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Review

New paradigms for synthetic pathways inspired by bioorganometallic chemistry

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Abstract

Two series of particular examples of reactions used in bioorganometallic chemistry are described. One based on a decomplexation–complexation reaction, indicates how, starting from a cymantrenyl derivative, a range of organometallic complexes bearing various metals can be prepared. The second one refers to the easy synthesis in water of the very versatile Alberto's reagent, which leads to new organometallic radiopharmaceuticals of Tc and Re. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Radiopharmaceuticals; Metal exchange reaction; Decomplexation–complexation; Aqueous carbonyl complexes; Hormones/antihormones; Rhenium; Technetium; Iron; Titanium complexes

1. Introduction

Life on earth rarely makes use of organometallic chemistry, which is defined by the formation of at least one direct metal–carbon bond in a given system. There is of course the well-known exception of the coenzyme of vitamin B12 and its derivatives, in which a direct cobalt–carbon bond is one of the features of the system [1]. But, although new organometallic metal enzymes are reported from time to time in the literature, these tend to be relics or variants of the evolutionary process, rather than part of a general phenomenon [2,3][1].

In fact, the emergence of bioorganometallic chemistry (by which is meant the use of organometallic complexes in real problems of biological interest, rather than merely the coordination of bioligands to metals, which is not by itself sufficient to justify use of the term)[2] has benefited from the enormous amount of synthetic work that has been done to prepare all kinds of organometallic compounds over the last half century [5–8]. The artificial nature of organometallic compounds, in comparison with naturally occurring biological phenomena, has led a number of groups to seek out these molecules in their efforts to solve problems of biological analysis, to study proteins or DNA fragments, or even to develop new classes of therapeutic agents. The maturation of this discipline has reached the point where this year a special issue of the Journal of Organometallic Chemistry was dedicated to the topic [9].

It is important to realize that access to compounds for use in bioorganometallic chemistry is not always achieved merely by adapting synthetic methods used in classical organometallic chemistry. For example, the

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[1] Interestingly, in Ref. [3] the term bioorganometallic enzymology has been coined for Fe–Ni hydrogenase and CO dehydrogenase possessing a Ni–C bond to take into account the original reactions occurring at the active site, as well as a novel enzymology important in environmental chemistry.

[2] It should be noted that recent work does not encompass this definition based on pioneering articles of the 1980s. The word has also been used to study the reaction of organometallic complexes with biomolecules preferably in water [4].

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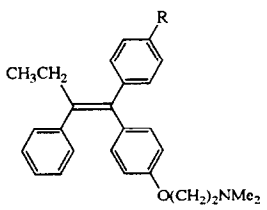
ultimate biological solvent, water, is often incompatible with metal–organic species. The synthetic strategy for these complexes must take account of this fact in all its ramifications. In biological research it is also often necessary to deal with multifunctional molecules, for which none of the known preparation methods may be suitable if one wishes to attach a given organometallic moiety. Finally, particularly in the area of radiopharmaceuticals, kinetics can be a crucial factor in determining the usefulness of the product. For all these reasons, bioorganometallic chemistry demands an exploration of unusual or previously unknown synthetic routes, and thus sometimes gives rise to the discovery of new reactions. It is our hope that the entire community of organometallic chemists will be able to benefit from these insights, even though their own objectives may be different. Since this domain is still in an early stage of development, we shall draw chiefly on our own work to illustrate how the imperatives of this new discipline may contribute to the enrichment of the synthetic repertoire.

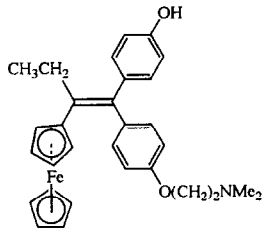
2. A novel and facile metal exchange reaction in the organometallic cyclopentadienyl series

2.1. Introduction: the need for a new reaction

In the Western world, breast cancer occupies a prominent position among hormone-related diseases, with one woman in nine likely to be affected in her lifetime, and a mortality rate close to 35%. Within the arsenal of drugs used to treat the disease, tamoxifen holds the primary position because, being fairly well tolerated, it can be administered at all stages of the disease, and recently has even begun to be used prophylactically. This antiestrogen, although possessing relatively low toxicity, has only moderate efficacy and does cause problems over the long term, notably in development of resistance and various undesirable side-effects. There are presently great advantages, both scientific and economic, to be gained in seeking substitutes. The goal of such a search is to find a different antiestrogenic molecule that would be more cytotoxic than tamoxifen as well as possessing a higher level of efficacy.



1a : R = H, tamoxifen
1b : R = OH, hydroxytamoxifen



2 : hydroxyferrocifen

Scheme 1.

With this aim in mind, we explored a novel route based on modifying the base skeleton of tamoxifen **1a** and its active metabolite **1b** (Scheme 1) using organometallic moieties that in some cases have recognized cytotoxicity [10]. In this way we hoped to gain access to molecules whose antiestrogenic properties (i.e. the ability to block the estrogen receptor) are preserved or even improved, but which also show increased cytotoxicity.

We next prepared and studied hydroxyferrocifen **2**. The ferrocene moiety η^5-$(C_5H_5)_2Fe$ does indeed have some toxicity in vivo owing to its oxidation to a radical cation, η^5-$(C_5H_5)_2Fe^+$, known as ferricinium, which interacts with DNA. The cytotoxicity of **2** was evaluated on human cells derived from breast cancer and possessing estradiol receptor sites (MCF7 ATCC). In vitro, this molecule exhibits an antiestrogenic effect comparable with that of hydroxytamoxifen [11], but in vivo on T47-D xenografts in nude mice, **2** acts as a weak estrogen [12]. The explanation may well involve many factors, but it led us to examine the effect of the length of the chain in the derivatives of **2** (with $n = 3$, 4, etc.), for which the ferrocenyl group is bulkier than the phenyl in the β position present in **1b**. This was particularly timely since the X-ray structural analysis of the ligand binding domain (LBD) of the estrogen receptor-α (Erα) had just been published [13] (Scheme 2).

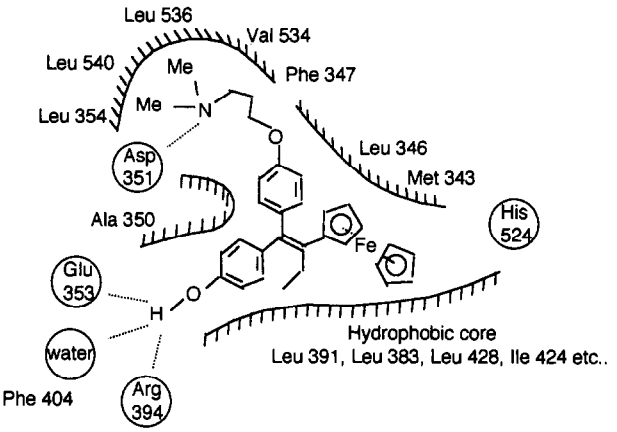
Whatever the true explanation may be, it is noteworthy that the antiproliferative effects in the ferrocifen series **2** become superior to those of tamoxifen **1**, both in vivo and in vitro, with a longer basic arm ($n = 3$, 4, 5) [12]. These early results are already sufficient to show that the combination of an antiestrogen vector with an organometallic fragment is a promising route to follow in the search for tamoxifen substitutes.

In the above series of ferrocifens **2**, the organometallic ferrocenyl unit is fairly robust and can be introduced at an early step of the synthesis. This is not always possible, for example in molecules such as **3** and **4**. Preparation of **3** and **4** necessitated a search for novel synthetic strategies, since Re must be used in radioactive form and titanium must also be introduced in the final step (Scheme 3).

The challenge presented by **4** was of particular interest since it has been reported in the literature that Cp_2TiCl_2 is a good antitumor reagent [14] and also that complicated metallocenes of Group IV are useful polymerization catalysts [15].

2.2. Description of the principle of the decomplexation–recomplexation reaction

The cyclopentadienyl (Cp) ligand, because of its size, robustness and electron count, is one of the most useful coordinating ligands in organometallic chemistry. Its almost ubiquitous nature has led to a number of arti-



Recovery of an antagonist effect. Owing to the bulky Ferrocenyl group with respect to a phenyl substituent as in the antagonist 4-OH tamoxifen, the basic chain might not firmly bind to Asp 351 with a 2 C arm but the antagonist effect has been recovered with a longer (3C, 4C) arm.

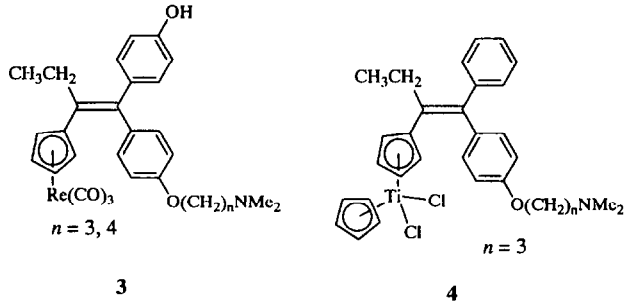
Scheme 2. Antiestrogen site with ferrocifen **2** ($n = 3$) shown within it.

cles on its functionalization [16–23]. Among the principal strategies employed are the Friedel–Crafts reaction, whenever the organometallic substrate permits, which unfortunately is frequently not the case [24]; an extension of Thiele's reaction [25–27], which involves electrophilic attack on the nucleophilic cyclopentadienyls of sodium, lithium or thallium [28,29]; the action of bases on fulvenes [30–33] and of nucleophiles on diazocyclopentadiene η^5-($C_5H_4N_2$) [34–38]; the transformation of cyclopentenone [39–41]; and the construction of substituted rings by means of various coupling methods [42–44]. The variety of preparation methods utilized demonstrates both the absence of a dominant synthetic strategy suitable for all cases, and the necessity of seeking new approaches to deal with novel problems. The ever-expanding range of organometallic chemistry makes such research a Sisyphean task. Here we describe a new synthetic approach that gives access, starting from the same substrate, to different families of cyclopentadienyl organometallic complexes whose substituents possess a degree of complexity compatible with the production of fine chemicals [45]. This approach was dictated by the imperatives inherent in the new field of bioorganometallic chemistry, as illustrated above.

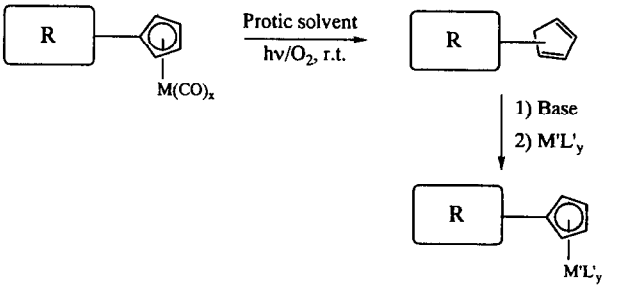
The principle of this new reaction allowing access to substituted cyclopentadienes and hence to the intended organometallic complexes is shown in the general scheme (Scheme 4). This reaction involves the photochemical decomplexation of easy to handle substrates in protic solvents. Thus it was possible to keep the unstable intermediate Cp^- in its stable form as a cyclopentadiene, with good yields. This is followed by recomplexation by another metal in the last step.

These hypothetical ideas have been applied to the cymantrene complex **5**, which was readily accessible in good yields (83%) via a McMurry cross-coupling reaction (Scheme 5). Compound **5** is a yellow solid, stable in air. It can be stored in the dark for several months with no trace of decomposition. This molecule was easily decomplexed by UV irradiation in air in a 2:1 ethyl ether–methanol mixture to give the cyclopentadiene **6** (88% yield, 30 min). Once pure, **6** proves relatively stable against polymerization, and it can be kept for several days under refrigeration. The ^{1}H-NMR spectrum showed that **6** is a mixture of at least two diene positional isomers. However, separation is not essential for the rest of the procedure.

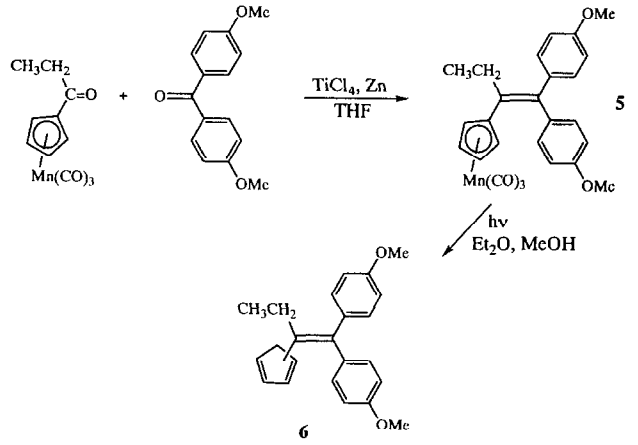
The organo-lithium reagent **7** reacts with $CpTiCl_3$, $BrRe(CO)_5$, $W(CO)_6$, and $FeCl_2$ to form compounds **8**,



Scheme 3.



Scheme 4.



Scheme 5.

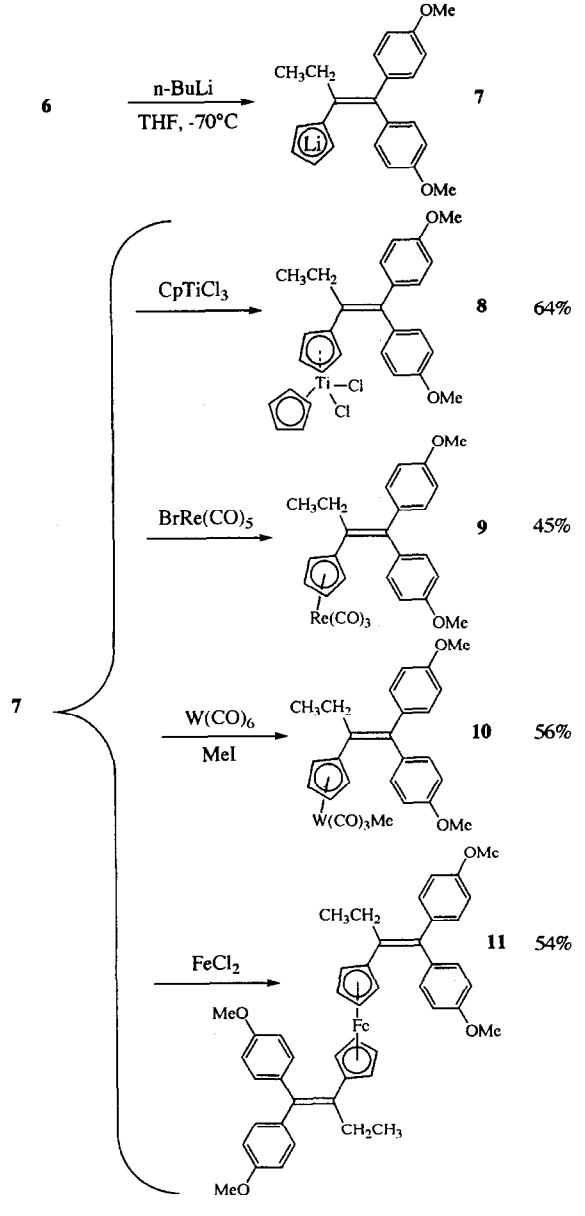
9, **10**, and **11**, respectively, in yields ranging from 45 to 64% (Scheme 6). Scheme 6 calls for several comments. It illustrates the fact that the cymantrene complex **5** is a stable and unique source for several series of organometallic complexes of varied interest. A complex of Re similar to **9** could be used, in radioactive form, as a radiopharmaceutical. There is a current revival of interest in the rapid fixation of isotopes 186 and 188 of Re, and even of ^{99m}Tc, onto bioligands (vide infra). A compound such as **10** containing a heavy metal could find an application in the X-ray structural determination of the receptor protein [13]. However, although **9** and **10** are obtainable by other routes, the same cannot be said for **8**, as access to **8** requires the construction of $Cp'CpTiCl_2$ in the last step of the synthesis, which is permitted by the strategy described here.

2.3. Application of the decomplexation–recomplexation strategy to the preparation of cyclopentadienyl titanium dichloride complexes attached to vectors for the estradiol receptor

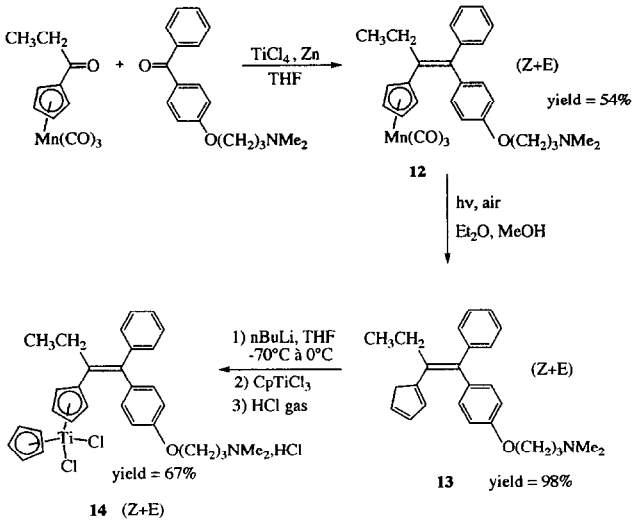
In addition to the ferricinium cation, the metallocene, Cp_2TiCl_2 has been described as a cytotoxic agent against several type of tumors [46]. Furthermore, the titanium molecule has been reported to be more efficient than the iron moiety. Therefore, a molecule such as **14** shown in Scheme 7 could be a useful target, since it is both a potential antiestrogenic vector to the estradiol receptor and a recognized cytotoxic molecule.

The synthesis of **14** was not an easy task and we realized that a good strategy required the introduction of $CpTiCl_2$ in the last step of the procedure. We therefore took advantage of the synthetic pathway described above. The complete synthesis of **14** is shown in Scheme 7. The preparation of the cymantrenic intermediate **12** by use of a McMurry cross-coupling reaction was carried out in 54% yield, while the photochemical decomplexation in air took place in 98% yield in the presence of methanol to produce cyclopentadiene **13**. From this molecule the introduction of $CpTiCl_2$ was performed (yield: 67%) by using ^nBuLi and $CpTiCl_3$. In order to stabilize and to increase the water solubility of the product, it was necessary to treat the amine chain with gaseous HCl.

The antiproliferative effects of **14** have been studied on an MCF7 cell line modified by the introduction of luciferase firefly genes into the cells in order to increase the rapidity of the test [47]. The results show that **14** does not act as an antiestrogen but behaves as a weak estrogen in vitro. The reasons for this must be sought in the transformation of this metallocene in biological media. Therefore, although **14** was an interesting synthetic challenge, it does not appear to be superior to the ferrocifen **2** ($n = 3$), described previously in terms of its



Scheme 6.



Scheme 7.

antiproliferative effects. However, since this synthetic strategy has been shown to be a viable one, and in view of the numerous applications for Ti, Zr metallocenes, this approach may well be useful to other chemists with different goals in mind.

3. Organometallic radiopharmaceutical compounds of Tc and Re

3.1. The premise

Group 7 transition metals (Tc, Re) are currently the subject of particular interest as radiopharmaceuticals, whether for diagnostic (^{99m}Tc) or therapeutic (^{186}Re, ^{188}Re) purposes [48–56]. Some of the physical characteristics of these elements are shown in Table 1. The major radioisotope used today in nuclear medicine is ^{99m}Tc. It is readily available in any hospital, is inexpensive and has very favorable decay properties for imaging purposes. The low-energy photons result in a low dose burden to the patient during diagnostic procedure [50]. On the other hand, ^{99m}Tc complexes for routine clinical use must be prepared directly in the hospital.

Since the synthesis must basically consist of one single step, organometallic complexes have not been seriously considered as markers. It has been generally believed that their preparation demands water- and air-free conditions. This myth was laid to rest by the preparation of the complex $[^{99m}Tc(CN\text{–}R)_6]^+$ (**15**) directly from $[(TcO_4)]^-$ in water in the early 1980s by Davison and co-workers [57]. Today, **15** is one of the most widely used ^{99m}Tc compounds for various indications. It represents, in fact, a proof of principle for a number of advantages possessed only by low-valent organometallic complexes. The monodentate ligands are perfectly stable in vivo due

Table 1
Physical characteristics of ^{186}Re, ^{188}Re and ^{99m}Tc

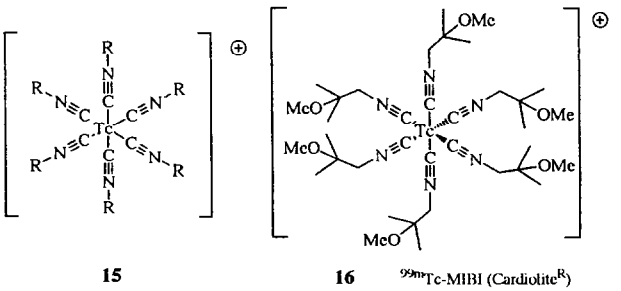
	$t_{1/2}$ (h)	β_{max} (MeV)	$\beta_{average}$	Energy γ (KeV)
^{186}Re	3.7 days	1.07	0.36	137 (9%)
^{188}Re	16.8	2.12	0.77	155 (10%)
^{99m}Tc	6			141 (89%)

to the high inertness of the d^6 electronic configuration. The isocyanides are not subject to protonation, which is the most strongly competing reaction in a biological environment, and the closed shell complex allows only dissociative or interchange dissociative substitution mechanisms, which depend solely on the off-rate of the inner sphere ligands. One disadvantage is that **15** is of such a high stability that ligand substitution does not occur under practical conditions, and is thus not a candidate for the so-called third generation of radiopharmaceuticals, the labeled targeting biomolecules.

Unlike in the case of the pertechnetate, it was not possible to prepare analogous compounds of rhenium starting from $[(ReO_4)]^-$, which proved to be a weaker oxidant than $[(TcO_4)]^-$.

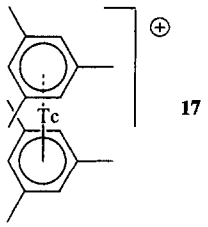
Based on the concept illustrated by compound **15** (Scheme 8), a range of commercial organometallic compounds has been developed [58]. A significant example is compound **16**, ^{99m}Tc-MIBI, Cardiolite®. This cationic myocardial perfusion agent, developed as an analogue of large positively charged ions such as K^+, Cs^+ and Tl^+, accumulates at the level of the cardiac muscle. Advantage is taken of its highly lipophilic character to obtain an acceptable level of specificity for that organ [52].

Attempts to improve the results were made using ligands derived from boronic acid [59] and organometallic complexes **17** of the $[Tc(arene)_2]^+$ type [60] (Scheme 9). The synthesis of [bis(arene)technetium (I)]$^+$ (**17**) was carried out starting from $^{99m}[TcO_4]^-$ with various aromatics that permit subtle modifications of the structure. The preparation methods make use either of ultrasound, Al, $AlCl_3$, or of Zn, HCl. Biodistribution studies in rats show substantial myocardial capture of these organometallic products, which are very stable in aqueous solution for a number of members of the



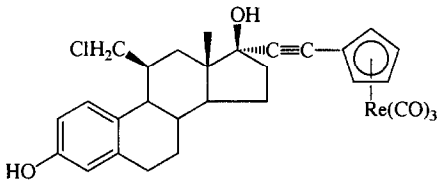
15 16 ^{99m}Tc-MIBI (Cardiolite®)

Scheme 8.



17

Scheme 9.



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Scheme 10.

series, particularly those that have four to six substituted carbons. The capture by myocardial tissue is linked to the lipophilicity of the complexes. Unfortunately at the present time myocardial accumulation in humans is inferior to that found in animals [60].

Keeping in mind the principle of using inert complexes for in vivo applications, our interest remained focused on the (+1) valency of Tc or Re. This oxidation state is ideal for organometallic chemistry since ligands with strong acceptor properties are stabilized by the electron-rich metal center.

An organometallic complex mentioned earlier, η^5-$(C_5H_4R)M(CO)_3$ (M = Re, Tc), small and robust, is of obvious preliminary interest, and in fact has been attached both to antibodies without losing recognition for them [61,62], and also to steroidal hormones [63,64]. In particular, the organometallic hormone **18** in Scheme 10 was shown to have remarkable properties [63].

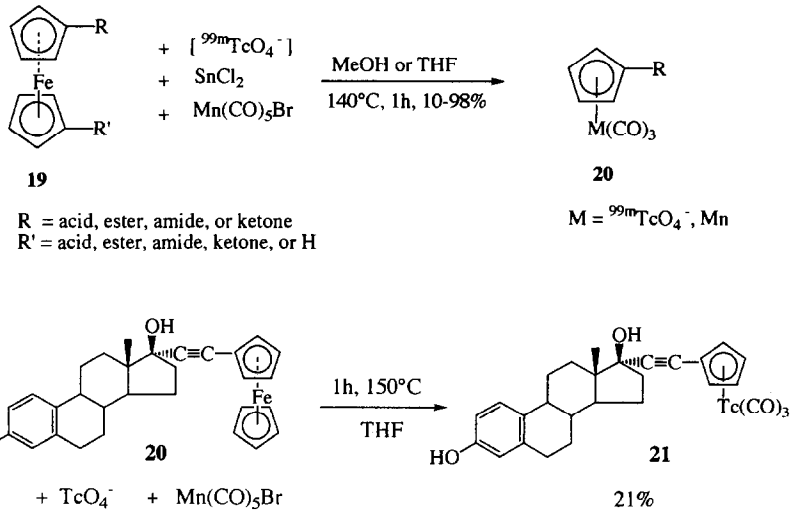
The relative binding affinity of this modified estradiol for its specific receptor is 172% at 25°C, compared with 100% for the natural ligand estradiol. This is the highest value ever found for an organometallic compound. The organometallic complex of $Re(CO)_3$ proves to be highly resistant to oxidation. In addition to this, the molecule enters the target cell more readily than the free ligand, and the residence time on the receptor site is 2 days, compared with 6–12 h for natural estradiol. These early results with Group 7 air- and water-stable organometallic complexes stimulated the search for new synthetic routes compatible with the chemistry of the radioactive isotopes of Re and Tc. Several groups independently developed different synthetic strategies that could in principle be applied to various biological vehicles.

3.2. The Wenzel approach by a double ligand-transfer (DLT) reaction

3.2.1. Improvements to the method

Wenzel published the transformation described in Scheme 11 in 1992 [65]. This reaction permits the one-pot synthesis of cyclopentadienyl complexes of technetium. It is noteworthy that the reduction, carbonylation and cyclopentadienylation of $^{99m}Tc(VII)$ in this system occur rapidly and under relatively mild conditions. This approach has been applied to derivatives of estradiol [66], as shown in Scheme 11.

A drawback to this reaction is that the analogous tricarbonylated complex of manganese is produced at the same time as that of Tc. This major contamination limits the interest of the procedure described above, as the separation of the two complexes is not always easy. This led Spradau and Katzenellenbogen in 1998 [67] to propose an improvement to Wenzel's reaction. The best conditions are obtained with $CrCl_3$ as the reducing



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R = acid, ester, amide, or ketone
R' = acid, ester, amide, ketone, or H

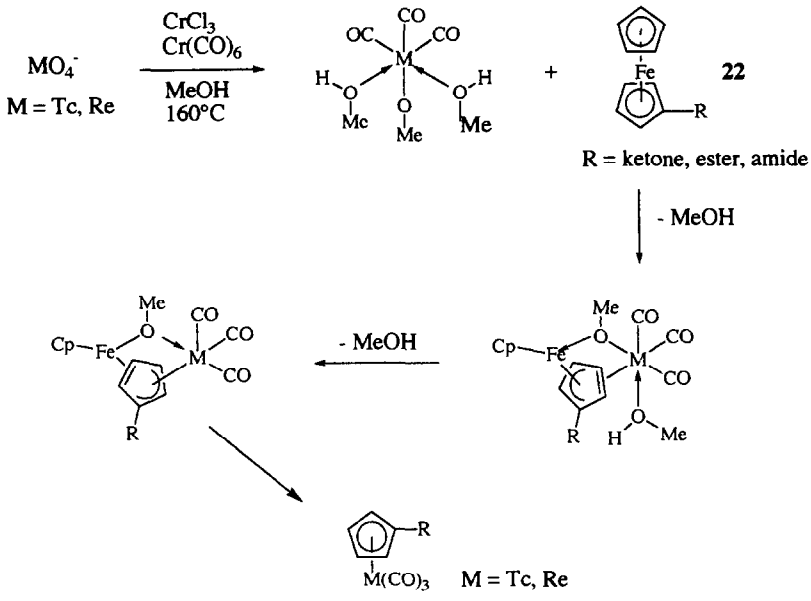
20

M = $^{99m}TcO_4^-$, Mn

20

21

Scheme 11.



23

Scheme 12.

agent, $Cr(CO)_6$ as the source of CO, methanol as the solvent and a reaction temperature of 160°C in a sealed tube. It is also necessary to substitute the cyclopentadienyl and the Re with a carbonylated R group. The mechanism proposed by the authors for this double ligand-transfer (DLT) reaction is shown in Scheme 12. It has been suggested that the $\eta^5-\eta^3$ ring slippage caused by an acylated chain is the key to this reaction.

This metal exchange also takes place when starting from nickelocene instead of ferrocene, although at present with lower yields. However, the high reaction temperature of 160°C should be noted. This reaction would be of greater interest if it could be carried out at 100°C in water.

Nevertheless, this strategy as it stands has been applied to analogues of phenyl-tropane labeled with ^{99m}Tc. Owing to their high affinity for the dopamine transporter (DAT), these compounds are a useful tool for imaging in disorders of this transporter system.

A recent example of this type of application is detailed in Scheme 13 [68].

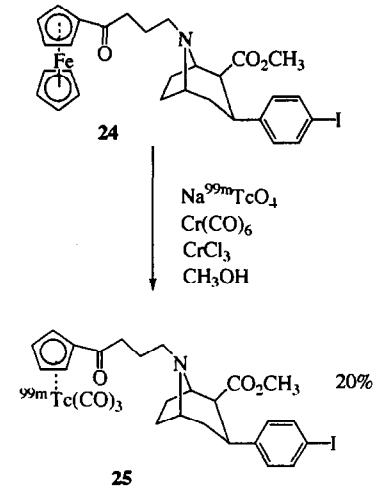
3.3. A mild and rapid new synthetic route to $M_2(CO)_{10}$; M = Tc, Re

3.3.1. Applications

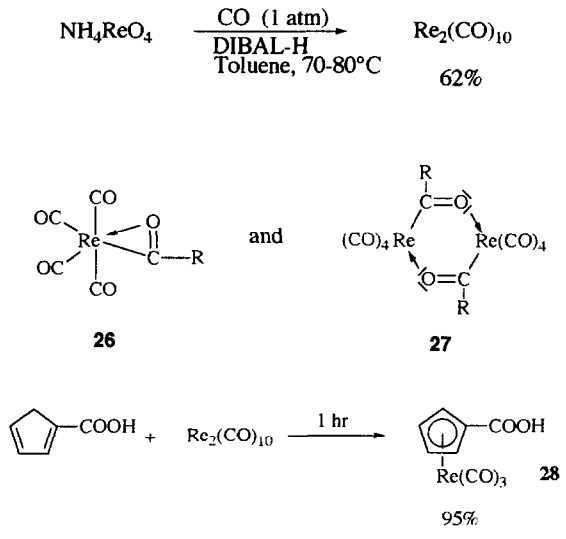
As ^{99m}Tc and ^{188}Re nuclides are commercially available in the form of pertechnetate and perrhenate generators in aqueous solution [56], these nucleides are normally used in the form of inorganic chelates (e.g. N_2S_2) in oxidation states (+ III) to (+ V). These species pose problems of synthesis, isomerization, stability and bulkiness [52]. An organometallic approach can be envisaged in terms of a new route to $Tc_2(CO)_{10}$ and $Re_2(CO)_{10}$, the basic precursors of this chemistry.

However, all the preparation methods described so far for these metal carbonyls are incompatible with the use of radioactive isotopes with short half-lives, with the notable exception of the method discovered by us [69]. It is in fact practically impossible in a laboratory setting to use radioactive isotopes in reactions that are slow (2–3 days) and also require high temperatures (200–300°C) and pressures (200–300 atmospheres). This synthetic obstacle is one of the major reasons for the lack of development in the organometallic approach to radiopharmaceutical chemistry.

There was therefore a need for a new reaction compatible with all the imperatives outlined above. This



Scheme 13.



Scheme 14.

reaction occurs in high yields, under low CO pressures and with short reaction times (1 h). It is shown in Scheme 14 for the case of Re.

It should be noted that this reaction is compatible with reduced-scale reagents (on the order of milligrams or less). It can also be extrapolated to Tc, although in this case the yields are slightly lower (53%). Moreover, such interesting reagents as $Re(CO)_5Br$ and $Re(CO)_5I$ can be obtained, in one pot, by simple addition of Br_2 and I_2 to the medium. Furthermore, still in the same pot, the addition of cyclopentadiene to $Re(CO)_5Br$ gives $CpRe(CO)_3$ almost quantitatively. A low-temperature study of the reaction shown in the scheme identified $RReO_3$ as the first intermediate, followed by previously unknown species such as **26** and **27** shown in Scheme 14, while the ketones RCOR are also to be seen in the reaction medium [70]. We were also able to prepare $(CpCO_2H)Re(CO)_3$ (**28**), directly in one step starting from $C_5H_5CO_2H$ and $Re_2(CO)_{10}$ in 1 h with yields on the order of 95% (Scheme 14) [71]. The success of this reaction is surprising since it occurs with an unprotected carboxylic acid. Normally, this function decomposes metal carbonyls. This is another indication of the high degree of stability of Re and Tc complexes. The strategy thus gives easy access to coupling reagents for antibodies, proteins, peptides and even hormones, providing these are modified with NH_2 groups.

With this method it was possible to label the estrynamide **30** with $CpRe(CO)_3CO_2CH_3$, as shown in Scheme 15 [64].

$Re(CO)_5Br$ was also used to label erythro-norhexestrol **32**, and still with $Re(CO)_5Br$ by the fulvene route, it is possible to label position 6 of estradiol [72]. Finally, an especially interesting route to explore with $Re_2(CO)_{10}$ depends on the new metal-exchange reaction [73]. Titanium proves to be a particularly easy metal to exchange with Re. This is due to a simple modification in the hapticity of the unsaturated 16-electron metallocene, **34**, which then reacts easily with $Re_2(CO)_9$, produced by heat activation of $Re_2(CO)_{10}$ [73]. Refinement of this strategy may permit access to antiestrogenic radiopharmaceuticals.

4. A water-soluble organometallic reagent: Alberto's reagent

4.1. Synthesis and reactivity

A more promising approach to the synthesis of organometallic radiopharmaceuticals, since it can be used in water, is that of Alberto et al. This work first became known in 1995 with the publication of the reaction shown in Scheme 16 [74]

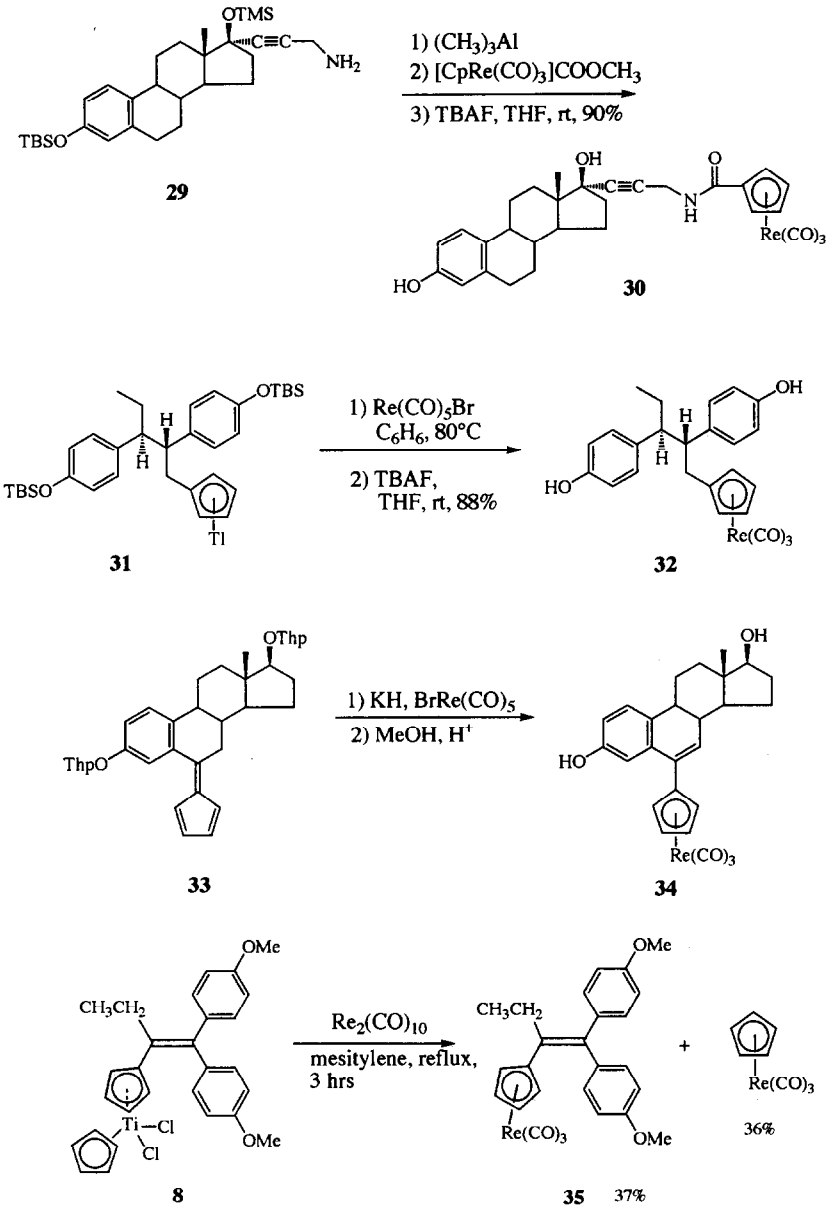
This is a new carbonylation method, at low pressure, of $[MOCl_4]^-$ and $[MO_4]^-$, with M = Tc, Re, in the presence of the non-coordinating reducing agent BH_3/$[N(Bu_4)]Cl$, giving $[(CO)_3MCl_3]_2^+$ after 6–10 h in yields between 45 and 74% [75]. The temperature of the reaction was 110°C. This reaction shares with the preceding method (Scheme 14, first reaction) [69] the advantage of permitting rapid reduction of perrhenate and pertechnetate under mild conditions.

However, a particular advantage of the trihalogeno tricarbonyl complex resides in the fact that ligand substitution is very easy. For example, in water the $[(H_2O)_3M(CO)_3)]^+$ (M = Re, Tc) complexes, **38**, are easily obtained. These aquo complexes are very stable in water in aerobic conditions. They react, among other things, with ligands such as isocyanides and sulfur chelates (Scheme 17) [74].

An application of Alberto's complex to steroids has been developed by Wüst et al. with $(NEt_2)_2[(MX_3(CO)_3]$ in aqueous methanol [76]. Products such as **41** are stable in aqueous media. They are unusual in being both inorganic chelates and organometallics (Scheme 18).

4.2. Preparation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$, its properties and its contribution to the application of organometallic compounds in medicine

Because of the importance of the ^{99m}Tc nuclide in nuclear medicine, a particular effort was applied to $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (**42**) (Scheme 19). Any attempt to apply **42** (as ^{99m}Tc) for nuclear medical purposes would require a one-step, one-pot synthesis. Since potential reducing agents must not be capable of coordinating the low-valent product (and thus blocking available labeling sites), only 'a-type' reducing agents can be used. As the products of $[BH_4]^-$ are boric acids, this proved to be a very versatile reducing agent in water to

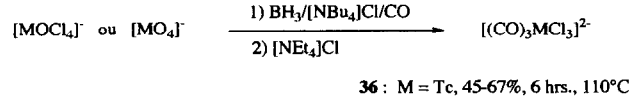


Scheme 15.

synthesize **42** in one step. In fact, filling a vial with one atmosphere of CO in the presence of $NaBH_4$ resulted in the quantitative formation of **42** directly from $[^{99m}TcO_4]^-$ (Scheme 20) [77].

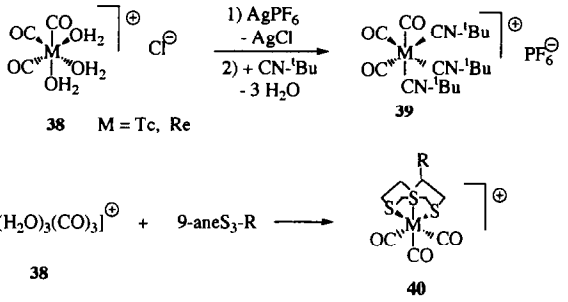
Although the reaction is straightforward, the mechanism by which the complex is formed is not clear. CO is not very soluble in hot water, nevertheless, three of these ligands must coordinate to the Tc(I) center once it is reduced. A hypothetical Tc(I) aqua-ion $[Tc(OH_2)_6]^+$ as a potential intermediate has not been characterized to date and its existence remains at least doubtful. Thus, some synergistic mechanism can be assumed, which must take place between the reducing agent and the CO.

Ligand substitution is basic to the question of whether **42** represents an adequate precursor for the labeling of biomolecules. The facially coordinated CO ligands are of high stability and could not be substituted by any other incoming ligand. Consequently, one hemisphere of the complex is chemically inert, while the



36: M = Tc, 45-67%, 6 hrs., 110°C
37: M = Re, 73-75%, 6 hrs., 110°C

Scheme 16.

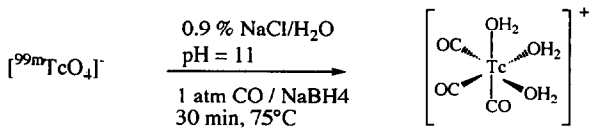


Scheme 17.

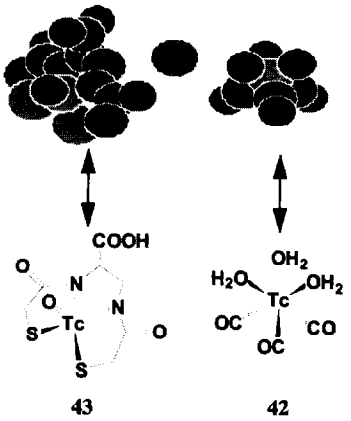
other is sufficiently substitution labile (owing to the *trans*-effect of CO) to allow the coordination by incoming ligands, i.e. those attached to a targeting biomolecule. The off-rate of water as a basic magnitude in such a substitution has been determined by $H_2^{17}O$ NMR exchange studies and the half life time of coordinated water found to be about 13 s, about 30 faster than in the corresponding rhenium complex [78]. Considering the Eigen–Wilkins relationship, the substitution rate by an incoming ligand should be on the same order of magnitude as the H_2O self-exchange.

Apart from these more practical properties, the physico–chemical characteristics of the final complex in relation to the targeting molecule are relevant for the retention of bioactivity. This is basically influenced by size, charge and the possibility of the complex itself interacting with the biological medium. Thus, the complex attached to the targeting molecule should have comparable properties with that molecule. This means specifically that it should be small in size, have comparable lipo- or hydrophilicity and show no formation of ionic or hydrogen-bond interactions. Comparing these basic properties between a typical Tc(V)=O complex **43** and **42** reveals the superior properties of the latter (Scheme 20).

Scheme 20 exhibits the significantly smaller size of **42** compared with **43**. In the space-filling model an iodine is shown for better comparison. Even the van der Waals radius of the latter is not much smaller than the imaginary sphere around **42**. It should also be borne in mind that the three H_2O ligands can be replaced by naturally occurring ligands integrated into a particular biomolecule. This reduces the additional size of the



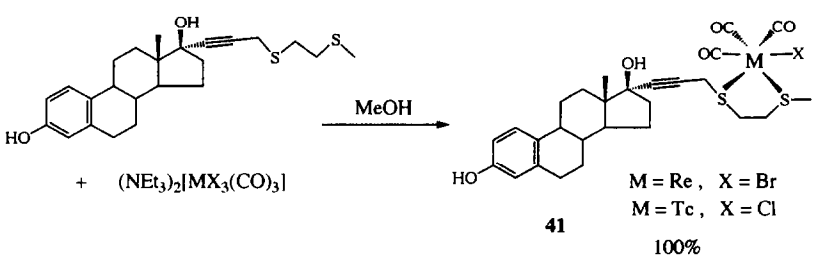
Scheme 19.



Scheme 20.

whole even more, since only half of the complex is added (see Scheme 20). In addition to the pure size comparison, it should also be noted that the different carbonyl groups in **43** can form H-bridges to other functional groups and thus interference with the receptor of the targeting molecule seems likely to occur. In contrast, the three COs are innocent and very unlikely to exhibit this kind of interaction with other functionalities.

If an additional ligand has to be introduced in a particular system, cyclopentadienyl (Cp^-) is no doubt the smallest one can imagine for the occupation of three facially situated coordination sites. The fact that this combination is almost ideal for application with highly lipophilic targeting molecules in combination with the innocent '*fac*-$M(CO)_3$' moiety (M = Tc, Re) is obvious from this article. However, at the present time two major obstacles still block the practical application of ^{99m}Tc compounds of this type in nuclear medicine. While the elegant approach described herein easily allows the introduction of cold '*fac*-$Re(CO)_3$' to the diene



Scheme 18.

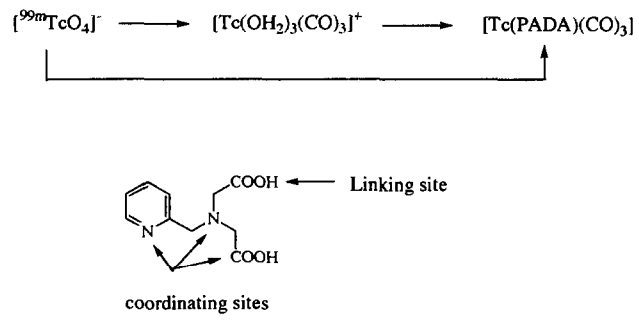
ligand attached to tamoxifen, the problem of performing the same synthesis from saline is still unsolved. So far, for the preparation of highly lipophilic complexes as required for e.g. CNS receptor ligand labeling, the versatility of the '*fac*-$^{99m}Tc(CO)_3$' must rely on other ligand systems, which are larger but provide similar properties to Cp^-.

A major advantage in labeling targeting molecules would be the general applicability of one single metal complex precursor. The '*fac*-$Tc(CO)_3$' in fact coordinates to a wide variety of different ligand types and, in particular, imidazole (i.e. from the histidine side chain) and pyridine groups form highly inert complexes with **42**. Besides these groups, practically all types of coordinating functionalities can be used as ligands. The high affinity for imidazole was applied to give convenient direct labeling of his-tagged scFvs [79]. Owing to its favorable properties, this one organometallic complex can provide access to a general labeling procedure for various biological macromolecules.

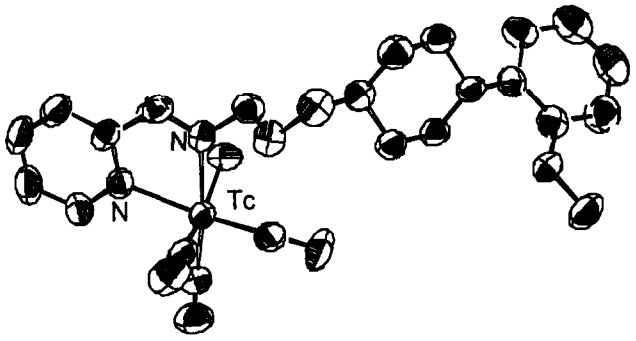
The rational design of ligands derived from the leading structure of the targeting molecule requires 'cloaking' of the metal moiety. A typical example for such a strategy is given in Scheme 21. Three of the four potentially coordinating atoms of the ligand pyridineamine-bis diacetic acid are used for coordination, while the pendant carboxylic acid group can be used for covalent attachment to a biomolecule.

The variability of ligand types is very large and ranges from hydrophilic polyamino–polycarboxylic acids to highly lipophilic groups such as Schiff base type ligands. The substitution of all these chelators even with strongly competing chelators adapted to the electronic requirements of the '*fac*-$Tc(CO)_3$' moiety is negligible on a realistic time and temperature scale.

As an example, the structure of a complex containing the $5HT_{1A}$ serotonergic subtype receptor derived with a simple Schiff base ligand is given below (Scheme 22) [80]. The derivation consists of a simple mixing of (commercially available) pyridinecarbaldehyde with the piperazine precursor. Labeling with **42** occurred at very low concentration. The concept of innocent labels is confirmed by the fact that the labeled receptor ligand



Scheme 21.



Scheme 22.

maintained its receptor affinity and its selectivity (5 ± 2 nM). For a number of other receptors the affinity constant was found to be higher than 1 μM.

The preparation procedure can be adjusted to a practical level. This last example shows that, in concrete terms, organometallic complexes or precursors can serve as very versatile tools in applications of nuclear medical diagnostics and/or therapy. The special characteristics of complex **42**, particularly its high inertness, give it a biologically inactive nature, which is the base for any application.

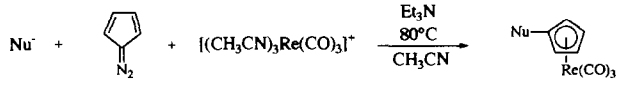
However, attempts to introduce Alberto's reagent directly onto cyclopentadienyl have already met with success [81]. It is clear that this type of work will be carried further.

4.3. A three-component synthesis of substituted cyclopentadienyl tricarbonylrhenium complexes

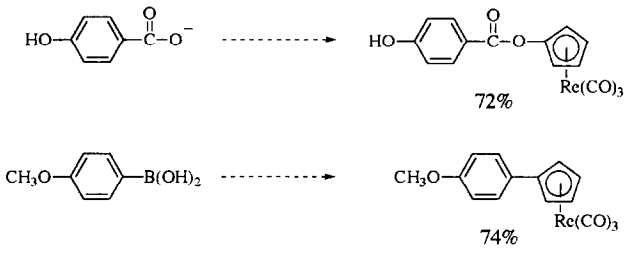
The principle of another route that permits rapid and efficient access to substituted cyclopentadienyl complexes of rhenium tricarbonyl is shown in Scheme 23.

The potential of the diazocyclopentadiene (η^5-$C_5H_4N_2$) as a precursor of Cp was noted by Herrmann [37] and Reimer and Shaver [35]. Alberto's reagent [77], which is much more reactive than the halogenides of $M(CO)_5$ with $C_5H_4N_2$, is used as the source of $Re(CO)_3$. Finally, the study of the nature of the nucleophile and of some of the mechanistic data is due to Minutolo Katzenellenbogen [82]. The most promising nucleophiles for application in nuclear medicine are the organic carboxylates and especially the boronic acids. These, like the halogenides, are moderately strong nucleophiles, while strong nucleophiles are inactive. Several significant examples are given below (Scheme 24).

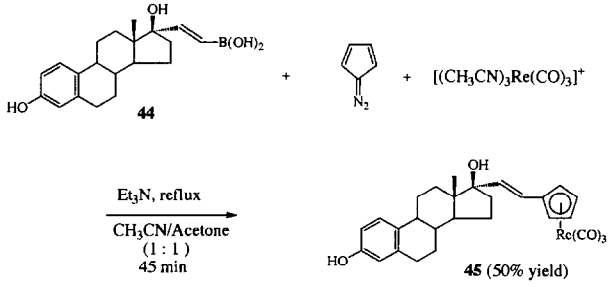
The one-pot synthesis of the estradiol analogue **45** (Scheme 25) starting from vinyl boronic acid **44** is a



Scheme 23.



Scheme 24.



Scheme 25.

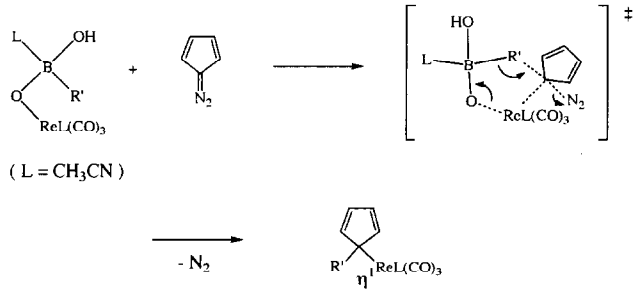
good example of the use of this reaction to functionalize a compound of biological interest with an organometallic Re moiety in a simple, rapid and efficient manner [82].

This shows the tolerance of the reaction towards functional groups such as alcohol and phenol. Also, no catalysts are required in this type of coupling for which a mechanism has been proposed.

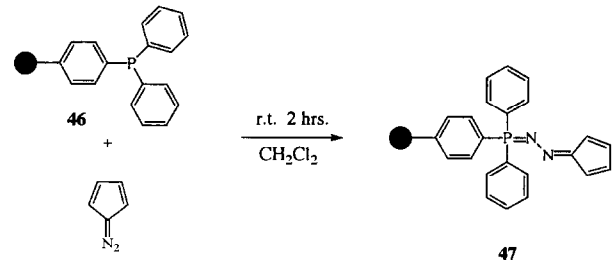
The most satisfactory mechanism proposed to date is shown for the case of boronic acids in Scheme 26. It introduces in the rate-determining step a concerted transition state of the S_N2 type, producing a short-lived η^1 complex that evolves to the final η^5 product via an η^3 species.

This mechanism implies that during the initial step there is a rapid association between the nucleophilic species and the metal tricarbonyl-type precursor.

An important practical limitation of this reaction for routine use is the high level of instability of $C_5H_4N_2$. This problem has been resolved using a phosphine-stabilized polymer technique (Scheme 27) [83].



Scheme 26.



Scheme 27.

The adduct **47** is very stable, dissociates from PPh_3 and gives the free diazo compound in acetonitrile. Besides very easy storage and handling, this precursor stabilized and strengthened by a cyclopentadienyl moiety also gives a high level of safety. This strategy can certainly be applied to other metallic moieties.

5. Conclusions

The examples given above are a good illustration of the stimulating effect on innovative organometallic synthesis that can be achieved by exploration of scientific avenues that lie off the beaten track, in this case bioorganometallic chemistry. The constraints that must be overcome in the attempt to reach new targets can provide a powerful spur to the imagination of the chemist. They may lead in directions that previously remained unexplored, whether through neglect or lack of information. In research such as this, considerations of stability of species in water and over time, the speed of the reaction process, the choice of appropriate metal moieties for attachment to complex multifunctional targets, are all a powerful driving force. It is very clear that the few aspects of the topic presented here do not represent an exhaustive panorama of this emerging field. They can only indicate some interesting directions, and point out some problem areas and possible solutions. Above all, they illustrate the fact that merely viewing a problem from a new angle can sometimes produce new and original chemical insights.

At the start of a new millennium that promises to bring with it an era of increasing complexity in chemistry, other zones of interaction for organometallic chemistry may also prove to be a source of creative energy. It is already possible to imagine the future gains to be harvested at the interface of the solid state, organometallics and biology, in the development of new analytical tools based on the specific properties of complexes at the junction of organometallics and medicine, in the advantages of new biosensors, and possibly in the use of organometallic metals in molecular computers.

Each interface will impose its own specifications and will thus inspire its own creative solutions. It is also likely that these new approaches will have repercussions

for other areas of organometallic chemistry, such as catalysis, illustrated here by new syntheses of Ti and Zr metallocenes, and will provide common ground for the expansion of the discipline as a whole.

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