Pergamon

Tetrahedron 55 (1999) 461–474

TETRAHEDRON

Use of the Stille coupling to label steroids with the ethynylcyclopentadienylmanganesetricarbonyl moiety

Angela Tuozzi,[a] Claudio Lo Sterzo,*[,a,†] Anna Sperandio[b], Gabriele Bocelli[c]

[a]Centro C.N.R. di Studio sui Meccanismi di Reazione,
[b]Centro C.N.R. di Studio per la Chimica delle Sostanze Organiche Naturali,
Dipartimento di Chimica, Box n°34-Roma 62, Università "La Sapienza"
Piazzale Aldo Moro, 5 - 00185 Roma (Italy),
[c]Centro di Studio per la Strutturistica Diffrattometrica del C.N.R.
Viale delle Scienze, 43100 Parma (Italy)

Received 10 June 1998; revised 23 October 1998; accepted 5 November 1998

Abstract

In order to use FT-IR spectroscopy as a technique for immunoassay analysis, the introduction of metallocarbonyl markers in steroids has been studied. By the use of the palladium-catalyzed cross-coupling reaction of steroidal triflates with [trimethyltin ethynyl (η^5-cyclopentadienyl)]manganesetricarbonyl it has been possible to label some steroids with the manganesetricarbonyl moiety. In the case of testosterone, by means of a properly designed synthetic route, it has been possible to introduce the organometallic marker minimizing the impact on the original structure and on the functional groups of the natural hormone thus making this labelled species particularly suitable for *CMIA* (Carbonyl Metallo Immuno Assay) purposes.

Keywords: Steroids; Labelling; Stille Coupling.

1. Introduction

In recent years, thanks to the pioneering work of Jaouen, new and fascinating uses of organometallics in biology have been disclosed [1,2]. In particular by the introduction of a metallocarbonyl fragment into biomolecules [3,4], a new immunoassay technique, indicated as *carbonylmetalloimmunoassay* (CMIA), has been developed. This technique essentially consists of the use of metal carbonyl complexes as FTIR markers in biological studies, as an alternative to radioactive markers in immunoassays. The strong infrared absorptions of the metal carbonyl moiety may in fact allow detection of picomole to femtomole quantities of metallocarbonyl labeled species. This sensitivity is comparable to that of radioisotopic methods [5]. The

† *E-mail: losterzo@uniroma1.it*

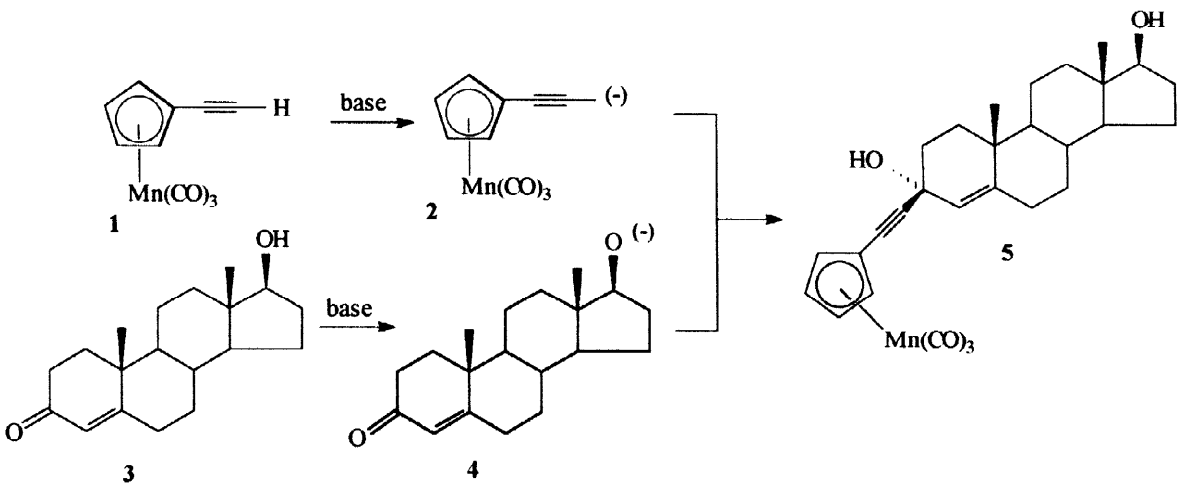
0040-4020/99/$ - see front matter
PII: S0040-4020(98)01045-X

development of these techniques has then required synthetic strategies for the introduction of metallocarbonyl fragments in steroids. Different approaches have been used to label biomolecules with metallocarbonyl tracers. Groups such as $Co_2(CO)_6$, $Mo_2Cp(CO)_4$ and $M_3(CO)_{12}$ (M=Ru, Os) were complexed on the triple bond previously introduced in the 17α-position of the steroidal D ring [6] or in position 3 of cortisol [7]. The $M(CO)_3$ (M=Cr [8], Mo [8], W [8], Mn [9]) fragments were complexed to the aromatic A ring of estrone and estradiol derivatives, and the $Cr(CO)_3$ fragment was also bound to the phenyl ring of a $C{\equiv}CC_6H_5$ moiety on the C(17) position of estradiol [10]. Moreover the $CpM(CO)_3$ (M=Mn, Re) group was attached on the C(17) position of estradiol in several different ways. i) through a CH_2 spacer by reaction of $(\eta^5\text{-}LiC_5H_4)M(CO)_3$ with the corresponding spirooxirane steroidal derivative [11], ii) by an alkyl chain linked to an amido functionality [11], iii) by an alkyne spacer, in two different fashion (a) upon attack of $(\eta^5\text{-}LiC{\equiv}CC_5H_4)M(CO)_3$ on the C(17)O carbonyl functionality of estrone [11], or (b) by Pd-catalyzed cross coupling of $(\eta^5\text{-}IC_5H_4)M(CO)_3$ with the tributyltin derivative of ethynylestradiol [11].

In this paper, we report on our studies on the introduction of the manganesetricarbonyl moiety on steroids.

In a first approach, miming the procedure used by Jaouen on estrone [12], we delivered the ethynyl cyclopentadienylmanganesetricarbonyl moiety on the C(3)O carbonyl moiety of testosterone (Scheme 1) by reacting the alkyne (**1**) and the steroid (**3**) after previous conversion into the corresponding lithium salts **2** and **4**.

Scheme 1



The 3β-{[ethynyl (η^5-cyclopentadienyl)]manganesetricarbonyl}-4-androstene-3α,17-diol (**5**) was thus formed, in 30% yield. It is important to note that although a stereogenic center has been generated in compound **5**, only formation of a single stereoisomer was observed[1] by NMR

[1] The number of lines displayed in the ^{13}C NMR spectrum of **5** matchs exactly the number of signals expected for a single stereoisomer. Although these data do not allow to establish its configuration, we believe that because of the sterical bulkiness of **2** only the β isomer has been formed

spectroscopy. However the limited yield of **5** imposed the search of a more efficient route to deliver a metallocarbonyl moiety in the steroidal frame.

In connection with our interest in Pd-catalyzed processes [13-15], we decided then to investigate the use of the Pd-catalyzed coupling of steroidal triflates with the (trimethyltin)ethynyl functionalized cyclopentadienyl manganesetricarbonyl complex (**6**) (Scheme 2). The Pd-catalyzed coupling of vinyl triflates with organostannanes-namely the Stille reaction-represents in fact one of the most versatile method for carbon-carbon bond formation [16-18], and, by the work of Cacchi ed others [19], a wide array of triflic derivatives of steroids have been made easily available.

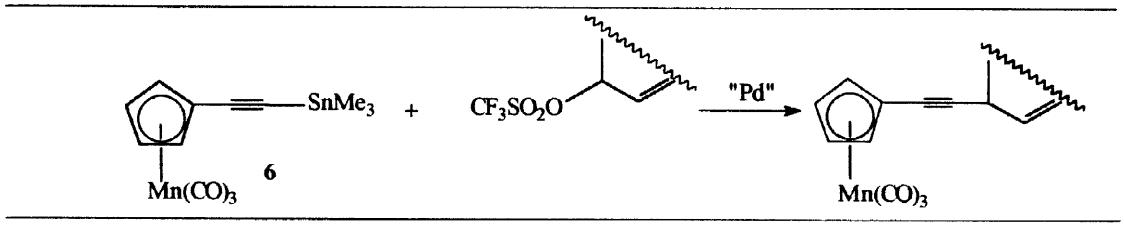
2. Results and discussion

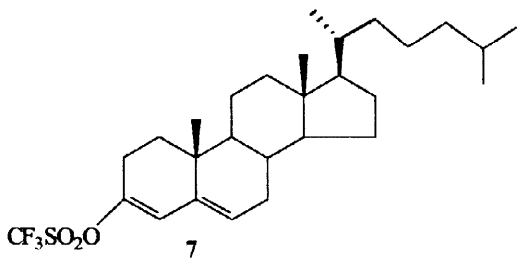
The steroidal triflate derivatives cholesta-3,5dien-3-yl- trifluoromethane sulfonate [19] (**7**), 17β-acetoxyandrosta-3,5dien-3-yl trifluoromethane sulfonate [19] (**8**), and, 3-[[trifluoromethyl) sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (**9**) [20] (Scheme 2) were readily available from cholest-4-en-3-one, (17α)acetyl protected testosterone, and estrone, respectively.

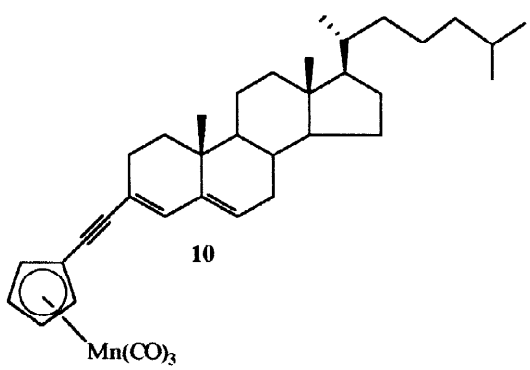
In a first series of experiments we attempted coupling reactions under mildest possible conditions, thus in the presence of the $(CH_3CN)_2PdCl_2$ catalyst (3-5%) and 3 equiv of lithium chloride at room temperature in DMF [17]. However under these conditions, only in the case of **8** it was possible to isolate the coupled product **11** in 40% yield, while in all the other cases starting materials remained unchanged and the subsequent chromatographic separation, while allowing the recovery of unreacted triflates, cleaved the trimethyltin functionality of **6** affording the manganese alkyne **1**. Longer reaction times and/or higher temperature had detrimental effect both on product yield and recovery of starting materials.

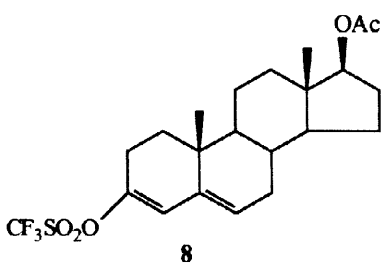
Switching to tetrahydrofuran (THF) as solvent and tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$, as catalyst, in the presence of 7 equiv of lithium chloride at reflux [17,21], coupling of vinyl and aryl triflates, **7-9** with {η^5-[(trimethyltin)ethynyl]cyclopentadienyl} manganesetricarbonyl (**6**) took place very efficiently. In the case of **7** and **8** starting materials were totally consumed in 12-14 hours, while for **9** reaction times needed to be prolonged to 20-24 hours in order to achieve complete conversion into the corresponding 3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}cholesta-3,5-diene (**10**), 17β-acetoxy-3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}androsta-3,5-diene (**11**), and, 3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}estra-1,3,5-trien-17-one (**12**). In all cases following workup and chromatographic separation, coupling products were isolated in the 80-85% yield range.

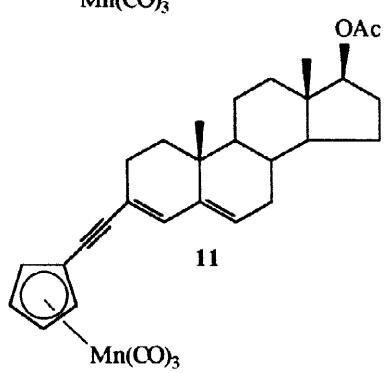
Scheme 2

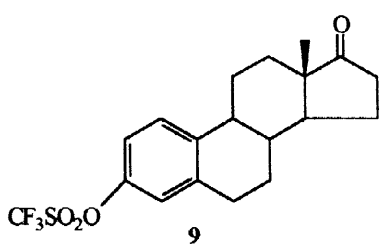


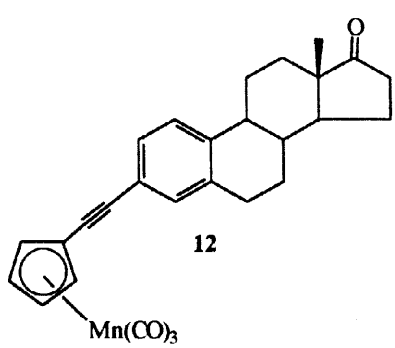






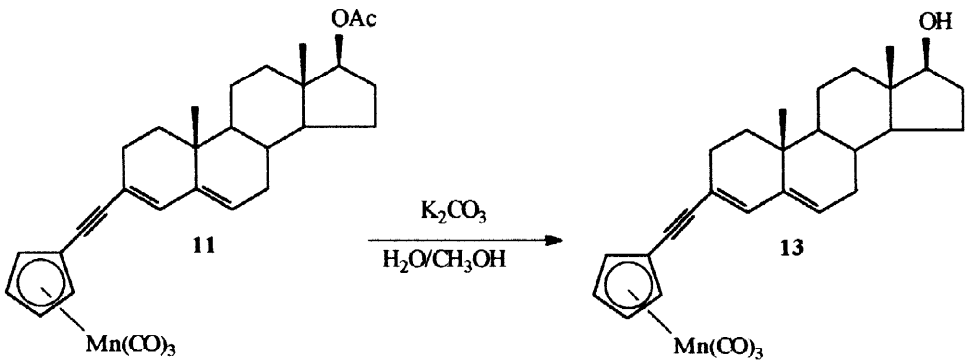






The acetyl protecting group of 17β-acetoxy-3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}androsta-3,5-diene **11** was subsequently easily and quantitatively removed upon dissolution of the compound in aqueous methanol and treatment with K_2CO_3 [22] (Equation 1).

Equation 1



The solid state structure of 3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl} estra-1,3,5-trien-17-one (**12**) was determined by X-ray diffraction methods. Figure 1 shows the crystal structure of the compound. The plot reveals that the $CpMn(CO)_3$ (Cp=cyclopentadienyl) moiety is spaced, by the acetylenic unit, 4.052 Å apart from the estrone skeleton. Because of this relevant distance, and the rigidity of the alkyne linkage -which do not allow any interaction of the $CpMn(CO)_3$ group with the estrone unit- this group does not exert any sterical influence on the steroidal frame, thus bond distances and angles are uninfluenced with respect of those found for the three pholimorph forms of estrone already analysed [23]. The same absence of steric influence by such kind of labelling group has been previously noticed in the case of the ethynyl cyclopentadienyltricarbonyl rhenium labelled estradiol [11]. While preventing sterical influences between the two groups, the acethylene spacer allows indeed electronic communication between the phenyl and the cyclopentadienyl rings. In this respect, although free rotation is allowed along the C17-C21 axis, the planes of the two aromatic rings, C14-C19 and C22-C26, are rotated of only 16.1(3)° thus revealing that to some extent electronic conjugation might exist-through the alkyne spacer-between the two systems. Both these aspects suggest that the introduction of the metallocarbonyl fragment on steroids by the η^1[ethynyl (η^5-cyclopentadienyl)] ligand might be the appropriate choice for the scope of CMIA. In fact, i) the ethynyl spacer prevents sterical influence of the cyclopentadienylmetallocarbonyl moiety on the steroidal frame, ii) electronic factors may be transmitted through the alkyne linkage and used to modify electronic properties of functional groups on steroids then influencing interaction with hormonal receptors. Of course it must be considered that, if attached to an unappropriate steroidal position, the $CpMn(CO)_3$ moiety itself can hinder the interaction with the hormonal receptor.

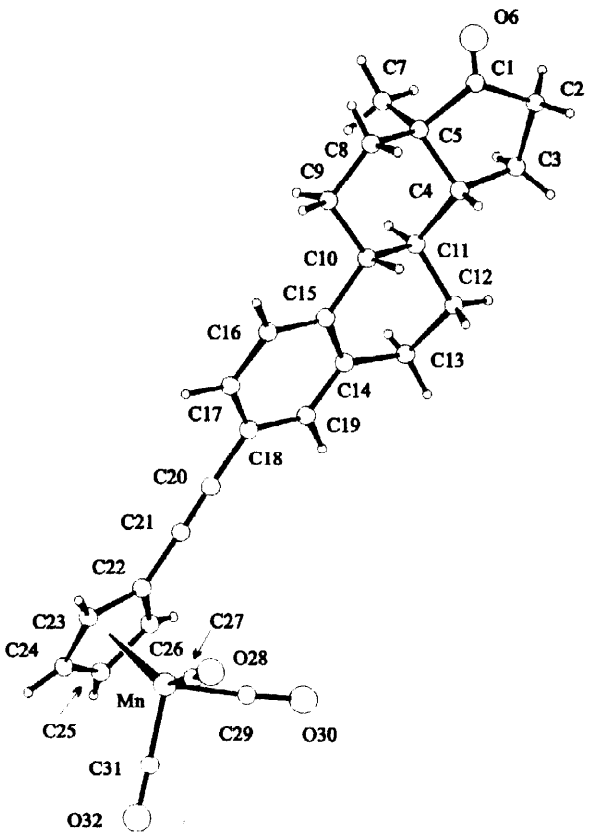


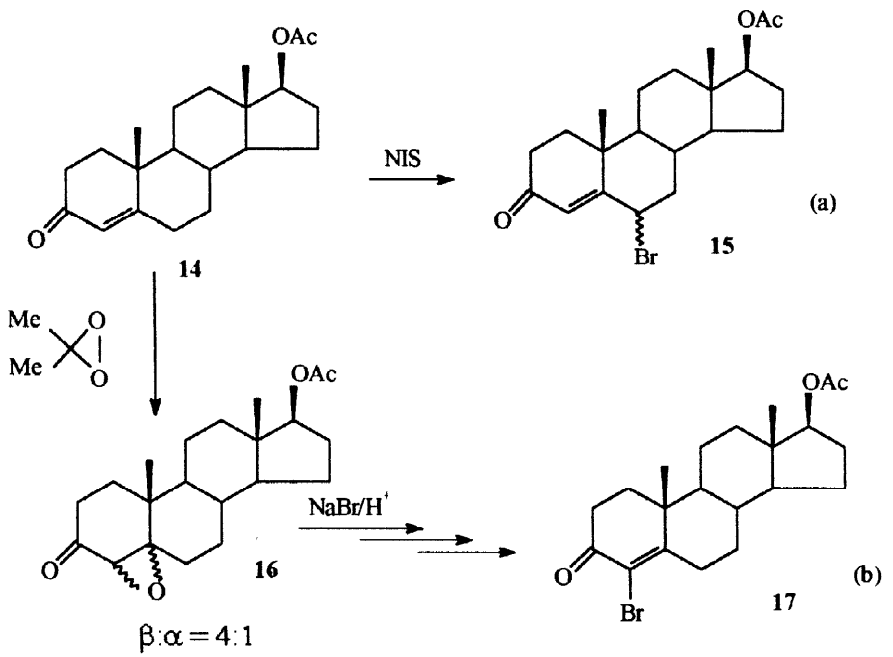
Figure 1. X-ray structure of **12**.

With formation of compounds **10-12** it has been established that the Pd-catalyzed coupling of steroidal vinyl and aryl triflates with trialkyltin acetylides may allow an efficient labelling of steroids with metallocarbonyl moieties. On the other hand the introduction of the triflic functionality significantly changes the original nature of the steroid itself by modification of functional groups, which might be essential for recognition of natural hormonal receptors. In general, although there are cases in which modified hormones have *better* binding affinity than the natural ones [11], the introduction of the marker should be planned in such a way as to minimize structural changes on natural hormones.

We therefore searched for synthetic routes that would transform a steroid in a suitable coupling partner for trialkyltinacetylides with minimum structural changes. Testosterone seemed to be a particularly suitable substrate to this purpose, especially in comparison with compound **8** (Scheme 3). In that case although **8** was originated from testosterone, the introduction of the triflic functionality destroyed the enone functionality, introducing a diene moiety in its place. We focused then on preserving as much as possible the structural features of testosterone, while introducing a modification that would make possible Pd-catalyzed coupling with tin acetylydes. Introduction of a bromine atom in position 4 or 6 of testosterone seemed to be appropriate since vinyl and allyl bromide derivatives are in general functionalities that well perform the Stille reaction [16,24,25]. 17β-acetoxy-6-bromo-4-androsten-3-one (**15**) was formed in 72% yield by treatment of 17β-acetoxy-4-androsten-3-one (**14**) with NBS [26] (Scheme 3a). Formation of the

17β-acetoxy-4-bromo-4-androsten-3-one (**18**) was instead performed by the procedure outlined in Scheme 3b. Treatment of 17β-acetoxy-4-androsten-3-one (**14**) with dimethyldioxirane (DMD) formed the epoxide **16**, shown to be a 8:2 mixture of β and α isomers by NMR analysis [27,28]. Subsequent treatment of the epoxide with NaBr in the presence of an acidic resin (Amberlyst 15) directly formed the vinyl bromide **17** [29], isolated in 75% overall yield.

Scheme 3

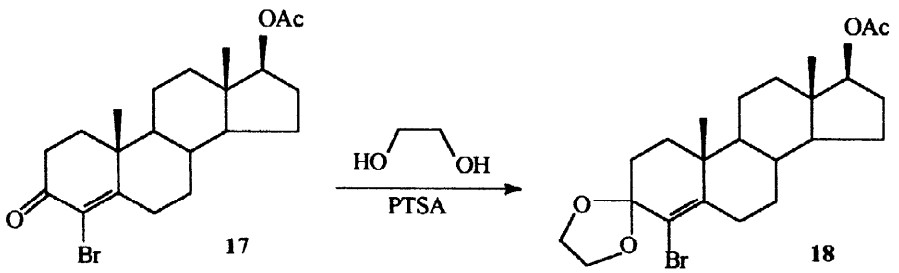


Despite several attempts, either the allyl and vinyl bromides **15** and **17** failed to couple with {η^5-[(trimethyltin)ethynyl]cyclopentadienyl} manganesetricarbonyl (**6**) in the presence of palladium. In the case of 17β-acetoxy-6-bromo-4-androsten-3-one (**15**), starting material were completely consumed, but a complex mixture of products were formed under typical conditions [25] used to couple allyl bromides and tin derivatives, such as the use of a combination of $Pd(dba)_2$ and PPh_3 as catalyst, in THF solvent at 50°C. By the subsequent chromatographic separation, no material corresponding to the desired product was identified beside a small amount of [η^5-(ethynyl)cyclopentadienyl] manganesetricarbonyl (18 %). An identical result was obtained by using $(CH_3CN)PdCl_2$ as catalyst in DMF at room temperature [17], while the use of $Pd(dba)_2/PPh_3$ as catalyst, in $CHCl_3$ solvent, at 65°C [24] left starting materials unchanged.

The vinyl bromide (**17**) showed the same disappointing behavior. Only variable amount of [η^5-(ethynyl)cyclopentadienyl] manganesetricarbonyl was recovered when coupling was attempted in the presence of $(CH_3CN)PdCl_2$ in DMF [17] either at 25 and 50°C, or with the same catalyst in the presence of $Pd(PPh_3)_4$/CuI in dioxane solvent [30,31]. No reaction occurs in the presence of $Pd(PPh_3)_4$ in boiling THF [17,21]. It is reported in the literature that α-bromoenones showed surprisingly inertness toward Pd-catalyzed coupling, but were successfully reacted upon transformation of the carbonyl functionality into the corresponding chetal [32]. The carbonyl functionality of **17** was then transformed by treatment with ethylene glycol in the presence of *p*-toluensulfonic acid (PTSA), and the corresponding 17β-acetoxy-4-bromo-4-androsten-3-ethylene

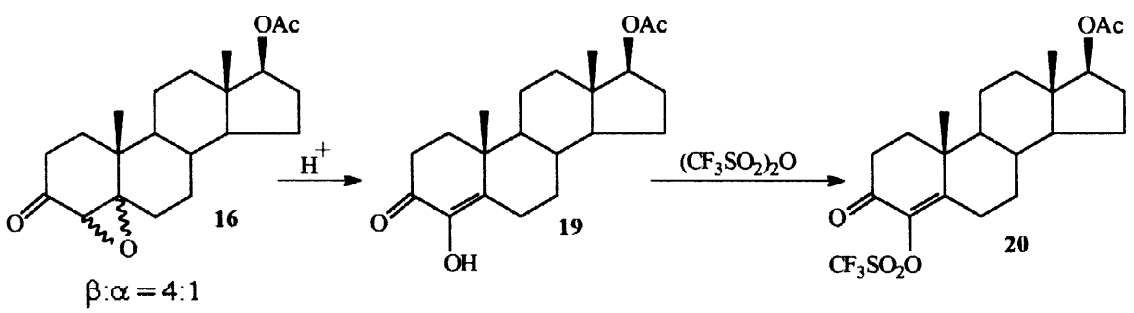
acetal (**18**) was recovered in a nearly quantitative yield (Equation 5). Unfortunately despite this modification further attempts to induce coupling of the modified vinyl bromide **18** with {η^5-[(trimethyltin)ethynyl]cyclopentadienyl} manganesetricarbonyl (**6**) were unsuccessful.

Equation 2



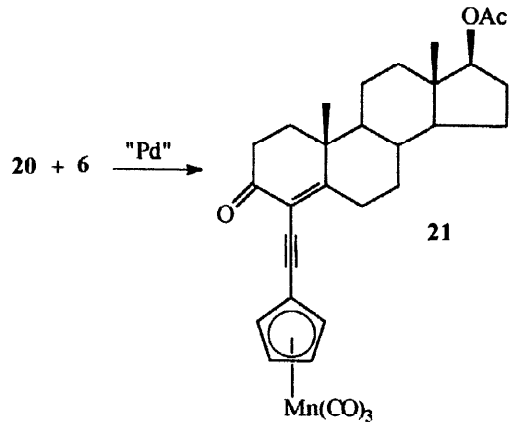
Although compounds **15**, **17**, and **18** revealed not to be suitable for Pd-catalyzed coupling with the tin acetylide **6**, in view of the successful coupling of triflate derivatives **7-9** (Scheme 2), we decided to modify the synthetic route used to form **17** to prepare the corresponding triflate and to test it in the coupling reaction with **6**. The epoxide **16** was then treated with an aqueous solution of sulfuric acid and the 17β-acetoxy-4-androstene-4-(hydroxy)-3-one [33,34] (**19**) was formed in 50% yield. Subsequent treatment of **19** with triflic anydride [20] afforded the 17β-acetoxy-4-androsten-4-[[trifluoromethyl)sulfonyl]oxy]-3-one (**20**) in 51% yield (Scheme 4).

Scheme 4



Coupling reaction of **20** with the tin acetylide **6** was carried out under the same conditions used for **7**, **8**, and **9**. In refluxing THF, using $Pd(Ph_3)_4$ as catalyst and 7 equiv of lithium chloride [17,21], starting materials were totally consumed after 20 hours, and following workup and chromatographic separation, the coupled product, 17β-acetoxy-4-androstene-4-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}-3-one (**21**) was isolated in 62% yield (Equation 3).

Equation 3



3. Conclusion

In this work, we have demonstrated that steroidal triflates efficiently undergo the Stille coupling with trialkyltin acetylides, and, by the use of this procedure it is possible to label steroids with metallocarbonyl fragments. Being relatively ease the introduction of the triflate functionality in the steroidal skeleton, the Stille protocol may become an interesting tool for the scope of CMIA. Moreover, X-ray structural determination carried out on an ethynylcyclopentadienylmanganesetricarbonyl labelled estrone has shown that this labelling group does not exert sterical influence -thus deformations- on the steroidal skeleton, while it is able to transmit electronic effects. This last aspect might be particularly appreciable in order to influence interaction with hormonal receptors. Finally chemical transformations outlined in this report might be extended also to technetium and rhenium metals, thus widening the biomedical use of these derivatives [35].

4. Experimental section

General

Elemental analyses were performed by the Servizio Microanalisi of the Area della Ricerca di Roma (C.N.R., Montelibretti). IR spectra were recorded on a Nicolet FT 510 instrument in the solvent subtraction mode, using a 0.1 mm CaF_2 cell. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC300P spectrometer at 300 and 75 MHz respectively. The ^1H NMR chemical shifts are reported in ppm downfield vs. Me_4Si, assigning the residual ^1H impurity signal in the solvent ($CDCl_3$) at resonance 7.24 ppm. The ^{13}C NMR chemical shifts are referenced to the ^{13}C triplet of $CDCl_3$ at 77.00 ppm. Solvents, including those used chromatograpy, and liquids were thoroughly degassed before use. Chromatographic separations were performed with 70-230 mesh silica gel (Merck).

Standard techniques, with Schlenk type equipment for the manipulation of air-sensitive compounds under a blanket of argon, were employed. All solvents were dried (sodium-potassium alloy for tetrahydrofuran (THF), sodium for Dioxane, CaH_2 for N,N-dimethylformamide (DMF)

and P_2O_5 for CH_2Cl_2) and argon-saturated prior to use. The following compounds were prepared by known methods: [η^5-(ethynyl)cyclopentadienyl] manganesetricarbonyl [14] (**1**), {η^5-[(trimethyltin)ethynyl]cyclopentadienyl} manganesetricarbonyl [14] (**6**) Cholesta-3,5dien-3-yl-trifluoromethane sulfonate [19] (**7**), 17β-acetoxyandrosta-3,5dien-3-yl trifluoromethane sulfonate [19] (**8**), 3-[[trifluoromethyl)sulfonyl]oxy] estra-1,3,5(10)-trien-17-one [20] (**9**), 17β-acetoxy-6-bromo-4-androsten-3-one [26] (**15**), $(CH_3CN)PdCl_2$ [36], $Pd(dba)_2$ [37] and $Pd(PPh_3)_4$ [38]. Cholest-4-en-3-one, and estrone were purchased from Aldrich, testosterone acetate was purchased from Sigma.

3β-{[Ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}-4-androstene-3α,17-diol (5)
In a 100 ml flask 0.51 g (2.23 mmol) of (η^5-HC≡CC_5H_4)$Mn(CO)_3$ dissolved in 20 ml of THF were treated with 1.73 ml (2.23 mmol) of *sec*-BuLi (1.3 M solution in hexane) at -70 °C. In the mean time a second flask was loaded with 0.65 g (2.23 mmol) of testosterone, 20 ml of THF and treated with 1.73 ml (2.23 mmol) of *sec*-BuLi (1.3 M solution in hexane) at -70 °C. After stirring both solutions for 30 min at low temperature the content of the second flask was slowly cannulated in the first one, and at the end of addition, the cold bath was removed and the mixture kept stirring overnight while allowed to warm at room temperature. After hydrolysis with ice water and extraction with brine the organic layer was dried over Na_2SO_4, filtered and evaporated under vacuum. Subsequent chromatographic separation, using a mixture of petroleum ether/THF (7:3) as the eluent yielded 0.3 g (30%) of product as white solid. mp 68-70 °C (dec.). IR (CH_2Cl_2): cm^{-1} 3600, 2955, 2024, 1940. ^{1}H-NMR ($CDCl_3$): ppm 6.29 (s, 1H, C_4-H), 5.51 (bs, 1H C_3-OH); 4.94 (t, 2H, J=2.01 Hz, Cp); 4.66 (t, 2H, J=2.01 Hz, Cp), 4.42 (s, 1H, C_{17}-OH), 3.63 (1H, C_{17}-H), 2.30-0.90 (m, 19H), 0.93 (s, 3H C_{19}-H), 0.76 (s, 3H C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.34, 141.19, 136.31, 126.71, 116.32, 90.98, 85.83, 83.89, 81.82, 81.09, 53.40, 51.36, 48.10, 42.82, 36.43, 34.50, 33.42, 31.69, 30.38, 26.61, 23.28, 20.55, 19.07, 11.03. Anal. Calcd for $C_{29}H_{33}O_5Mn$: C, 67.44; H, 6.44. Found: C, 67.77; H, 6.83.

Palladium-Catalyzed Coupling Reaction: General Procedure
3-{[Ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}cholesta-3,5-diene (10)
To a solution of 0.4 g (0.77 mmol) of 3-trifluoromethansulfonyl-3,5-cholestadiene in 10 ml of THF were added 0.3 g (0.77 mmol) of [η^5-Me_3SnC≡CC_5H_4]$Mn(CO)_3$, 0.25 g (5.6 mmol) of LiCl, and 0.014 g (0.0124 mmol) of $Pd(PPh_3)_4$. The resulting mixture was heated to reflux overnight, then after cooling the solvent was removed under vacuum and the residue redissolved in a mixture of 20 ml of THF and 60 ml of hexane. The solution was then transferred in a separatory funnel and washed with a 10% aqueous NH_4OH solution (3 X 30 ml). The organic layer was separated and the aqueous phase was extracted with ether (3 X 50 ml). The organic layers were then collected, dried over Na_2SO_4 filtered and evaporated to dryness under vacuum. The crude product obtained was purified by column chromatography using a mixture of petroleum ether/EtOAc (9:1) as the eluent to give 0.38 g (82%) of product as white solid. mp 128-130 °C (dec.). IR (CH_2Cl_2) cm^{-1} 3055, 2952, 2869, 2023, 1938. ^{1}H-NMR ($CDCl_3$): ppm 6.29, 5.52 (2 bs, 2H, C_4-H, C_6-H), 4.94 (bs 2H, Cp), 4.65 (bs 2H, Cp), 2.30-0.90 (m, 26H), 0.91 (s, 3H, C_{19}-H), 0.88 (d, 3H, J=6Hz, C_{21}-H), 0.84(d, 6H, J=6Hz, C_{26}-H, C_{27}-H), 0.67(s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.37, 141.13, 136.49, 127.24, 116.15, 91.11, 85.82, 83.99, 81.82, 80.96, 56.76, 56.05, 47.97, 42.39, 39.63, 39.46, 36.12, 35.76, 34.44, 33.41, 32.06, 31.62,

28.20, 27.98, 26.64, 24.12, 23.80, 22.82, 22.55, 20.93, 19.07, 18.66, 11.93. Anal. Calcd for $C_{37}H_{47}O_3Mn$: C, 74.73; H, 7.97. Found: C, 74.97; H, 7.70.

17β-Acetoxy-3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}androsta-3,5-diene (11)

This product was prepared from 0.8 g (1.7 mmol) of 17β-acetoxy-3-trifluoromethansulfonyl-3,5-androstadiene, 0.7 g (1.7 mmol) of [η^5-$Me_3SnC{\equiv}CC_5H_4$]$Mn(CO)_3$, 0.52 g (12 mmol) of LiCl and 0.032 g (0.027 mmol) of $(Ph_3P)_4Pd$ in 10 ml of THF with the procedure described for **(10)**. Following workup and chromatographic separation 0.8 g (85%) of product was isolated as yellow solid. mp 156-158 °C (dec.). IR(CH_2Cl_2): cm^{-1} 3431, 3055, 2024, 1939. ^{1}H-NMR ($CDCl_3$): ppm 6.29, 5.51 (2bs, 2H, C_4-H, C_6-H), 4.94 (bs, 2H Cp), 4.66 (bs, 2H Cp), 4.58 (t,1H, J=7.8 Hz, C_{17}-H), 2.40-0.80 (m, 17H), 2.02 (s, 3H, Ac), 0.94 (s, 3H, C_{19}-H), 0.80 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.42, 171.19, 141.16, 136.26, 126.57, 116.35, 90.97, 85.84, 83.87, 82.62, 81.82, 51.096, 47.93, 42.44, 36.62, 34.49, 33.37, 31.54, 27.47, 26.60, 23.42, 21.16, 20.42, 19.06, 11.99. Anal. Calcd for $C_{31}H_{33}O_5Mn$: C, 68.88; H, 6.15. Found: C, 69.02; H, 6.20.

3-{[Ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}estra-1,3,5-trien-17-one (12)

Following the procedure described for **(10)**, this product was prepared from 0.6 g (1.5 mmol) of 3-trifluoromethansulfonylestrone, 0.59 g (1.5 mmol) of $Me_3SnC{\equiv}CC_5H_4$]$Mn(CO)_3$, 0.46 g (10.8 mmol) of LiCl, and 0.028 g (0.024 mmol) $Pd(Ph_3P)_4$ in 30 ml of THF, but in this case reaction time was prolonged to 24 h. Following workup the product was purified by column chromatography using a mixture of petroleum ether/EtOAc (5:1) as the eluent to give(0.58 g 80%) of product as white solid. IR (CH_2Cl_2): cm^{-1} 2027, 1949, 1744. ^{1}H-NMR ($CDCl_3$): ppm 7.40-6.90 (m, 3H, Ar), 5.02 (bs, 2H, Cp), 4.69 (bs, 2H, Cp), 3.00-1.40 (m, 15H), 089 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.2, 220.68, 140.75, 136.68, 132.10, 128.98, 125.40, 119.56, 88.97, 86.08, 83.22, 81.98, 80.80, 50.43, 47.89, 44.42, 37.87, 35.79, 31.48, 29.04, 26.25, 25.52, 21.54, 13.79. Anal. Calcd for $C_{28}H_{25}O_4Mn$: C, 70.00; H, 5.24. Found: C, 69.82; H, 5.30.

17β-Hydroxy-3-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}androsta-3,5-diene (13)

In a 100 ml flask 0.4 g (0.74mmol) of **11** were dissolved in 10 ml of methanol and 5 ml of H_2O. While stirring a catalytic amount of K_2CO_3 were introduced in the flask, and the stirring continued at room temperature for 5 h. Following evaporation of the solvent, the residue was redissolved in a 50 ml mixture of petroleum ether /EtOAc 7:3 and filtered over a glass frit covered with a silica pad. Following removal of the solvent under vacuum the product is quantitatively recovered as white solid. mp 78-80 °C (dec.). IR (CH_2Cl_2) : cm^{-1} 3606, 2954, 2024; 1940. ^{1}H-NMR ($CDCl_3$): ppm 6.29, 5.51 (2bs, 2H, C_4-H, C_6-H), 4.94 (bs, 2H, Cp), 4.66 (bs, 2H, Cp), 4.10 (bs, 1H, C_{17}-OH), 3.63 (bs, 1H, C_{17}-H), 2.40-0.70 (m, 17H), 0.93 (s, 3H, C_{19}-H), 0.76 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.34, 141.18, 136.31, 126.71, 116.30, 90.98, 85.86, 85.81, 83.89, 81.82, 81.10, 51.36, 48.10, 42.82, 36.43, 34.50, 33.40, 31.69, 31.60, 30.41, 26.61, 23.27, 20.55, 19.07, 11.03. Anal. Calcd for $C_{29}H_{31}O_4Mn$: C, 69.87; H, 6.27. Found: C, 69.81; H, 6.37.

X-ray Crystallographic Analysis of 12

The diffraction-quality single crystal of **12** were obtained by vapor diffusion of pentane into a concentrated THF solution of the compound at room temperature. The single crystal used on the Philips PW1100 diffractometer was a prism of about 0.17 x 0.23 x 0.31 mm. Cell parameters were obtained as part of the alignment process of the crystal on the diffractometer using the angular values of 47 reflections ($5.3<\theta<16.3°$) automatically centered. The intensities of the reflections were collected in the 3-27° θ range following the Lehman and Larsen notation [39]. A reference reflection, measured every 100, was without variations during the data collection time. All the collected reflections were used to solve the structure with SIR97 [40] program and the refinement was performed with SHELX93 [41]. Most of the hydrogen atoms were localized in ΔF map computed after the anisotropic refinement, the remaining were put in their calculated positions and refined isotropically.

Crystal data for **12**; $C_{28}H_{25}O_4Mn$, M=480.44. Crystal system; orthorhombic, Space group P 2_1 2_1 2. Cell parameters; a = 15.527(2) (Å), b = 23.306(2) (Å), c = 6.763(4) (Å),V 2447.3 (Å^3), Z = 4, D_c = 1.30 (g cm^{-3}), μ = 5.46 (cm^{-1}), F(000) = 1000, T = 294(1) (K), Index h, k, l ranges = 0/19, 0/29, 0/8. Collected reflections = 3080. Independent reflections = 3058. Number of refined parameters = 399. Final R(R_w) = 0.031(0.11). Weighting scheme w =1./[$\sigma^2(F_o)^2$+(0.08 P)^2], where P =[max(F_o^2,0)+2F_c^2]/3. Goodness of fit = 48. Δρ min/max = –0.13/0.14 (e/Å^3).

Details of the crystal structure investigation may be obtained from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, GB-Cambridge CB2 1EZ(UK), on quoting the full journal citation.

17β-Acetoxy-4-bromo-4-androsten-3-ethylene acetal (18)

In a 50 ml flask, equipped with a Dean-Stark trap were introduced 0.4 g (0.97 mmol) of 17β-acetoxy-4-bromo-4-androsten-3-one (**17**), 0.24 ml (3.9 mmol) of ethylene glycol, 0.05 g of PTSA and 50 ml of toluene. The resulting mixture was refluxed for 24 h, then after cooling, the solution was filtered over K_2CO_3 and evaporated to dryness in vacuo. Compound **18** was quantitatively recovered as white solid. mp 140-143 °C (dec.). IR (CH_2Cl_2): cm^{-1} 2026, 1932, 1726. ^{1}H-NMR ($CDCl_3$): ppm 4.55 (dd, 1H, J=9.0Hz, J=7.7Hz, C_{17}-H), 4.20-3.89 (4H, OCH_2CH_2O), 3.03-2.98 (1H, ddd, J=14.4Hz, J=3.8Hz, J=2.6Hz, C_2-Ha), 2.3-0.8 (m, 18H), 2.01 (s, 3H, Ac), 1.06 (s, 3H, C_{19}-H), 0.78 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 170.72, 149.06, 121.181, 105.96, 82.18, 65.73, 64.82, 53.72, 49.89, 40.96, 36.38, 36.24, 34.88, 33.64, 31.20, 30.47, 27.16, 23.07, 20.81, 20.38, 17.72, 11.70. Anal. Calcd for $C_{23}H_{33}O_4Br$: C, 60.93; H, 7.34. Found: C, 60.52; H, 7.37.

17β-Acetoxy-4-androstene-4-[[trifluoromethyl)sulfonyl]oxy]-3-one (20)

This compound was prepared in 51% yield from 17β-acetoxy-4-androstene-4-(hydroxy)-3-one (**19**) (0.2g, 0.57 mmol), 2,6-lutidine (0.3 ml), and triflic anhydride (0.097ml, 0.57 mmol) in 20 ml of CH_2Cl_2, with the method described in the literature [20]. IR (CH_2Cl_2): cm^{-1} 3057, 2949, 2928, 2876, 2858, 1726, 1702, 1422, 1272. ^{1}H-NMR ($CDCl_3$): ppm 4.57 (t, 1H, J=8.4 Hz, C_{17}-H), 2.87 (dt, 1H, J=15.1Hz, J=2.7 Hz, C_2-H_a), 2.56-2.50 (m, 2H), 2.30-0.9 (m, 16H), 2.02 (s, 3H, Ac), 1.25 (s, 3H, C_{19}-H), 0.81 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 188.46, 171.02, 159.60, 139.41, 82.16, 53.58, 49.88, 42.31, 40.24, 36.35, 34.63, 33.86, 32.98, 30.20, 27.35, 24.76, 23.26, 21.04, 20.41, 17.51, 11.89. Anal. Calcd for $C_{22}H_{29}O_6F_3S$: C, 55.22; H, 6.11. Found: C, 55.47; H, 6.17.

17β-Acetoxy-4-androstene-4-{[ethynyl (η^5-cyclopentadienyl)] manganesetricarbonyl}-3-one (21)

This product was prepared from 0.13 g (0.29 mmol) of 17β-acetoxy-4-androstene-4-[[trifluoromethyl)sulfonyl]oxy]-3-one (**20**), 0.15 g (0.29 mmol) of $Me_3SnC{\equiv}CC_5H_4]Mn(CO)_3$, 0.089 g (2.1 mmol) of LiCl, and 0.01 g (0.008 mmol) of $(Ph_3P)_4Pd$ in 20 ml of THF with the procedure described for **(12)**. Following workup the product was isolated by column chromatography using a mixture of petroleum ether/EtOAc (5:1) as the eluent to give 0.1 g (62%) of product as white solid. mp 110-112 °C (dec.). IR (CH_2Cl_2) : cm^{-1} 3055, 2956, 2934, 2874, 2858, 2024, 1938, 1731, 1682. ^{1}H-NMR ($CDCl_3$): ppm 5.02 (bs, 2H, Cp), 4.67 (bs, 2H, Cp), 4.58 (bs, 1H, C_{17}-H), 3.16 (m, 1H, C_2-H_a), 2.60-0.80 (m, 18H), 2.02 (s, 3H, Ac), 1.22 (s, 3H, C_{19}-H), 082 (s, 3H, C_{18}-H). ^{13}C-NMR ($CDCl_3$): ppm 224.28, 195.19, 175.40, 171.14, 128.02, 87.17, 86.49, 82.93, 82.37, 82.01, 81.77, 53.84, 50.18, 42.43, 36.56, 35.06, 34.52, 33.67, 31.11, 30.78, 29.67, 27.46, 23.41, 21.14, 20.55, 17.85, 12.03. Anal. Calcd for $C_{31}H_{33}O_6Mn$: C, 66.90; H, 5.98. Found: C, 67.12; H, 5.87.

5. Acknowledgement

We thank Dr. Paolo Bovicelli and Dr. Giuliana Righi for advice in the preparation of 17β-acetoxy-4-bromo-4-androsten-3-one (**17**).

6. References and notes

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