UNIVERSITY OF OVIEDO

FACULTY OF CHEMISTRY

CHEMICAL MODIFICATION OF HORMONE ESTRONE: SYNTHESIS AND REACTIVITY OF AN ARYLBORONIC ACID DERIVED FROM ESTRONE BY HYDROXIL GROUP BORYLATION

FINAL THESIS IN CHEMISTRY

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1. INTRODUCTION

1.1 OBJECTIVE

In this experiment the goal is the obtention of estrone derivatives via Suzuki reactions "**A**" (figure 1), being R any organic group. This reaction is important as it is a very versatile strategy for obtaining derivatized natural products.

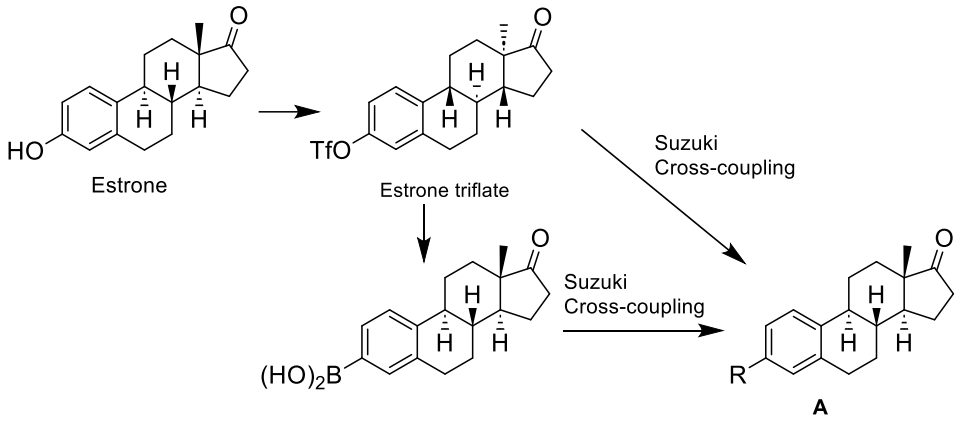


Figure 1

The plan to obtain estrone derivatives was to first obtain the boronic acid from estrone and then perform Suzuki reactions. However, the access to this boronic acid was not possible. Therefore, a second strategy, also based on Suzuki reactions, was developed, and now, the estrone trifate is used as a reagent.

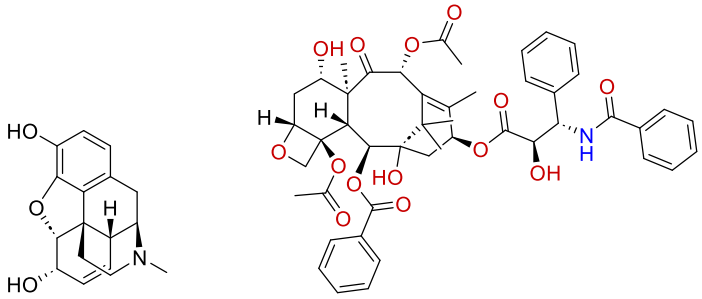
1.2 GENERAL VIEW

Throughout history, natural products have been powerful allies of medicine. Nature plays an important role in the development of new treatments aimed at curing ailments in the population. Antibiotics such as penicillin or erythromycin, antitumor agents like trabectedin, and painkillers like morphine share a common thread. They have each revolutionized the field of medicine, coming directly from plants, microorganisms, or marine animals. This is where chemistry intervenes, transforming initially undervalued elements into indispensable resources for hospitals worldwide. Chemistry facilitates the isolation of active compounds and enhances their applications, thereby advancing medical treatments. "Chemical synthesis has made available to us many natural-origin medications in the necessary quantity for therapeutic uses, even though their presence may be scarce in nature", explains the "Instituto de Productos Naturales y Agrobiología (IPNA-CSIC Spain)" chemist Antonio Hernández Daranas ("La naturaleza que inspiró los fármacos – January 19th 2021" (IPNA-CSIC Spain)).

Nowadays, drugs originating from plants keep a prominent status allowing significant advancements in the field, including developments in synthesis, combinatorial chemistry, and biotechnological methods like microbial fermentation. Remarkably, 66%* of recently sanctioned medications exhibit a direct association with the structure of a natural product, a proportion that escalates based on the particular medical condition being

addressed. Notably, in the realm of anticancer medications, a striking 88.3%* either bear a structural relation to natural products or are directly derived from them **(1)**.

Two examples of drugs derived from natural products are morphine, a powerful, widely used analgesic and obtained from "*Papaver somniferum*", and paclitaxel, a chemotheraphy medication used against different kind of cancer, derived from bark of the Pacific yew, *"Taxus brevifolia"* (figure 2).



Morphine Paclitaxel

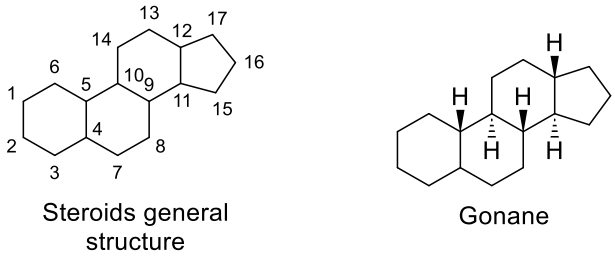
Figure 2

The economic possibilities that the research of these products allow are endless and not restricted uniquely to the pharmaceutical sector. Commercial applications of natural products range from manufacturing disinfectants for the chemical industry to creating new sweeteners and flavorings in the food sector, including the production of pesticides and crop protectants, and even extending to the creation of new cosmetic fragrances.

In this project, our objective is to find derivatives of estrogens, estrone specifically, in order to create new products with a similar structure for potential applications in the medical field.

1.3 ESTROGENS

Estrogens are steroids as they consist on the cyclopentaneperhydrophenanthrene structure a four carbon rings structure with sixteen carbon atoms, the simplest molecule with this structure is gonane (figure 3)



Steroids general structure Gonane

Figure 3

Specifically, estrogen refers to a group of hormones that are primarily responsible for the development and regulation of the female reproductive system and secondary sexual characteristics in women. These hormones are also present in smaller amounts in men and play essential roles in various physiological functions.

Estrogens are used as therapy for some diseases or conditions. Some of the main indications for the use of estrogens are: Hormone Replacement Therapy (HRT) in menopause, as estrogens help alleviate the symptoms; osteoporosis prevention as estrogens can help maintain bone density in postmenopausal women, reducing the risk of bone fractures; treatment of conditions related to estrogen deficiency, in some cases, such as premature ovarian insufficiency or certain conditions causing estrogen deficiency, estrogens may be prescribed to compensate for this hormonal deficiency and can also be used as part of the treatment against some cancers, although generally are avoided as they can produce the overgrowth of tumors.

However, applying estrogens as medical treatments has also disadvantages and not always can be used. Recommendations propose cyclic administration of estrogens (3 out of 4 weeks) while considering periodic dosage reductions or suspensions every 3-6 months to reassess the necessity for ongoing usage **(2)**.

Estrogens are predominantly produced in the ovaries, although they are also synthesized in smaller quantities in other tissues such as the adrenal glands, fat cells, and testes (in males) **(3)**. The main types of estrogen include estradiol, estriol and estrone (figure 4), among others.

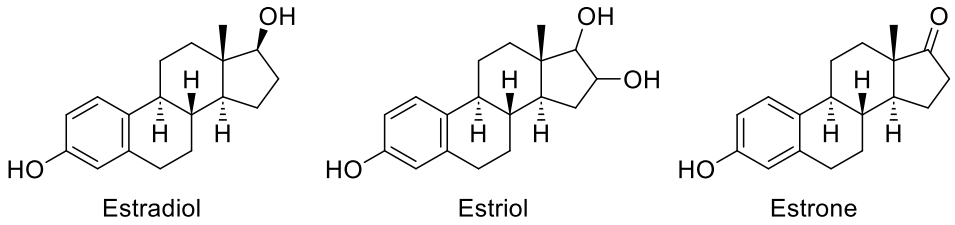


Figure 4

1.4 ESTRONE

In this experiment, our focus will be the derivatization of the estrone. As said before, estrone is a naturally occurring hormone and one of the three main types of estrogen produced in the human body. It is primarily synthesized and secreted by the ovaries in women. Additionally, estrone is generated in peripheral tissues through the conversion of androstenedione (figure 5), a precursor hormone, into estrone via the action of the enzyme aromatase.

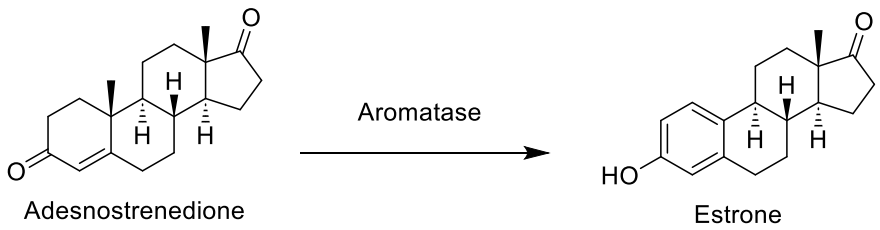


Figure 5

In females, estrone plays a significant role in the menstrual cycle and menopause, contributing to the development and maintenance of secondary sexual characteristics, regulating the menstrual cycle, and supporting ware productive functions. It is also found

in men, in a significant lower amount, where it contributes to various physiological processes such as bone health and brain function.

Estrone levels fluctuate throughout a woman's life, notably rising during menopause due to decreased ovarian estrogen production. Moreover, estrone can be synthesized artificially for medical purposes, such as hormone replacement therapy for menopausal symptoms or as part of certain medications **(4)**.

1.5 SUZUKI REACTION

The Suzuki reaction (figure 7) is a type of carbon-carbon bond-forming chemical reaction widely used in organic synthesis to create biaryl compounds (**5**). It was developed by Japanese chemist Professor Akira Suzuki in the 1970s and has since become one of the most versatile and commonly employed reactions in organic chemistry.

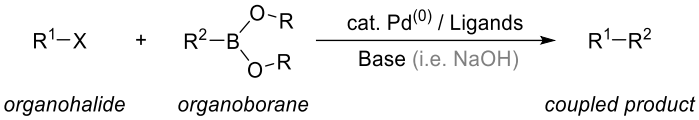


Figure 6

The reaction involves the coupling of an aryl halide (such as an aryl bromide or aryl chloride) with an organoboron compound, usually an arylboronic acid or an arylboronate ester **(6)**. This coupling is catalyzed by a palladium catalyst in the presence of a base and typically occurs under mild reaction conditions, making it valuable for synthesizing complex organic molecules **(7)**.

The Suzuki reaction's prominence in various industries, including pharmaceuticals, agrochemicals, and materials science, is due to several factors:

- Efficiency, as it enables the synthesis of complex molecules in a step-wise manner.
- Selectivity, as it allows the formation of specific bonds; operation at mild reaction conditions and versatility to construct diverse organic products with complex structures.
- Sustainability, as it has not harmful emissions.

Its widespread use in drug development, materials science, and other fields showcases its value as a crucial synthetic tool for creating compounds with desired properties and

functionalities **(8)**. Suzuki´s reaction mechanism (figure 7):

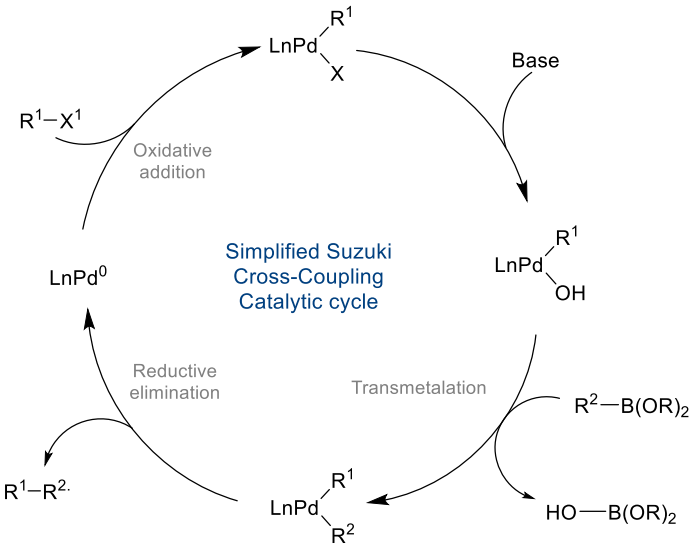


Figure 7

The mechanism of the Suzuki reaction involves the following steps:

1. Oxidative Addition: The catalytic cycle begins with the oxidative addition of a palladium (0) species, often a Pd(0) complex such as $Pd(PPh_3)_4$, to the aryl or vinyl halide substrate. This step leads to the formation of a Pd(II) species and generates the first organopalladium intermediate.

2. Transmetallation: In this step, the organopalladium intermediate formed in the previous step coordinates with the boronic acid or boronate ester substrate. This coordination facilitates the transfer of the organic group from the boron compound to the palladium center, leading to the formation of a new organopalladium species.

3. Reductive Elimination: The final step involves reductive elimination, where the carbon-carbon bond formation occurs. This process leads to the formation of the desired biaryl or alkenyl product and regenerates the Pd(0) catalyst, which can re-enter the cycle for further reactions. **(9)**

1.6 BORYLATION OF PHENOLS.

The borylation of phenols consists on the substitution of the –OH group with a $–B(OH)_2$ group. To do so, it is first necessary to convert the alcohol into a good leaving group, so then, via palladium catalysis under basic conditions, the boronic acid can be formed (figure 8).

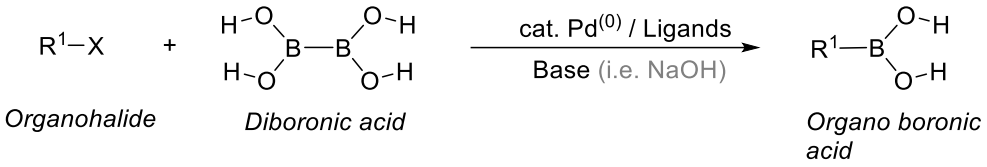


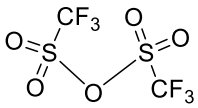
Figure 8

This reaction is quite similar to the Suzuki reaction as it is a cross coupling reaction with palladium as catalyst. It follows the same three steps as the previously explained Suzuki reaction (figure 8). The difference in this reaction is that, instead of using an arylboronic acid in the transmetallation step, a diboronic acid is used, then, instead of the formation of a C-C bond, as it happens in Suzuki, a C-B bond will be formed, producing the desired arylboronic acid (that can be used to perform a Suzuki reaction).

In this specific case, as the starting point is the estrone, the first step is the modification to get a good leaving group (X) instead of the to get an R-X type molecule. To do so, we will perform a esterification of the phenol with triflic anhydride (figure 10) via pyridine (figure 9) which activates the reagent **(10).** Triflates can participate in oxidative addition reactions like halides, and therefore can be used in Pd-catalyzed cross-couplings. This characteristic allows a great yield on the reaction.

Pyridine

Figure 9



trifluoromethanesulfonic anhydride

Figure 10

Following this sulfonation reaction (figure 11) we obtain our desired R-X type molecule:

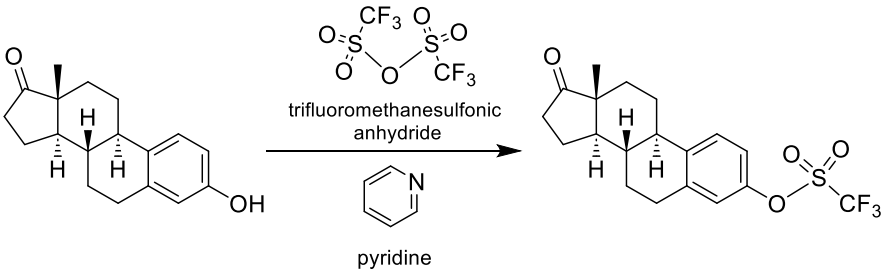
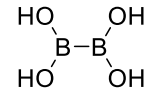


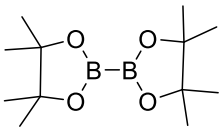
Figure 11

After this reaction, now we have a very good leaving group capable to coordinate with palladium and perform the catalyzed cycle. As said before we will use diboronic acid (figure 12) to obtain the arylboronic acid (**9**). Other way to introduce the boronic group is using bis(pinacolato)diboron (figure 13) (**11**). The divergences between these two methods are the number of steps used to obtain the final product **A**, as after the introduction of the borate group a deprotection of the OH groups must be performed. Due to this fact, the reagent used will be diboronic acid as its requires less steps and provide a better yield.



Diboronic acid

Figure 12



Bis(pinacolato)diboron

Figure 13

Therefore, the catalyzed reaction that will take place follows the following scheme (figure 14):

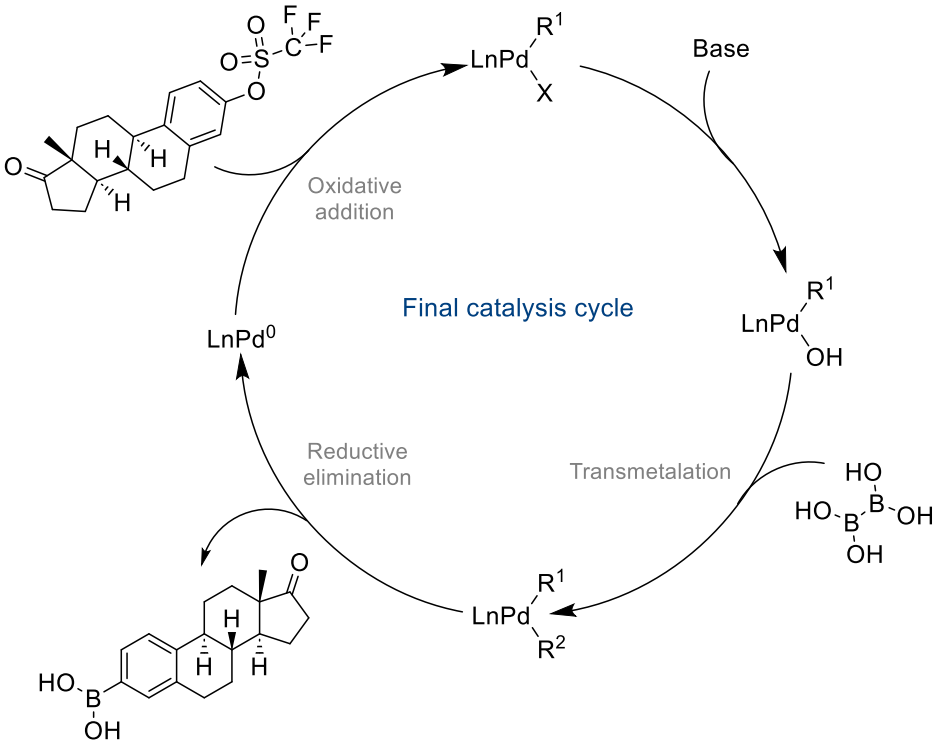


Figure 14

Where the symbols in the cycle mean (figure 15):

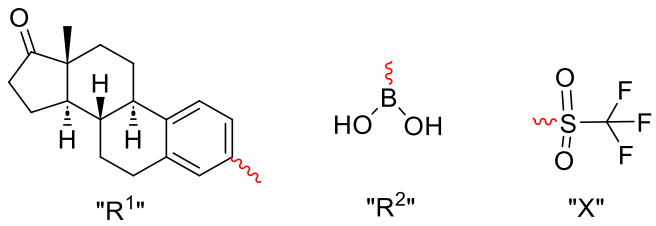


Figure 15

There are two ways to obtain estrone derivatives through Suzuki reaction. Estrone can either be used as a boronic acid derivative, and treat it with another molecule containing an halide (ie.: triflate), or the halide-like group can be in the estrone and be treated with another organic molecule containing a boronic acid group. These two options will lead to the same product.

In this experiment we perform the reaction using estrone with a halide-like group, triflate. Two different estrone derivatives are formed, the (8*R*,9*S*,13*S*,14*S*)-3-((*E*)-5-chloropent-1-en-1-yl)-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-17H-cyclopenta[a]phenanthren-17-one **(P1)** and (8*R*,9*S*,13*S*,14*S*)-3-(benzo[*b*]thiophen-2-yl)-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-17H-cyclopenta[*a*]phenanthren-17-one **(P2)** (figure 16).

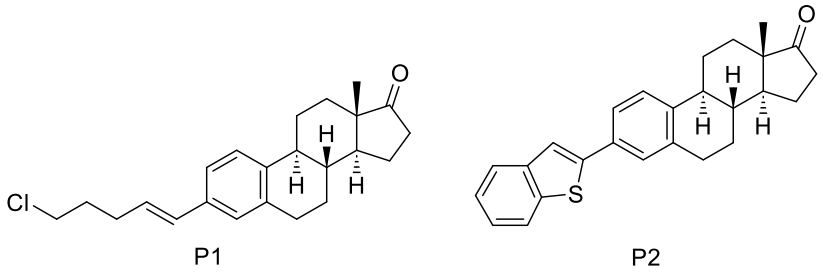


Figure 16

2. EXPERIMENTAL PROCEDURES

2.1 General considerations:

All reactions were performed by conventional heating; the bath was controlled by digital temperature regulator. Reactions were carried on in Schlenk tubes. All reactants used are commercial. NMR spectra were recorded in $CDCl_3$ and CD_2Cl_2 at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 282 MHz for ^{19}F with tetramethylsilane as internal standard for ^{1}H and the residual solvent signals as standard for ^{13}C. The data is being reported as s= singlet, bs= broad singlet, d=doblet, dd= double doublet, t= triplet, dt =double triplet, q= quatriplet and m= multiplet or unresolved, chemical shifts in ppm and coupling constant(s) in Hz. Melting points are uncorrected and were measured in a Gallenkamp apparatus.

2.2 Synthesis of the estrone triflate ((8*R*,9*S*,13*S*,14*S*)-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl trifluoromethanesulfonate)

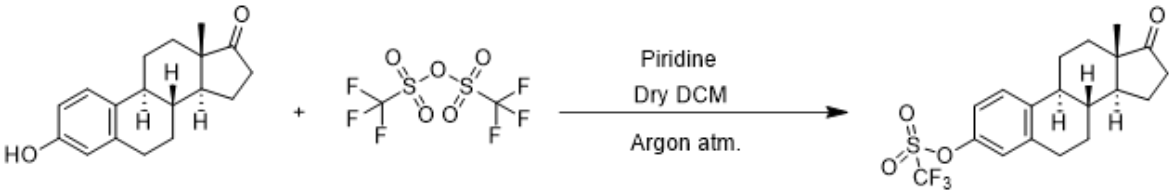


Figure 17

To a Schlenk tube equipped with a stirring bar were added 0.5 g of estrone (1.85 mmol), then the Schlenk was sealed and evacuated/back filled with argon 3 times. 10 mL of dry DCM (dichloromethane) and 0.49 mL of pyridine (3 equivalences) are added successively to the Schlenk through a counter flow of argon. Then, the tube is cooled with an ice bath to 0 ºC. With a syringe, added dropwise 0.19 mL (1.05 equivalence) of triflic anhydride and after the addition finished, the reaction was stirred at 0 ºC for 60 minutes and 18 hours at room temperature. Then to finish the reaction 15 mL of 1 M HCl were added to the solution. After finishing the reaction, the organic compounds were extracted with 5 mL (3 times) of DCM. The combined organic layers were washed with 5 mL (3 times) of brine, dried with Na_2SO_4 and concentrated under reduced pressure **(9)**. The white solid was purified through a silica gel column with Hex/AcOEt 5:1 eluent obtaining 0.639 g of estrone triflate (86% yield) as a white solid. This compound showed value for Rf= 0.47 for Hex/ AcOEt 5:1 eluent.

The signals for 1H obtained for the estrone triflate were:

1H NMR (300 MHz, $CDCl_3$): δ 7.34 (d, J = 8.6 Hz, 1H), 7.21 – 6.99 (m, 1H), 7.00 (t, J = 4.2 Hz, 1H), 2.93 (dt, J = 9.6, 4.8 Hz, 2H), 2.59 – 2.12 (m, 4H), 2.15 – 1.90 (m, 3H), 1.76 – 1.35 (m, 5H), 1.26 (dt, J = 14.0, 6.8 Hz, 1H), 0.92 (s, 3H) ppm.

^{13}C NMR (75 Hz, $CDCl_3$): δ 220.5, 147.7, 140.4, 139.4, 127.3, 121.4, 118.9, 118.4, 50.5, 48.0, 44.2, 37.9, 35.9, 31.6, 29.5, 26.2, 25.8, 21.7, 13.9 ppm.

^{19}F NMR (300 Hz, $CDCl_3$): δ -73.1 ppm.

2.3 Synthesis of Estrone boronic acid.

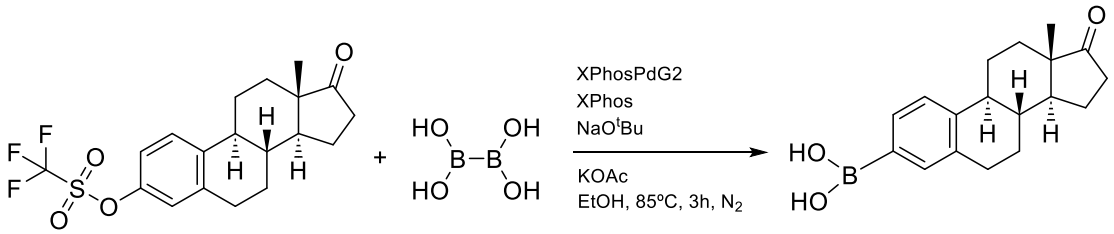
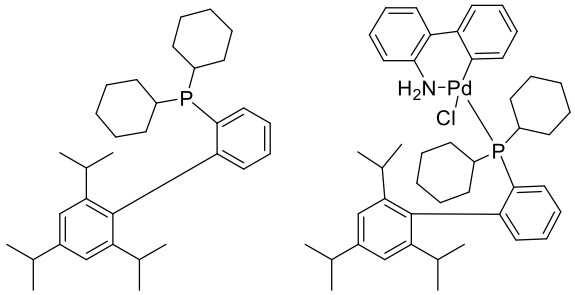


Figure 18

Estrone boronic acid was prepared by the following method. To a 50 mL Schlenk flask equipped with a stirring bar added estrone triflate (0.150 g, 0.497 mmol, 1.00 equivalence), hypodiboric acid (0.189 g, 0.745 mmol, 1.5 equivalence), XPhos-Pd-G2 (figure 19) (3 mg , 0.005 mmol, 1% mol), XPhos (figure 19) (5.1 mg, 0.01 mmol, 2% mol), sodium tert-butoxide (0.65 mg, 0.01 mmol, 2% mol), and potassium acetate (0.113g, 1.49mmol, 3 equivalence). Ethanol (10 mL) was added under N_2 atmosphere. The reaction was heated at 85 °C for 3 hours. Then the flask was cooled to room temperature, the reaction mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in 5 mL of dichloromethane and washed with brine in a separatory funnel **(9)**.

However, although all steps done were performed as the literature wrote, the reaction did not take place as, when performing the ^1H NMR for the crude organic residue, the signals coincide with the ones for estrone triflate.



XPhos XPhos-Pd-G2

Figure 19

2.4.A Synthesis of Estrone pinacolato borate, method A

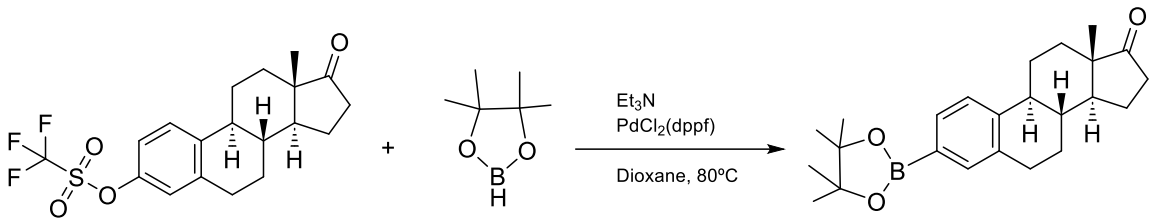


Figure 20

To a tube equipped with a stirring bar added 0.094 mL of Et_3N (0.666 mmol, 3 equivalence (trimethylamine), 0.31 mL of 1-4,dioxane (solvent), 89.3 mg of estrone triflate, 0.048mL of Pinacolborane (0.333 mmol, 1.5 equivalence) and 8.1 mg (5%mol) of $PdCl_2$(dppf) ([1,1'-Bis(diphenylphosphino)ferrocene] dichloropalladium). The mixture was heated to 80 ºC for 12 hours, then extracted with 5 mL (3 times) of Et_2O and washed the combined organic layer with brine (5 mL x 3 times). Then the organic layer was dried with Na_2SO_4 and the filtered residue solvent was removed in vacuo **(12)**.

However, although all steps done were performed as the literature wrote, the reaction did not take place as, when performing the ^{1}H NMR for the crude organic residue, the signals coincide with the ones for estrone triflate.

2.4.B Synthesis of Estrone pinacolato borate, method B.

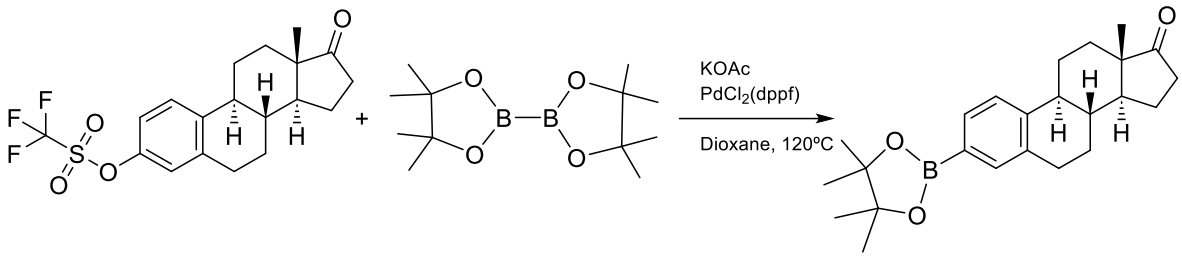
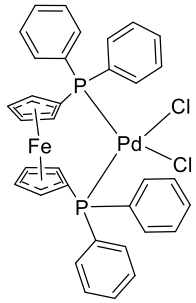


Figure 21

To a sealed tube equipped with a stirring bar, 0.300 g of estrone triflate (0.745 mmol), 0.0218 g of $PdCl_2$(dppf) (4% mol), 0.378 g B_2Pin_2 (1.49mmol, 2 equivalence), 0.2193 g KOAc (2.235 mmol, 3 equivalence) were added. Then a N_2 atmosphere was created inside the tube and 17.1 ml of dioxane were added. The mixture was heated to 120 ºC for 12 hours and extracted with AcOEt (5 mL x 3 times). The combined organic layers were washed with brine (5 mL x 3 times) and dried over Na_2SO_4, then solvent was eliminated in vacuo **(13)**. The solid residue was analized in ^{1}H NMR and the spectrum coincide with the one for estrone triflate, the reaction did not proceed.



$PdCl_2$(dppf)

Figure 22

This method was repeated with new reagents, but the reaction did not take place either.

2.5 Synthesis of (E-5-Chloro-1-penten-1-yl) estrone.

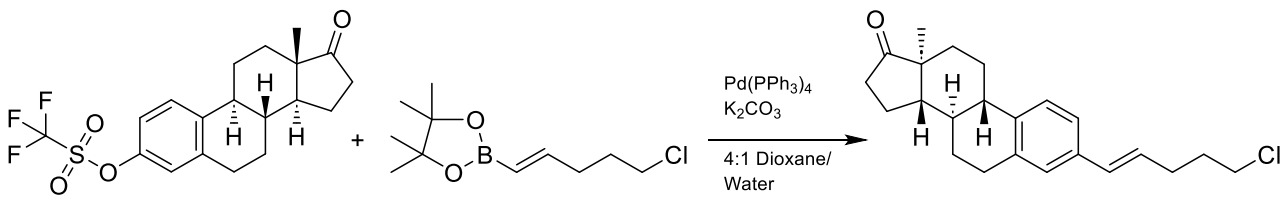


Figure 23

To a sealed flask equipped with a stirring bar, 0.114 g of estrone triflate (0.283 mmol), 0.1173 g of K_2CO_3 (0.849 mmol, 3 equivalence), 0.033 g of $Pd(PPh_3)_4$ (10% mol), 0.074 mL of (***E***)-2-(5-chloropent-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.322mmol, 1.15 equiv) and 4 mL of 4:1 dioxane/water as solvent. The mixture was sealed and heated to 100 ºC for 18 hours. The reaction was cooled to room temperature and extracted with AcOEt. The combined organic layers were washed with brine and dried over Na_2SO_4, then the solvent was removed under vacuo. The crude solid product was then dissolved into dichloromethane and purified by column chromatography using Hex/AcOEt 5:1 as eluent **(14)**. The white solid obtained weighted 0.095 g yielding 94%.

RF=0.25 in 5:1 Hex/AcOEt, melting point of 101.1-102.3 ºC and the recorded NMR spectra showed the following signals:

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.2 (d, J = 8.1 Hz, 1H), 7.2 (dd, J = 8.0, 1.9 Hz, 1H), 7.1 (d, J = 1.9 Hz, 1H), 6.4 (d, J = 15.8 Hz, 1H), 6.1 (dt, J = 15.8, 7.0 Hz, 1H), 3.6 (t, J = 6.6 Hz, 2H), 2.9 (dd, J = 9.0, 4.2 Hz, 2H), 2.6 – 2.3 (m, 5H), 2.2 – 2.1 (m, 1H), 2.1 – 2.0 (m, 2H), 1.9 (dq, J = 13.8, 6.8 Hz, 3H), 1.7 – 1.3 (m, 6H), 0.9 (s, 3H) ppm.

^{13}C NMR (75 MHz, $CDCl_3$): δ 221.0, 138.8, 136.6, 135.1, 130.9, 128.1, 126.6, 125.6, 123.4, 50.5, 48.0, 44.4, 44.3, 38.2, 35.9, 32.1, 31.6, 30.1, 29.4, 26.5, 25.7, 21.6, 13.9 ppm.

135 DEPT ^{13}C NMR (75 MHz, CDCl3) δ 130.9, 128.1, 126.6, 125.6, 123.4, 50.5, 44.4, 38.2, 13.9 ppm.

2.6 Synthesis of estrone benzo[*b*]tiophene.

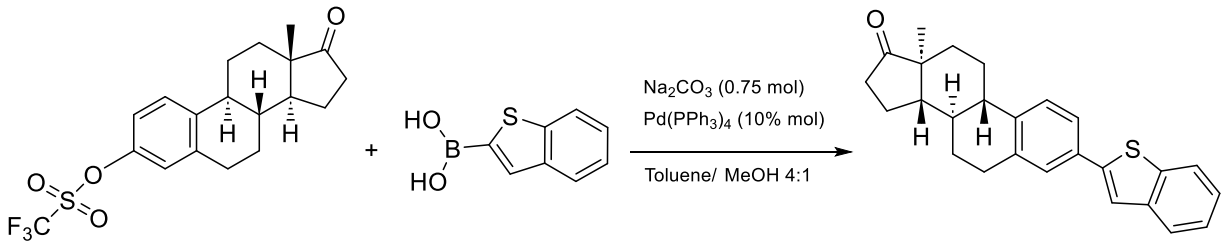


Figure 24

To a sealed tube 0.153 g of estrone triflate (0.380mmol), 0.02953 g of Na_2CO_3 (3 equivalence), 0.04437 g of $Pd(PPh_3)_4$ (10% mol), 0.06763 g of benzo[*b*]thiophen-2-ylboronic acid (1.5 equivalence) and 5 mL of toluene/MeOH 4:1 as solvent. The mixture was stirred heated to 100 ºC for 18 hours. The mixture was extracted with AcOEt and the organic layer was washed with brine. The combined organic layer was dried with Na_2SO_4 and solvent was eliminated in vacuo. The resulting solid crude was dissolved in dichloromethane and purified by column chromatography using Hex/AcOEt/CHCl2 10:1:1 as eluent **(15)**.

The resulting pale orange solid showed a Rf=0.38 in Hex/AcOEt/CHCl2 5:1:1 and 0.1202 g were obtained, yielding 82%.

The melting point of the product was 200.1-201.3 ºC.

The sample was analyzed by NMR and the obtained signals were:

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 – 7.98 (m, 2H), 7.95 – 7.66 (m, 3H), 7.66 – 7.50 (m, 3H), 3.22 (dt, J = 9.3, 6.1 Hz, 2H), 2.80 – 2.49 (m, 3H), 2.45 – 2.11 (m, 4H), 1.96 – 1.66 (m, 6H), 1.19 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 131.31, 129.81, 128.71, 127.90, 126.44, 126.12, 125.53, 125.32, 124.10, 120.77, 56.11, 55.75, 55.39, 55.03, 54.66, 52.36, 49.75, 46.37, 39.97, 37.69, 33.54, 31.32, 28.30, 27.62, 23.41, 15.59.

3. RESULTS AND DISCUSSION

3.1 Synthesis of the estrone triflate.

The first step required to develop the project was the transformation of estrone into the triflate by reaction with triflic anhydride which consists on the addition of a triflate group to the estrone through triflic anhydride and pyridine under an argon inert atmosphere (figure 25).

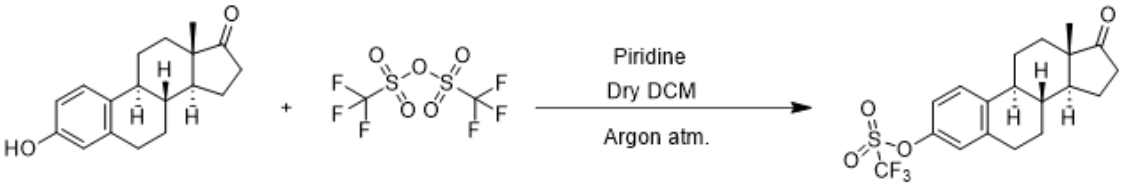


Figure 25

Mechanism (figure 26):

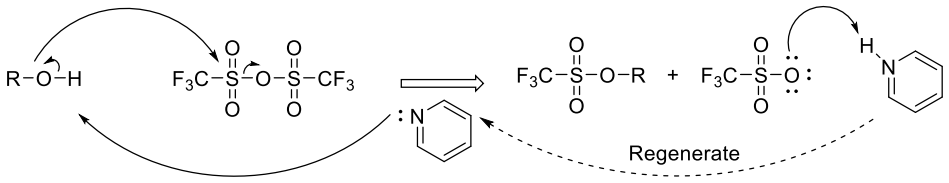


Figure 26

This reaction worked as expected according to the literature precedents **(9)**. It was obtained an 86% yield of the triflate. The key point of this reaction was the creation of an inert atmosphere (with argon) and the use of dry solvents as the triflic anhydride can be easily hydrolyzed by water or air. Due to the toxicity of pyridine, this reaction had to be prepared in a fume hood. The reaction is carried on in an ice bath to keep 0 ºC during the time it is working. The reason for this is that this reaction is exothermic and the boiling point of the solvent (DCM) is quite low (39.6 ºC, 1 atm. The mixture presents a turbid pale orange color when all reagents are added. Once the reaction time ended, the

mixture presents a clear pale brown color. The reaction is finished when adding an acid, HCl in this case. The addition of an acid produces the hydrolysis of the triflic anhydride that remains in solution, making it unable to keep reacting with estrone and also easier to extract as the trifluoromethanesulfonic acid is prone to go to the aqueous phase.

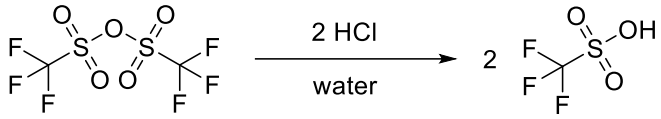


Figure 27

The final product, the estrone triflate, presents 21 Hydrogen atoms, 19 Carbon atoms and 3 Fluor atoms. The presence of a distinguishable signal in the 19F NMR indicates that the formation of the triflate has indeed taking place.

^{1}H NMR (300 MHz, $CDCl_3$):

Due to the complexity of the signals, there is not 100% confident in the assignment of them all. However, there are some characteristic signals that can be assigned. The singlet at 0.9 ppm corresponds to the hydrogens of the methyl. The signal around 2.9 ppm for 2 atoms correspond to the 2 hydrogens in the carbon next to the carbonyl and they are the most de-shielded in the non-aromatic area due to this functional group. The aromatic part presents signal for 3 atoms, one more de-shielded (31) and two almost equivalent (32 and 33) next to the carbon substituted with the triflate. The rest of the ^{1}H NMR can be seen at figure 28.

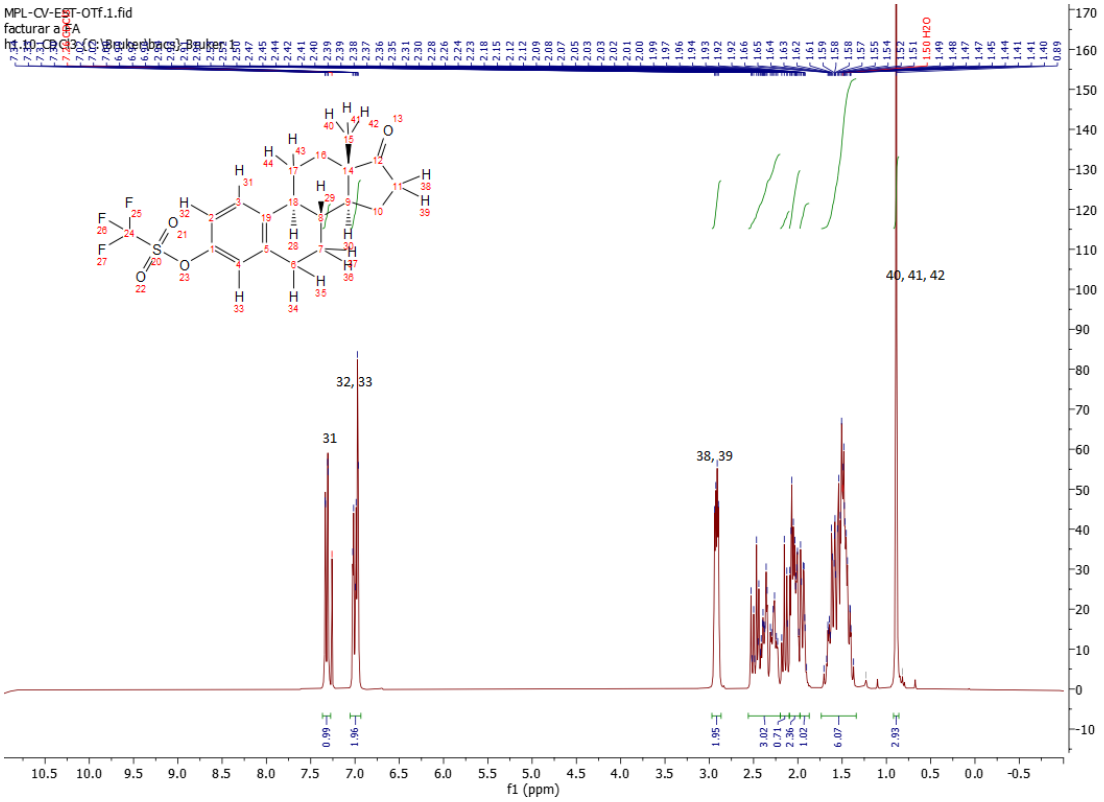


Figure 28

^{13}C NMR (75 MHz, $CDCl_3$):

This spectrum is also difficult to analyze but the main signals are the carbonyl which signal appears to the left in the spectrum and the methyl which appear to the right.

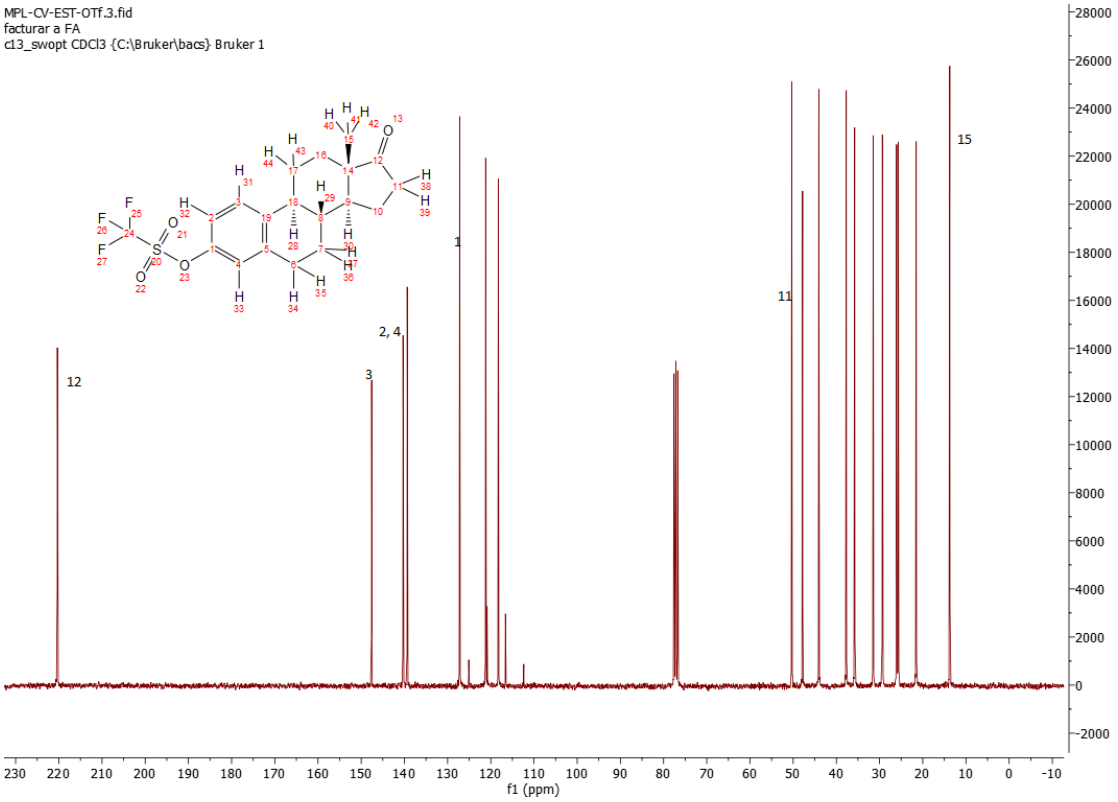


Figure 29

This assignment is borne out by the DEPT analysis for the positive (+y) signals:

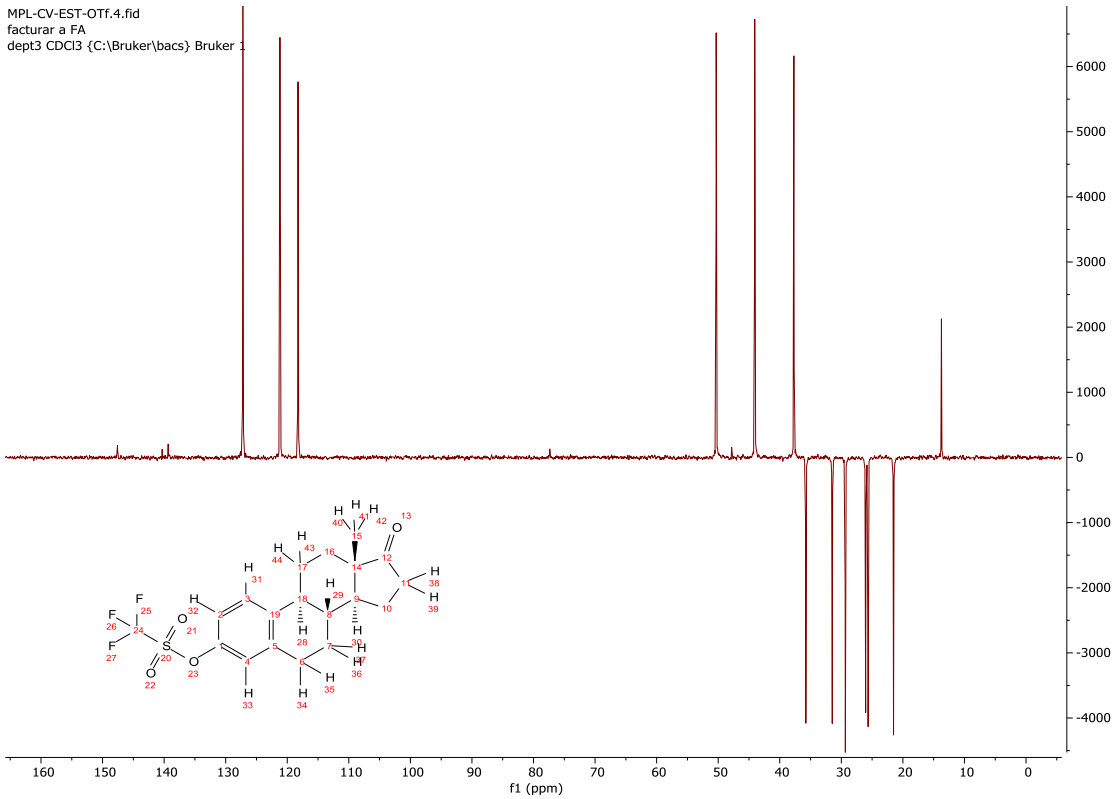


Figure 30

In DEPT analysis the signals for CH_2 grow towards the bottom part of the spectrum and the quaternary carbons do not appear.

As the molecule presents 3 atoms of Fluor as substituents for the same carbon, the signal is a singlet for three atoms in the ^{19}F NMR spectrum.

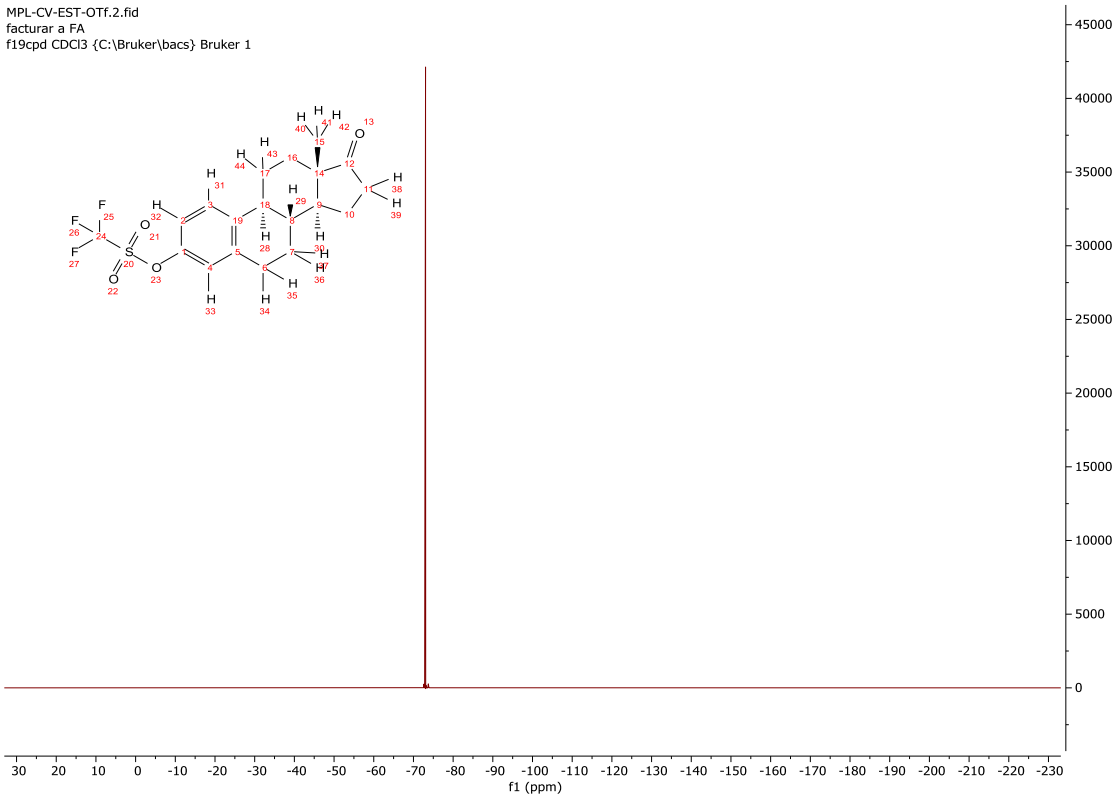


Figure 31

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3.2 Substitution of the triflate.

To perform the substitution three different reactions were performed. All of these reactions follow the Suzuki mechanism with a palladium species as catalyst. The first reaction (figure 32):

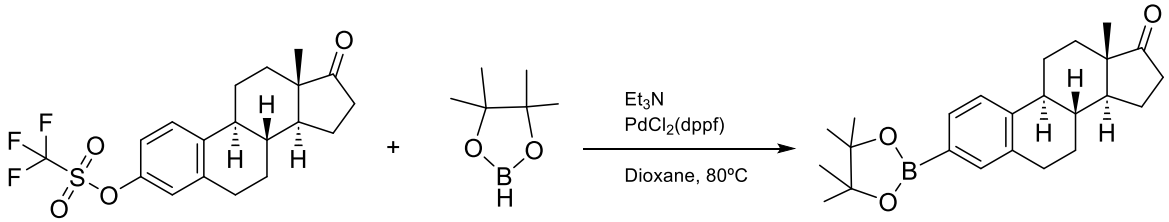

Figure 32

The second reaction (figure 31):

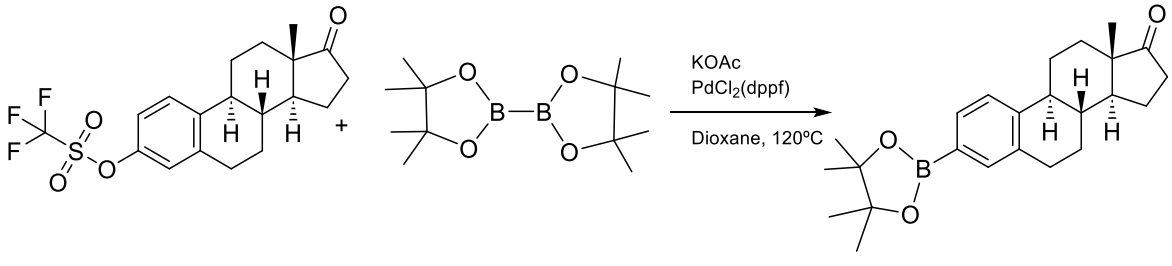

Figure 33

And the third reaction was the same as the last one but changing the conditions, we used NaOAc as base and the working temperature was 110ºC.

Unfortunately, none of them showed a positive result as the spectrum obtained was the same for all three and equal to the estrone triflate previously prepared.

The possible reasons for the incompletion of the reactions are 2: the bad state of the K(OAc) used, which we had to dry as it was very wet, that may introduced some water to the mixture; and the wrong control of the temperature of reaction, as the thermal controller stopped working at certain temperature.

I can determine they are all the same compound as all the spectra show the same peaks at the aromatic region. As for the three reactions, I expected three different products, the shifts and couplings must differ in this region.

3.3 Synthesis of (E-5-Chloro-1-penten-1-yl)estrone.

An alternative to prepare derivatives of estrone is to perform a directly Suzuki reaction between the estrone triflate and a boronic acid. To illustrate this concept, the reaction between an alkenylboronic acid ((*E*)-2-(5-chloropent-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane) and estrone triflate was performed. The reaction was carried out employing $Pd(PPh_3)_4$ as the catalyst and K_2CO_3 as the base and performed under inert atmosphere (nitrogen) to avoid degradation of the palladium catalyst.

Again, this reaction follows the Suzuki mechanism:

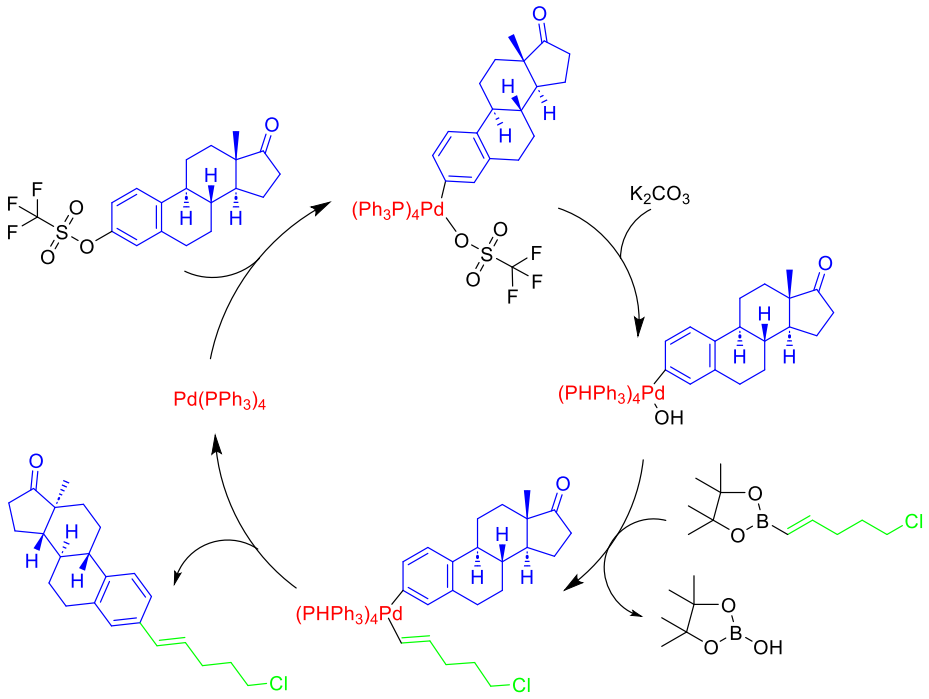

Figure 34

The yield of the experiment was 94%, similar to the yields reported in the literature for similar cross-couplings.

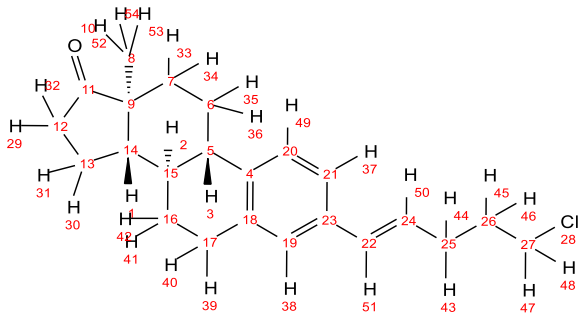

Figure 35

Analysis of the ^{1}H and ^{13}C NMR spectrum indicate that the coupling reaction occurred as expected. In particular, the signals between 6.13 and 6.39 ppm in the 1H NMR spectrum are a clear indication of the presence of a *trans* double bond 6.39 (dt, J = 15.8 Hz) 6.13 (dt, J = 15.8, 7.0 Hz). Other representative signal corresponds to the triplet that appears at 3.58 ppm, which corresponds to the CH_2 group bonded to the chlorine atom and the signal that appears as a doublet of doublets at 2.91 ppm corresponds to the hydrogens in the carbon (12) next to the carbonyl group. The last characteristic signal is a singlet at 0.91 ppm that corresponds to the hydrogens on the methyl group. The rest of the signals in the ^{1}H and ^{13}C NMR spectrum are assigned in figure 35 and 36.

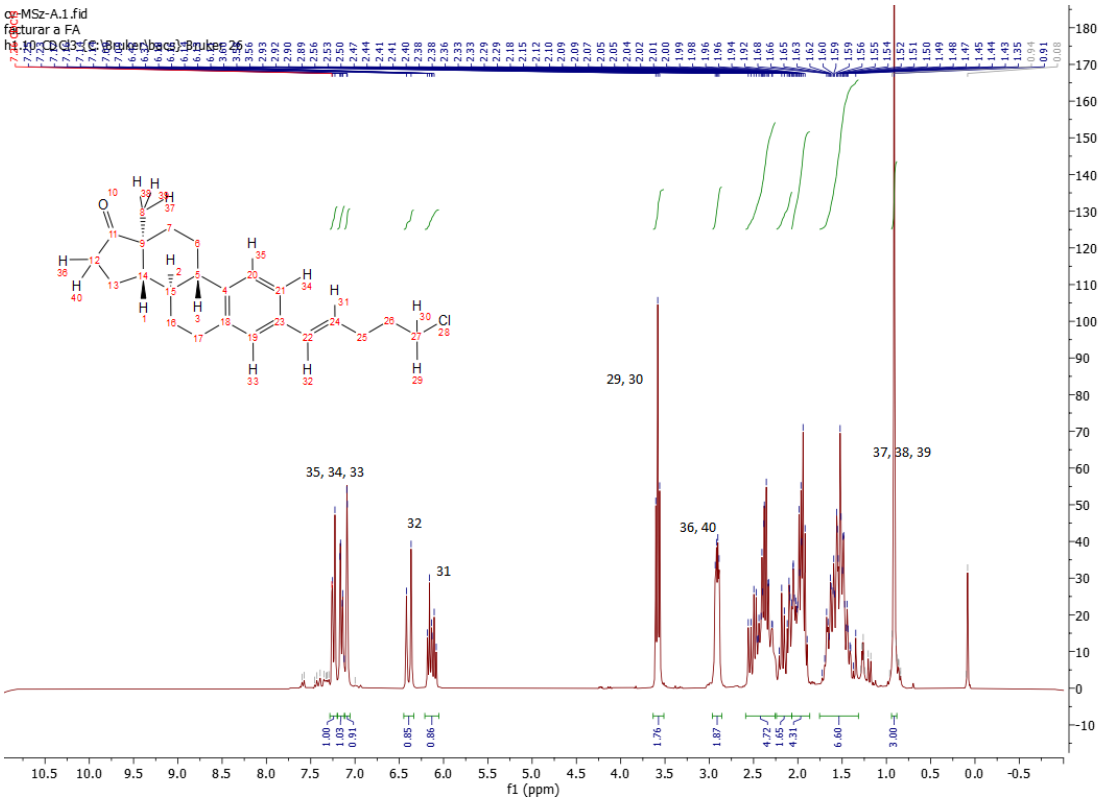

Figure 36

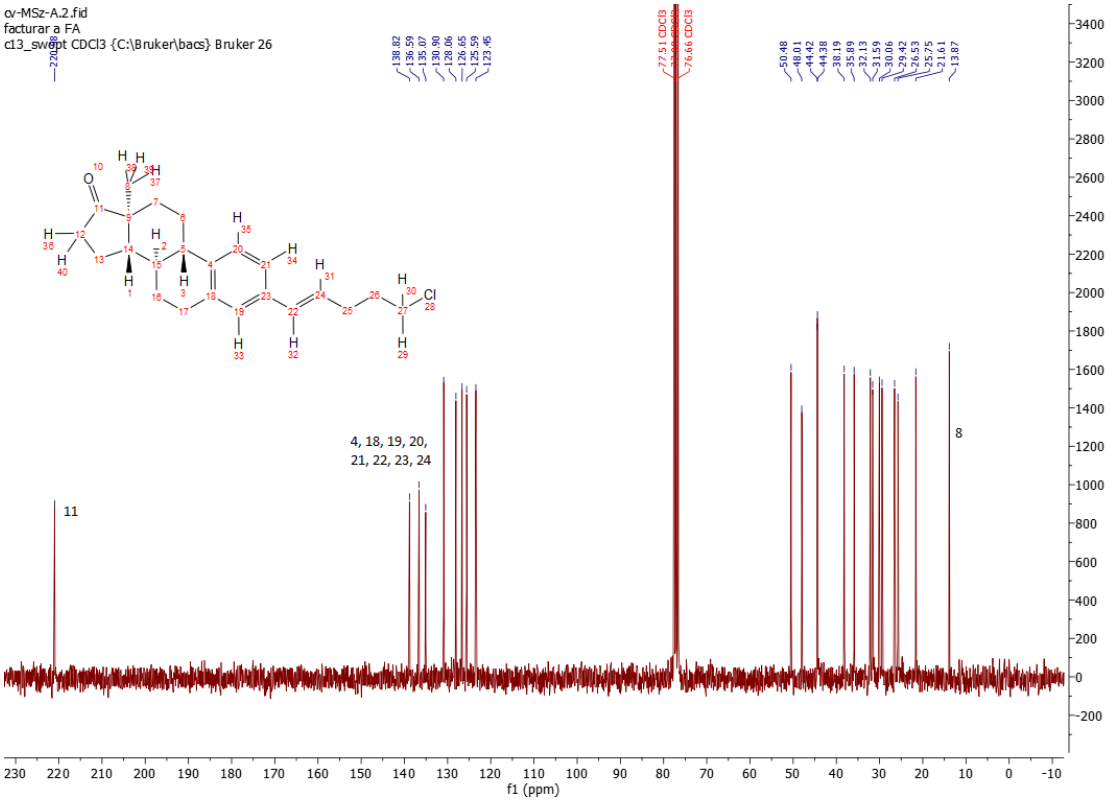

Figure 37

In the ^{13}C NMR spectrum can be highlighted the signal at 220 ppm that belongs to the carbonyl and eight signals between 120 and 140 ppm, six aromatic Carbons and the two C atoms of the alkene, and the signal at 13.7 ppm corresponds to the carbon of the methyl group.

3.4 Synthesis of (benzo[*b*]tiophene-2-)estrone.

A second Suzuki reaction was attempted employing benzo[*b*]thiophene boronic acid, to attach a heterocycle to the aromatic ring of the steroid. The reaction conditions were similar to those employed for the reaction above. However, in this case, the solvent used was a mixture of toluene/MeOH 4:1 and the base was Na_2SO_4. The mechanism of this reactions also follows the Suzuki coupling scheme.

The product obtained was an orange solid with a melting point of 200.1-201.3 ºC. This value is quite high compared with other estrone derivatives that shown a mp around 105-120 ºC. The increase of the melting point can be explained with the presence of the S atom and the size of the molecule. This reaction gave a yield of 81 %after purification by column chromatography .

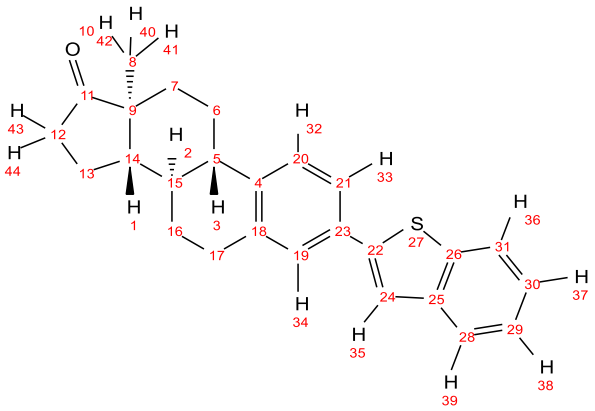

Figure 38

A quite complex set of signals, corresponding to 8 hydrogens, appears in the aromatic region, due to the overlap of the three signals that correspond to the estrone and the five signals that belong to the benzothiophene. A signal for 2 hydrogens appear around 2.9 ppm and corresponds to the hydrogens (43 and 44) next to the carbonyl. The last characteristic signal is the one that appears at 0.87 ppm as a singlet for 3 hydrogens and corresponds to the hydrogens in the methyl group. The rest of the signals can be seen in the next figure (figure 39).

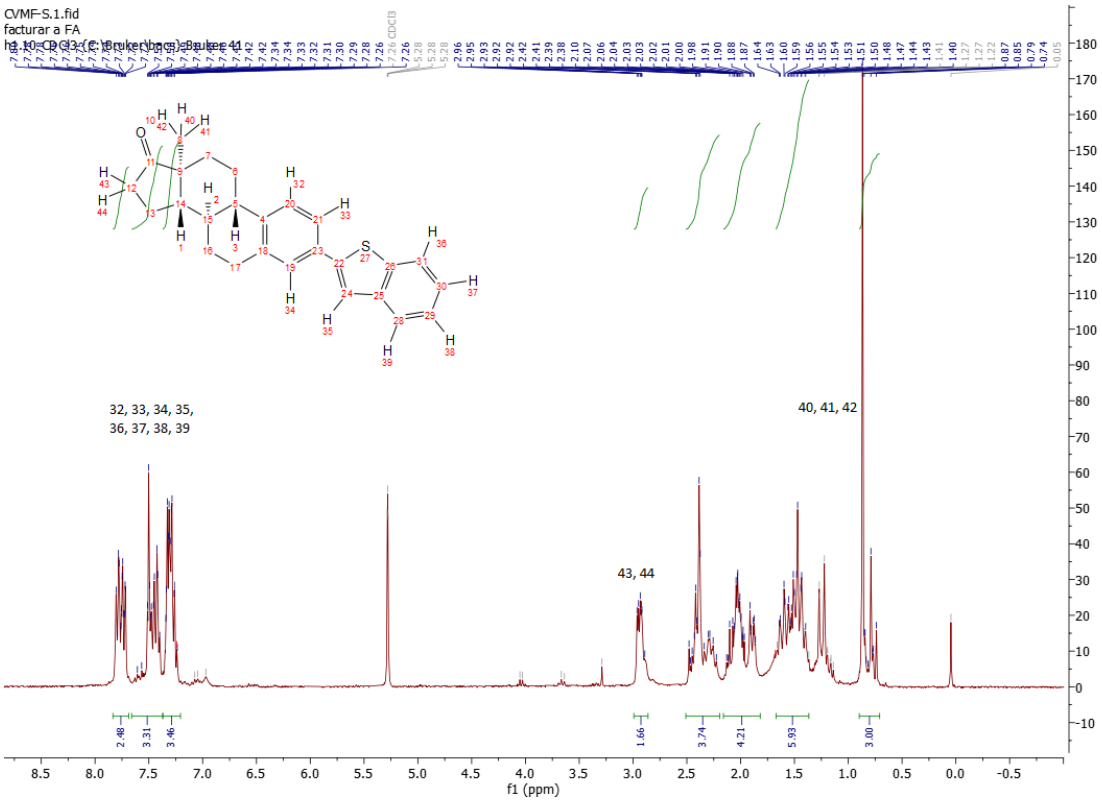

Figure 39

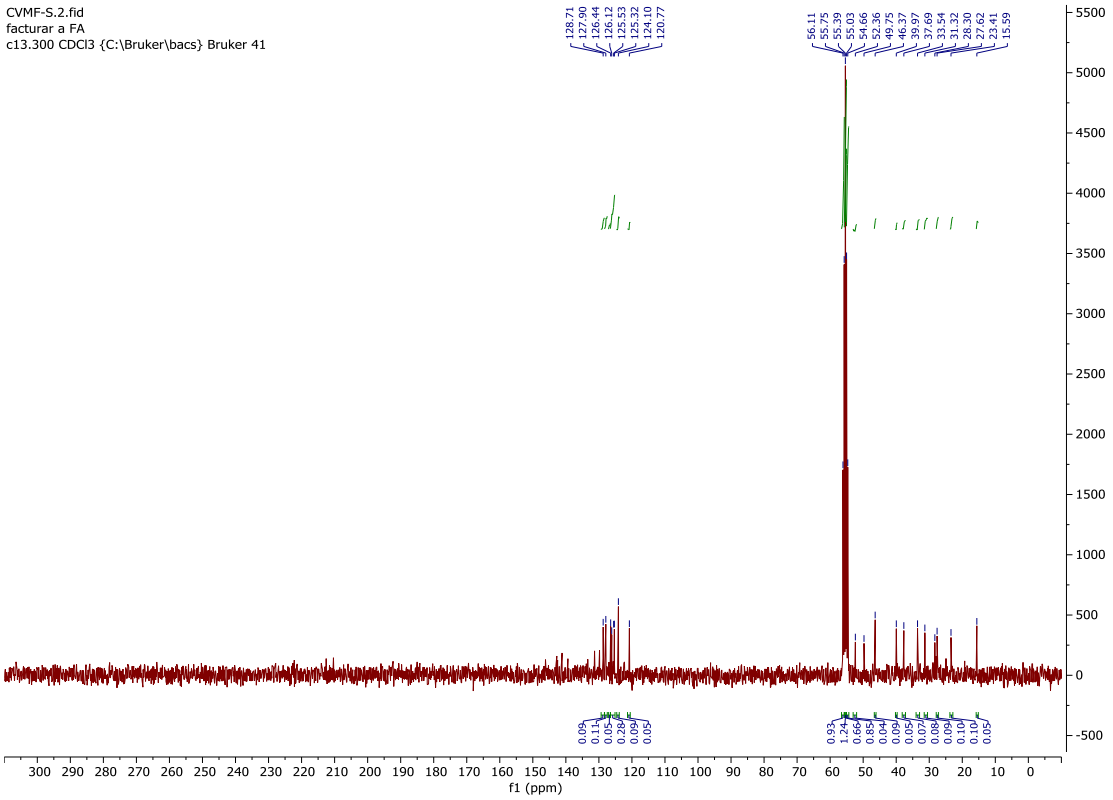

Figure 40

The signals for the carbon in position 11 (carbonyl) and carbon in position 22 appear with a small band as the sample was analyzed in low concentration. This low concentration also produces the high ratio noise/signal in the spectrum.

^{13}C NMR DEPT 135 (75 MHz, $CDCl_3$) δ 128.69, 127.90, 126.43, 126.11, 125.53, 125.31, 124.09, 120.76, 52.37, 46.37, 39.97 ppm.

This signals appear in the upper part of the spectrum and represent the aromatic carbons (signals to the left) and the carbons C-H carbons (5, 14, 15), represented in the three signals between 40 and 55 ppm. The data obtained in this spectra coincides with the one obtained in the ^{13}C NMR.

4. CONCLUSSIONS

The experiments conducted in this study aimed to synthesize various derivatives of estrone using different methodologies and reaction conditions. The results yielded significant insights into the reactivity and challenges associated with these processes.

a) Synthesis of Estrone Triflate:

The synthesis of estrone triflate was successful, achieving an 86% yield, close to the expected 88%. The key to this success was maintaining an inert atmosphere with argon and using dry solvents to prevent the hydrolysis of triflic anhydride. The reaction's exothermic nature required an iced bath to maintain the temperature at 0ºC.

b) NMR Characterization:

Detailed NMR characterization provided valuable data on the hydrogen and carbon environments within the synthesized estrone triflate. This precise assignment of NMR signals is crucial for confirming the structure and purity of the compound.

c) Challenges in Boronic Acid and Pinacolato Borate Syntheses:

The attempts to synthesize estrone boronic acid and estrone pinacolato borate encountered difficulties. Despite following literature protocols, the reactions did not proceed as expected, as confirmed by 1H NMR spectra which showed unreacted starting materials. This highlights the need for further optimization of reaction conditions or alternative catalytic systems.

d) Successful Synthesis of *(E)*-5-Chloro-1-penten-1-yl)estrone:

The synthesis of *(E)*-(5-Chloro-1-penten-1-yl)estrone was achieved with a 94% yield. This high yield underscores the efficacy of the selected conditions, including the use of $Pd(PPh_3)_4$ as a catalyst and the reaction setup under a nitrogen atmosphere. The NMR data confirmed the successful formation of the product.

e) Reaction Mechanisms and Conditions:

Throughout the experiments, the importance of reaction conditions was evident. Factors such as inert atmospheres, temperature control, and the use of specific solvents played critical roles in the success and reproducibility of the reactions.

f) Implications for Future Research:

The findings suggest several avenues for future research. For instance, exploring alternative catalysts or reaction conditions might improve the yields and success rates of boronic acid and pinacolato borate syntheses. Additionally, further studies could investigate the application of these synthetic methods to other steroidal compounds.

To sum up, this study provided valuable insights into the synthesis of estrone derivatives, highlighting both successes and challenges. The detailed NMR characterizations and the high-yield synthesis of *(E)*-(5-Chloro-1-penten-1-yl)estrone represent significant achievements. However, the difficulties encountered in other synthetic routes underscore the complexity of these reactions and the need for ongoing optimization.

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