies using methyl methanethiosulfonate (MMTS), a highly specific reagent for sulfhydryl groups. We found ~90% inactivation of estradiol receptor with MMTS,[30] suggesting that a cysteine was involved in receptor inactivation with the bulky organometallic hormones. The acidic character of cysteine may result from either sulfur protonation or divalent cation (e.g., Zn^{2+}) coordination to the thiolate function.[31] In order to obtain more evidence for cysteine being located at the inactivation site, we undertook a study of the reactions of several cysteine and histidine derivatives with (η-$C_5H_4CH(OH)CH_3$)Fe(η-C_5H_5). This 1-ferrocenylethanol molecule is stable, is easy to prepare, and is a suitable model for the organometallic moiety of estradiol labeled with a ferrocenyl substituent

(26) Vessières, A.; Top, S.; Vaillant, C.; Osella, D.; Mornon, J. P.; Jaouen, G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 753.
(27) Vessières, A.; Vaillant, C.; Gruselle, M.; Vichard, D.; Jaouen, G. *J. Chem. Soc., Chem. Commun.* **1990**, 837.
(28) Nicholas, K. M. *Acc. Chem. Res.* **1987**, *20*, 207.
(29) Gruselle, M.; Cordier, C.; Salmain, M.; El Amouri, H.; Guérin, C.; Vaisserman, J.; Jaouen, G. *Organometallics* **1990**, *9*, 2993.
(30) Vessières, A.; Vaillant, C.; Salmain, M.; Jaouen, G. *J. Steroid Biochem.* **1989**, *34*, 301.
(31) Kumura, E.; Koike, T. *Comments Inorg. Chem.* **1991**, *11*, 285.

Scheme IV



Scheme V



at the 17α-position (8). Three key experiments were performed with equimolar amounts of (1) CF_3CO_2H (product yield 70%), (2) $ZnCl_2$ in H_2O/THF (30 h at 25 °C; product yield 60%), and (c) $MgCl_2$ in H_2O/THF (20 h at 25 °C; product yield 60%) (Scheme IV). No attack occurred at the nitrogen atom of the amino acid. In addition, there was no reaction either with $CaCl_2$ or with histidine protected at the acid function. These results confirmed the special nucleophilicity of sulfur when compared to nitrogen. The possibility of effecting coupling, not only with protons but also with Lewis acids such as Zn^{2+} and Mg^{2+}, was clearly demonstrated. It seemed reasonable, therefore, to implicate a Lewis acidic metal in the irreversible fixation of the organometallic-labeled hormones to a cysteine located near the active site. The primary sequence of estradiol receptor has recently been determined, and there are indeed four cysteines (381, 417, 447; 530) situated in the hormone association domain.[32]

We next determined the effect of incorporating positively charged organometallic fragments on the binding properties of estradiol. The presence of a charged complex on the A ring of the steroid did not significantly affect receptor recognition, but when the charge was introduced at the 17α-position, there was no recognition at all (Scheme V).[33] The effects on the binding affinities were presumably due to electrostatic repulsion and suggest that the binding partner in the natural receptor is also positively charged. In fact, cysteine 530 is located between two lysines (529, 531) which are protonated (NH_3^+) at physiological pH. Similarly, cysteine 447 is positioned close to another lysine (449).

A likely scenario at this stage is that a cysteine is located near the hormone association site, a protonated lysine near the hormone recognition site, and a metal in the hormone binding region, and the association site is close to the protein surface. These conclusions follow in part from our biochemical results with the bulky estradiol–alkyne clusters. The Lys-Cys-Lys sequence (529–531) is quite probably located near the estradiol receptor binding site since these external residues should be quite readily accessible.[26] In addition, radiosequencing studies of peptide fragments, truncation experiments, and mutations have implicated the Cys (530) region in the binding of estradiol.[34–36] More recent work on mutagenesis in the steroid binding domain of estradiol receptor has pinpointed the Ile–Met sequence (514–522) in the association region.[37] Furthermore, the activity of the receptor is coupled to a dimerization step whereby it can be attached to DNA. The dimerization zone is more extensive than is the association site, but together the residues encompass Arg–Val (507–534). Finally, the existence of putative binding sites for metal cations (e.g., Zn^{2+}) in the hormone binding domain has been suggested on the basis of immobilization experiments for divalent metal ions on iminodiacetate–Sepharose.[38] The presence of a metal cation in the hormone binding domain would favor fine-tuning fixation to DNA, since two zinc fingers are known to be required in the DNA binding domain.[39] A crucial zone in the C terminal part of the estradiol binding domain has the sequence shown below.

His- Ile- Arg- His-Met- Ser-Asn-Lys-Gly- Met-Glu-His-Leu-Tyr-Ser-Met-Lys-Cys-

513-514-515- 516-517-518-519- 520- 521-522-523-524-525-526-527-528-529-530

Lys-Val-Val-

531- 532-533

This sequence is preserved irrespective of the species involved (the numbering refers to human estradiol).

A molecular modeling study of the estradiol receptor site was undertaken bearing all the above points in mind and using the hydrophobic cluster analysis procedure together with crystallographic data for the structure of α_1-antitrypsin (α-AT).[40] The results of these calculations (Figure 5) were striking in that they revealed that histidines (516, 524) and cysteines (530, 381) were ideally located for metal coordination. The metal still remains to be identified, but Zn^{2+} is a good candidate. It should also be emphasized that the zones utilized for hormone association, receptor dimerization, and metal coordination are astonishingly close to one another. Whether or not these processes function in concert is an interesting thought. In any event, a combination of organometallic chemistry and molecular modeling

(32) Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon, P. *Nature* **1986**, *320*, 134.
(33) El Amouri, H.; Vessières, A.; Vichard, D.; Top, S.; Gruselle, M.; Jaouen, G. *J. Med. Chem.* **1992**, *35*, 3130.
(34) Fawell, S. E.; Lee, J. A.; White, R.; Parker, M. G. *Mol. Endocrinol.* **1989**, *3*, 1002.
(35) Harlow, K. W.; Smith, D. N.; Katzenellenbogen, J. A.; Greene, G. L.; Katzenellenbogen, B. S. *J. Biol. Chem.* **1989**, *264*, 17476.
(36) Reese, J. C.; Wooge, C. H.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1992**, *6*, 2160.
(37) Fawell, S. E.; Lee, J. A.; White, R.; Parker, M. G. *Cell.* **1990**, *60*, 953.
(38) Medici, N.; Minucci, S.; Nigro, V.; Abbondanza, C.; Arnetta, I.; Molinari, A. M.; Pucca, G. A. *Biochemistry* **1989**, *28*, 212.
(39) Schwabe, W. R.; Neuhaus, D. *Trends Biochem. Sci.* **1991**, *16*, 291.
(40) Lemesle-Varloot, L.; Ojasoo, T.; Mornon, J. P.; Raynaud, J. P. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 369.
(41) Lemesle-Varloot, L. Ph.D. Thesis, Paris VI, 1991.
(42) Top, S.; Vessières, A.; Jaouen, G. Manuscript in preparation.

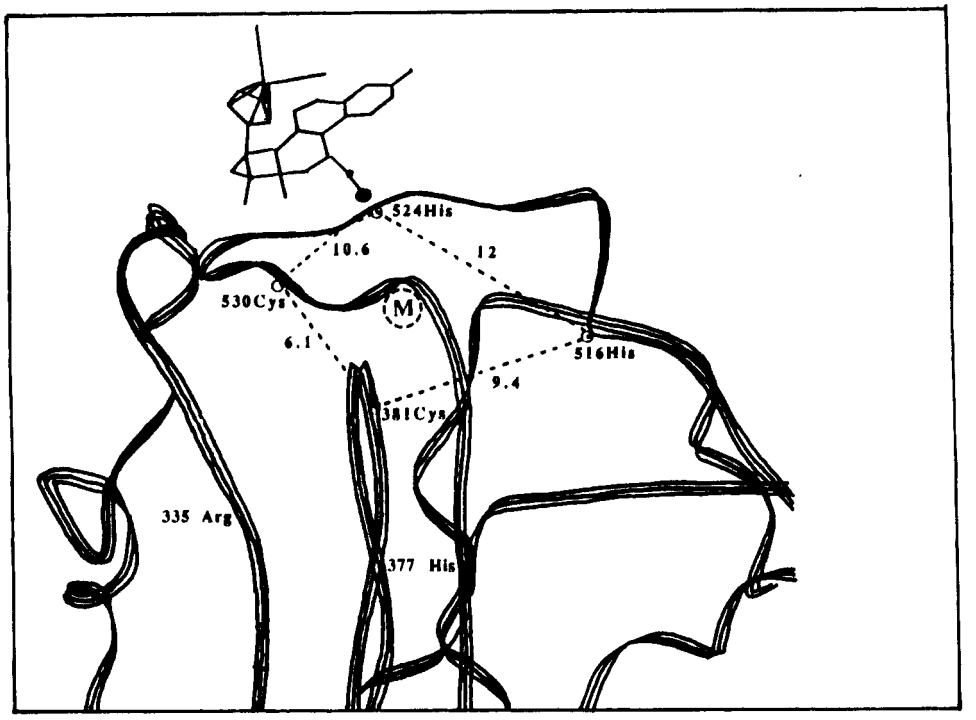
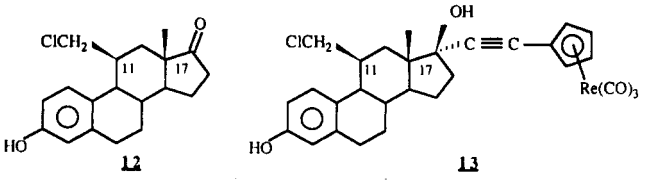


Figure 5. Close-up of the functional domains of the hormone binding domain (HBD) of E_2R as modeled by using the HCA (hydrophobic clusters analysis) method.[41] The region of the postulated binding site of estradiol shows a spatial proximity between two cysteines (Cys 381, 530) and two histidines (His 524, 516) compatible with the possible complexation of a metal cation (e.g., Zn^{2+}). The distances (Å) are given on an approximate basis, and the hormonal skeleton of **13** is shown to specify the scale. Upon hormone binding, heat shock protein (HSP 90) may uncover the functional areas to allow the functional sites to operate.

Scheme VI

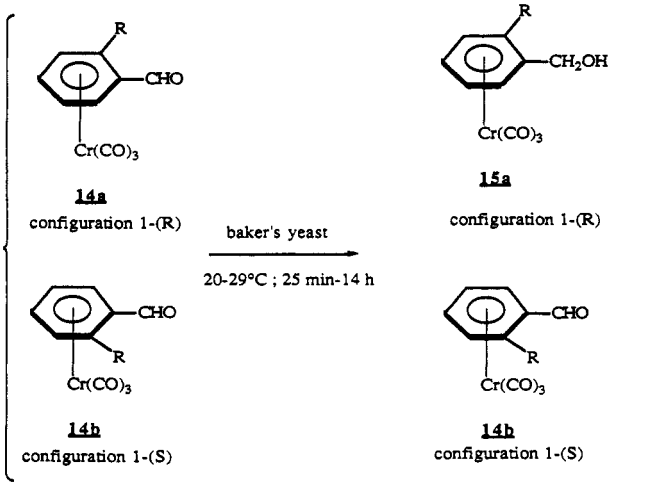


affords a powerful new approach for studying protein receptors. This approach should also prove useful in many other biochemical areas amenable to similar bioorganometallic studies.

The modeling studies shown in Figure 5 open new perspectives for the use of organometallic complexes. The chemistry of the nuclear medicine radioisotopes ^{99m}Tc, ^{186}Re, and ^{188}Re is essentially that of chelate coordination (bidentate N and S ligands). The chief disadvantages of these compounds are their volume and instability in biological media. A Re-containing organometallic fragment can be grafted onto a model hormone such as **12** (Scheme VI) to form a compound that is stable in solution and retains an exceptional affinity for the labeled complex. For example, the RBA is **13** for cytosolic estradiol receptor at 25 °C is 172%, which is the highest value ever obtained for an organometallic-labeled hormone. The residence time of 11β-(chloromethyl)estradiol on the receptor is greater than 50 h instead of 6 h for natural estradiol. Thus, by using radioactive Re isotopes, ideal compounds for anticancer therapy should be produced.

The presence of the CH_2Cl group at 11β, with p-electrons capable of coordination, fits within the scope of the preceding model. This approach can certainly be extrapolated to other receptors (mutatis mutandis), e.g., the progestatif RU 486 and the progesterone receptor.

Scheme VII



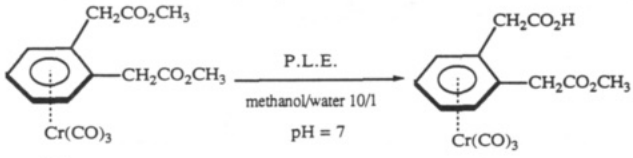
R	OMe	Me	Cl	F	$SiMe_3$
e.e. (%) for 14b	81	96	87	52	75

Conclusions and Future Directions

Several examples of the use of metal carbonyl fragments in immunological analyses based on the detection of the ν(CO) bands of the fragments by FT-IR spectroscopy have been described above. Atomic absorption[10] is another promising analytical method, and so is the electrochemical analysis of organometallic complexes[43,44] based on the recent development of new microelectrodes.[45] Both approaches are well suited to environmental problems, and the preliminary results

(43) Osella, D.; Fiedler, J. *Organometallics* **1992**, *11*, 3875.
(44) Bond, A. M.; Mocellin, E.; Pascual, C. B.; Wedkanjana, P.; Jaouen, G. *Appl. Organomet. Chem.* **1990**, *4*, 557.

Scheme VIII



16 17 e.e. > 99%

of the development of FT-IR immunological multi assays constitute a major advance in analytical chemistry. Organometallic complexes should also play a role in the resolution of the protein structures. Covalent markers of heavy metals can already be introduced selectivity onto the target residues of proteins.[17] A well-chosen marker, which is stable in biological media, could modify favorably the crystallization potential of a protein and thereby help in its structural resolution. The labeling can be directed toward functional and/or nonfunctional targets on the protein under investigation.

It is interesting to note that one can take advantage of the interactions between an enzyme and organometallic complexes to improve significantly the access to chiral organometallic precursors for use in organic synthesis. Overall, this constitutes an inversion of the previous perspective, i.e., the enzyme may be used to modify the organometallic, rather than vice versa. The degree of tolerance of enzyme catalysts for non-natural systems is quite remarkable.[1] Moreover, chiral organometallic systems constitute a class of synthons whose potential in synthesis is now well established.[46] Unlike organic compounds, whose chirality is usually due to carbon centers, an organometallic fragment can be grafted onto a prochiral ligand in the complexes. Molecules of this type also feature a chiral plane. Curiously, such complexes not only are able to penetrate into the cells[47] but also allow a kinetic resolution. For

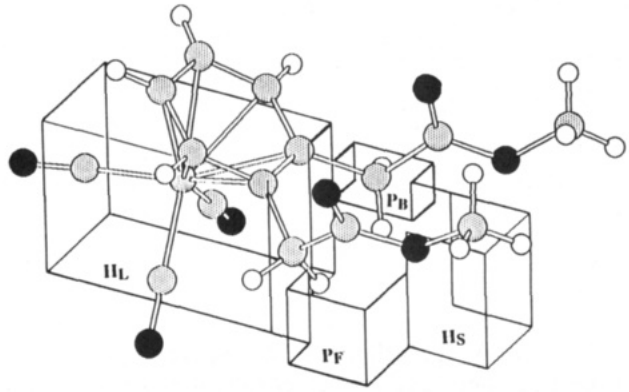


Figure 6. Jones model[50] for the active site of the enzyme PLE with the organometallic compound **16** located in the binding pockets.

example, in the presence of bakers' yeast, the racemic aldehydes in Scheme VII form equivalent amounts of alcohols as optically-active aldehydes with enantiomeric excesses ranging from acceptable to very high (52–96%).[48] Chiral planes can be enantiogenerated as shown in Scheme VIII. The action of pig-liver esterase (PLE) on the compound *meso*-**16** forms the acid ester **17** with an enantiomeric excess of 99%.[49] The best model of the active site of PLE is that proposed by Jones. This model, shown in Figure 6, is based on an arrangement of five cubic regions of space; H_L and H_S represent two hydrophobic zones. The large H_L pocket may accommodate weakly polar heteroatoms such as halogen or ether or ketal oxygen if necessary. Two other sectors (P_B and P_F) accept strongly polar or hydrophilic fragments. The essential catalytic region involves the serine residue near P_B which initiates hydrolysis by attack at the reactive ester group. Figure 6 shows clearly that compound **16** fits perfectly into the model and leads to the experimentally found configuration.[49] Experiments of this type can be conducted on multigram scales and extended to other organometallic systems of synthetic interest, e.g., dienes of $Fe(CO)_3$.[51] In conclusion, the potential of bioorganometallic chemistry is clearly quite considerable, and this new field holds considerable promise for the future.

We thank our graduate students, postdoctoral fellows, and other co-workers, without whom little would have been accomplished. The research was supported by the following: CNRS, MRT, Alliance program, Région Bourgogne (France); Medgenix (Belgium); NSERC (Canada), FCAR (Quebec). Numerous missions under the auspices of the France–Quebec (Biotechnology) Exchange Program are gratefully acknowledged.

(45) Limoges, B.; Degrand, C.; Brossier, P.; Blankespoor, R. *Anal. Chem.* **1993**, *65*, 1054.
(46) Jaouen, G. In *Transition Metal Organometallics in Organic Synthesis*; Alper, H., Ed.; Academic Press: New York, 1978; Vol. II.
(47) Vessières, A.; Top, S.; Ismail, A. A.; Butler, I. S.; Louer, M.; Jaouen, G. *Biochemistry* **1988**, *27*, 6659.
(48) Top, S.; Jaouen, G.; Baldoli, C.; del Buttero, P.; Maiorana, S. *J. Organomet. Chem.* **1991**, *413*, 125 and references therein.
(49) Malezieux, B.; Jaouen, G.; Salaün, J.; Howell, J. A. S.; Palin, M. G.; McArdle, P.; O'Gara, M.; Cunningham, D. *Tetrahedron: Asymmetry* **1992**, *3*, 375.
(50) Toone, E. J.; Werth, M. J.; Jones, W. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946.
(51) (a) Alcock, N. W.; Crout, D. H. G.; Henderson, C. M.; Thomas, S. E. *J. Chem. Soc., Chem. Commun.* **1988**, 746. (b) Howell, J. A. S.; Palin, M. G.; El Hafa, H.; Top, S.; Jaouen, G. *Tetrahedron: Asymmetry* **1992**, *3*, 1355.