UNIVERSITÀ DEGLI STUDI DI PAVIA

DOTTORATO IN SCIENZE CHIMICHE E FARMACEUTICHE

XXIX CICLO

Coordinatore: Chiar.mo Prof. Mauro Freccero

NOVEL TITANOCENE DERIVATIVES FOR DIAGNOSIS AND THERAPEUTIC PURPOSES.

Tutore

Chiar.mo Prof. Giuseppe Zanoni

Tesi di Dottorato di

ANDREA GANDINI

a.a. 2015- 2016

Contents

Chapter 1: Introduction

1.1 Organometallic chemistry

Organometallic chemistry is an important area of chemical sciences that studies compounds containing at least one bond between a carbon atom of an organic compound and a metal, including alkaline, alkaline earth, transition metal, and other cases[1]. Moreover, some related compounds such as transition metal hydrides and metal phosphine complexes are often included in discussions of organometallic compounds. The organometallic chemistry research field combines aspects of traditional inorganic and organic chemistry[2]. Organometallic compounds are widely used both stoichiometrically in research and industrial chemical reactions, as well as in the role of catalysts to increase the rates of such reactions (as in uses of homogeneous catalysis), where target molecules include polymers, pharmaceuticals, and many other types of useful compounds. In recent decades new types of organometallic compounds have been isolated. One of the first compounds, has a typical structure with two Ciclopentadienyl (Cp) rings centered on an iron atom . The name of that structure is Ferrocene (Figure 1) and is considered the founder of the family of Metallocenes.[3]

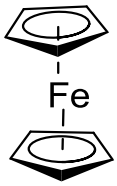


Figure 1: Structure of Ferrocene

These compounds are frequently called “sandwich complexes” for their typical symmetry.[5] Structurally, the bis-cyclopentadienyl complexes can be classified into two classes, namely, the “classical” ones with parallel Cp rings and the “bent” metallocenes,

[1] Robert H. Crabtree, The Organometallic Chemistry of the Transition Metals, Wiley, 2005, p. 560
[2] O. José; E. Christoph; 2006, Organometallics. Weinheim: Wiley
[3] A. Federman Neto, A. C. Pelegrino, V. A. Darin; Trends Organomet. Chem.; 2002, 4, 147-169

which have other ligands (generally two halides atoms) bonded to the metal in addition to the Cp-rings (Figure 2).[4]

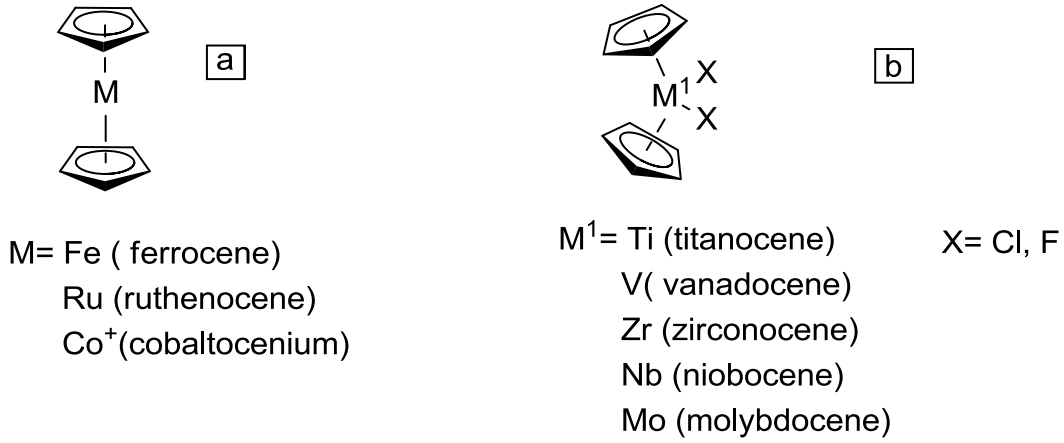


Figure 2: Differences of Classical(a) and Bent(b) metallocenes

The metallocenes used for medicinal applications contain metals from the iron and cobalt triad, with Fe, Ru, and Co . The bent metallocenes typically comprise elements from the early transition metals, most importantly Ti, Zr, V, Nb, Mo in a medicinal context. Interestingly, all metallocenes important in medicine field have a cis-dihalide motif as depicted above, which is similar to the cis-dichloro motif of the well-known anticancer drug cisplatin.[5]

[4] Wilkinson, G. J. Organomet. Chem. 1975, 100, 273.

[5] K€opf-Maier, P.; Hesse, B.; Voigtl€ander, R.; Kopf, H. J. Cancer Res. Clin. Oncol. 1980, 97, 31

1.2 Titanium and Titanocenes

Titanium is an element of the periodic table with atomic number 22 and symbol Ti. Titanium is one of the most abundant elements in the Earth's crust (ninth position, fourth position for metals) and is known for its high resistance to corrosive agents and for the high strength-to-weight relationship.[6]

Titanium was discovered in 1791 by the English chemist and mineralogist William Gregor and named in 1795 by the German chemist Martin Heinrich Klaproth. The metal is widely distributed in practically all rocks, sand, clay, and other soils but, the two prime commercial minerals are ilmenite and rutile.[7] Titanium is composed of 5 stable isotopes; ^{46}Ti, ^{47}Ti, ^{48}Ti, ^{49}Ti and ^{50}Ti, and the most abundant is ^{48}Ti.

Titanium has different oxidation states; but, while the chemistry of the Ti^{2+} is particularly restricted, +3 and +4 oxidation states are more widespread and are used in various fields. In fact $TiCl_3$ is widely used as a catalyst in the stereospecific polymerization of propylene to make the commercially valuable polymer polypropylene.[8]

Ti (IV) is the most stable oxidation state and the dioxide, TiO_2, is the most important compound. This nontoxic, pure and white powder is used extensively as a pigment in paints, enamels, and lacquers.[9] Of greatest interest, however, for chemical-biological research, is its biocompatibility through the oxide layer that forms on the surface, which is able to interact with the bone tissue. For this reason, pure titanium (CP4) and titanium alloy Ti_6Al_4V are used in hip and knee prosthetic components for the manufacture of surgical sutures and clips in dentistry for dental implants.[10]

As described above, the metallocene compound titanium-centered is titanocene. This compound presents two Cp-rings bonded on opposite on a titanium atom as in ferrocene, but moreover has two other ligands (normally two halides atoms) that can be varied to change the stability of the entire system.(Figure 2b)[11]

---

[6] M. R. Jr Donachie; TITANIUM: A Technical Guide. Metals Park, OH: ASM International, 1988.
[7] Titanium: Encyclopædia Britannica. 2006.
[8] a) K. S. Collins, M. P. McDaniel; 2009, WO2009042149; b) M. P. McDaniel, K. S. Collins, 2009, US 20090082197
[9] Krebs, Robert E. (2006). T ed.). Westport, CT: Greenwood Press
[10] *Oshida Y, Tuna EB, Aktören O, Gençay K; Int J Mol Sci, 11, 4, 2010, pp. 1580-1678,*
[11] Bochmann, Manfred. Organometallics 2; Oxford University Press: New York, 1994; pp 46, 47.

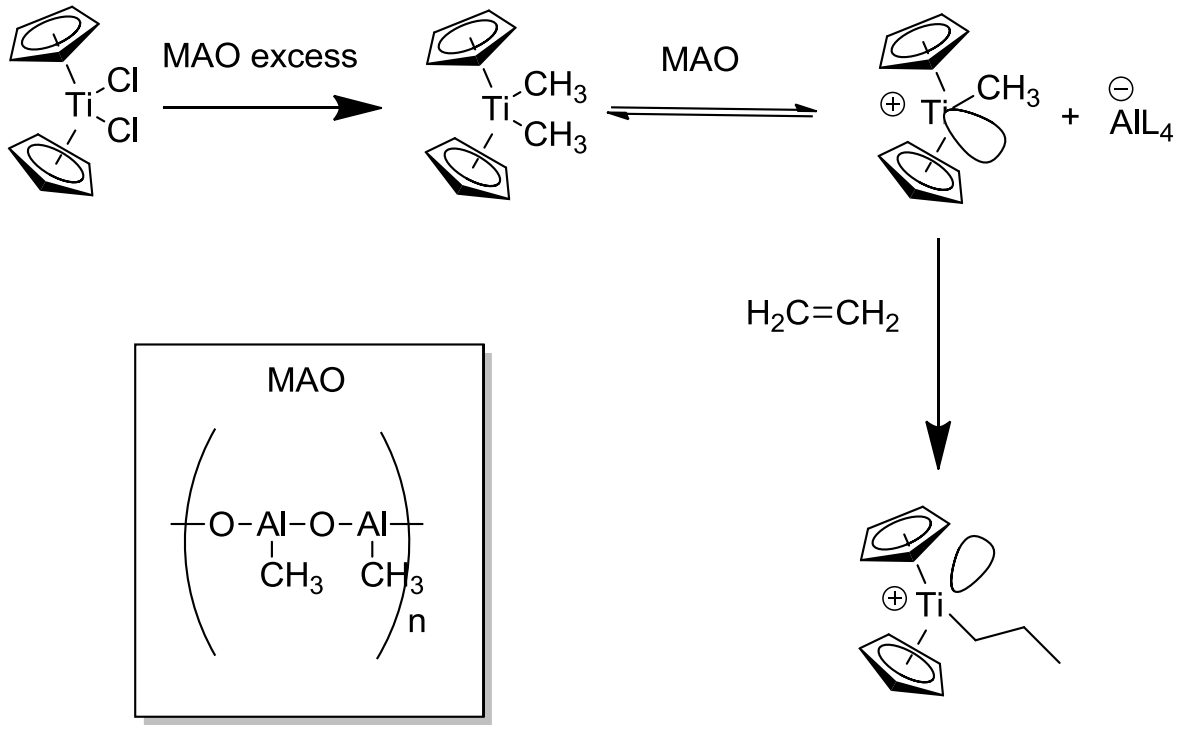
Titanocene complexes were synthesized, in a classical pathway, from the titanium tetrachloride with two equivalent of an alkali metal cyclopentadienide salt. (Scheme 1).

Scheme 1: Classical synthesis of titanocenes

Titanocenes derivatives are used in various fields of application, from plastic industry to medical oncology and, in particular, many commercially important processes have been developed to take advantage of organometallic complexes as catalysts.[12] The mechanism for the process of polymerization using titanocene catalysts is thought to be the same of Cossee–Arlman mechanism proposed in the 1960s for traditional Ziegler–Natta systems.[13] In toluene, with the cocatalyst methylalumoxane (MAO) (Scheme 2), the titanocene complex undergoes a fast ligand exchange, followed by removal of one methyl anion by MAO, to produce the active catalyst and a cocatalyst anion that stabilizes the charged system. Ethylene then reacts as a ligand with this active catalyst through coordinate bonding of its π electrons to titanium (Scheme 2).

[12] G. Wilkinson, J. M. Birmingham; J. Am. Chem. Soc., 1954, 74, 4281-4284

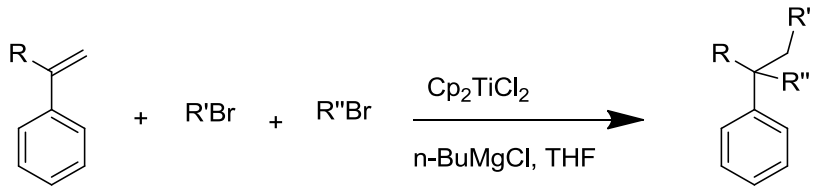
[13] Hartwig, J. F. Organotransition Metal Chemistry, from Bonding to Catalysis; University Science Books: New York, 2010.



Scheme 2: mechanism for the process of polymeration of polyhetylene using titanocene

The methyl group from titanium migrates to one of the carbons of the ethylene. At the same time, the π electrons of the ethylene ligand are used to form a σ bond between the other carbon and titanium: After thousands of repetitions, polyethylene is formed.[14]

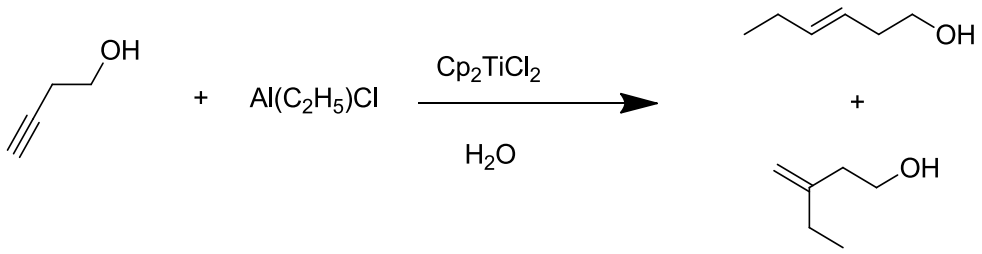
As already mentioned, titanocenes are largely used in organic synthesis and some examples are reported here. In fact, Titanocene dichloride can be used for alkylation reaction (a double alkylation) of styrene using mild conditions. Thus, is possible to prepare a wide range of alkyl-substituted benzenes (Scheme 3).[15]



Scheme 3: Titanocene in an alkylation reaction.

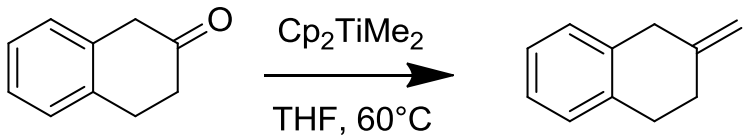
[14] Y. Quan, J. Huang, M. Bala, B. Lian, H. Zhang; Chem. Rev. 2003, 103, 2672-2673.
[15] J. Terao, K. Saito, S. Nii, N. Kombe, N. Sonabu; J. Am. Chem. Soc.; 1998, 120, 11822-11823

Titanocene dichloride is also used for alkylation of alkylols with an organoalluminium reagent. In particular, this protocol afford generally two products in a ratio 50:50. (Scheme 4)[16]



Scheme 4: alkylation of an alkyne-alchol

Other derivatives of titanocene are also used in organic synthesis: dimethyl titanocene, (also known as Petasis reagent) for example, has been used in a Wittig type reaction for an enolizable ketone, very difficult to run with classical reagents. (scheme 5) [17]



Scheme 5: Wittig-like reaction using Titanocene

Finally, the potential of titanocene as antitumor agent will be described in detail in a specific chapter of this thesis, in particular in chapter 5.

[16] J. D. Parrish, D. R. Shelton, R. Little; Org. Lett., 2003, 5, 3615-3616
[17] A. N. Petasis, E. I. Bzogej; J. Am. Chem. Soc., 1990, 112, 6392-6393

Chapter 2: Aim of the Thesis

The titanocenes, as described before in the introduction chapter, have been used in different fields of application. In particular, in the organic synthesis and in the industrial sector, we have confirmation of the pratical use of Titanocene derivatives. On the other hand, in the biological application the use of Titanocene derivatives and in particular the use of Titanocene dichloride (Cp_2TiCl_2) have not led to expected results.[18] Although are well known anticancer tests of Cp_2TiCl_2 *in vitro* and *in vivo*, the following tests on humans have been locked in phase two for poor efficiency.[18] Otherwise, further studies on the modification of this derivative were published and in this field is possible to insert this thesis work.

Indeed, in these three years we focused our attention on the synthesis of new Titanocene derivatives suitable for conjugation with biomolecules. In particular, we can divide this work in three main sections:

- Synthesis of a Titanocene bioconjugate for the biological application.
- Synthesis of cold fluorinated Titanocenes derivatives for the identification of a potential PET probe candidate.
- Solid Phase Synthesis of Titanocenes in order to explore new synthetic strategies and to reduce the purification problems in bioconjugation steps.

In practice we can summarize these concept in the structure presented in Figure 3

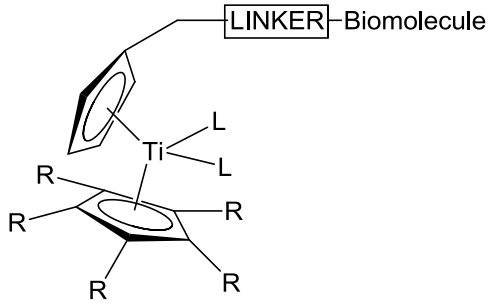


Figure 3: General structure of a modified Titanocene

We modified groups on a ciclopentadienil ligand (R-groups in Figure 3) to improve the hydrolytic stability of the complex, a necessary characteristic for biological application.

[18] P. Kopf-Maier and H. Kopfs Chem. Rev. 1987, 87, 1137-1152

Then, we worked on the ligand on titanium (L groups in Figure 3) to further influence the stability of the system and to find a binder that can be displaced rapidly by fluorides (important for PET applications). Finally we worked on the functionalization on the other Cp ring with a linker for the conjugation with biomolecules to improve solubility, efficiency, and selectivity.

Chapter 3: Bioconjugation

One of the main goals of this PhD Thesis was to combine the Titanocene core with a biomolecule, in order to prepare more bioavailable scaffolds and increase its biological activity. In recent decades the attention of the scientific world has increased towards the production of increasingly selective molecules for a specific purpose. In particular, in the field of anticancer therapy research, the development of efficient tumor-selective approaches remains a primary challenge. This necessarily led to achieve the development of different approaches. For example, is possible to change the compound of interest combining it with a biologically active molecule to improve their effectiveness and selectivity. Another approach is the ligation *in vivo* between a suitably modified cell surface and the compound of interest.[19]

This approach is very interesting, but, as solves one problem, it opens up a new challenge in another part of the process. In details, the requirements for a reaction to be suited for this process and to be considered a "bioorthogonal ligation reaction" are highly diverse and extensive. Namely, they have to be highly selective in order to specifically label only the intended target and minimize background labeling in a varied biological environment. Their products and educts have to be inert and remain stable toward any unwanted biological or chemical interactions. The reaction kinetics have to be favorable so that the ligation occurs before and the reaction partners can be cleared from the biological system. Furthermore, it has to be biocompatible, meaning that it has to work in physiological conditions and no toxic component must be present. Last but not least, the functional groups for the ligation reaction have to be as small as possible and should be readily available to enable the incorporation into biomolecules.

Throughout this series of issues we decided to develop the first approach considering later if it could be improved in the second one.

First of all we decided to synthesize new titanocene derivatives for the conjugation with biomolecules and, to do this we tried to evaluate conjugation-protocols that included mild conditions. Having regard to the considerable number of opportunities we chose

[19] E. Saxon, C. Bertozzi; Science, 2000, 287, 2007

certain types of Protocol, usually used in bioconjugation chemistry, that are compatible with the chemistry of titanocenes.

In detail, we focused our attention on:

- Staudinger Approach (Staudinger Ligation).
- “Click chemistry “ Approach.

In both cases we started with commercially available titanium compounds shown below. (Fig.4)

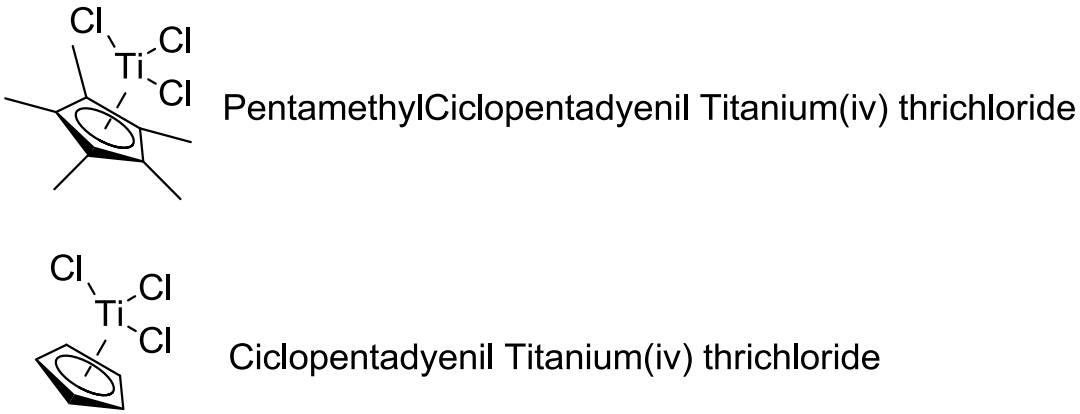
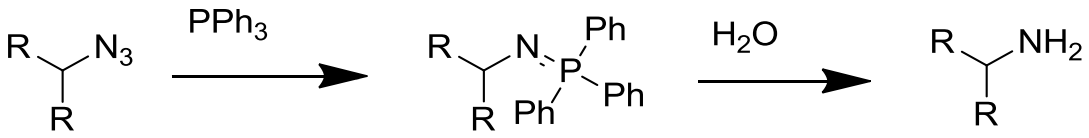


Figure 4: Titanium complexes used in this work

3.1.Staudinger Approach

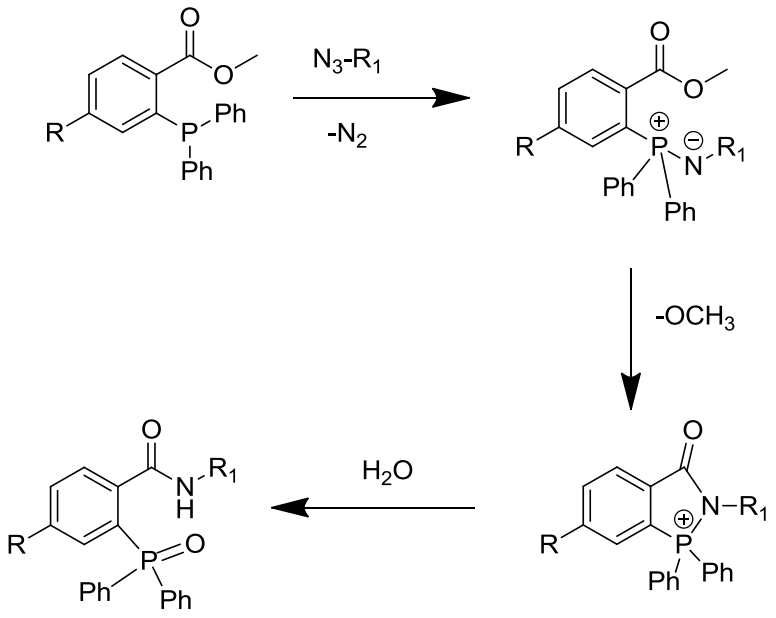
The classical Staudinger reaction is a reduction of an organic azide in to amine, triaryl posphine mediated.

The reaction of azide and posphine produce an amminoposphorane intermidate that, after aqueous work-up leads to the corresponding amine.[20] (Scheme 6)



Scheme 6: Classical Staudinger reaction

Unfortunately, initial covalent adduct, the aza-ylide, is not stable in water. The solution to this problem was to design a phosphine that would enable the rearrangement of the unstable aza-ylide to a stable covalent adduct. Bertozzi et Co-workers[21] had envisioned that an appropriately situated electrophilic trap, such as a methyl ester, within the phosphine structure would capture the nucleophilic aza-ylide by intramolecular cyclization leading to the formation of an amidic bond.[22] (Scheme 7)



Scheme 7: Staudinger Ligation mechanism

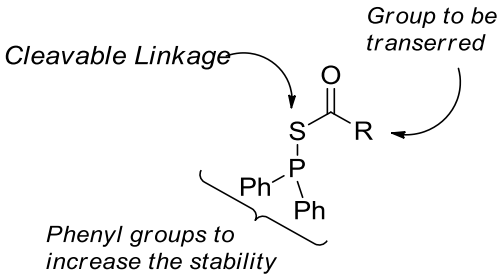
[20] W. Q. Tian , Y. A. Wang; J. Org. Chem. 2004, 69, 4299-4308.

[21] E. Saxon, J. I. Armstrong, C. R. Bertozzi; Org. Lett., Vol. 2, No. 14, 2000

[22] Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.

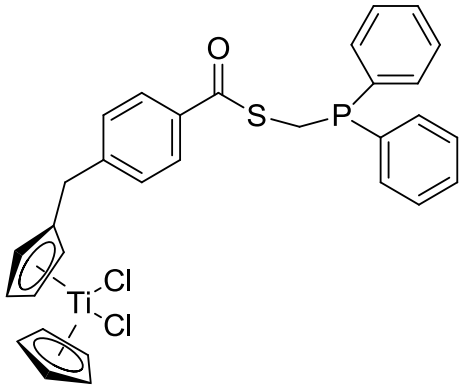
The phospine and the azide react with each other rapidly in water at room temperature in high yield. Both are abiotic and essentially unreactive toward biomolecules inside or on the surfaces of cells. Thus, in its modified form, the so called "Staudinger Ligation" reaction meets many of the criteria required of a chemoselective ligation in a cellular environment. [21]

Subsequently, modifications of Staudinger Ligation were developed to improve applications in organic synthesis[20]: the aim was to produce an amide bond without the intervening of the triarylposphine oxide group. For this application was developed by Bertozzi and co-workers a new posphine reagent, and this variant is known as "traceless Staudinger Ligation" (Scheme 8).[20]



Scheme 8: Posphine designed by Bertozzi

Despite not having direct information on titanocenes, in literature, works on ferrocene moiety are presented [23]. And seen the similarity between ferrocene and our compound we selected a target molecule that is shown in the figure below

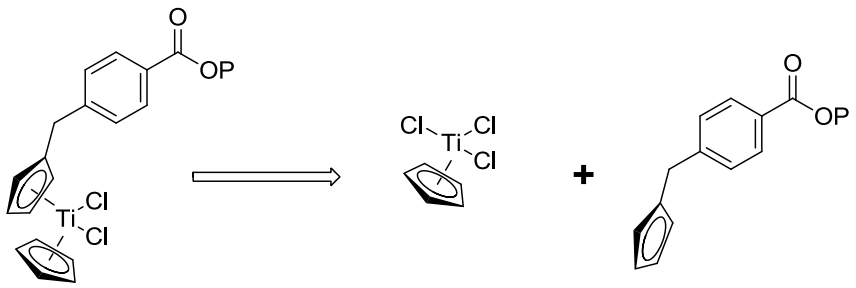


6a

Figure 5: Target compound for Staudinger ligation reaction.

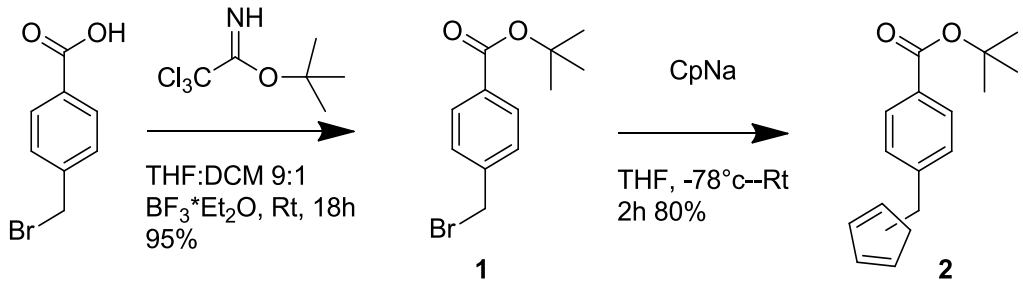
[23] I. Kosiova, A. Janicova and P. Kois; Beilstein Journal of Organic Chemistry 2006, 2, No. 23.

This structure shows the carboxylic function that could be used as a precursor of Phospinic derivative (imagine a tio-ester derivative), for Staudinger ligation and also for the formation of an amidic bond with a terminal amine. The interesting compound was easily prepared from the corresponding Titanocene complex commercially available (Scheme 9). Compound 6 was prepared in six passages .



Scheme 9: Retrosynthetic approach.

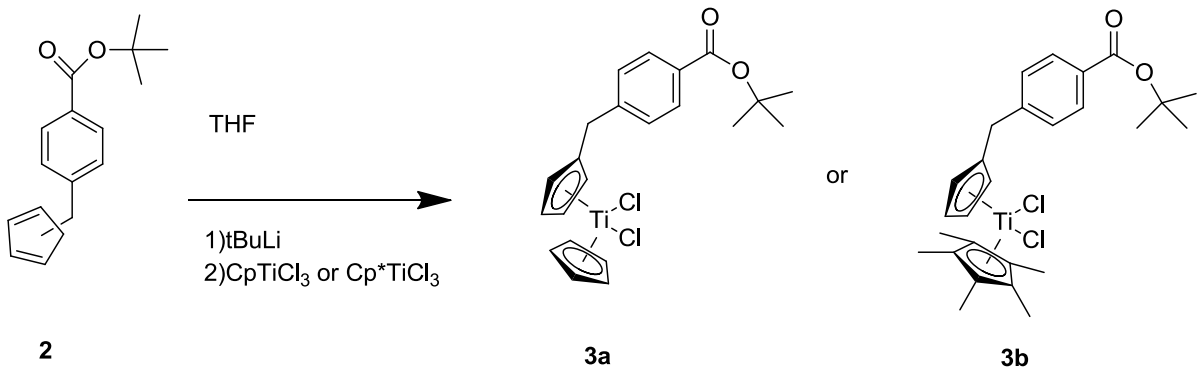
Cp derivative was prepared from the corresponding bromide after protection of Carboxylic group and subsequently nucleophilic substitution with CpNa (Scheme 10).



Scheme 10: Synthetic pathway that afford precursor (3) of Titanocene

The substiution of Bromine atom with Cp moiety leads to an intermediate easily editable. In fact, in this case , two commercial Titanium (IV) complexes were used , obtaining derivatives described below (Scheme 11)

Experimentally, we observed that the yields of the last synthetic step were very influenced by different condition, for this reason we decided to do a methodological study in order to obtain the best possible conditions. (Table 1)



2 **3a** **3b**

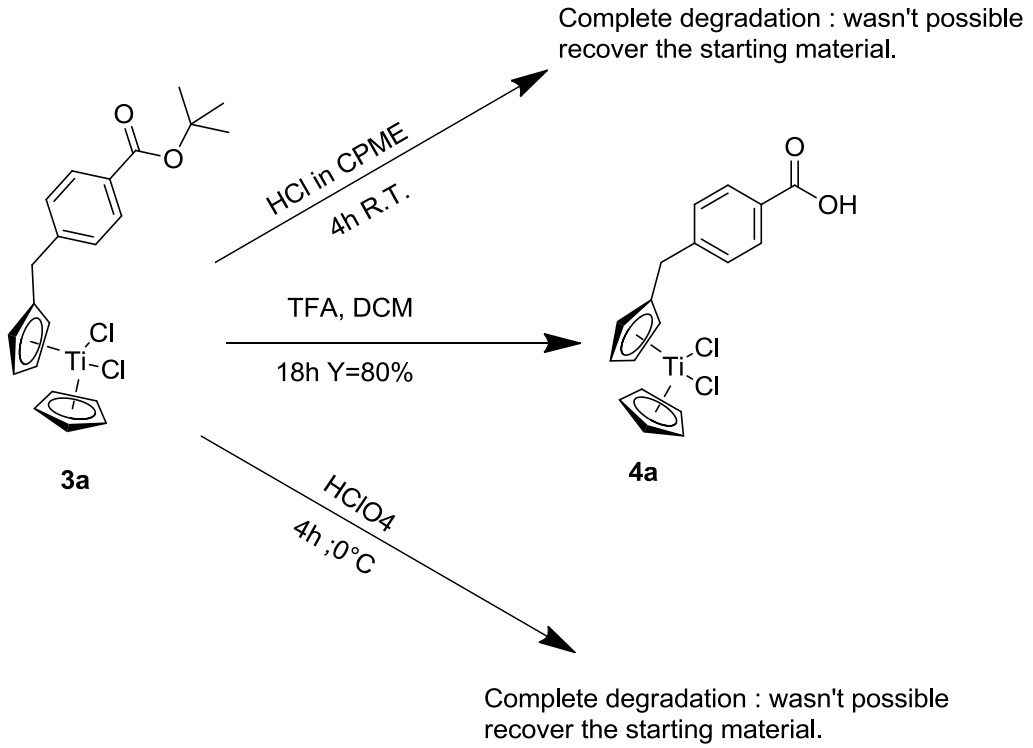
Scheme 11:reaction with different Titanium complexes

Entry	Ti Complex[a] (equiv.)	T (°C)	Time (h)	Yield (%)[b]
1	0.70	-78 to -30	18	30
2	0.80	-78 to -30	18	35
3	1.0	-78 to -30	18	35
4	1.2	-78 to -30	18	28
5	0.85	-78 to -10	18	35
6	**0.85**	**-78 to -10**	**8**	**55**

Table 1: a= all the data are reported for CyclopentadienylTitaniumtrichloride, for the other complex best conditions were used to obtain a yield of 62% . b= isolated yield

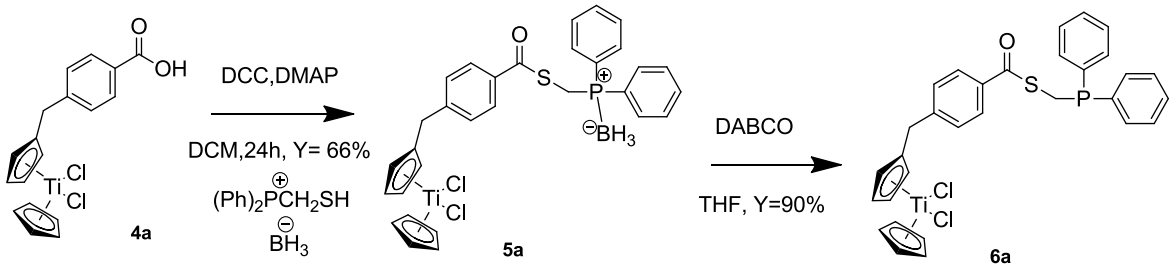
As can be seen from the datas in the table, use in slight excess of the titanium complex does not lead to significant increases in yield and, as well as the reaction time, has only negative effect and this is explained by the increased risk of entry of moisture in the reaction mixture. Conversely, shorter exposure times and slightly higher temperatures lead to optimal conditions.

The target compound was obtained after deprotection of the tert-butyl group using acidic conditions: the scheme below shows that trifluoroacetic acid (TFA) leads to the target compound while $HClO_4$ and HCl causes the complete degradation of starting material. (Scheme 12)



Scheme 12: Hydrolysis attempts of t-Butyl ester; for derivative 3b an Y=82% was obtained

The presence of a carboxyilic function in compound 4a pave the way for subsequent bioconjugation Indeed, the carboxylic function was used to prepare a posphinic derivative for the Staudinger ligation (Scheme 13).



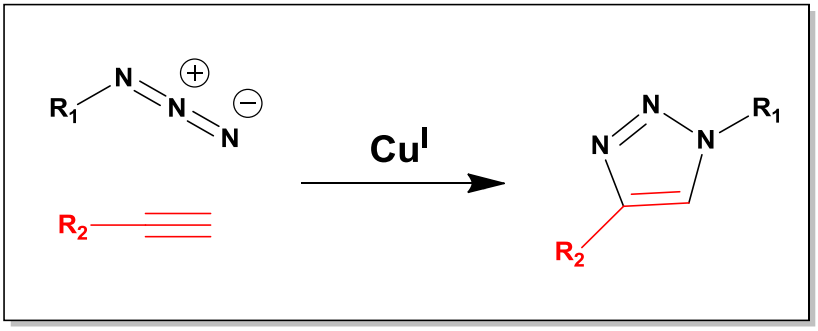
Scheme 13: Synthesis of bioconjugation precursor

Compounds 6a will be used unaltered for further functionalization with biomolecules.

3.2.Click chemistry approach

The use of various systems of conjugation expands the possibility of obtaining more selective derivatives and, in particular, the click chemistry is nowadays one of the most used, with quick and easy protocols, for conjugation of synthetic compounds with biomolecules or markers.[24,25] Indeed, a lot of Biomolecules are commercially available already azido-modified. To explore new ways of conjugation, we decided to use these protocols for the functionalization of titanocene .

In literature, there are many work in which a terminal alkyne react with an azide group in a [3+2] cycloadditions with a Cu^{I} catalyst.[26,27,28] In this reaction is involved an alkyne, that with a copper (I) catalysis, react with an azidic group to obtain a triazole moiety.[9] (Scheme 14)



Scheme 14: Classical reaction scheme for alkyne-azide cicloaddition

The CuAAC (Cu^{I} catalyzed alkyne−azide cycloaddition) reaction proceeds considerably faster than the Staudinger ligation in physiological settings.[29] However, the use of Cu^{I} catalyst is not without problems, cause Cu^{I} may be toxic for living systems[30] and a decrease in copper concentration is generally accompanied by a large decrease in reaction rate.[31]

[24] K. Lang and J. W. Chin; Chem. Biol. 2014, 9, 16−20
[25] M. King and A. Wagner; Bioconjugate Chem. 2014, 25, 825−839827
[26] V. V.Rostozev, L. G. Green, V. V. Fokin, and K. B. Sharpless; Angew. Chem., Int. Ed. 41 (14), 2596−2599
[27] C. W. Tornøe, C. Christensen, and M.Meldal; (2002) J. Org. Chem. 67 (9), 3057−3064.
[28] I. A. Maretina, B. I. Ionin, Alkynes in cycloadditions, wiley, 2014
[29] N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo and C. R. Bertozzi, (2006) Chem. Biol. 1 (10), 644−8.
[30] V. Hong, N. F. Steinmetz, M. Manchester, and M. G. Finn; (2010) Bioconjugate Chem. 21 (10), 1912−1916.
[31] V. O. Rodionov, S. I. Presolski, D. Díaz, V. V. Fokin and M. G. Finn, (2007) J. Am. Chem. Soc. 129 (42), 12705−12712

Furthermore, using this approach, we noticed another problem:

- The presence of a Copper catalysis is discrepant with the chemistry of particular ligands on titanium (See chapter 4).

For the reasons mentioned above, we decided to choose a different type of protocol. In fact the bioconjugation with alkyne−azide cycloaddition is accelerated respect to the uncatalysed reaction by introducing ring strain into the alkyne (rather than using metal catalysis[32]), creating a reaction named "strain-promoted alkyne−azide cycloaddition" (SPAAC). A series of cyclooctyne-based probes have been developed to react with azides, and these reactions were used to label abundant biomolecules within complex biological systems, including live mammalian cells[33] and animals[34]. These considerations have led us to assume our target compound shown below (Figure 6).

Figure 6:Target compounds for Click chemistry apprach

In detail, the linker for the titanium complexes is (Figure 7)

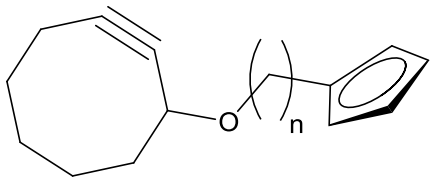


Figure 7: Linker for Titanocene with the necessary cyclooctine mojety

This linker has three main charachteristics:

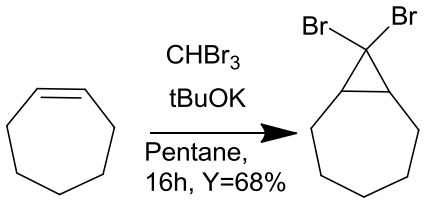
[32] N. J. Agard, J. A. Prescher and C. R. Bertozzi; (2004),J. Am. Chem. Soc. 126 (46), 15046−15047.
[33] J. M. Baskin, J. A. Prescher,S. T. Laughlin, N. J. Agard, P. V. Chang, and C. R. Bertozzi, (2007) Proc. Natl. Acad. Sci. 104 (43), 16793−16797.
[34] S. T. Laughlin, J. M. Baskin, S. L. Amacher and C. R. Bertozzi; (2008) Science 320 (5876), 664−667.

1. The cyclooctine moiety necessary for the Alkyne-Azide cicloaddition strain promoted.
2. The aliphatic spacer that can change the stability and the rigidity of the entire molecule.
3. The cp ring, necessary for condensation with titanium complexes.

With this background, in this thesis work, we tried to optimize the various reactions in order to obtain a stable Titanocene derivative, functional for both imaging and antitumor activity aspects.

To obtain the general compound shown in Fig. 6 the first step is the generation of the cyclooctine moiety. In literature, there are several ways to obtain this typical structure. One of the most common use the cycloheptene as starting material and, after an oxidative insertion Silver mediated and an elimination of bromine leads to the desired product.[35,36]

Dibromobycicloheptane can be prepared from an electrophilc addition of dibromo carbene on the double bond of cycloheptene. (Scheme 15)



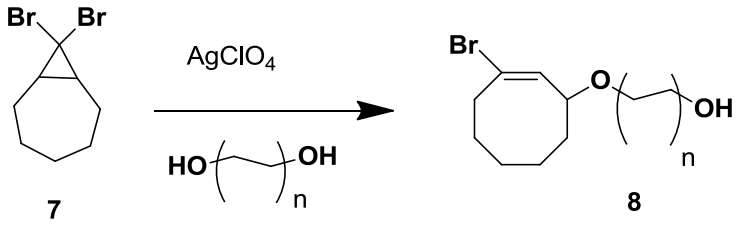
7

Scheme 15: Synthesis of dibromobycicloheptane

The reactive dibromocarbene is generated *in situ* by the action of Potassium tert-butylate on Bromoform. Then the *gem* dibromide is treated with Ag^{I} and a terminal diol to obtain product 8.(Scheme 16)

[35]A. B. Neef and C. Schultz; Angew. Chem. Int. Ed. 2009, 48, 1498–1500

[36] C. Ornelas, R. Lodescar, A. Durandin, J. W. Canary, R. Pennell, L. F. Liebes and M. Weck; Chem. Eur. J. 2011, 17, 3619–3629.



Scheme 16: Oxidative insertion of an aliphatic diol

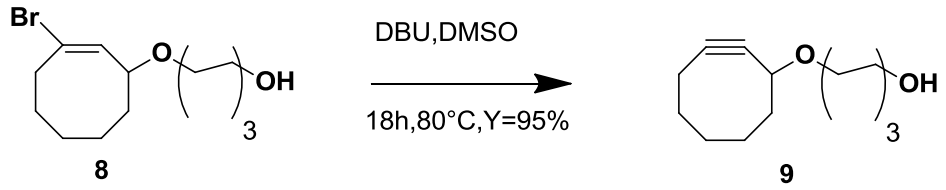
This reaction is the first key step of the synthesis and we decided to improve initially yield with a methodological study described below (Table 2). The improvements of yield and other features of this reaction were obtained changing the size and the amount of diol, together with other reaction conditions.

Entry	AgClO4	n	DIol	Solvent	T (°C)	Y[a]
1	2 equiv.	1	10 equiv	Toluene/Py 10:1	110	20
2	3 equiv.	1	20 equiv.	Toluene/Py 10:1	110	22
3	3 equiv.	3	10 equiv.	Toluene/Py 10:1	110	35
4	5 equiv.	3	30 equiv.	Toluene/Py 10:1	110	36
5	3 equiv.	3	10 equiv.	Aceton	r.t	50
6	**3 equiv.**	**3**	**30 equiv.**	**Aceton**	**r.t**	**62**
7	2 equiv.	3	30 equiv.	Aceton	r.t	40

Table 2:: Attempts to generate bromo-olefin 9a. a= isolated yield

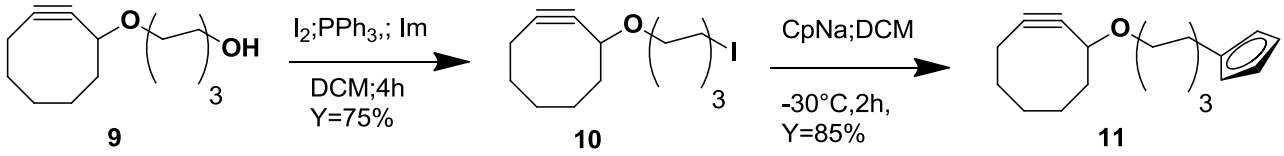
The table shows that best results were obtained using 1,6-Hexandiol as reaction partner and acetone as solvent. All these experiments were performed in a dark reaction vessel to limit photo-degradation of Silver Complex.

From this compound is possible to obtain easily the cyclooctyne moiety with an elimination of the last bromine atom. (Scheme 17)



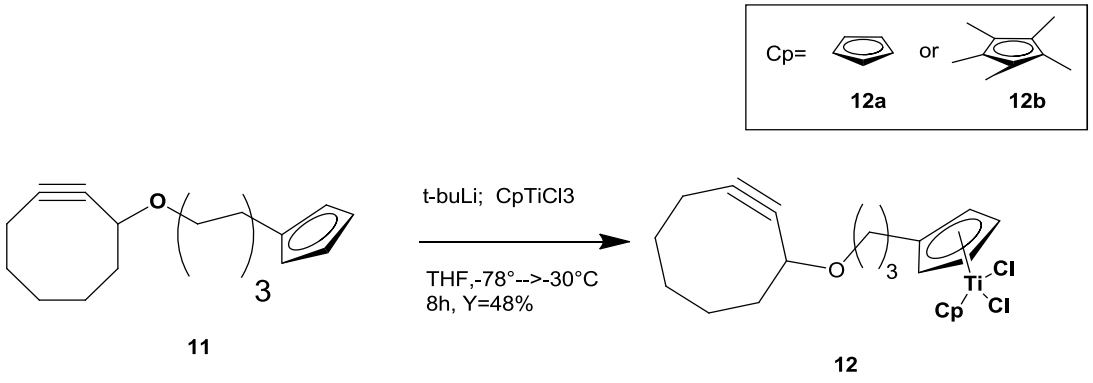
Scheme 17: Elimination of Bromine atoms

Compounds 9 was transformed in the corresponding iodo-derivative trough a Mitsunobu-Like reaction and, subsequently, after a nucleophilic substitution using CpNa we synthesized compound 11 (Scheme 18).



Scheme 18: Preparation of precursor of Titanocene derivative

Compound 11 is treated with *t*-butyl-Lithium, as described in the previous paragraph (3.1) with a titanium complex to afford compound 12. (Scheme 19) As in the previous Staudinger approach we used two different titanium complexes to obtain two different Titanocene derivatives.



Scheme 19: synhtesis of Titanocene target

Synthesized derivatives 6a, 6b, 12a and 12b were used subsequently to obtain bioconjugates with different biomolecules. In the third part of this chapter, the choice of biomolecules and the protocols of bioconjugation are discussed.

3.3.Biomolecules and Bioconjugation

Nowadays, there are many possibilities when choosing a biomolecule rather than another. In this work we focused our interest to biomolecules already used as tumor markers or biological-vectors. In particular, as already mentioned, the choice of biomolecule is crucial to give to the final compound a good bio-selectivity. In fact, the conjugation of a transition metal complex with a biomolecule leads to reduction of toxicity, an increase of aqueous solubility, and increase of biocompatibility. Furthermore, if the biomolecule is a target for tumor cell receptors, selectivity of the drug and its cytotoxicity may be increased.

One of the biomolecules widely used in this field is Folic Acid (FA) because the folate receptor is a promising tumor target for folate conjugates since folate receptors are overexpressed in a wide variety of cancer cells.[37] A number of folate conjugates have been synthesized for application in several fields from tumor therapy to tumor diagnosis.[38] Unfortunately, one of the worst problems in the synthesis of FA-drug conjugates is around the selectivity of protocols of conjugation. In fact FA (Figure 8) presents two carboxylic function and, a direct attempt of modification of this structure results on mixture of both the inactive α-derivative[39a], the active γ-derivative[20a], often accompanied with the bis-functionalized derivative.[20b]

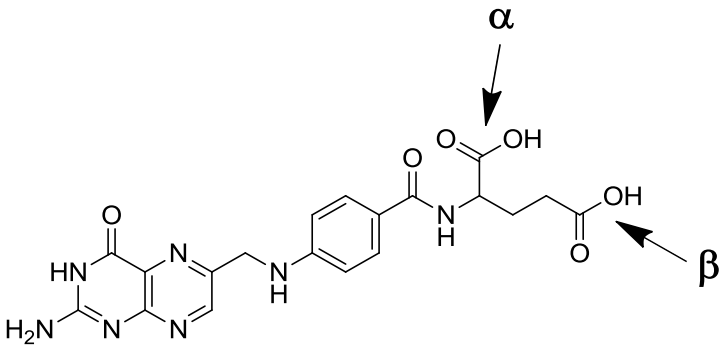


Figure 8: Structure of Folic Acid

[37] S.D. Weitmann, R. H. Lark, L. R. Coney, D. W. Fort, V. Frasca, V. R. Zurawski; Cancer Res. 1991,52, 3396
[38] E. I. Sega, P. S. Low; Cancer Metastasis Rev.; 2008, 27, 655
[39] a) C.P. Leamon, P. S. Low; J. Biol. Chem., 1992, 267, 24966. b) C. P. Leamon, P. S. Low, I. Pastan; J. Biol. Chem., 1993, 268, 24847

However, views the potential of this type of conjugates, several research groups have developed a series of protocols that can differentiate α and γ positions.[40,41] For our purposes, we identified the ideal target in the compound in figure 9 that present an Azido Function that can be used both for the Staudinger ligation and for the Click chemistry approaches. (Figure.9)

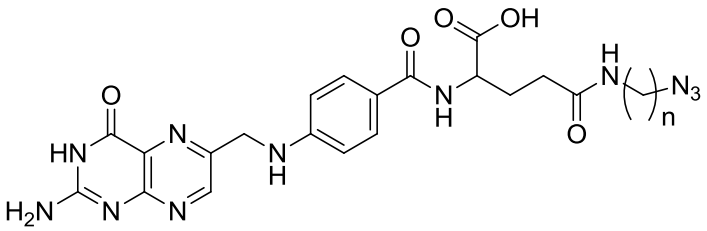
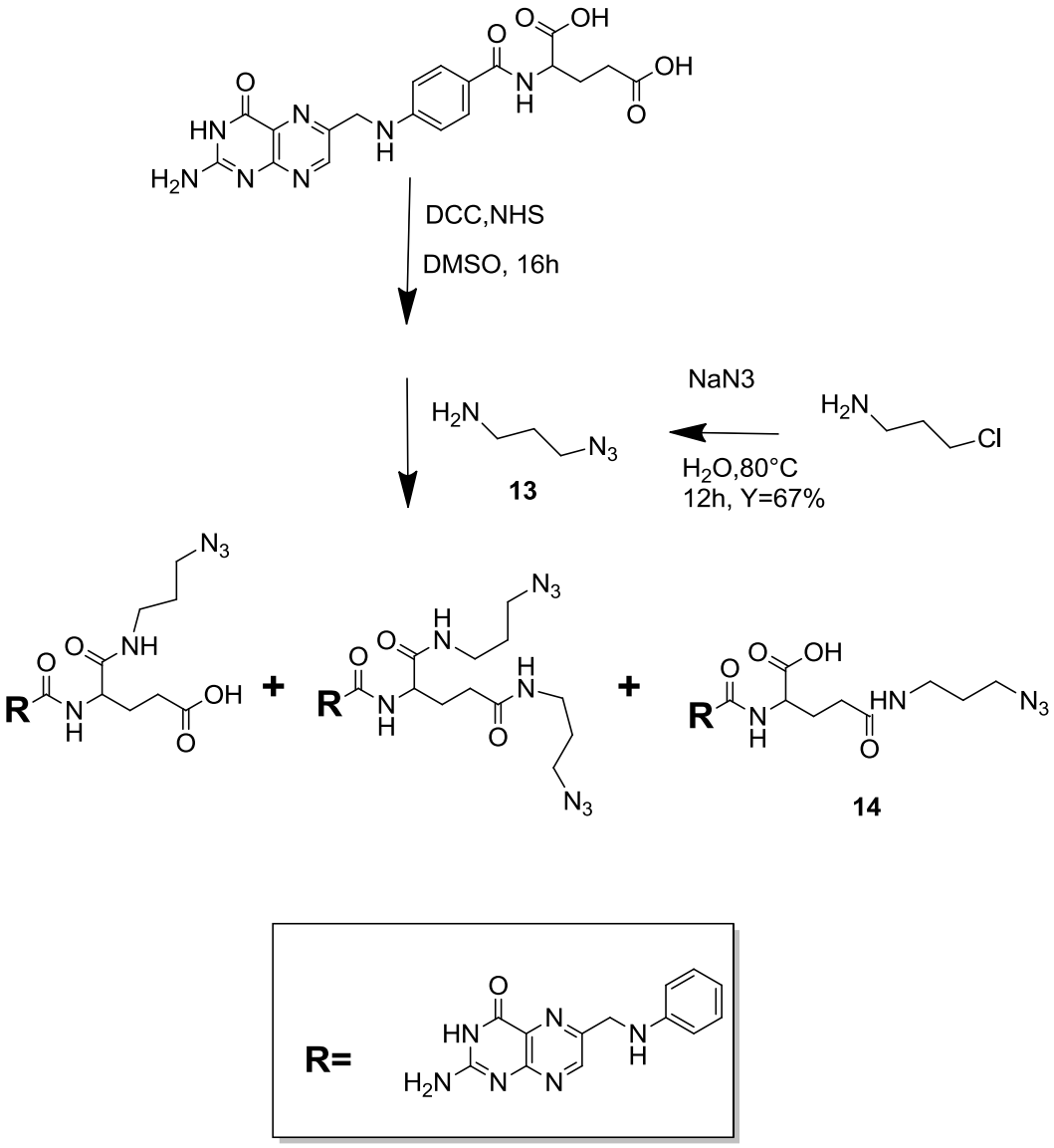


Figure 9: Azido derivative of FA

Initially, we decided to activate FA, as described in literature,[42] with a direct activation of the carboxylic function *via* NHS-derivative, coupled with amminopropyl-azide [43](Scheme 20). Unfortunately, this reaction afforded in poor results because we obtained a mixture of α,γ and bis-functionalized derivatives.

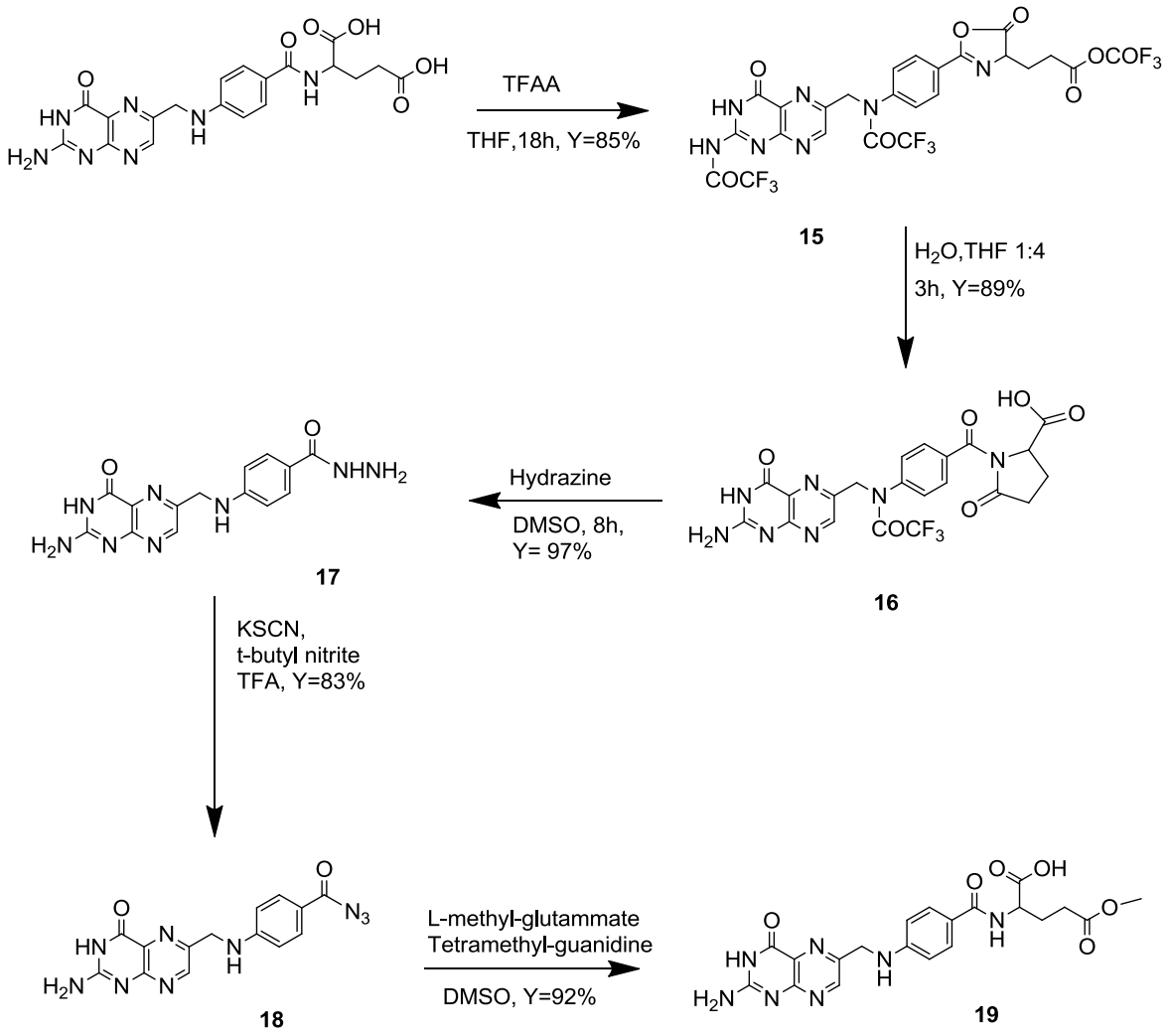
[40] L. li and others; Carbohydrate Polymers, 86,2011, 708-715
[41] V. Groehn, R. Moser, T. L. Ross, T. Betzel, C. Muller, R. Schibli, S, Ametamey; Synthesis, 2011, 22, 3639-3648.
[42] M. Fani, M.L. Tamma, G. P. Nicolas, E. Lasri and H. R. Maecke; Mol. Pharmaceutics 2012, 9,1136
[43] M.Di Antonio, G. Biffi, A. Mariani, E.A. Raiber, and S. Balasubramanian Angew. Chem Int. Ed. 2012 ;51(44):11073-8.



Scheme 20: Activation and subsequently coupling with amino propyl azide previously prepared

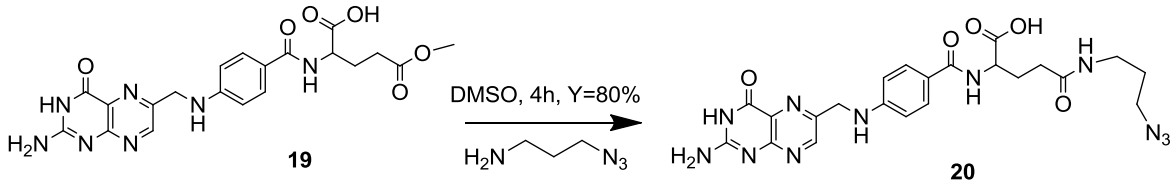
With these results in our hands we decided to continue with the approach of Fuchs[44] within the key-passage is the hydrolysis of the FA to afford Terrace Acid and subsequent condensation with a glutammic derivative that leads to the intermediate 19 .(Scheme 21)

[44]J. Luo, M. D. Smith, D. A. Lantrip, S. Wang and P. L. Fuchs; J. Am. Chem. Soc,1997,119,10004-10013



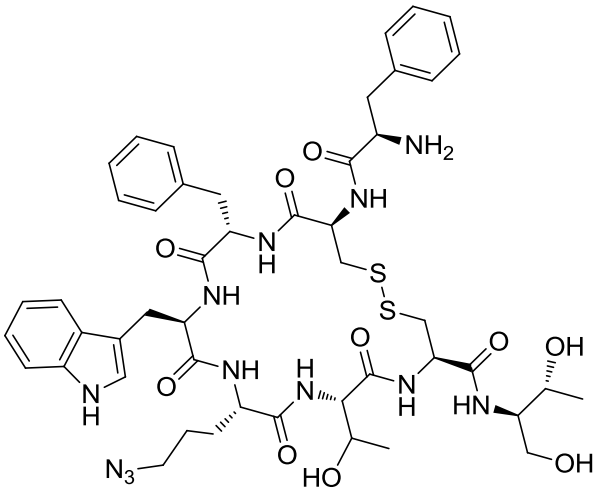
Scheme 21: differentiation of alpha and gamma positios of folic acid

This derivative presents the α-position as a carboxylic group and the γ position as an ester derivative,(Scheme 22) position more active for the next derivatization with aminopropyl-azide to afford compound 22. This compound was used for the next conjugation reaction.



Scheme 22: synthesis of azido modified Folic Acid

To test the wide range of use of our Titanocene derivatives we decided to synthetise new conjugates with others biomolecules as Octeotride. Octeotride (commercially available as Sandostatin) is an octapeptide that mimics the behavior of somastatin (Figure.10). This molecule is also used in nuclear medicine imaging by labelling with ^{111}In to non invasively image neuroendocrine and other tumours expressing somatostatin receptors;[45,46] recently was labeled also with 11C that enable the use of PET.[47]

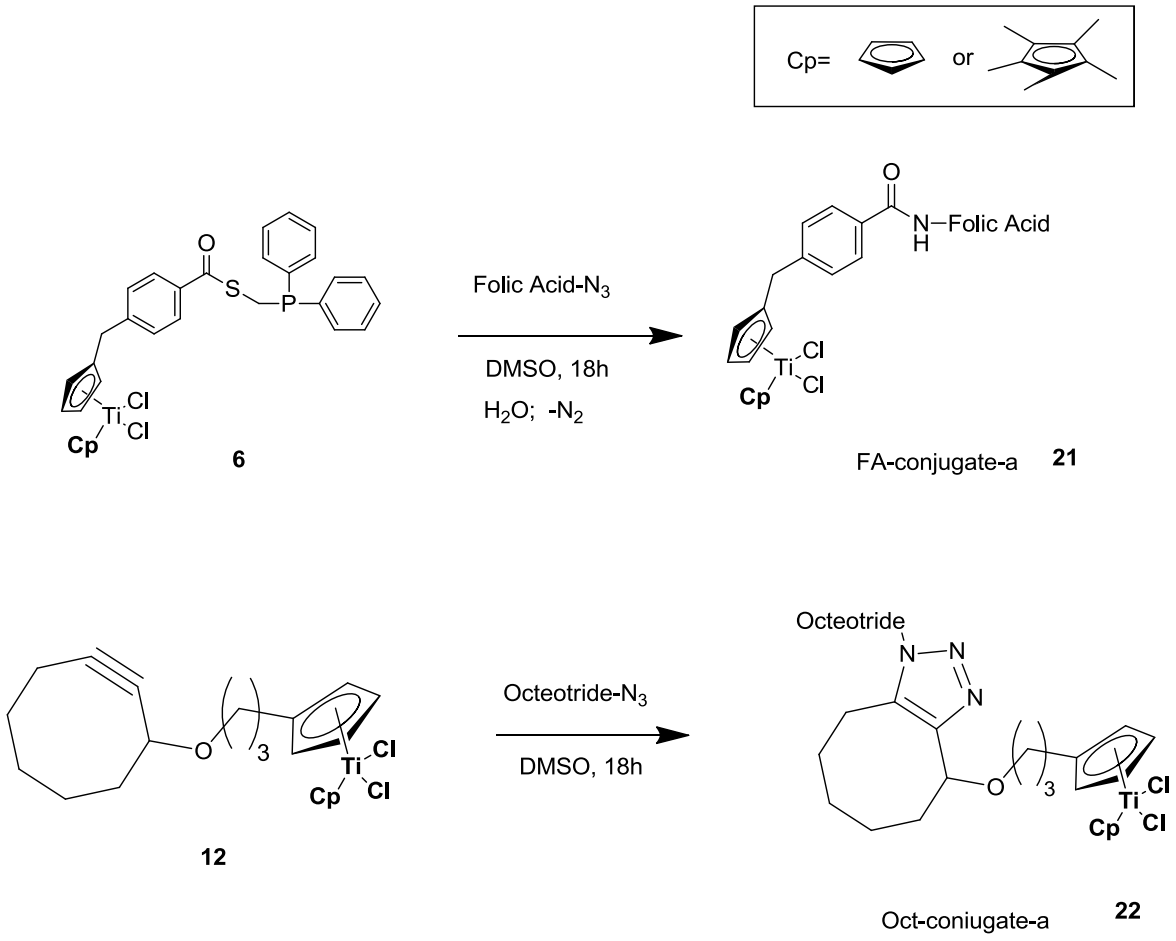


Octreotide-N_3

Figure 10: Structure of Octeotride azido derivative

[45] M. E. Valsecchi; M. Coronel; C. M. Intenzo; M. Kim Sung ; A. Witkiewicz and T. Sato;Melanoma res., 2013, 23, 33.
[46] V. Rufini; M. Salvatori; I. Saletnich; V. Valenza; L. Troncone, IAR, 1995, 39, 140-4.
[47] J. Chin, M. Vesnaver, V. Bernard-Gauthier, B. Wängler, C. Wängler, R. Schirrmacher; *Amino Acids*. 2013 Aug 7

Later, it was decided to couple the derivatives 7 and 13 with biomolecules, in order to obtain target compounds.(Scheme 23)



Scheme 23: Synthesis of bioconjugates

Unfortunately, the condensation between Titanocenes derivatives and selected biomolecules, have highlighted a very important issue: the difficulty of purification for the desired product to the starting material. This problem is not solved even by adding an excess of titanocene to the reaction mixture. To solve this problem we decided to start a ligand study in order to identify a binder suitable for anchoring the Titanocene on a solid phase to change purification procedures. In addition the ligand study is necessary to develop a protocol for a fast Ligand-Fluorine exchange, necessary for PET applications.

Chapter 4: P.E.T.

4.1.The PET Technique

Recent advances in noninvasive imaging techniques have opened endless opportunities for molecular diagnostic and therapeutic procedures.[48] Molecular imaging may be used for early detection, characterization, and "real time" monitoring of disease, as well as investigating the efficacy of drugs. There is an agreement, among experts in the field, that one of the most sensitive molecular imaging technique is the radionuclide-based positron emission tomography (PET), that shows the sensitivity needed to visualize most interactions between physiological targets and ligands such as analogs of cellular nutrients, neurotransmitters, tumor markers and brain receptors.[149]

PET imaging agents are radiolabeled with positron emitting radionuclides, which decay by the emission of a positively charged particle, the positron (e^+). After traveling a short distance in the electron-rich tissue, the positron recombines with an electron in a process called annihilation: the masses of both positron and electron are converted in two high-energy photons (511 keV γ rays), which are approximately 180° placed and allow to approximate location of the emitting source within the organism by the detectors of a PET scanner.[50] (Figure 11a). The longer is the distance traveled by positron before annihilation, the larger is the loss in spatial resolution.[51] (Figure 11b)

[48] S. M. Ametamey, M. Honer and P. A. Schubiger; Chem. Rev., 2008, 108, 1501–1516.
[49] T. Reiner, E. J. Keliher, S. Earley, B. Marinelli, and R. Weissleder; Angew. Chem. Int. Ed. 2011, 50, 1922-1925.
[50] T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson; Chem. Rev. 2010, 110, 2858–2902.
[51] R. Gong, X. Cheng, W. Han; Imaging 2005, 32, 325-345.

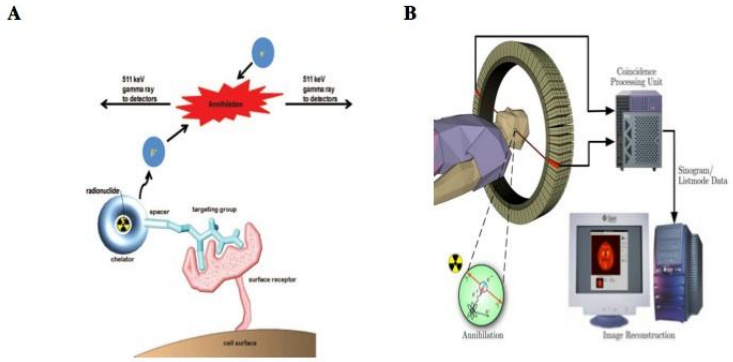


Figure 11:The positron emission tomography (PET). A: the e^+ emission and annihilation process of a new generation radiolabeled PET-imaging agent bound to an extracellular target; B: Data collection by a PET camera allowing to identify the position of annihilation

4.2.Applications of Positron Emission Tomography

PET technique is used in several areas of clinical-medical field, in particular Oncology, Cardiology and Neurology.

Oncology is undoubtedly one of the most significant applications of PET imaging. It is acknowledged that the chances of success in cancer treatment depend significantly from an early diagnosis of the disease.[52] PET molecular imaging allows us to identify and study specific biological characteristics of early stage cancer and identify changes in bio-functional pathways, so is possible to make an almost entirely non-invasive diagnosis and/or take control of the evolution over time. A further application of PET in the Oncology's field is the tumor staging, which allows, in some cases, to choose the most appropriate treatment plan, studying directly how the disease responds to treatment and enable the possibility to make the appropriate corrections or modifications.[53]

Cardiology has significant importance for the PET applications: in this field, the technique is used for both vascular flow and metabolism studies. In particular PET is widely used to assess the vitality of cardiac tissues.[54]

In Neurology, the PET is increasingly used to study the Physio-pathology of the brain, an hard-to-reach organ using conventional methods.[55] In particular, regarding psychiatric diseases, numerous studies have shown that neurodegenerative pathologies can produce significant imbalances in the cerebral glucose metabolism, detectable with PET. These discoveries allowed the obtaining of important information on neurodegenerative diseases such as Alzheimer's.[56]

[52] Weber G.; N Engl J Med. 1977;296:541–551
[53] Michael E. Phelps; J Nucl Med 2000; 41:661–681
[54] R.O Bonow ; Circulation, 83 (**1991**), pp. 26-37.
[55] Farde, L. Trends Neurosci. (**1996**) 19, 211–214
[56] J. C. Mazziotta, R. S. J. Frackowiak , M. E. Phelps; Semin Nucl Med. **1992**;22: 233–246.

4.3.PET Probes: State of the art

The radio-tracers are molecules used in nuclear medicine for therapeutic purposes, that are marked by appropriate diagnostic radionuclides; some of radio medicines belongs to the family of PET tracers. An "ideal" PET tracer, with the aims of being employed in the human body, needs to:

- -Be easily detectable; for this purpose contributes the choice of an appropriate radionuclide and the development of an efficient method of marking;
- -Not interfere with the metabolism of the substance studied;
- -Be characterized by a completely kinetics similar to that of the marked substance;
- -Show high affinity and selectivity for the molecular site of interest. This point is important, in particular when the target is a specific enzyme or a receptor subclass. For the same reason, it must have a low non-specific binding to various cellular components.[57]
- -Have a good ability to cross biological barriers. If the molecule, for example, is designed to monitor a brain process, it must be able to pass the blood-brain barrier (BBB); thus must have suitable lipophilicity (LogP between 1.5-2) to pass through passive diffusion or active transport carrier-mediated. In this case, the presence of substances that can compete with the radiopharmaceutical drug for carriers has to be evaluated.[58]
- -Have a suitable metabolic degradation. This is a crucial element because these drugs can produce radioactive metabolites which can interfere and hinder the interpretation of the images. Most drugs are metabolized in the liver, but the metabolism can take place even in kidney, intestine and lung. Metabolism may be influenced by factors such as dose, route and schedule of administration, physiological conditions, nutritional status and interaction with other drugs.[59]
- -Reach the necessary concentration in the tissue of interest. The anatomical distribution and concentration of the molecular targets should also be adapted in the spatial resolution and sensitivity of the scanners[7].

[57] Halldin, C., et al., Quarterly J. Nucl. Med., **2001**, 139-152.
[58] Moresco, R.M., et al., Pharmacol. Res., **2001**,151-159.
[59] Moerlein, S.M., et al., Nucl. Med. Biol., **1985**,353-356.

- -Have a high specific activity (Ci/µmole) that allows to limit the amount of tracer injected (µmol), to reduce the risk of toxicity and to decrease the possibility of saturation of binding site (for receptor tracers). It is the concept of the tracer dose, so the amount of radioactive molecules administered are below µmole[6,7].

4.4.Radionuclides

In the table below, (Table 3) most common radionuclides used for PET are presented[60]:

Isotope	Production		Decay			
	Nuclear	(MeV)	Decay product	$t^{½}$ (min)	β^+(%)	$E^{\beta+}$ **max** (MeV)
^{18}F	$^{20}Ne(d,\alpha)^{18}F$ $^{18}O(p,n)^{18}F$ $^{16}O(3He,p)^{18}F$	14 → 0 16 → 3 41 → 14	^{18}O	109.8	96.9	0.693
^{11}C	14N(p,α)11C 11B(p,n)11C 10B(d,n) 11C	13 → 3 10 → 0 10 → 0	^{11}B	20.38	99.8	0.96
^{13}N	$^{12}C(d,n)13N$ $^{13}C(p,n)^{13}N$ $^{10}O(p,\alpha)^{13}N$	8 → 0 10 → 0 16 → 7	^{13}C	9.96	100	1.19
^{15}O	$^{14}N(d,n)^{15}O$ ^{15}N (p,n) ^{15}O $^{16}O(p,pn)$ ^{15}O	8 → 0 10 → 0 26 → 16	^{14}N	2.03	99.9	1.72

Table 3: most common radionuclides for PET applications

In particular ^{18}F is the most useful PET radionuclide due to its physical and chemical characteristics. ^{18}F can be produced through different nuclear reactions;[61] the most widely used, involved the reaction with ^{18}O (p, n) to ^{18}F, that consists in bombing a target liquid material of H_2O enriched in ^{18}O (> 95%) with a protons beam, to an energy of 16 MeV. This production method is the most advantageous for reaching high yields of reaction, high specific activity and because any radioactivity produced is potentially usable for the marking of the desired ligand.[62] The only drawback is the cost of the target material, which is rather high and takes therefore to develop methods for retrieving the enriched water after the entrapment of ^{18}F.

Furthermore, the maximum energy associated with the positron emitted by ^{18}F is only 0.633 MeV, which translates into an average radius of tissue penetration of 0.6 mm (the

[60] Dewey, S.L., et al., J. Neuroscience, 1992,3773-3780.

[61] Yigit, M.; Tel, E. From Annals of Nuclear Energy (2014), 69, 44-50

[62] *S. M. Ametamey; M. Honer; P. A. Schubiger; Chem. Rev.* ***2008****, 1503*

lowest among all radionuclides normally used) making it the radionuclide which guarantees the highest image resolution.[63]

The half-life of ^{18}F is 109 minutes, makes it ideal to monitor many biological processes also with slow kinetics. Being less ubiquitous carbon fluorine, 18F labeled radiotracers can be obtained with high specific activity. Finally, Its chemical properties can be used to block the metabolism and/or studying biological processes.[64] The most widely used PET-tracer with ^{18}F is an analogue of glucose: the 2-[F-18] Fluoro-2-Deoxy-D-glucose (FDG) (Figure 12). This FDG is retained by cells through glucose transporters and is an esokinasi substrate, an enzyme that phosphorylates glucose in position 6. Because the molecule doesn't have the hydroxyl group in position two, the molecule cannot continue in the metabolic process of glucose degradation and remains trapped in the cell; cancer cells, characterized by higher consumption of glucose, will pick up the molecule marked and will be revealed.[65]

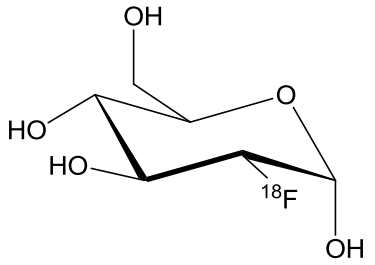


Figure 12: Structure of the labeled FDG

[63] O. Jacobson, D. O. Kiesewetter, and X. Chen; Bioconjugate Chem., 2015, 26 (1), pp 1–18
[64] R. Chakravarty, H. Hong, and W. Cai; *Mol. Pharmaceutics*, 2014, 11 (11), pp 3777–3797.
[65] Bustamante and Pedersen, PNAS, 2005, 74 (9): 3735

4.5.PET and Titanocenes.

A new frontier of radio pharmaceuticals and radio tracers is based on the use of radio metals or substrates based on a metal complex capable of carrying a radionuclide such as gadolinium[66]. In this area is possible to insert the use of Titanocene derivatives because the structure of this compound presents some important characteristics necessary for each pet probes:

- The bond strength between Titanium an Fluorine is higher than the bond strength between Fluorine and Carbon.[67] This characteristic produce two main effects: on one side the fluoride release should be slower, allowing the radionuclide to reach the affected area and dispose of fluorides into the surrounding tissue; on the other side there is the increase of hydrolityc stability of the entire complex due to the greater difficulty of water molecules to displace the atoms of fluorine.
- The possibilities, as described in chapter 2, to bioconjugate Titanocene with a specific tumor marker to improve selectivity of the PET tracer.
- The use of Titanium as a radionuclide[68] because this element presents an half-life time of 3.09h and a maximum energy of 1.04 MeV. This radionuclide can be produced in a diagnostic cyclotrones using ^{45}Sc as a target.[69]

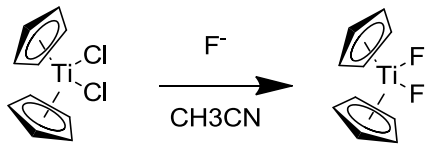
In this work we focused our attention on the synthesis of cold-derivatives of Titanocenes with two atoms of fluorine as ligands of the complex. Furthermore, to evaluate the chance to use a Titanocene derivative as a PET probe, we collected preliminary experimental data in the fields of:

- Hydrolytic stability
- Ligend-Fluorine exchange evaluation to obtain the fastest protocol, necessary for good results.

[66] I. Velikyan I. Med Chem. 2011 Sep;7(5):345-79
[67] T. L. Cottrell, The Strengths of Chemical Bonds, 2d ed., Butterworth, London, 1958
[68] A. L. Vavere, M. J. Welch, J Nucl Med April 1, 2005 vol. 46 no. 4 683-690.
[69] Vāvere AL. et al *Nucl Med Biol. 2005 Feb;32(2):117-22.*

4.6.Ligands Study

As described above, one of the most important characteristics of a PET tracer is the possibility to have the fastest protocol for the insertion of a radionuclide, in order to have the maximum resolution once the tracer was tested in the patient. In our case this feature is focused to the fastest exchange between the ligands on Titanium complex and the fluorine atoms. First of all we tested the exchange between commercially available Titanocenes dichloride and fluorine sources modulating reaction conditions (Scheme 24 and Table 4)



23

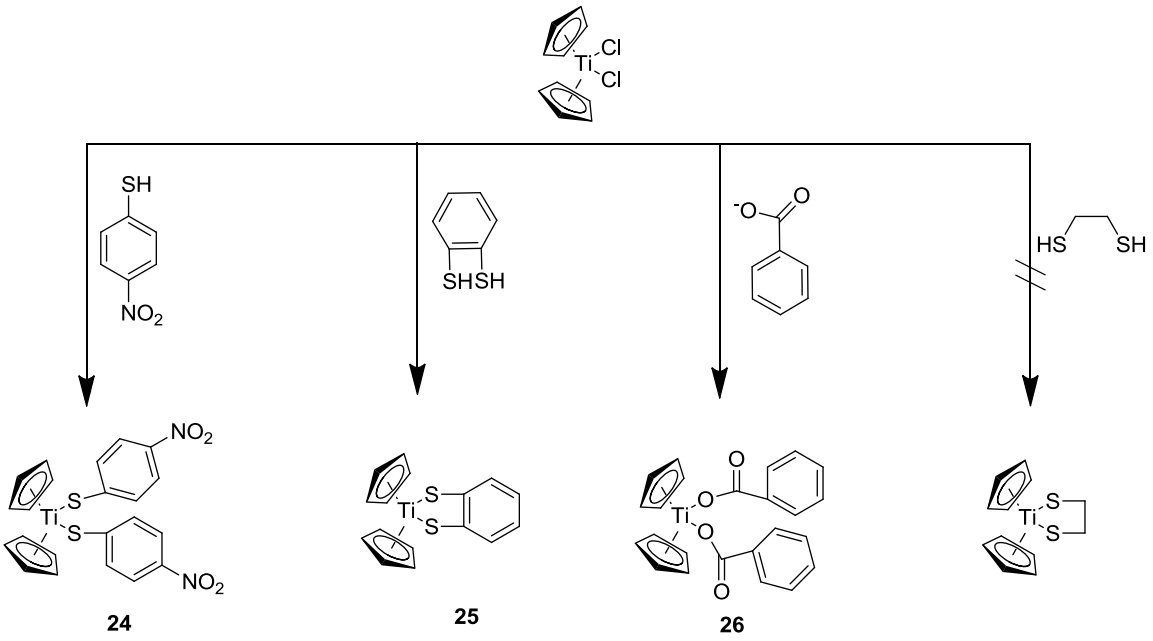
Scheme 24: chlorine-fluorine exchange evaluation

Entry	Source of F^- [a]	T(°C)	Additives[b]	Time (min)[c]
1	NaF	25	Et_3N	120
2	NaF	50	Et_3N	x
3	KF	35	Et_3N,Kryptofix	35
4	KF	25	Et_3N	45

Table 4: a) was used 10 equivalents of the salts; b) 3 equivalents of Et3N; c) the time is referred to complete conversion of dichloride monitored with an UV-Vis spectrometer

As shown in Table 4, only entry 3 presents a good result. In fact the others are not compatible with the half-life of ^{18}F. So, we decided to change ligands in order to increase the speed of exchange and to synthesize many derivatives with different ligands, taking advantage of the natural bonding-strenght of Titanium with other elements of the periodic table.[70] We synthesized derivatives with ligands based on Oxygen and Sulfur. (Scheme 25)

[70] a)T. L. Cottrell, The Strengths of Chemical Bonds, 2d ed., Butterworth, London, 1958; b) B. deB. Darwent, National Standard Reference Data Series, National Bureau of Standards,31, Washington, 1970; c) S. W. Benson, J. Chem. Educ. 42, 502, 1965 ; d) J. A. Kerr, Chem. Rev. 66, 465, 1966.



Scheme 25: preparation of different Titanocene derivatives for ligand-fluorine exchange evaluation

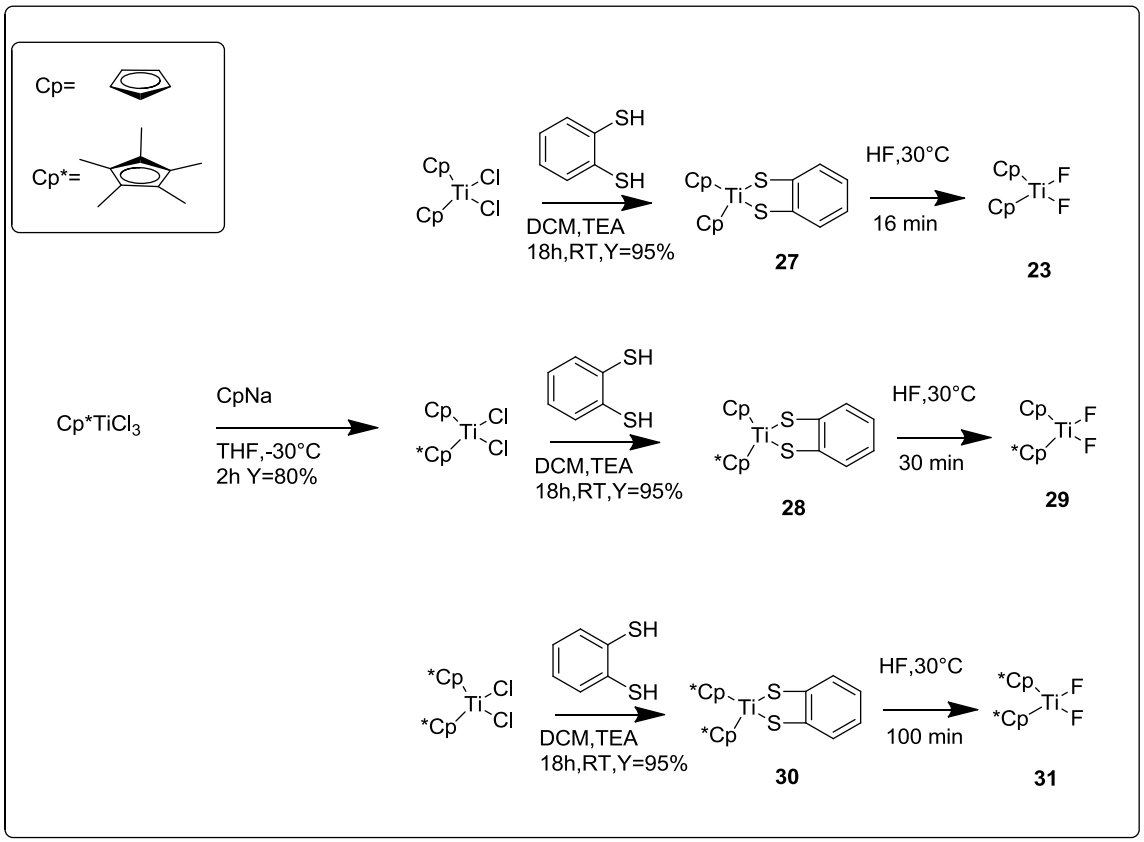
All the compounds synthesized were tested for Fluorine exchange and results are presented in the table below (Table 5).

Entry	Compound	T(°C)	Source of F^-	Time (min)[a]
1	24	25	KF	>200
2	24	25	HF	120
3	24	40	HF	120
4	25	25	HF	40
5	25	40	HF	25
6	26	25	KF	>200
7	26	25	HF	-

Table 5: exchange evaluation: a:complete conversion of starint material checked through TLC, b:degradation of the compound.

After this experiment we have seen that compound 26 (with a 1,2-dithiolic aromatic system) presented the characteristics to become a good candidate for ^{18}F-marking. The results of these various compounds are in agreement with our consideration; in fact the bond Ti-O is stronger than the bond TI-S and shows a lower speed of displacement.

After this data we decided to modify the Titanocenic core; indeed, in literature is known that the presence of methyl groups on cycopentadienyl ligand increases the stability of the entire complex .[71]In our work we decided to test different titanium complexes with 0, 5, or 10 methyl groups on Cp rings respectively, in the ligand-fluorine exchange. (Scheme 26)



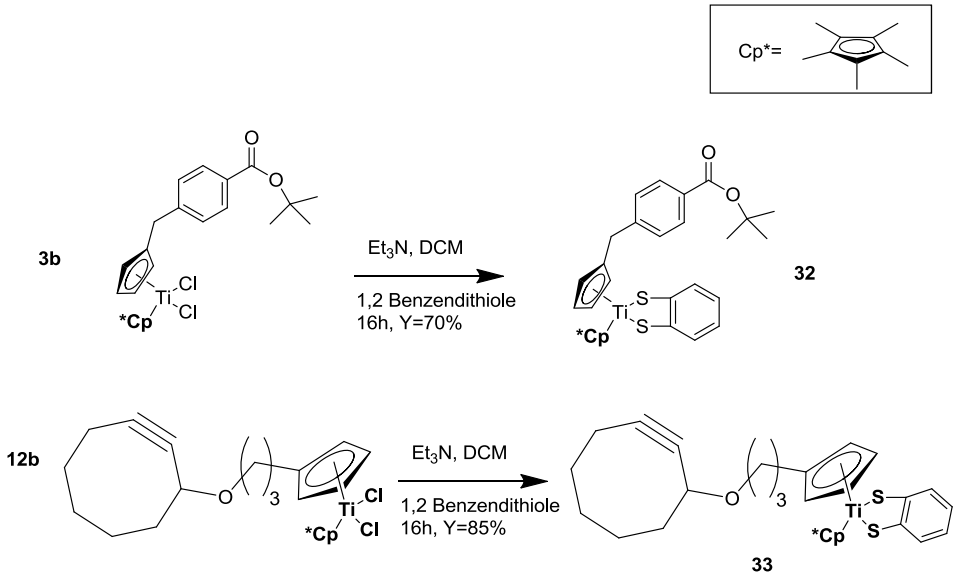
Scheme 26: Ligand-fluorine exchange with different Titanium complexes

Scheme 26 shows that the use of Decametil derivative is not appropriate for two reasons:

- The exchange speed Cl-F is lower than others, approaching to half-life of ^{18}F.
- The presence of ten methyl groups blocks the further modification with a linker, important for the selectivity and bioactivity of the compound.

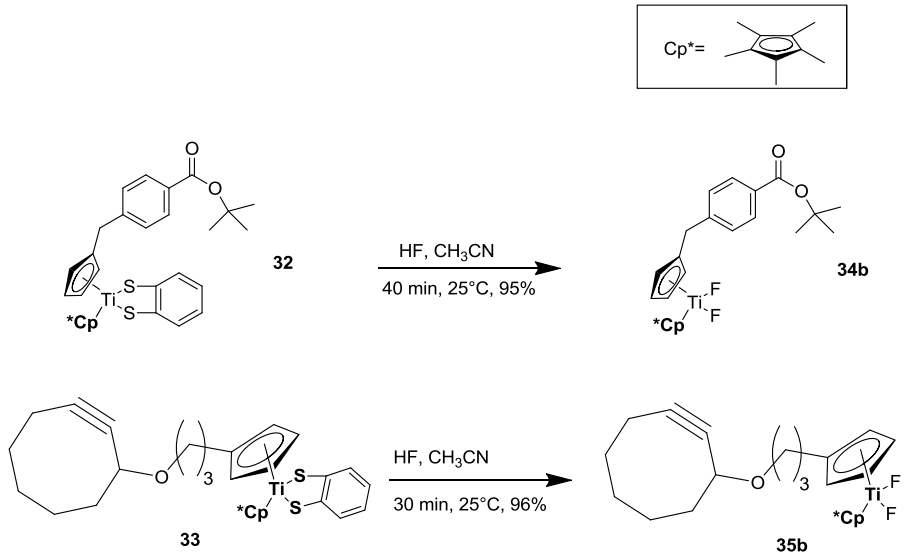
Thanks to these results we decided to modify the derivatives 3b and 12b in two compounds, 32 and 33 that show the dythiolic system. This approach was in according with the late stage fluorination for PET-probes.[72]

[71]K. Mach, V. Vojtech; Journal of Organometallic Chemistry, 1988, 347, 85-92.
[72] C. N. Neumann and T. Ritter; Angew. Chem. Int. Ed. 2015, 54, 3216–3221.



Scheme 27: Synhtesis of Titanocene modified with 1,2benzendithiol system.

Indeed we decided to synthetize derivatives 34 and 35 with two fluorine atoms to test the exchange protocol. (Scheme 28)



Scheme 28: Synthesis of Titanocene Fluoro-modified

4.7.Hydrolitic stability

In order to study the application of these derivatives *in vitro* and *in vivo*, we collected stability data in physiological solution of the Compound 34a. Results are presented below (Figure 13).

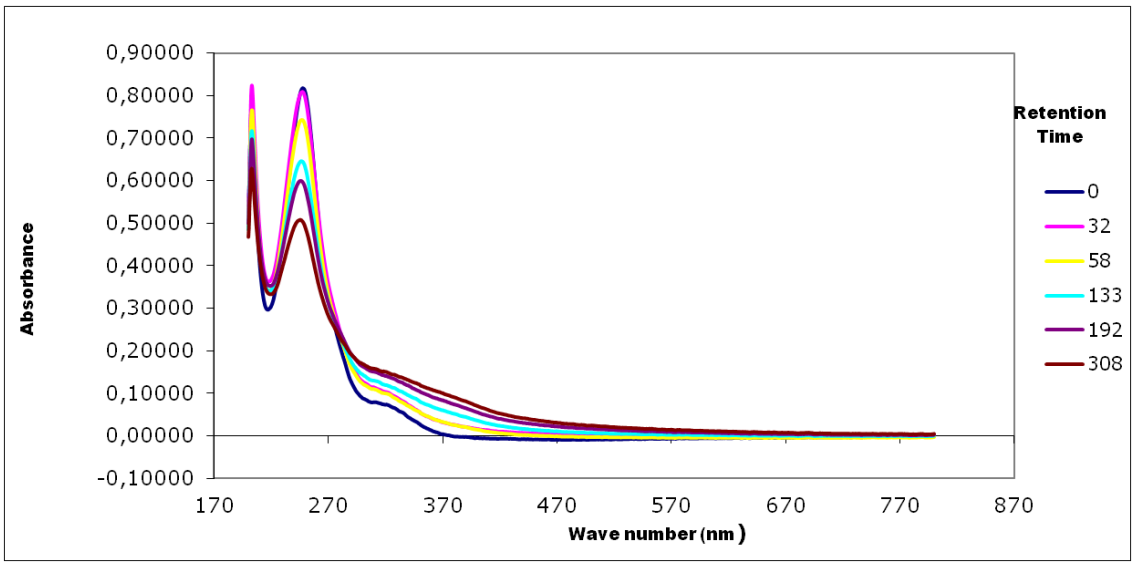


Figure 13: the figure shows the hydrolitic stability of compound 32a, maximum UV Absoprtion: 247 nm

Data reported shows a degradation of this derivative of 40% after 308 min. of analysis. This value was calculated assuming at $time_0$ the stability at 100% and maximum absorbance. Variations from the maximum value of absorbance at different times lead to degradation rate.(Figure 14)

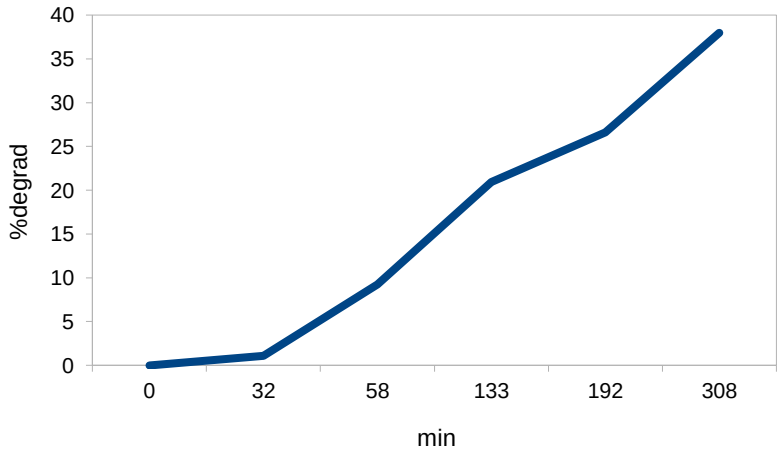


Figure 14: Degradation of 34a on time

In order to evaluate the effect of the substitution grade on a Cp ring we decided to test the derivative 34b and results are presented below.

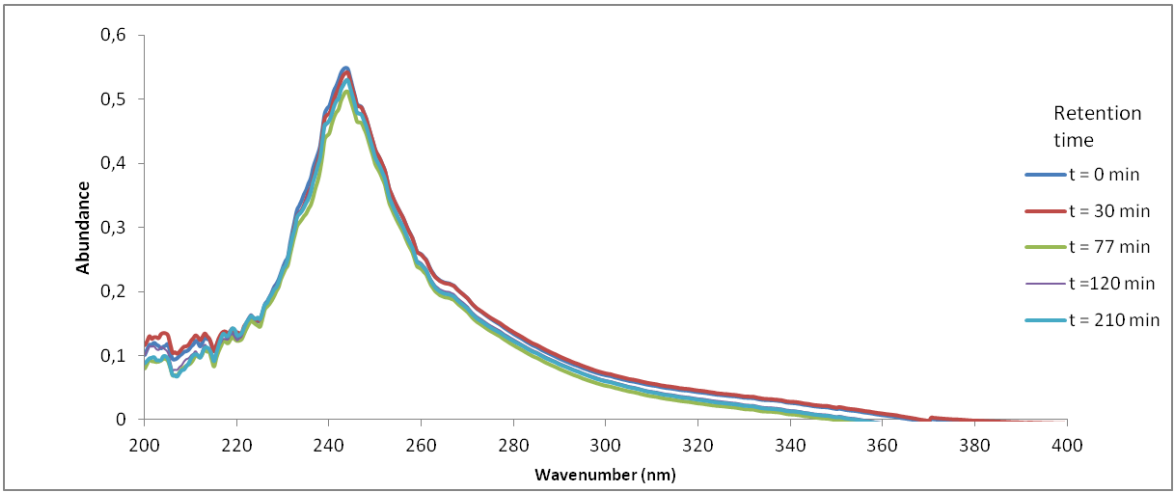


Figure 15: Absorbance of 34b

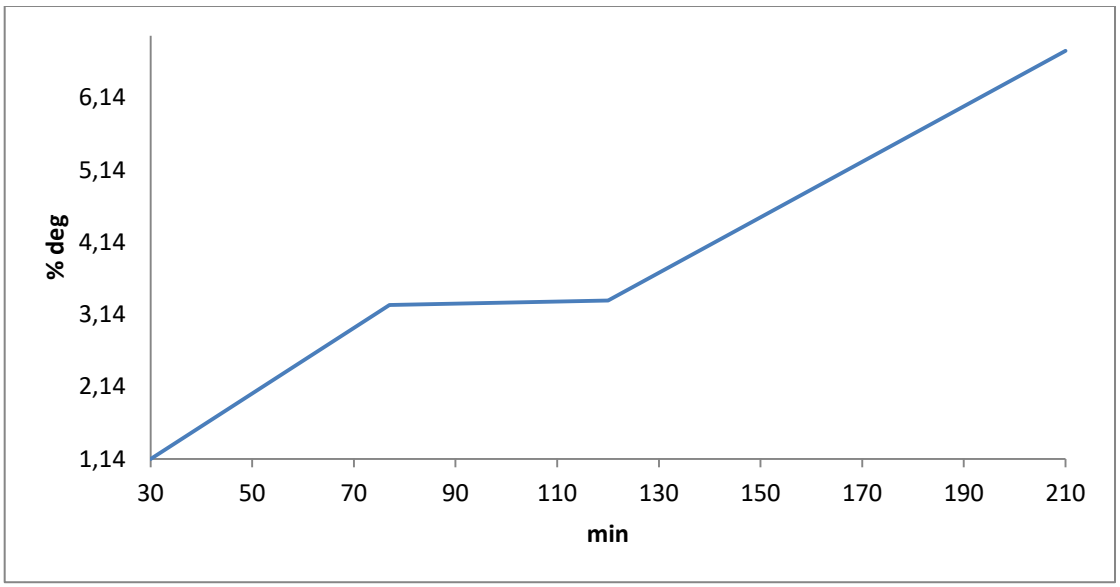


Figure 16: Degradation of 34b on time

Figures 15 and 16 show that the addition of five methyl groups on the ciclopentadienyl ring, increase the stability of the entire complex; the degradation value change from the 27% (192 min) of Cp_2TiF_2 to the value of 5.3% (191 min) for $CpCp^*TiF_2$

Chapter 5: Antitumoral Applications

5.1.History of Antitumor Agents metal-based

The recent history of metal-containing antitumor agents began with the detection of antitumor activities of inorganic compound the cis-diaminedichloroplatinum(II) (cisplatin, Figure 12) in 1969.[73] The great success of this compound against various types of cancer(in particular urogenital tumors and carcinomas of the head and the neck) opened a broad search for further antitumor organometallic compounds.[74,75] Even thought these compounds showed high antitumor activities against a wide range of human tumors, their use was often associated with dangerous side-effects and, this problem represented the principal limitation to their therapeutic efficiency.[76]

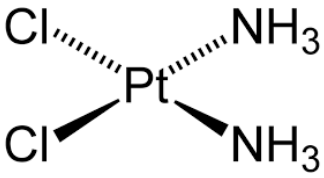


Figure 17: structure of cisplatin

In this context must be inserted studies on titanium-based drugs. For example, Budotitane (Figure 18a , [cis-diethoxybis(1-phenyl-1,3-butanedionate)-titanium (IV)], proved to be very promising in the preclinical evaluation phase, but did not pass phase I clinical trials, due to its rate of hydrolysis[77].

[73] Rosenhere. B.: Van Camo. L.: Trosko. J. E.: Mansour. V. H. Nature (hdbn) 1969,222, 385
[74] P. Kopf-Maier and H. Kopfs; Chem. Rev. 1987, 87, 1137-1152.
[75] V. Cepeda, M. A. Fuertes, J. M. Jerez Castilla; Anti Canc. Agents Med. Chem. 2007, 7, 3
[76] Kater, L.; Claffey, J.; Hogan, M.; Jesse, P.; Kater, B.; Strauss, S.; Tacke, M.; Prokop,A. Toxicol. in vitro 2012, 26, 119.
[77] Scilling, T.; Keppler, B. K.; Heim, M. E.; Niebch, G.; Dietzfelbinger, H.; Rastetter,J.; Hanauske, A. R. Investig. New Drugs 1996, 13, 327.

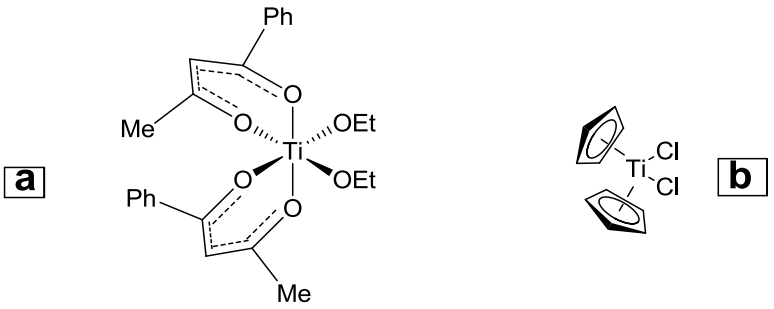


Figure 18: Structures of Budotitane (a) and Titanocene dichloride (b)

Titanocene dichloride (Cp_2TiCl_2), (Figure 18b.), is much more resistant in this aspect, shows moderate antiproliferative activity in vitro, and promising results in animal model reaching phase II clinical trials.[78] Unfortunately, its efficiency in patients with metastatic renal cell carcinoma or metastatic breast cancer was too low to be pursued.[79] Even thought these results weren't outstanding, the potential of this compound led the global scientific community to continue studies in order to synthesize the most effective derivatives.[80] In fact the research group of Prof. Tacke designed a range of heteroaryl and *ansa* titanocene derivatives to improve the hydrolytic stability and the efficiency of the drug[81]. In particular, a study of the cellular effects of the p-methoxybenzyl derivative (Titanocene Y in Figure 19) on prostate cancer cells showed that it was more capable to induce apoptosis than cisplatin in a dose-dependent manner[82]

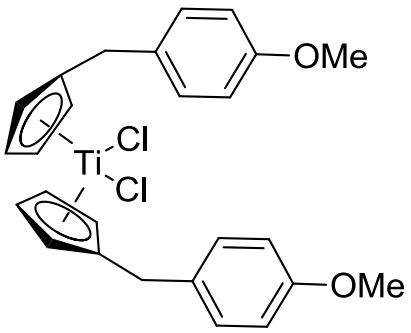


Figure 19: structrure of Titanocene Y

[78] Kröger, N.; Kleeberg, U. R.; Mross, K.; Edler, L.; Saß, G.; Hossfeld, D. Onkologie, 2000, 23, 60.
[79] Melendez, E. Crit. Rev. Oncol. Hematol. 2002, 42, 309.
[80] M. M. Harding and G. Mokdsi; Current Medicinal Chemistry 2000, 7, 1289-1303
[81] Strohfeldt, K.; Tacke, M. Chem. Soc. Rev., 2008, 37(6), 1174-1187.
[82] O'Connor, K.; Gill, C.; Tacke, M, N.; Fitzpatrick, J.M.; Watson,Apoptosis, 2006, 11, 1205-1214

5.2.Mechanisms of Cytotoxicity of Anticancer Titanocenes

Cancerostatic activity and tumor inhibition by application of Cp2TiCl2 against Ehrlich ascites tumor (EAT) cells in a CF1 mouse model was first described by Köpf-Maier in 1979.[83] It has been proposed that such complexes may interact with DNA and inhibit cell cycle, although the antitumor mechanism depends on the transport and delivery of Ti species into cancer cells. Furthermore, the hydrolysis rate plays a major role for the tumor-inhibiting potency, because it is correlated to the interaction with nucleic acids, proteins and other potential intracellular targets.[84] Interactions of Cp2TiCl2 with human serum transferrin, and the interactions of Ti^{2+}-transferrin with adenosine triphosphate (ATP), could allow the Transferrin to mediate titanium delivery to tumor cells.[85] This mechanism requires hydrolysis of labile Cp–Ti bonds on a time scale to pass titanium to transferrin. Generally, transferrin takes Fe^{3+} up from blood plasma and delivers it to cells via receptor mediated endocytosis. Therefore, this protein has been implicated as shuttle for a number of metal ions and anticancer drugs. In support of the important role of transferrin in the biological chemistry of titanium (IV). Tinoco and Valentine demonstrated that titanium(IV) binds to human serum transferrin more tightly than $Fe3^{+}$.[86] Bound titanium(IV) is then complexed with ATP at cellular endosomal pH values, possibly facilitating transport to the nucleus and interaction with DNA. On the other hand, titanocene derivatives that presents Cp–Ti bonds stable under physiological conditions may exert different action mechanisms. Thus, titanocenes in which the metal–Cp bonds are stabilized by substitution of the Cp rings (altering electronics, polarity and solubility) and also permits the release of titanium (IV) in order to bind transferring, are expected to exhibit biological profiles similar to Cp2TiCl2[87]. To confirm these hypothesis, different derivatives were synthesized and tested.[88] Otherwise, the two main problems of the titanocene dihalides, as poor aqueous solubility and hydrolitic instability, were both addressed in recent years by chemical synthesis.[89]

[83] Köpf, H.; Köpf-Maier, P.. Angew. Chem. Int. Ed. Engl., 1979, 18, 477-478. b) Köpf-Maier, P.; Hesse, B.; Köpf, H. Cancer Res. Clin. Oncol., 1980, 96, 43-51.
[84] Kostova, I. Anti-Cancer Agent Me 2009, 9, 827.
[85] Guo, M.; Guo, Z.; Sadler, P.J.; J. Biol. Inorg. Chem., 2001, 6(7), 698-707.
[86] Tinoco, A.D.; Eames, E.V.; Valentine, A.M.; J. Am. Chem. Soc., 2008, 20, 130(7), 2262-2270
[87] U. Olszewski and G. Hamilton Anti-Cancer Agents in Medicinal Chemistry, 2010, 10, 302-311
[88] Kelter, G.; Sweeney, N. J.; Strohfeldt, K.; Fiebig, H.-H.; Tacke, M. Anti-Cancer Drugs 2005, 16, 1091–1098
[89] Allen, O. R.; Croll, L.; Gott, A. L.; Knox, R. J.; McGowan, P. C. Organometallics 2004, 23, 288–292

Our titanocenes derivatives developed for PET imaging shows interesting characteristics for the antitumoral application (Figure 20) :

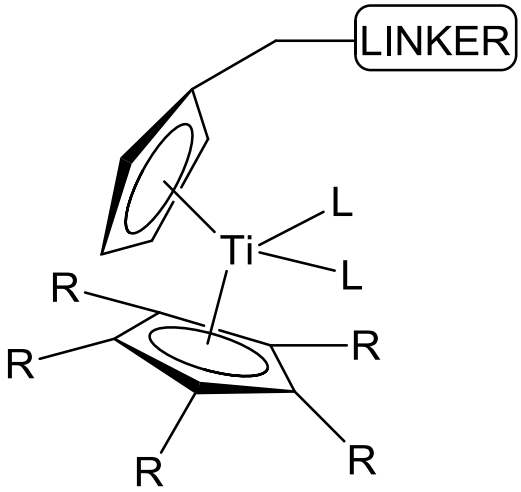


Figure 20: general structure of modified titanocene

- The use of different titanium complexes could allow a different titanocene with different grade of Cp-functionalization (here shown as R-group)
- The use of different L (ligands) as Fluorine, chlorine or thiolic, can also affect the hydrolytic stability of the system.
- The linker that could be used for the conjugation with various biomolecules to improve both the selectivity and efficiency of titanocene derivative.

For these reasons, we decided to submit to biological evaluation different complexes in wich the linker was modified with a carboxylic function to improve solubility. Furthermore, two different cyclopentadienyl ligands for Titanium were chosen: the classic one and the modified with five methyl groups. Finally, three different ligands (L-groups in figure 20) as chlorine, fluorine and 1,2-benzendithiole were selected.

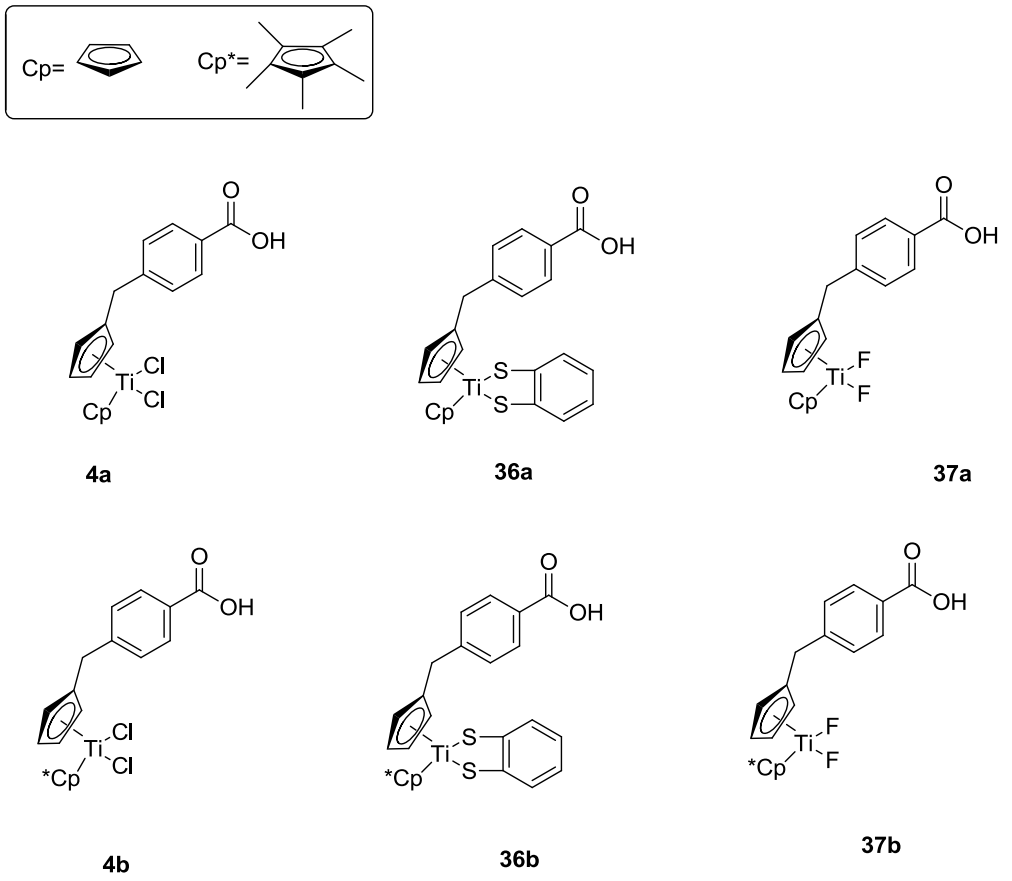


Figure 21: Compounds submitted for biological evaluation

Below is presented the biological evaluation of compounds descrive above: (Table 6)

Compound	MCF7	SkBr3	IST-MES1	A549	BG-1	Ishikawa
Cisplatin	17 (±4)	10 (±2)	10(±3)	13(±2)	12(±3)	12(±2)
4a	>50	>50	>50	>50	>50	>50
4b	35 (±4)	18 (±4)	18 (±5)	15 (±2)	17 (±3)	20 (±5)
36a	>50	>50	>50	>50	>50	>50
36b	>50	>50	>50	>50	>50	>50
37a	>50	>50	>50	40 (±8)	38 (±2)	37 (±8)
37b	>50	23 (±3)	20 (±2)	30 (±5)	30 (±5)	33 (±3)

Table 6: Biological Evaluation

The cytotoxic activity of compounds tested after 24 h treatment, as determined by MTT assay. IC50 values were calculated by probit analysis ($P<0.05$, $\chi 2$ test). Cytotoxic activity of tested compounds on breast MCF7 and SkBr3, mesothelioma IST-MES1, ovarian BG-1, endometrial Ishikawa, and lung A549 cancer cells after 48 h treatment, as determined by using the MTT assay. As shown in table 6, no derivatives presented good antitumoral activity, but this study permits some considerations:

- As mentioned above the antitumoral effect of a titanocene derivative is directly related to the hydrolytic stability of the complex, in fact, more stable is the complex most difficult is the formation of side-products such as titanium dioxide.
- However, the derivative should not to be too stable to allow the action of Transferrin.

Moreover, the simultaneous presence of five methyl groups on the Cp-ring (increased stability) with two chlorine atoms as ligands translate into betterl conditions for anticancer-treatment. Furthemore, it is clear that the presence of dithiolic ligands (less strenght of the Ti-S bond) is not compatible with acqueous or serum solutions. Otherwise, the presence of two fluorine atoms results in a low activity of the molecule, due probably, to the lower capacity of Transferrin action. Finally we think that the the lack of effectiveness is due to the linker inserted: we suppose that this derivative, without further coniugation, doesn't permit a good solubility, resulting in a decrease of the bioavailability of the Titanium.

Chapter 6: Solid Phase Synthesis

To solve purification problems of Titanocene-Biomolecules conjugates, we decided to change the purification process, taking advantage, as described in the previous chapters, the ability of particular dithiolic ligands to form very-stable Titanium-complexes. In particular, we selected solid-phase synthesis as a useful tool for ours purposes. We hypothesized to take advantage of the binding sites occupied by L in Figure 15 for the purification of these complex, in fact, with an appropriate bi-dentate ligand anchored in solid phase we can imagine to easily remove impurities (filter the solution and retrieve the resin) leading to a rapid purification of the entire complex.

6.1.Commercial ligands

A potential candidate for our applications could be a system Tio-Aza-2-mercapto aromatic (Figure 22 a) which is already commercially available supported on polystyrene base. In addition, this derivative mimic the behavior of dithiolic ligands (Figure 22b) used previously in this thesis work to synthesize stable Titanium complexes..

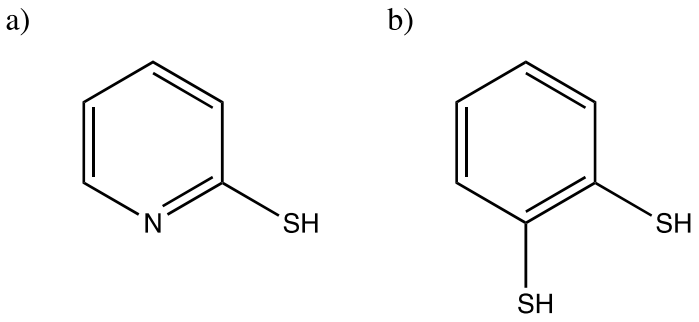


Figure 22: a)2-thiopyridine; b) 1,2 benzendithiole

The key point of this type of synthesis is the characterization of the derivatives. To solve various problems we decided to use the scanning electron microscope (SEM) techniques with Energy Dispersive x-ray Spectrometry (EDS). The analyses were made both on the

resin bonded in their natural state or after Browning needed to increase its conductivity. Figure 23 shows the polystyrene resin in solid phase on which we performed the analysis. In Figure 20 are reported quantitative results of EDS pattern. Unfortunately, the micro elemental analysis conducted to SEM-EDS on a commercial resin, compared with the resin with titanocene Cp_2TiCl_2 (Figure 24) were not able to provide results clear enough to guarantee that there is a correlation between the traces of titanium, already present on some beads, and the tests already carried out. Therefore it was decided to continue this work by designing a new resin.

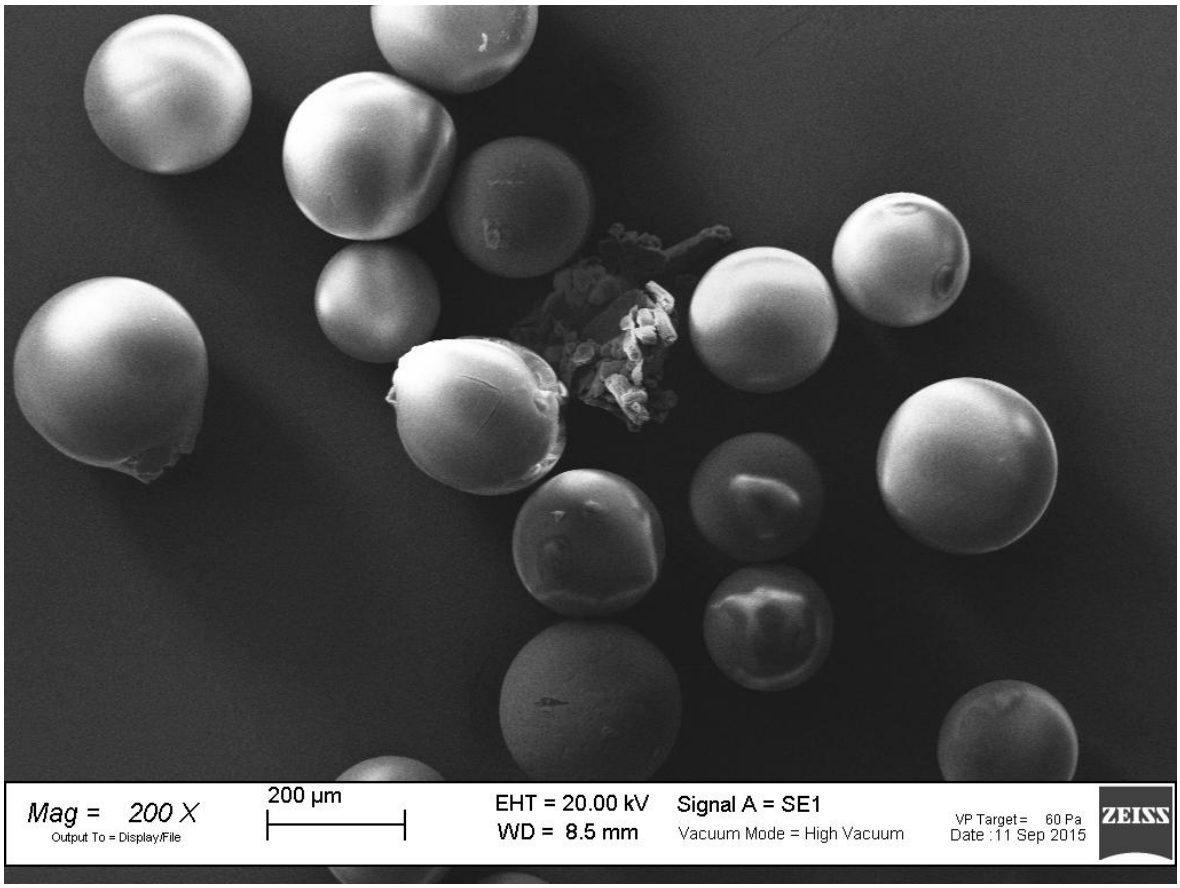


Figure 23: SEM carachterization of titanocene bonded on the resin

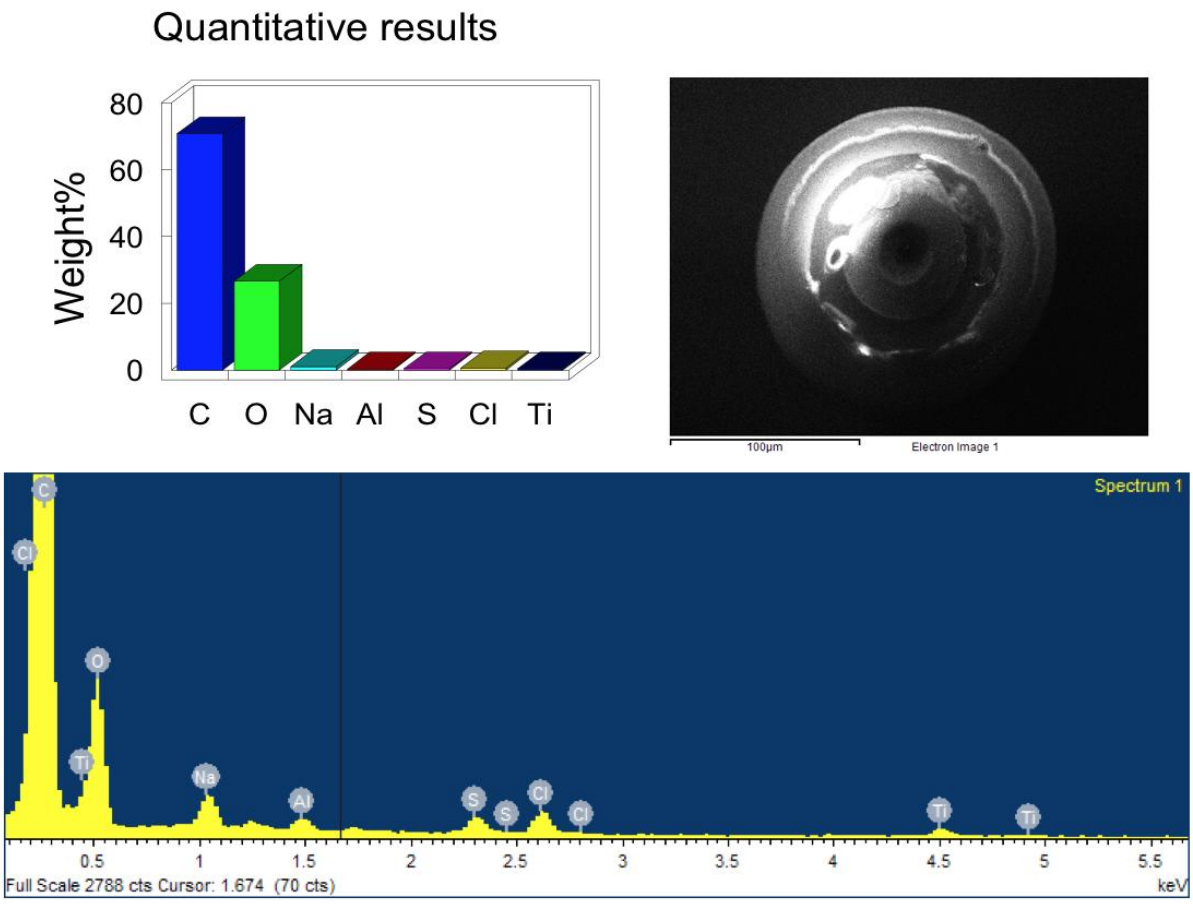


Figure 24: EDS analisys on commercial resin

6.2.Development of the dithiolic-ligand

Combining doubts of EDS pattern interpretation, with the fact that the resin has been withdrawn from the market at the beginning of this thesis work~~;~~ we decided to synthetize a new ligand and to link this ligand to a commercially available resin such as Tentagel-NH2. This choice was based on previous studies produced in our laboratory in which we focused on dithiolic linkers for titanium. This choice was motivated by the fact that these ligands~~,~~ showed good stability (Ti-S 426 kJ/mol) and a good kinetic of exchange with fluorides or other halogens, features that are mandatory for subsequently applications. First of all we thought to modify our tiol-ligand with a third functional group (Figure 25b) suitable for linking with the resin.

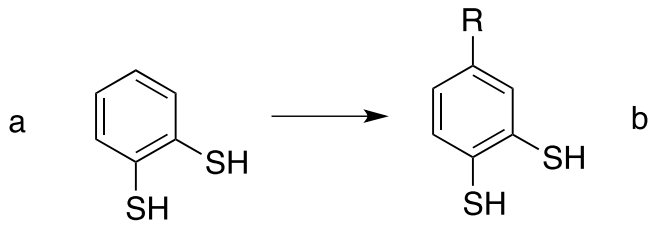


Figure 25:(a) Benzendithiole, (b) modified benzendithiole

In order to link our compound, we decided to use an amidic-bond, which, would not interfere with the reactions carried out on supported -titanocene. Therefore, we decided to functionalize the compound in Figure 21a as carboxylic acid~~-~~, therefore, our synthetic target, became the acid 3.4-dimercaptobenzoic (Figure 26).

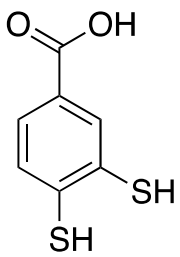
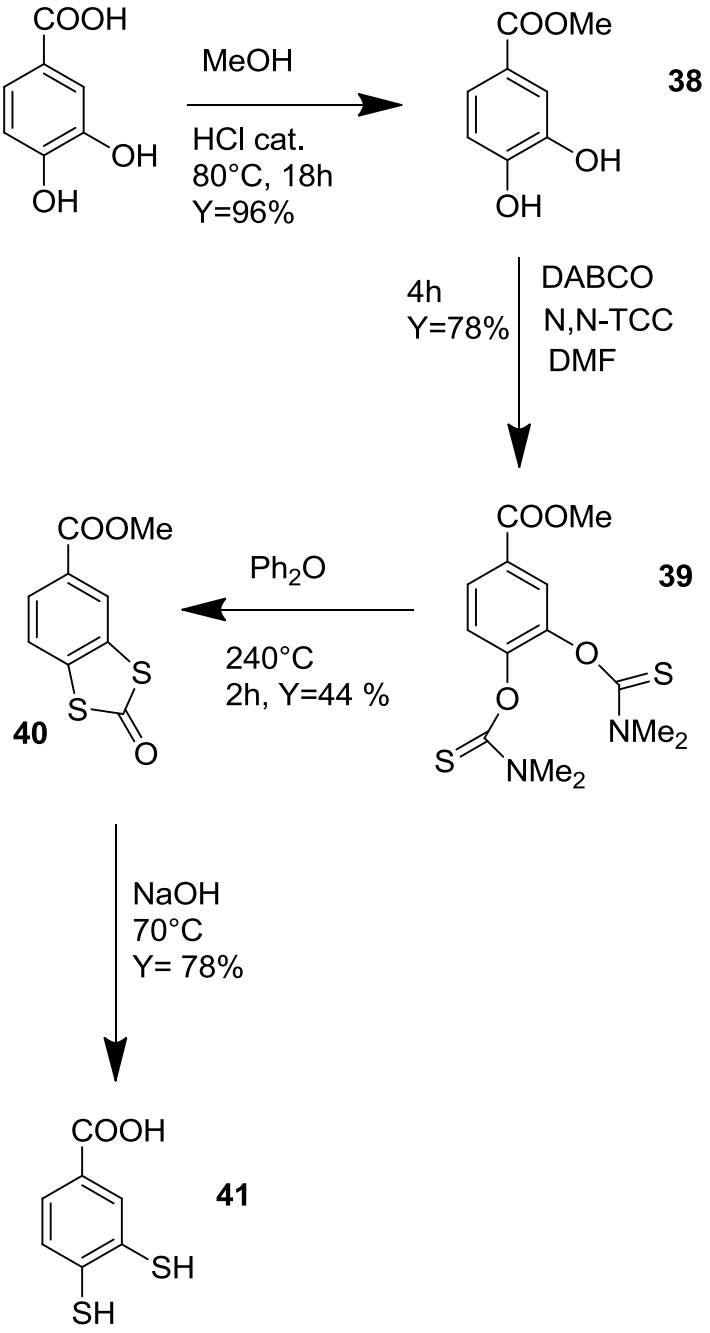


Figure 26: 3,4 dithiobenzoic acid

Below is depicted the synthetic pathway in order to obtain the target compound following the procedure of Geer and co-workers[90]. (Scheme 29)

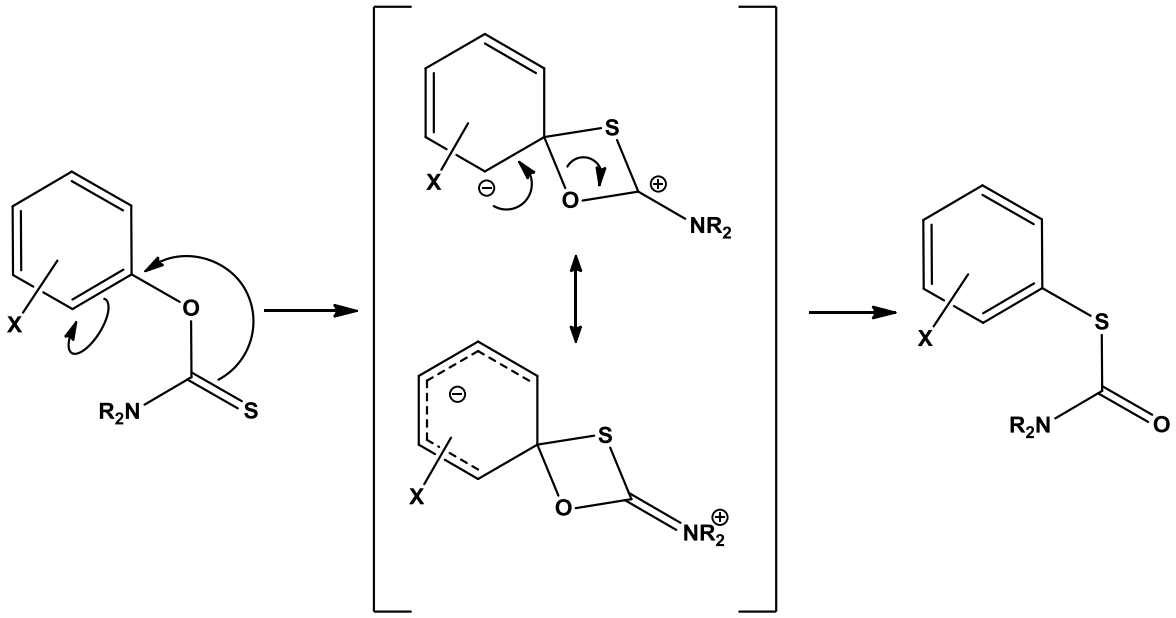


Scheme 29: Synthesis of 3,4 dithiobenzoic acid

As starting material the commercially available 3.4-dihydroxybenzoic acid has been used, which, after a classical Fisher HCl mediated esterification, afforded the corresponding

[90] Y. Adaickapillai, A. Vuong, D. Aebisher, Y. Gong, A. Greer; J. Org. Chem., 2010,75,5549-5557.

methyl ester which was used in the next synthetic step. Compound 38 is then treated with N,N-thiocarbamoyl-chloride, in the presence of a suitable base, affording product 39, wich is the substrate for the key step of the synthesis: the Newman-Kwart (NKR) rearrangement the mechanism of which is illustrated in Scheme 30.



Scheme 30: Newman-Kwart rearrangement

The reaction mechanism shows how is possible to replace an aromatic oxygen with an aromatic thiol, through an high temperatures mediated rearrangement, using an high-boiling solvents such as diphenyl ether (Ph2O). The reaction was, in fact, performed at 240° C to obtain the product (40) which in our case derived from a double NKR rearrangement. Since we need to obtain the free thiol functions for the Titanium complexation, we treated compound 40, with NaOH (Concentrazione) our target compound (41) with a 78% isoltaed yield after a final water recrystallization.

Once we had the ligand, before to start with the solid phase chemistry, we encompass few solution phase experiments using the commercial available 2-(3.4-dimetossifenil-ETAN-1-amine) as a model. This model share some common features with the chemical functionality present of the resin. (Figure 27)

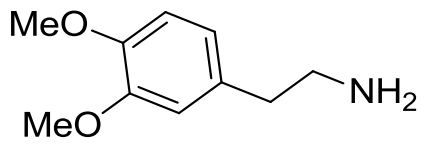
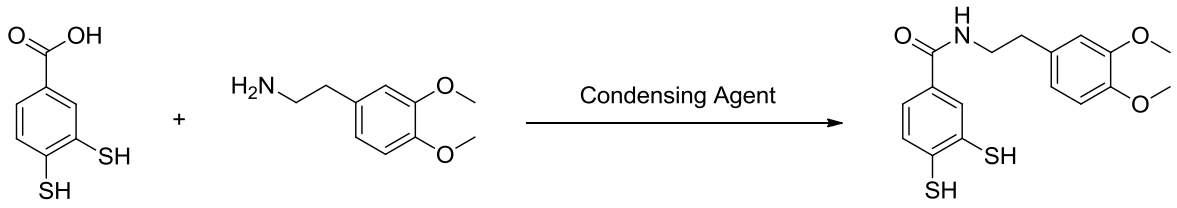


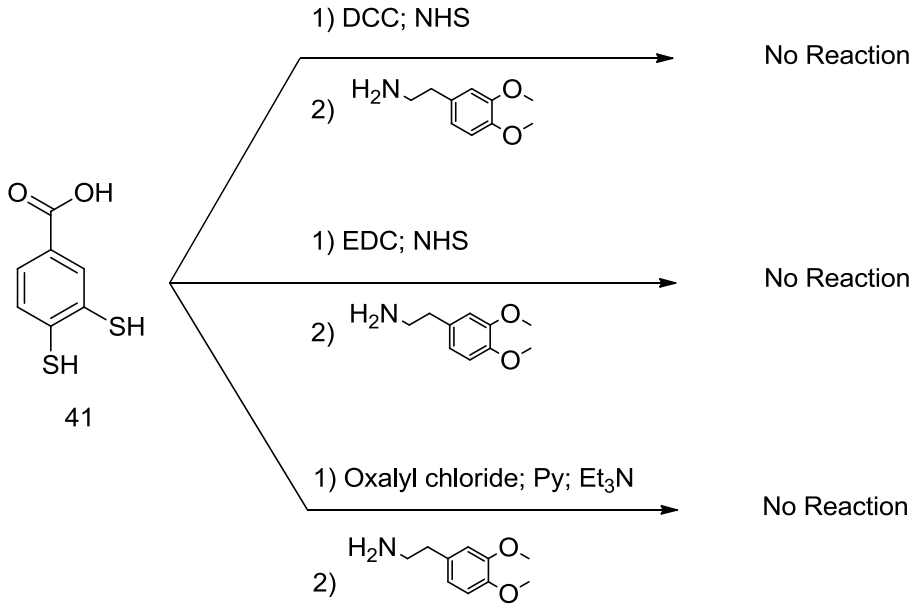
Figure 27: Structure of 2-(3,4-dimetoxyphenyl)ethan-1-amine

6.3.Omogeneous Phase Synthesis

In Scheme 31 and 32 are illustrated our attempts to find out the best reaction conditions for the key amide bond formation.

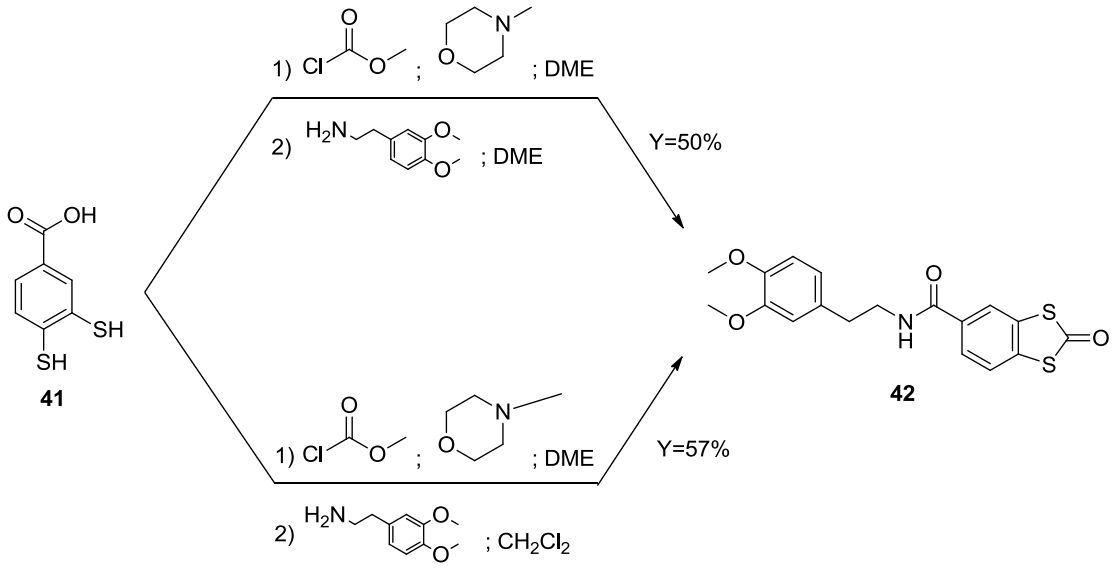


Scheme 31: Amidic Bond formation



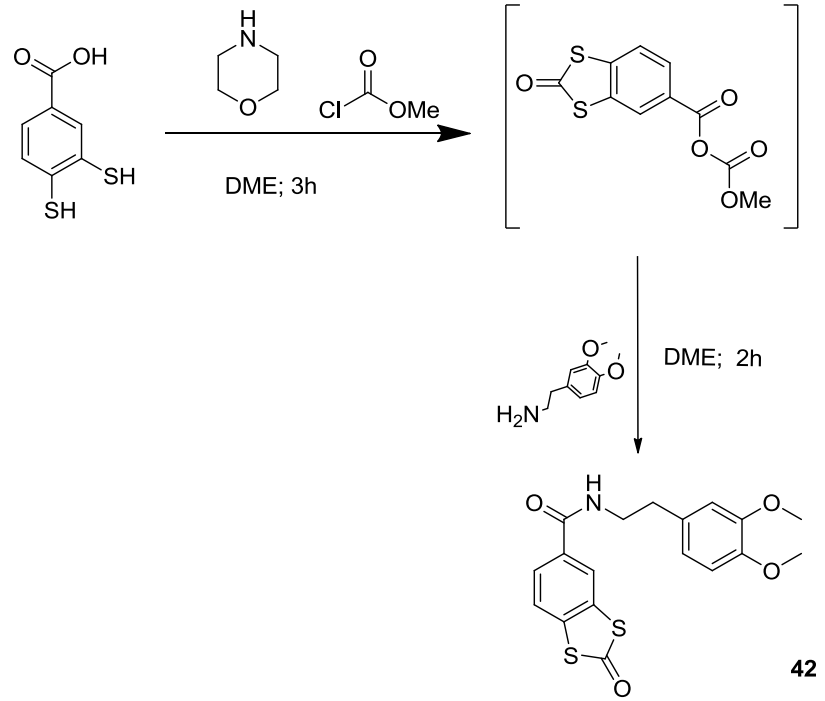
Scheme 32: Condensation attempts, was not possible to determine the product formation

The first three experiments (Scheme 32), include the use of condensanting agents commonly used in peptide synthesis, but this failed to provide the desired product. In all the reactions we checked through TLC (and LC-MS analysis) a spot formation, that we assumed to be the desired product, but after work-up, it was not possible to isolate the desired compound . Probably, it was due to the free SH functions, acting as nucleophiles, that could interfere with the synthesis of amide bond. Then, was decided to change the synthetic tactic (Scheme 33).



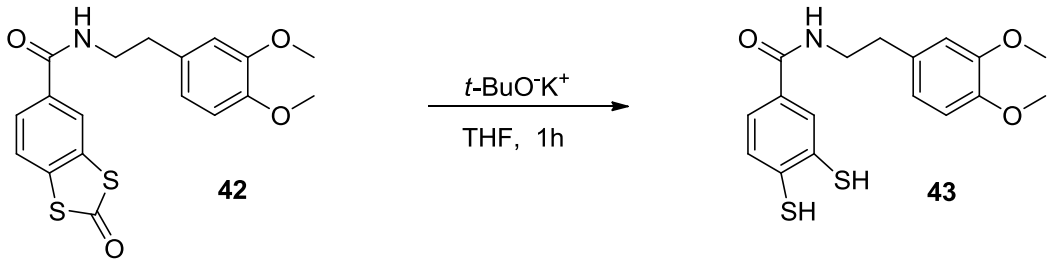
Scheme 33: Different condensation protocols

In the showed approach we used the mixed anhydride tactic protocols to afford the desired product. In the event, compound 41 was treated with Methyl-cloroformate in the presence of N-methyl-morpholine as a base in a suitable solvent affording compound 42.



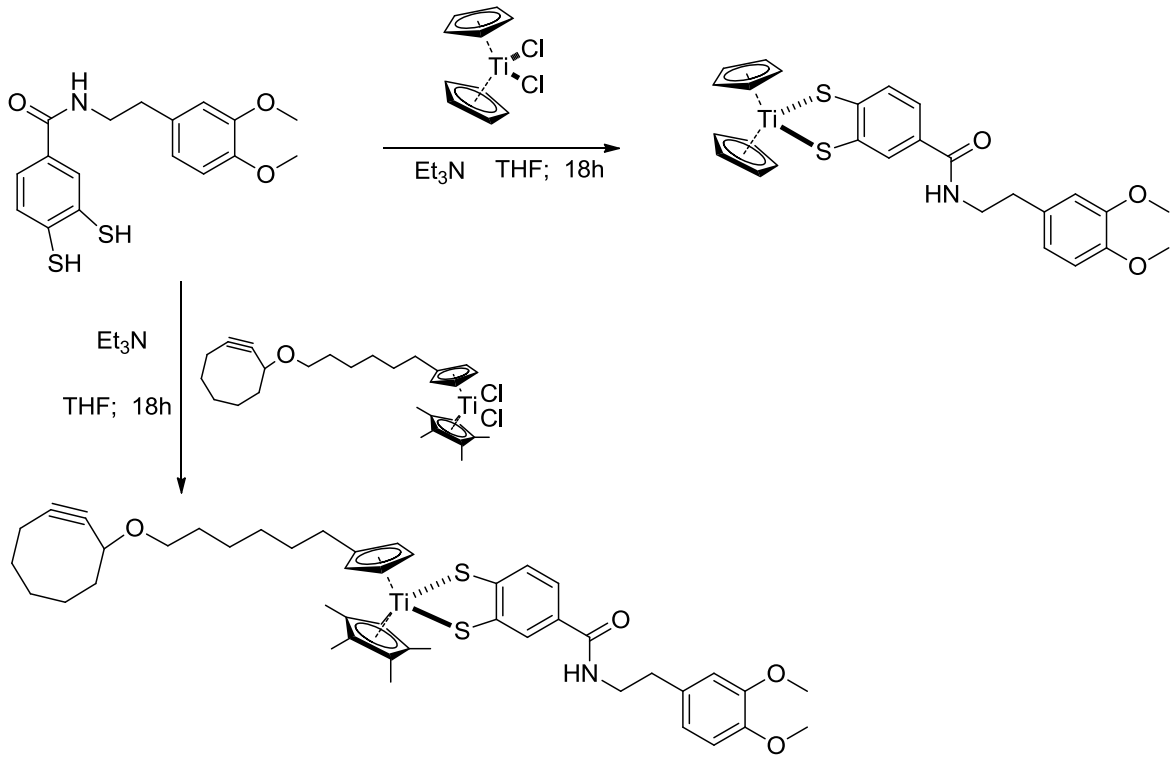
Scheme 34: Synthesis of 42 via active carbonate

The scheme 34 shows that the Thiocarbonate function act as a protecting group for free thiols prevent their nucleophylicity. Thus, the activated carbonate reacted with the amine providing the desired product (42) with a yield of 50%. In order to further optimize the procedure for the subsequently solid phase synthesis, the DME, which is known to be unable to swell the resin, was replaced with DCM, a powerful swelling solvent. Under this conditions, we obtained the desired product in a yield of 57%, the thiol deprotection which is encessary to permit the coordination with the titanocene, was performed with potassium Tert-butylate as a base (Scheme35).



Scheme 35: Deprotection reaction

Formation of the desired product was confirmed by NMR analysis of the crude product, but was not possible to separate it from the starting material. To solve this problem, we firts tried to add the Titanium Complexes, Cp_2TiCl_2 or our Titanocene 12, (Scheme 36) to promote the condensation between our Ligand and the metallic centre. Unfortunately we were not able to extract the desired complex from the reaction mixture. However, the good news was that we were able to find the complex 44 in the reaction mixture.



Scheme 36: Attempts of isolation of Titanium complexes

Seen the failures due to classical purification methods we moved ahead to heterogeneous solid-phase synthesis.

6.4.Solid-phase synthesis: General procedure

After having developend the protocol for the solution phase chemistry, we moved to heterogeneous phase to get the Functionalized resin, our target.

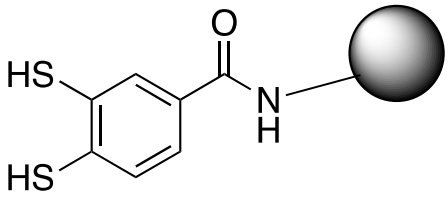


Figure 28: Dithiolic Resin Target

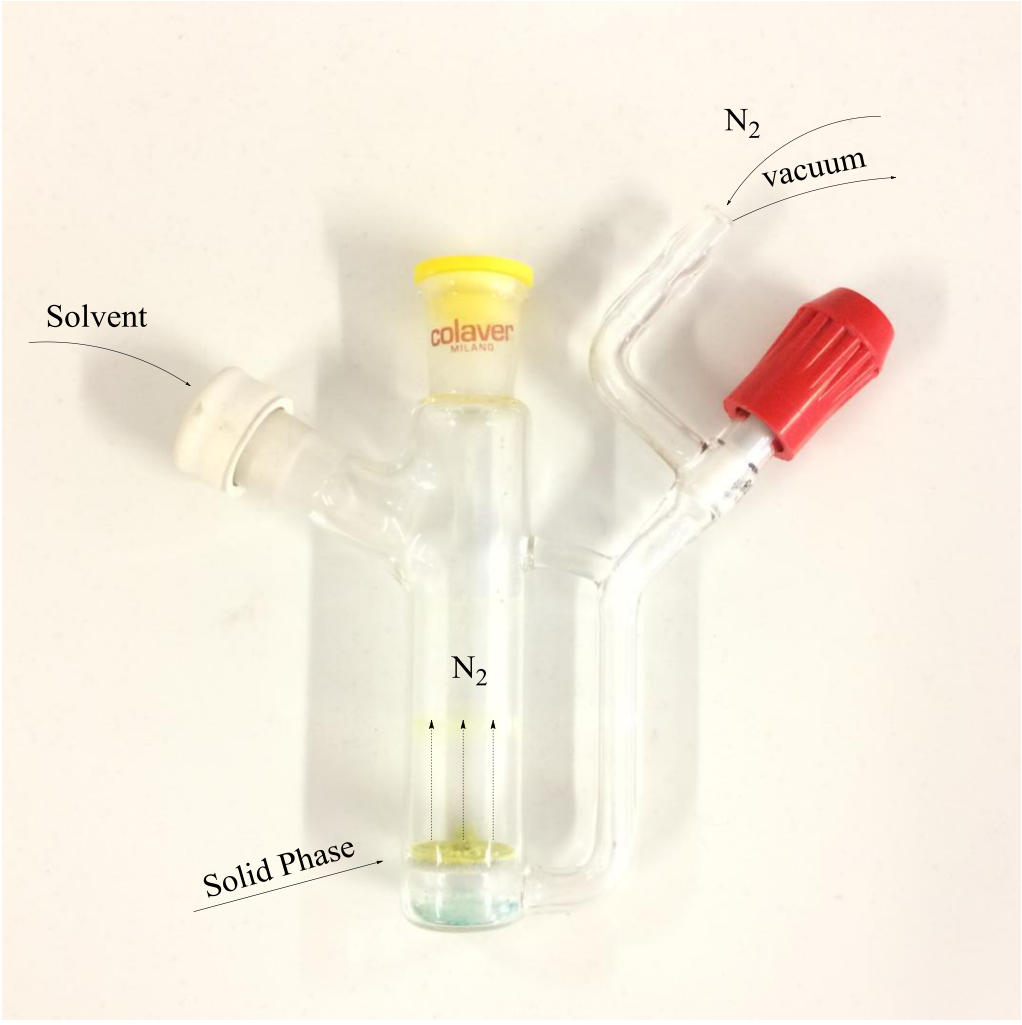


Figure 29: Reactor used in solid phase synthesis

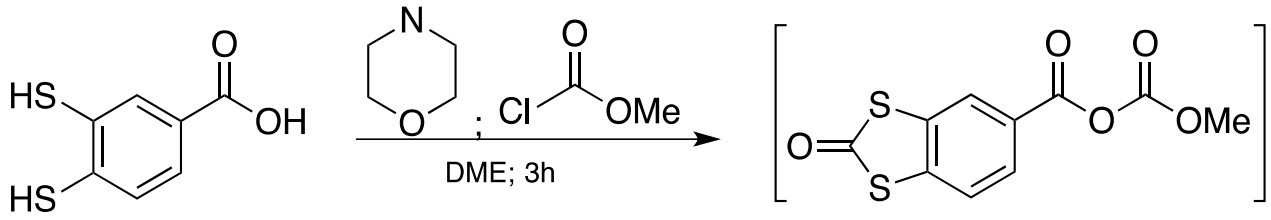
This reactor (Figure 29) allows, through the valve on the right side, to connect a dry nitrogen flow which, flowing through the sintered glass, maintains the inert atmosphere necessary to prevent the titanium complex degradation and allowing the stirring . Indeed, the use of magnetic stir-bar is not advisable because the friction between the bar and the reactor walls, could irreversibly damage the resin by milling the beads. Before each synthetic step, the resin was prepared, enabling it to swell in an appropriate solvent. The preparation took about an hour under dry nitrogen stream and, after this time, we performed the reaction. Once the reaction is completed, the nitrogen flow was replaced with an attack on a vacuum pump, to eliminate the solvent leaving the resin solid phase on the sintered filter. After each reaction the resin has been flushed from 6 to 9 times with different solvents, usually in the order (of polarity) DMSO or DMF, THF and finally CH_2Cl_2. Each washing cycle included:

- -Removal of the solvent in the reactor via a vacuum
- -Addition of the washing solvent
- -Shaking for about 15 minutes under a flow of dry nitrogen
- -Removal of the solvent from the reactor with vacuum.

These steps will be omitted from now on in this chapter but are given in the experimental section. Solid-phase synthesis started with forming the amide bond, testing the only procedure which gave us good results in homogeneous phase.

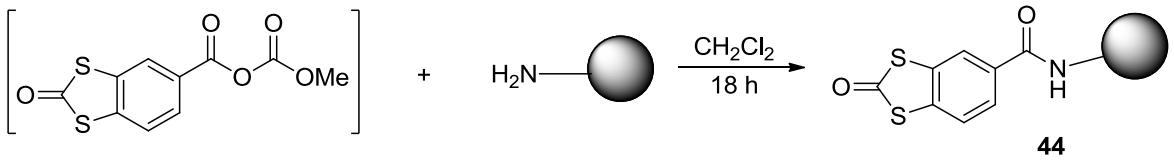
6.4. Synthesis of Target Resin

The 3,4-mercaptobenzoic acid previously prepared, was treated with N-methylmorpholine and methyl chlorofomate to afford the activated anhydride. (Scheme 37)



Scheme 37: Synthesis of Activated carbonate

Without further purifications, the compound activated was dried under vacuum, dissolved in dichloromethane and subsequently added *via cannula* to a suspension of the resin swelled in DCM in the solid–phase reactor.(Scheme 38)



Scheme 38: Synthesis of dithiocarbonate resin modified

The reaction product 44 was obtained as a spongy solid and was characterized with SEM-EDX microscopy and IR spectroscopy. Furthermore was analyzed the commercially available Tentagel-NH_2 resin as a blank. SEM-EDX and IR analyses of the commercial resin are shown in the Fig 30 and 31. In these images we can observe only the presence of carbon and oxygen, according with our expectations.

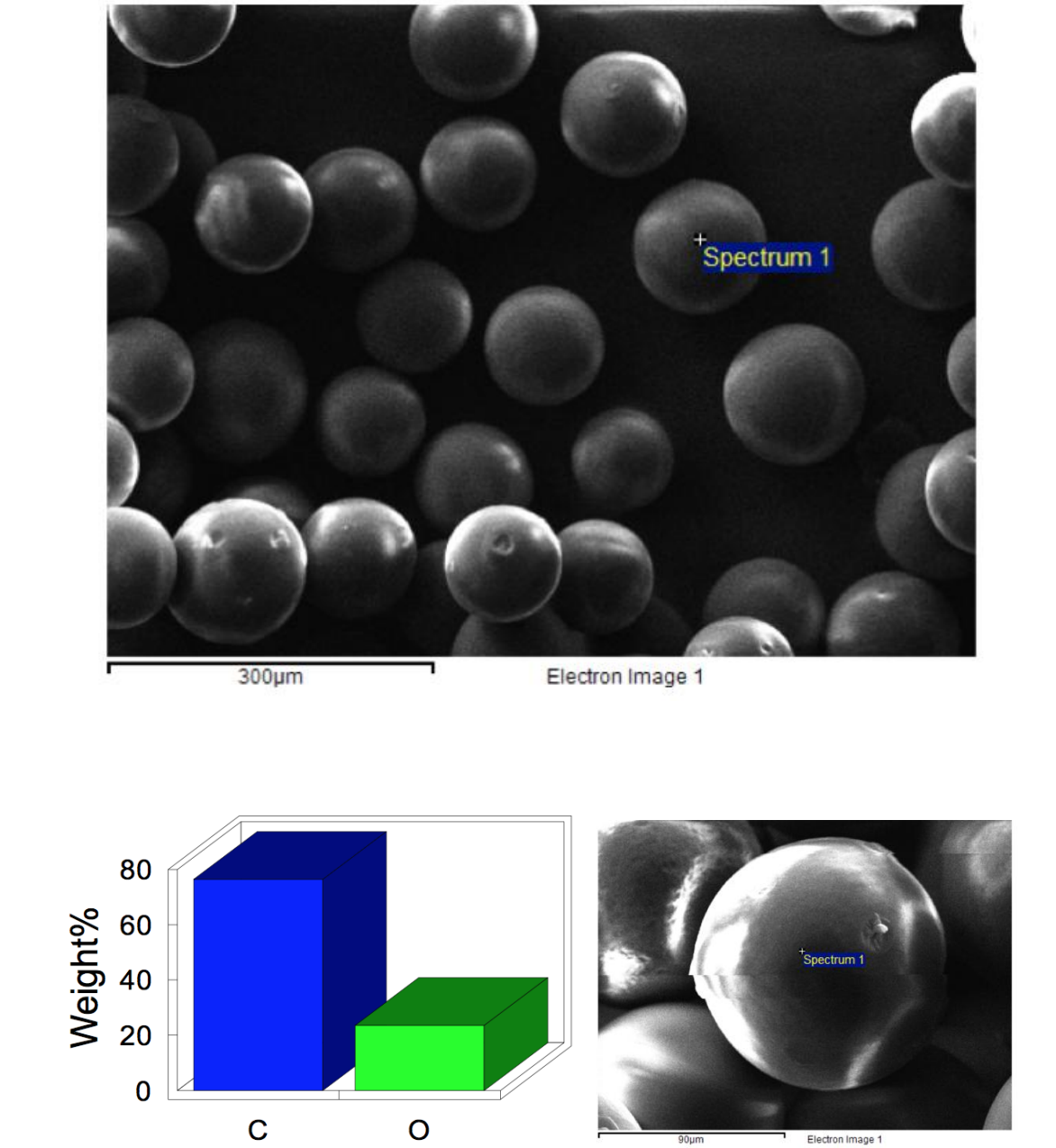


Figure 30: SEM-EDX analyses of commercial resin Tentagel-NH2

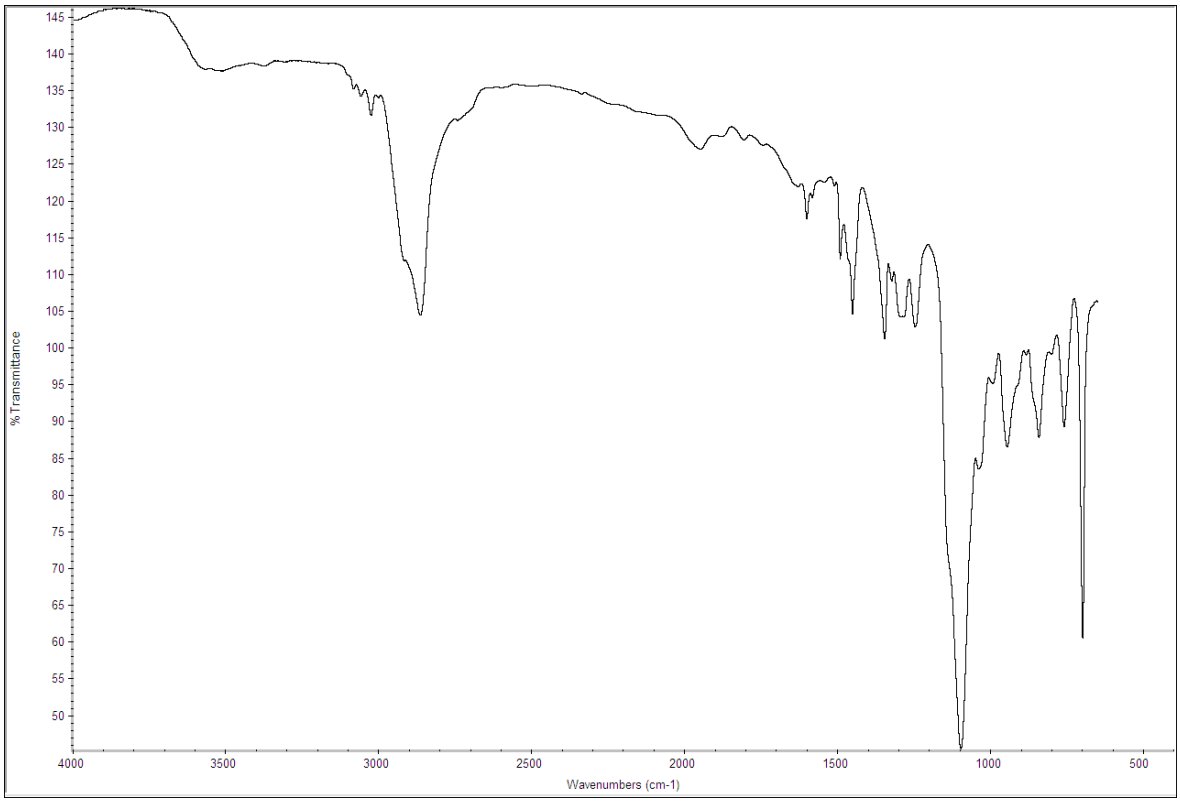
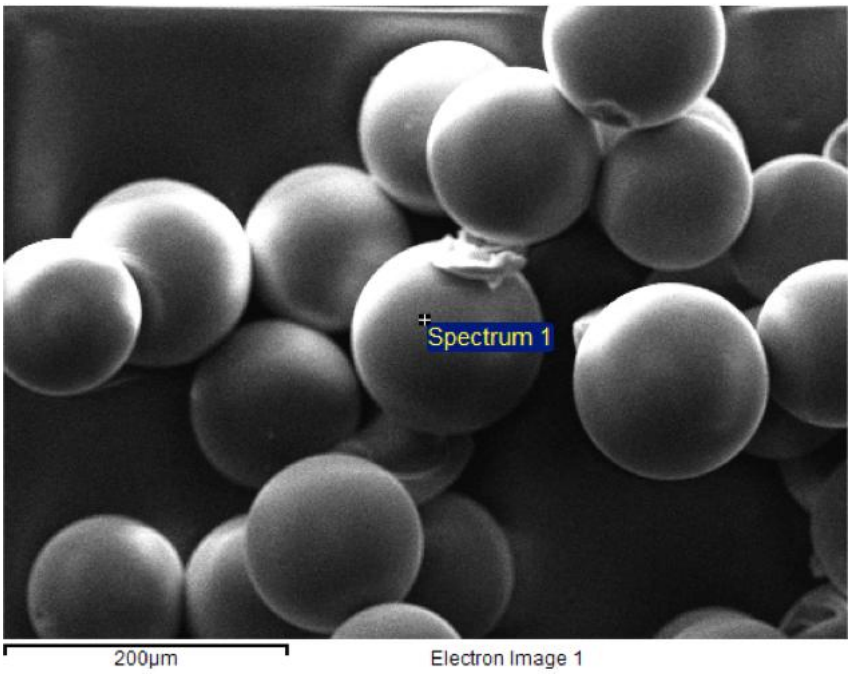
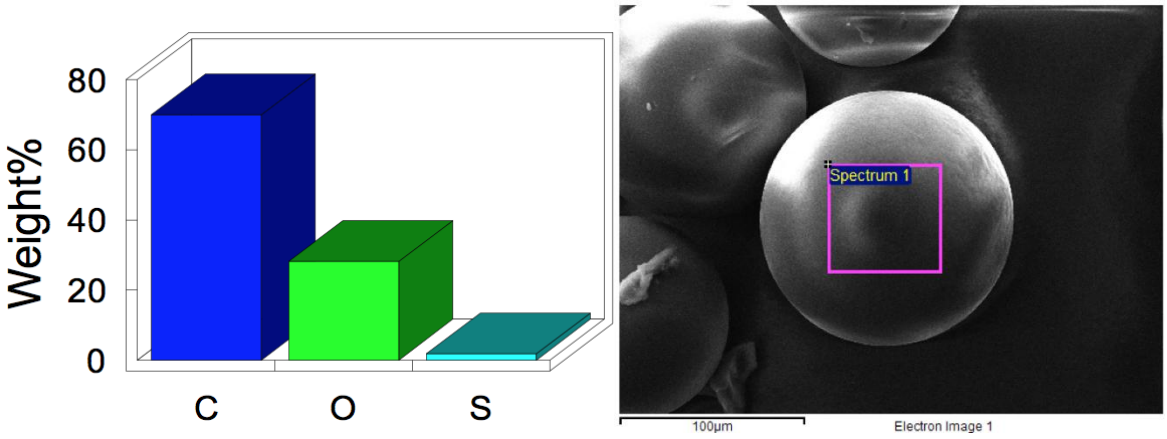


Figure 31: IR-spectra of Tentagel-NH2

The analyses of the modified resin are shown below (fig xx and xx). EDX analysis shows the presence of sulfur. In order to determine a reaction yield, a sulfur weight percentage average was evaluated. These data (Figure 32), permitted us, to obtain a sulfur percentage of 2.2% and after the correlation with the resin loading capacity (0.4 mmol/g of resin) the yield was 85%.





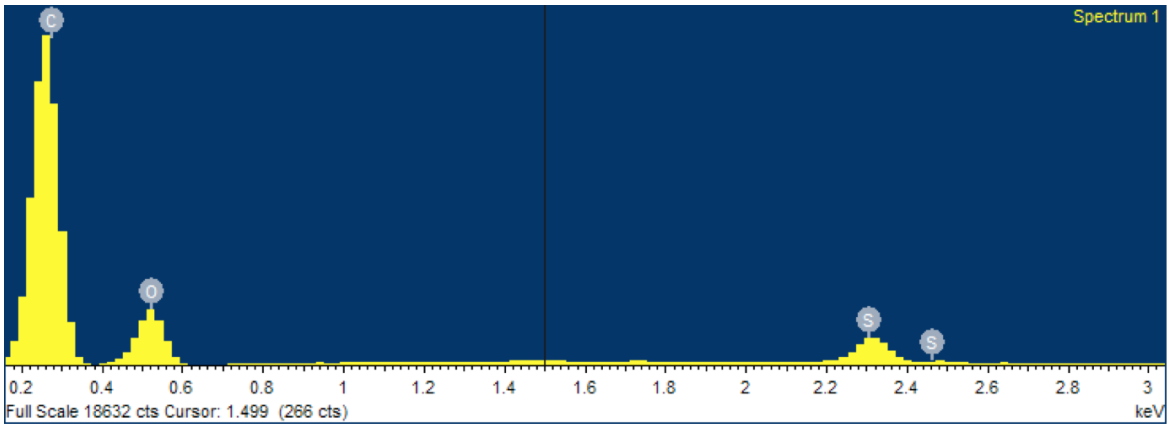


Figure 32: SEM-EDX analyses of 44

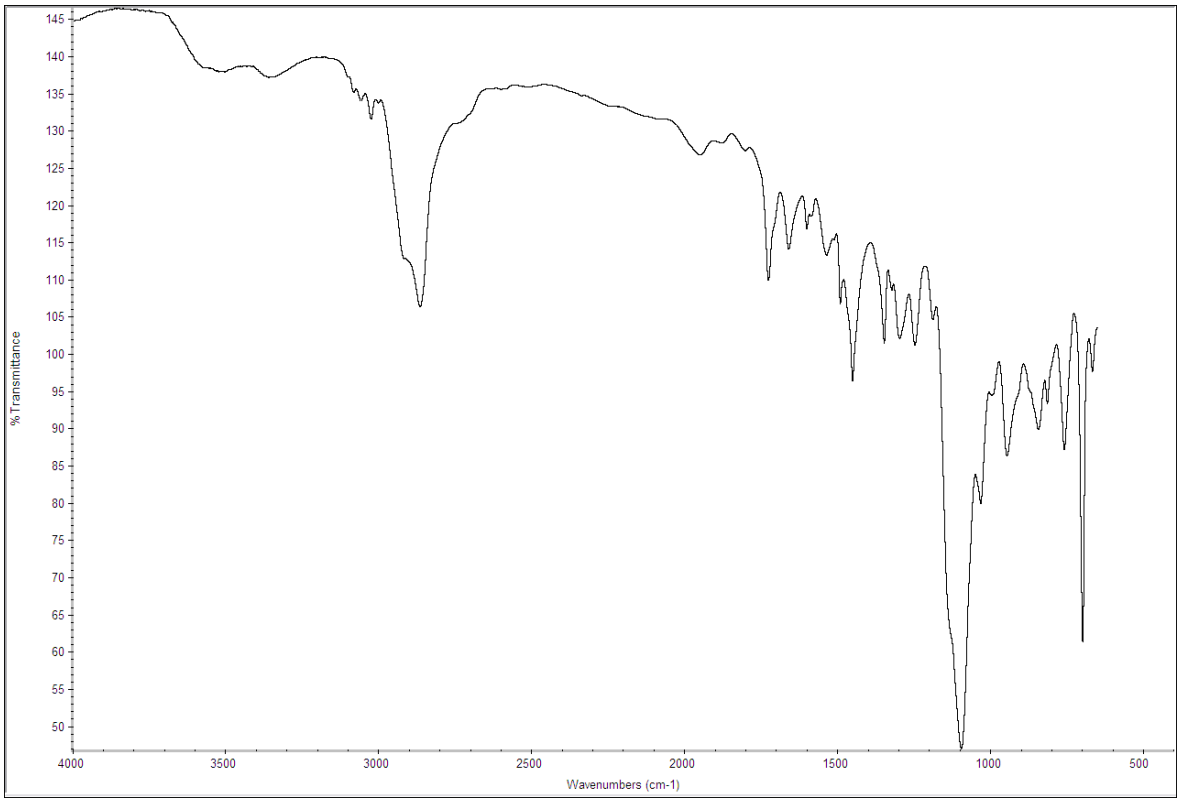
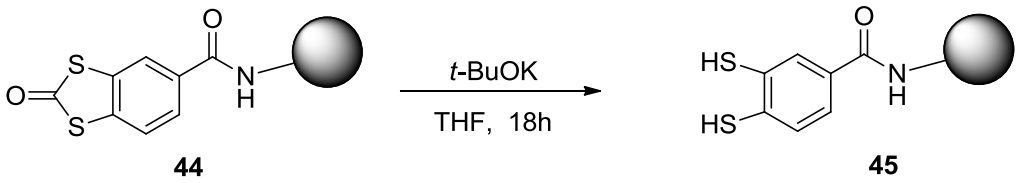


Figure 33: IR analysis of 44

After the confirmation of the sulfur presence on the resin we moved to the deprotection reaction in order to obtain free thiolic functions, for the subsequently titanocene complexations. These functions have been deprotected using Potassium ter-butylate (Scheme 39).



44 **45**

Scheme 39: SOlid Phase deprotection reaction

The product 45 was isolated as a yellow solid and was analysed as described above. The EDX analisys shows that the sulfur percentage was not changed after the reaction while de IR analysis showed characteristic peaks of the S-H functions.

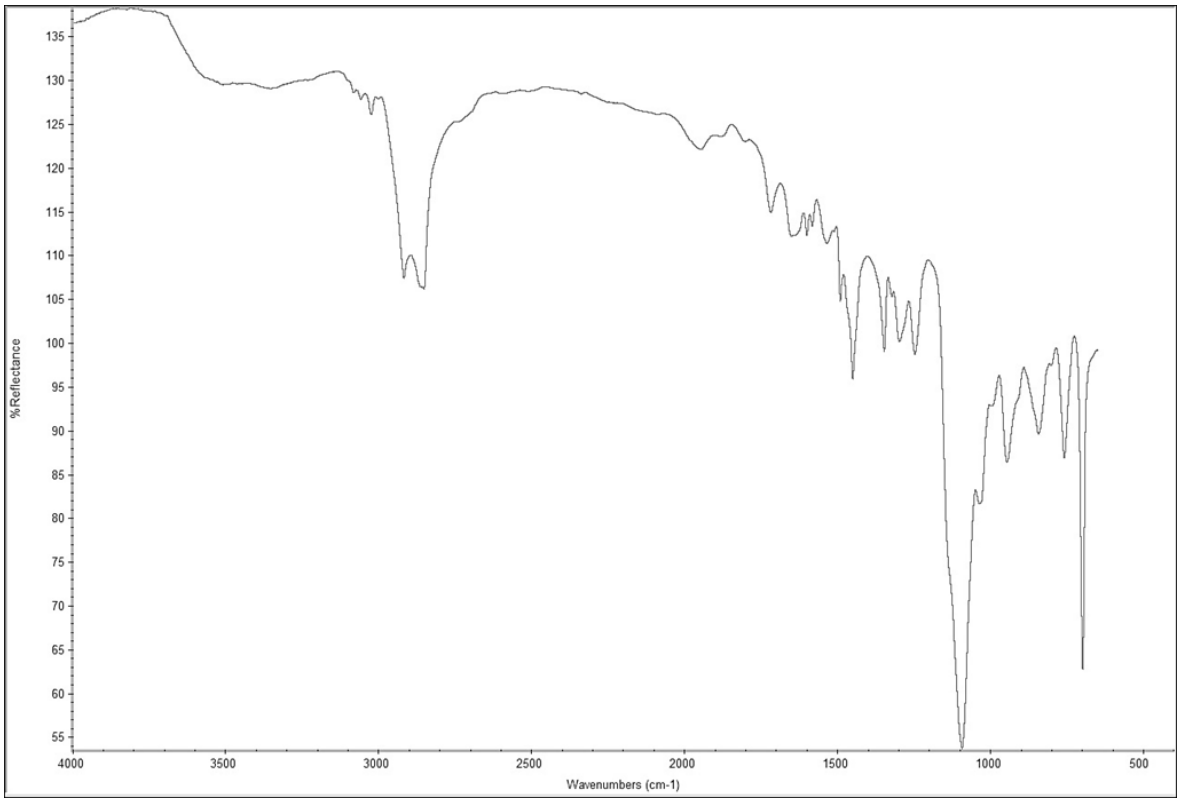
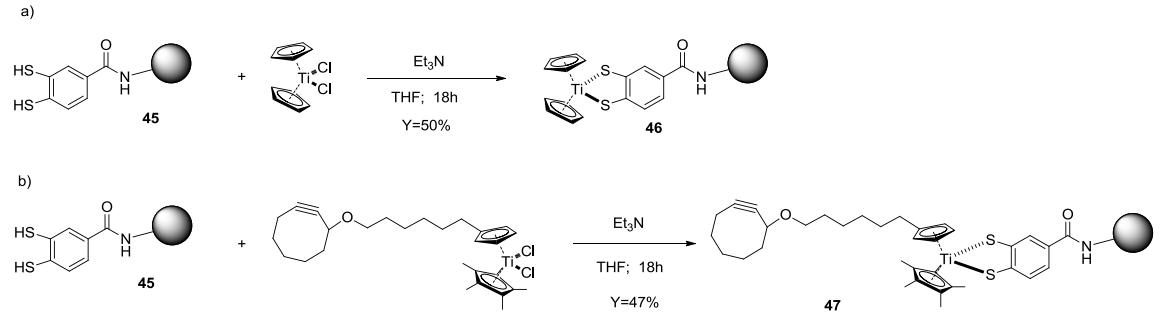


Figure 34: IR-Spectra of 45

- 2800cm^{-1}-3000cm^{-1} peak of the secondary amine NH, double tip;
- 1500cm^{-1}-1600cm^{-1} peak of the carbonyl group C = O of the amide bond;
- 1650cm^{-1}-1700cm^{-1}-3100 cm^{-1} peaks of S-H bond belonging to the thiols of deprotected resin.

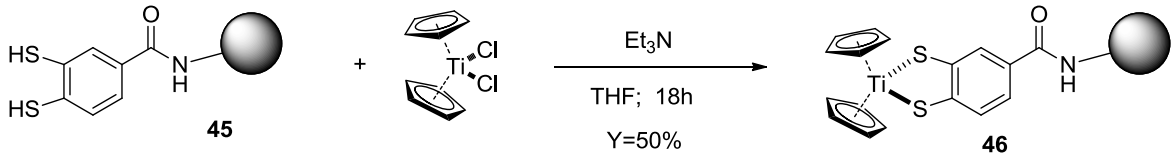
6.5. Study on Dithiolic Resin

The validation of deprotection reaction, allowed us to test the chelation activity of our modified resin with metal center . (Scheme 40)



Scheme 40: Solid Phase synthesi of Titanocene, a) Titanocene dichloride, b)Modified Titanocene

Firts of all, we tested the commercially available Cp_2TiCl_2 and subsequently we tested our modified titanocene. The yields presented in Scheme 40 were calculated using the Sulfur-Titanium ratio. The following procedure for the synthesis of modifide dithiolic-titanocene was already developed from our reasearch laboratory with good results.[91]Following analyses of 45 are presented. (Scheme 41)



Scheme 41: Synthesis of Titanocene -dithiolic resin

The product (45) was obtained as dark yellow solid and Images taken by electron microscopy and related to this compound are shown in Figure 35. The quantitative analysis of the compound (45) performed through EDX confirmed the presence of titanium in the sample. Also in this case in order to evaluate the yield of the reaction, an average of the Titanium percentage weights of the resin was performed and produced

the result of 0.95% (Histogram in Figure 35). Considering that the average of the percentages of S is the 2.2% the yield of reaction was 50%. This result was in according with previous tests performed in homogeneous phase in our laboratory.

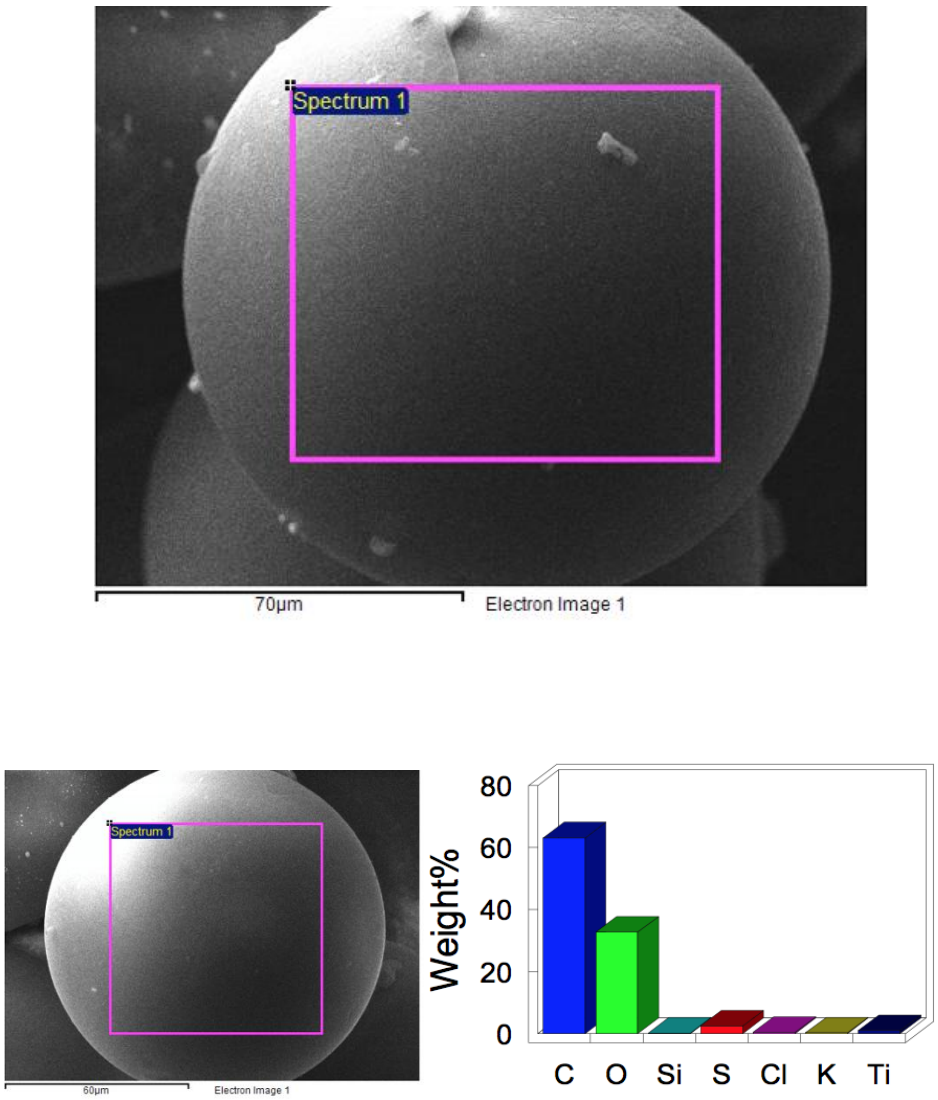


Figure 35: SEM-EDX analyses of 45

After confirmation that Titanocene moiety was attached to the resin, we attempted to eluite it in order to record an NMR spectrum of the eluate. This allowed us to verify purity of the reformed titanocene dichloride and the restoration of the ditiolic ligands in solid phase (for possible reuse). In order to elute the titanocene di-thiolic complex from the solid phase as dichloro derivative, we carried out a reaction using HCl dry in a suitable organic solvent such as the commercially available HCl solution in CPME (Cyclopentyl methyl ether). Elution was carried out in a chromatographic column packed with the resin and dry HCl in CPME (1M) was added. The eluate, resulted as Cp2TiCl2, was collected. It was subsequently performed a characterization by NMR and 13 cC-NMR spectroscopy, 1 H-that confirmed our hypothesis or the presence of titanocene dichloride without impurities.

Subsequently, we performed the reaction between the modified resin and our titanocene derivative developed in this work (Figure 36). In particular, we chose the Pentamethyl titanocene cyclooctine derivative, for the improved purification characteristics. Furthermore this derivative with the cyclooctine moiety allow us, after condensation, to perform a conjugation reaction with biomolecules.

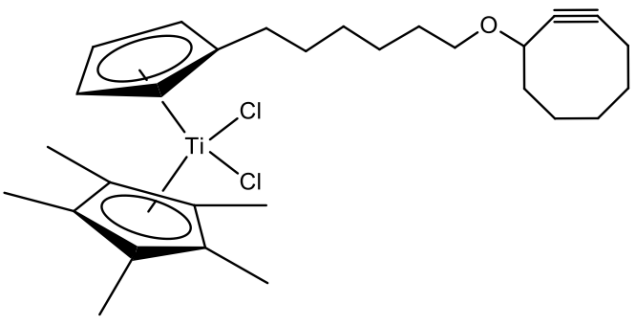
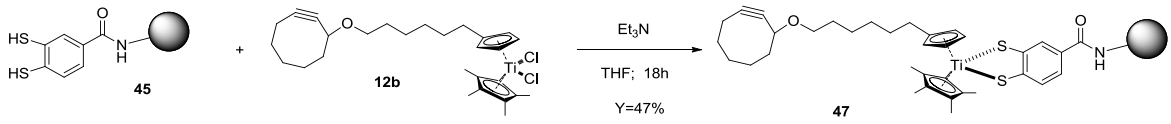
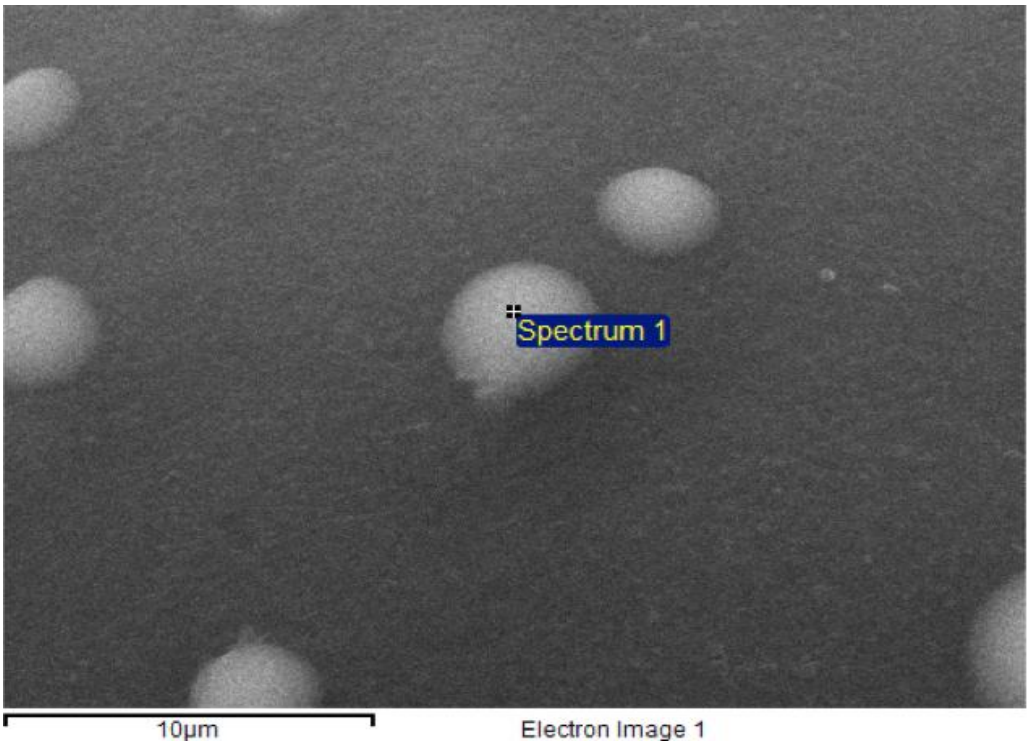


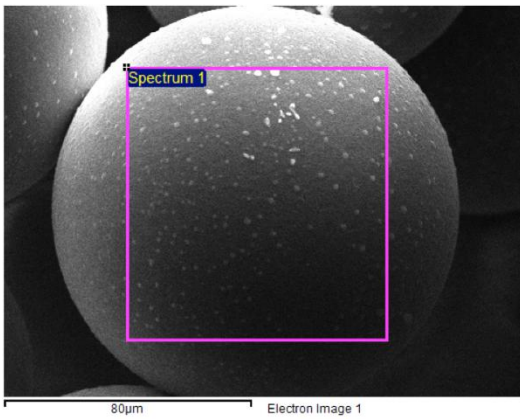
Figure 36: Selected Titanocene for biomolecules conjugation

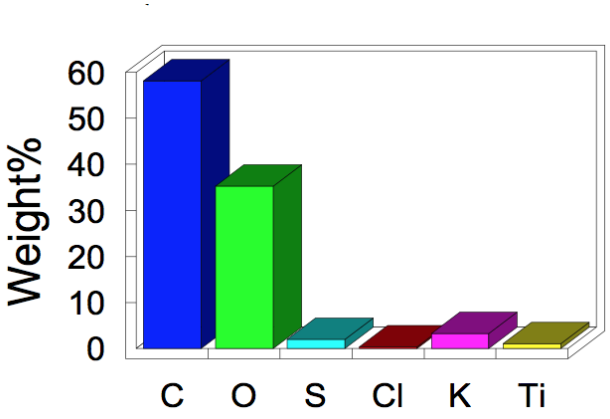
The scheme 42 show reaction conditions. Following SEM, EDX and IR analyses are presented.



Scheme 42: Titanocene anchored on solid phase







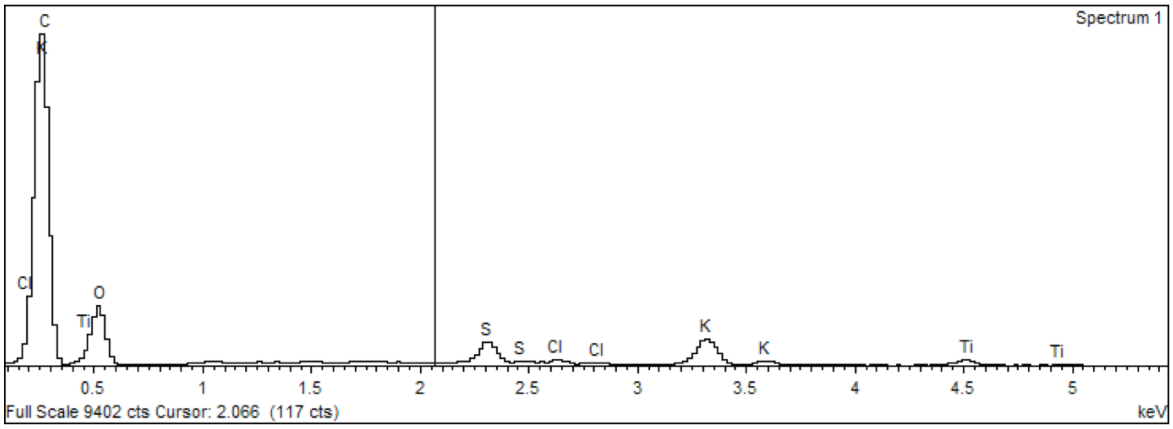


Figure 37: SEM-EDX analyses of our resin and modified Titanocene

Images taken by electron microscopy related to the compound (46) are shown in Figure 37 The quantitative analysis of the compound 46 performed through EDX confirmed the presence of titanium in the sample. Also in this case, in order to calculate the reaction yield, an average of the percentage weights of titanium was made and the result was 0.89% (Histogram in Figure 37). Considering that the average of the percentages by weight of S is the 2.2% the yield of reaction was 47%. This important result permitted us, to star future studies on conjugation with biomolecules.

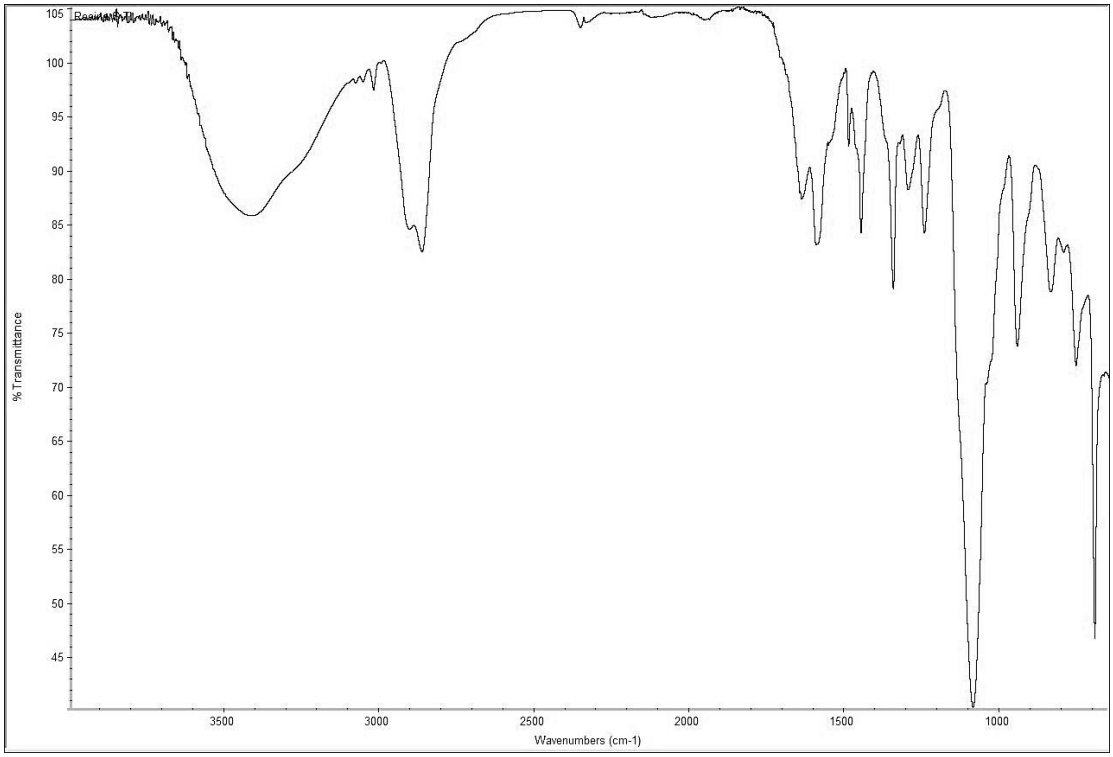
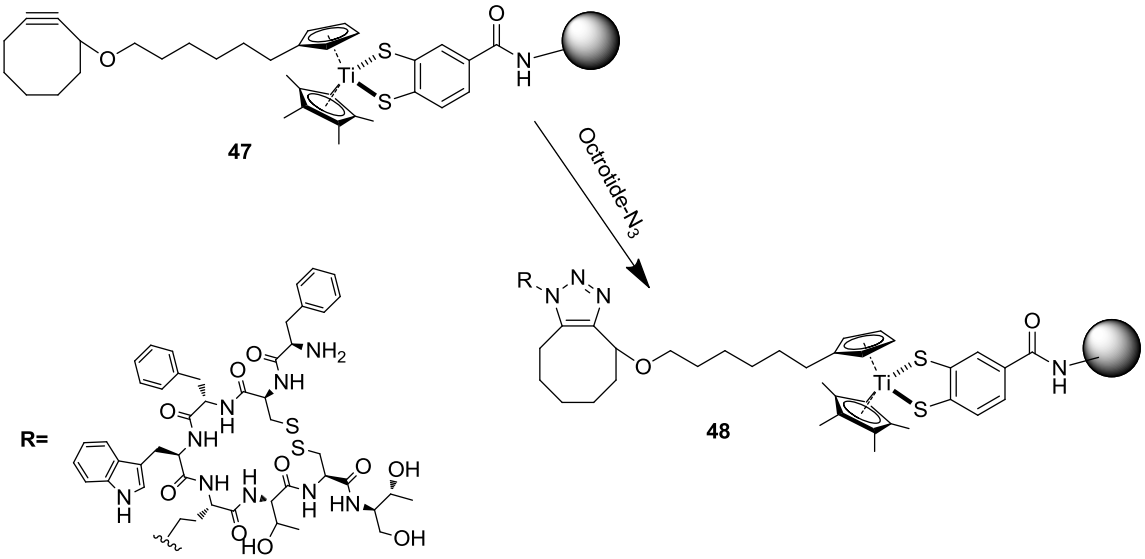


Figure 38: IR-spectra of Modified Titanocene 12b

Indeed we decided to test our biocnjugation protocol between compound 46 and the azido modified octeotride as biological partner. (Scheme 43)



Scheme 43: Bioconjugation reaction

While the SEM and EDX analysis showed the presence of Titanium in the same percentage, the IR analysis showed the presence of typical peaks of hydroxyl groups of Octeotride. (Figure 39)

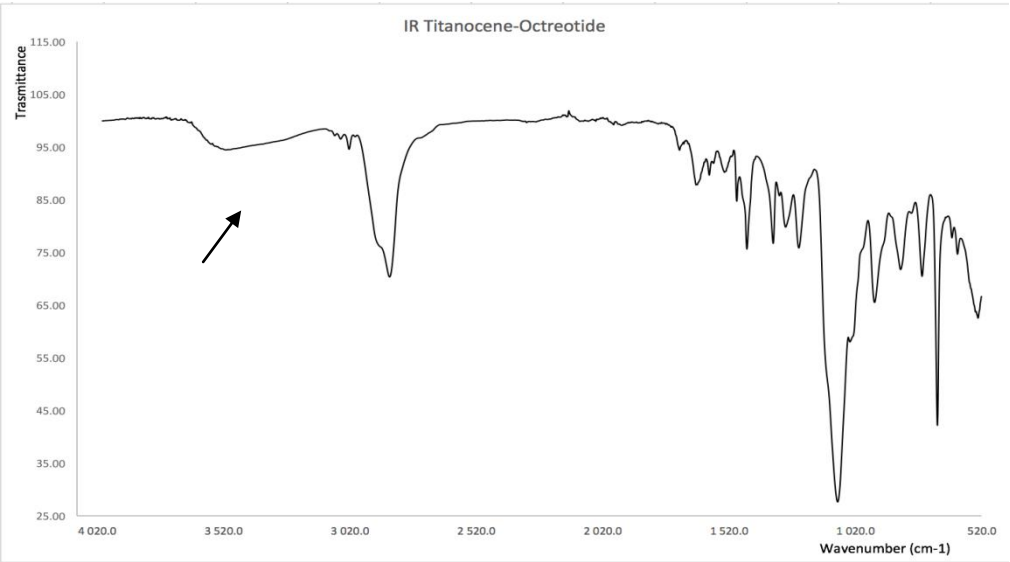
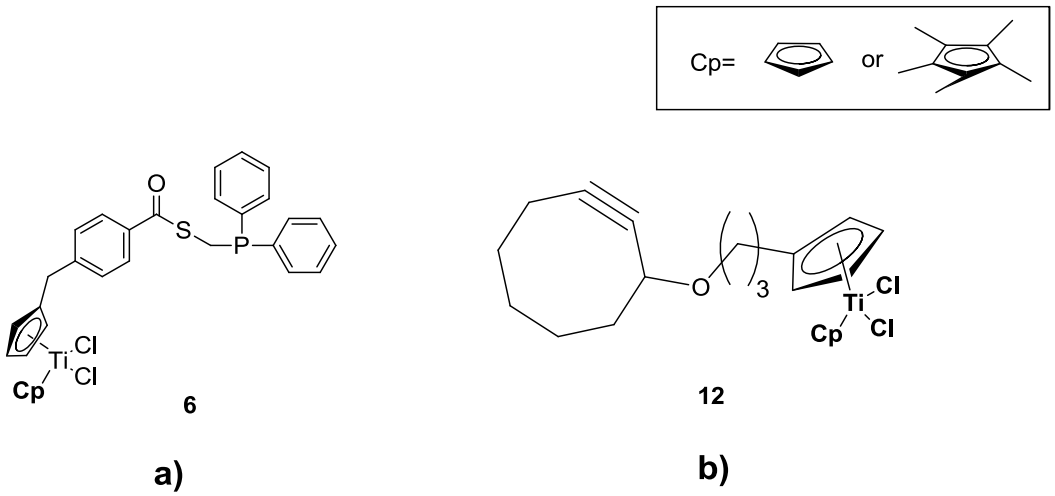


Figure 39: IR-spectra of 47, the arrow shows the typical broad band of -OH

Chapter 7: Conclusions

In this PhD work, we focused our attention on the synthesis of many Titanocene derivatives for bioconjugation reaction. Thus, we focused on two different approaches affording two new derivatives. (Scheme 44)



Scheme 44: Titanocene derivatives synthesized in this work; compound a) for staudinger reaction and compound b) for click chemistry reaction

Because the direct purification of the bioconjugate of these derivatives with biomolecules as Folic-Acid azido modified and Octeotride azido modified was not possible, we decided to develop a solide phase synthesis to solve these problems, and we obtained a Titanocene derivative solid phase supported. (Figure 40)

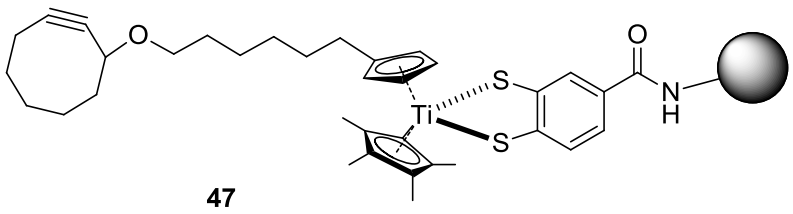


Figure 40: Titanocene solid phase supported.

Furthermore, to explore other applications of ours compounds, we started a ligand study with two objective :Evaluation of Ligand-Fluorine speed exchange to identify a ligand for a subsequently PET probe application.

- Evaluation of different substitution grade on Cp rings in order to improve the hydrlitic stability of the complex, important characteristic for biological applications.

Indeed, we identified the 1,2benzendithiole as the most important ligand for the exchange with fluorine. (Figure 41)

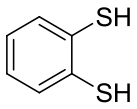


Figure 41: Structure of 1,2 benzendithiole

Although, we have tested the biological activity of different compounds, we obtained only poor results; otherwise, we identified that the simultaneous presence of two chlorine atoms and a modified ciclopentadyenil (with five methyl groups) as Titanium ligands afforded bioactivity resulst that paves the way for the further modification of Titanocene and a further investigation of this compound as anticancer drug.

Chapter 8: Experimental Section

8.1.General Methods

The practical work was performed by the aid of the following instruments

For the characterisation of compounds

- Brucker NMR AV 300 MHz spectrometer
- Brucker NMR AV 200 MHz spectrometer
- "Thermo scientific" LTQ-XL with HESI source
- "Thermo scientific"Focus GC-DSQ II

Cromathographic techniques

- Kieselgel 60 Merck flash (230-400) mesh
- Silica gel GF-254 Merck (0,25 mm) for TLC analysis
- Preparative HPLC with phenyl silica column and column C18
- ESI-MS thermo LTQ
- HUPLC-MS: mass LTQ-XL; UHPLC JASCO XLC seies 31. Pumps XLC 3080DG, column oven 3067 CO, autosampler 3159 AS. Column Agilent Poroshell 120 EC-18, particle size 2,7 micron. Eluents H_2O-MeCN 0.2% HCOOH
- MPLC Isolera One Biotage Flash Cromatography for chromatographic purifications of raw materials of more than 500 mg
- Fluorescence lamp 254-366 nm,
- $KMnO_4$ (solution in acetone) Vanilline (solution in H_2SO_4-EtOH) and Phosphomolibdic Acid (solution in EtOH), for the stain detection on TLC plates.

All preparations involving anhydrous conditions and inert atmosphere, were carried out under atmosphere of argon, into dried reactors dried in oven at 120 ° C for one night.
Most of the solvents used were dried by distillation with an appropriate drying agents under an argon atmosphere.
In particular: the toluene was distilled from sodium metal; tetrahydrofuran, diethyl ether, and dioxane from sodium and using benzophenone as indicator for H_2O; acetonitrile by

azeotrope distillation with water under reduced pressure; other solvents were used as well as commercially available.

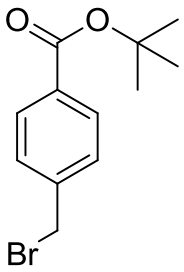
The IR spectra were reported using the wave numbers expressed in cm-1. The NMR spectra were tabulated bringing the chemical shift (δ) in ppm and the coupling constants (J) in Hz. The multiplicity of the signals are abbreviated thus: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broadened signal). For the 13C-NMR, the number of carbon atom bound to any hydrogen atoms was determined by DEPT experiments.

8.2.Glossary

AcOEt	Ethylacetate
Brine	Saturated Acqueous solution of NaCl
t-BuOK	Potassium tert-butylate
BuLi	Butyl Litium
t-BuLi	ter-Butyl Litium
Cp	Cyclopentadyenil
Cp*	Pentamethyl ciclopentadyenil
DABCO	Diazabicyclooctane
DBU	Diazabicycloundecene
DCC	Dicycloesylcarbodiimide
DCM	Dichloromethane
DME	dimetoxyethane
DMF	Dimethyl formamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	Dimethyl sulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et_2O	Diethyl Ether
EtOH	Ethanol
FA	Folic Acid
MeOH	Methanol
NHS	N-hydroxy succinimide
Ph_2O	Diphenyl Ether
TEA	Triethyl amine
TFA	Trifluoro acetic acid
TFAA	Trifluoroacetic
THF	Tetrahydrofurane

8.3.Synhtetic procedures

Synthesis of compound 1



Chemical Formula: $C_{12}H_{15}BrO_2$
Exact Mass: 270,03

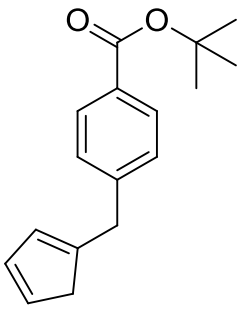
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, 4-(bromomethyl)benzoic acid (1.0 Equiv., 1120 mg, 5.05 mmol) was added and dissolved in a mixture of ciclohexane (9 ml), CH_2Cl_2 (5ml) and THF (10 ml). A solution of tert-butyl 2,2,2-trichloroacetimidate (2.0 Equiv. 10.1 mmol, 3.3 M in ciclo-hexane) was added via cannula and then catalytic BF_3Et_2O was added. The reaction mixture was stired at room temperature for 18 h. The reaction mixture was quenched with 1g of $NaHCO_3$, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by liquid chromatography (AcOEt:Hexane 9:1 as eluent) to afford the product 1 (yield = 95%).

MS (EI): two peaks at 270.14 and 272.15

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7,98 (2H, d, J=8Hz); 7.45 (2H, d, J=8.10Hz); 4.51 (2H, s); 1.61 (9H, s)

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 165.20 (s); 142.11 (s); 132.00 (s); 129.93 (d); 128.88 (d); 81.25 (s); 32.41 (s); 28.20 (q).

Synthesis of compound 2



Chemical Formula: $C_{17}H_{20}O_2$
Exact Mass: 256,15

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, 4.8 ml of a solution of CpNa 0.8 M in THF was added. The reaction mixture was cooled to -78 °C and compound 1 (1.1 Equiv., 1168 mg, 4.3 mmol) dissolved in dry THF (9 ml) was added via cannula. the resulting mixture was covered with an alu foil and allowed to warm to r.t. and react for 2 h. Reaction was monitored with TLC (AcOEt:Hexan 2:8) to observe the complete conversion of the starting material. The reaction mixture was quenched with a satured solution NH_4Cl then Hexan (5 ml was added). Layers were separated in a separatory funnel and the aqueous layer was extracted three times with Et_2O (3x30 ml). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (2:98 AcOEt:Hexano as eluent) to afford the product 2 (yield = 80%).

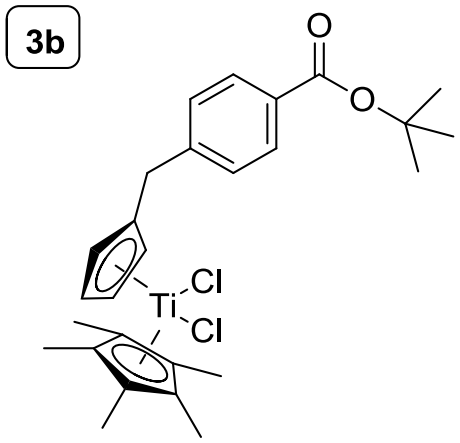
MS (EI): 256.45

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.98 (2H, d, J=8.16Hz); 7.26 (2H, d, 7.65Hz); 6.45-6.04 (4H, m); 3.78 (2H, d, J=8.15Hz); 3.01-2.86 (1H, m); 1.64 (9H, s)

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm):165.65; 147.16; 145.67; 145.04; 134.14; 132.20; 131.55; 129.82; 128.61; 127.80; 80.54; 42.96; 41.23; 37.22; 36.26; 28.11.

Synthesis of compound 3a and 3b

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 2 (1.0 Equiv., 152 mg, 0.593 mmol) was added and dissolved in dry THF (1 ml). The reaction mixture was cooled to -78 °C and *t*-BuLi (1.05 Equiv. 0.37 ml, 1.7 M) was added dropwise. The reaction mixture was stirred for 1h at r.t. A solution 0.85 M of Cp*$TiCl_3$ (0.77 Equiv. 136 mg) in THF was added via cannula and reaction mixture was stirred for 16h at -30°C. The solvent was evaporated by vacuum obtaining the crude product. This crude was purified by liquid chromatography (1:9 to 2:8 AcOEt:Hexan as eluent) to afford the product 3b (yield = 62%).

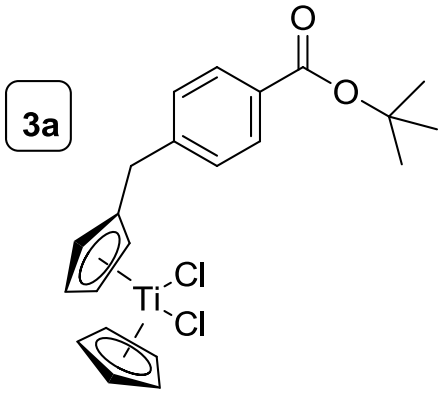


Chemical Formula: $C_{27}H_{34}Cl_2O_2Ti$
Exact Mass: 508,14

MS (HESI): 509.87 ($M+H^+$); 531.87 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.36 (2H, d, J=8.12 Hz); 6.15-6.08 (4H, m); 4.14 (2H, s), 2.11 (15H, s); 1.64 (9H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 166.13; 146.19; 135.72; 130.85; 129.55;124.01; 116.048; 81.33; 42.21; 37.47; 30.17; 28.64; 14.02.



Chemical Formula: $C_{22}H_{24}Cl_2O_2Ti$
Exact Mass: 438,06

Yield=55%

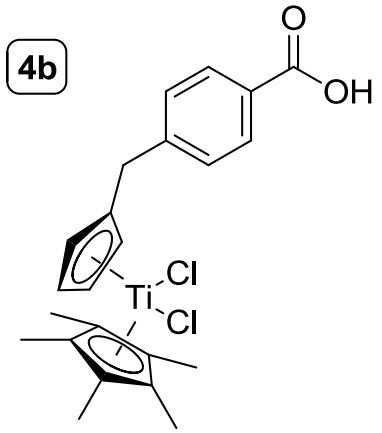
MS (HESI): 439.25 ($M+H^+$); 461.25 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.36 (2H, d, J=8.12 Hz); 6.28-6.02 (8H, m); 4.14 (2H, s); 1.64 (9H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 166.13; 146.19;133.45; 132.28 130.85; 129.55;124.01; 116.048; 81.33; 42.21; 37.47; 30.17; 28.64;

Synthesis of compound 4b and 4a

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 3b (1.0 Equiv., 62 mg, 0.122 mmol) was added and dissolved in dry DCM (1 ml). Reaction mixture was cooled to 0°C and TFA (30 Equiv, 3.66mmol, 280 µl) was added. After 20 minutes the resulting solution was stirred overnight at 5°C. The reaction mixture was checked trough TLC (AcOEt:Hexan 3:7) to observe the complete conversion of the starting material. The solvent was evaporated by vacuum obtaining the crude product. This crude was purified by liquid chromatography (3:7 AcOEt:Hexane as eluent) to afford the product 4b (yield = 82%).

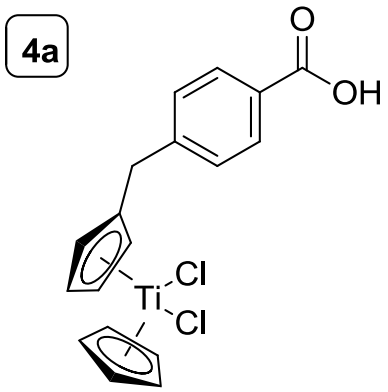


Chemical Formula: $C_{23}H_{26}Cl_2O_2Ti$
Exact Mass: 452,08

MS (HESI):450.89($M\text{-}H^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.36 (2H, d, J=8.12 Hz); 6.15-6.08 (4H, m); 4.14 (2H, s), 2.11 (15H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 164.23; 146.19; 135.62; 130.25; 128.25;124.51; 114.68; 42.21; 37.47; 30.17; 14.02.



Chemical Formula: $C_{18}H_{16}Cl_2O_2Ti$
Exact Mass: 382,00

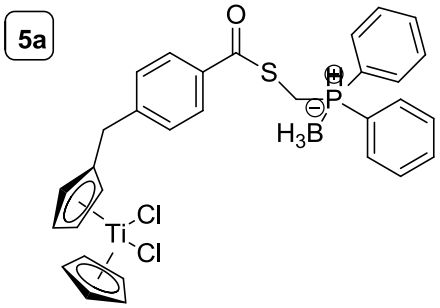
Yield=80%

MS (HESI): 381.05 ($M\text{-}H^+$).

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.94 (2H, d, J=8.40 Hz); 7.34 (2H, d, J=8.11 Hz); 6.35-6.08 (8H, m); 4.12 (2H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 165.18; 146.45; 135.62; 133.67; 132.81 130.75; 128.95;124.01; 116.048; 42.21; 36.41; 29.47.

Synthesis of compound 5a



Chemical Formula: $C_{31}H_{30}BCl_2OPSTi$
Exact Mass: 610,07

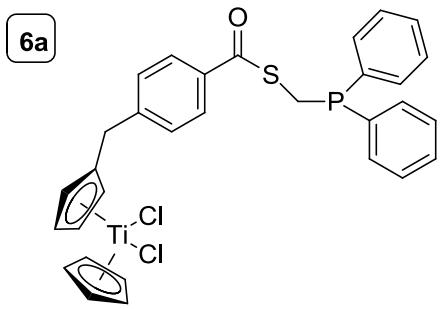
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 4a (1.0 Equiv., 50 mg, 0.13 mmol) was added and dissolved in dry DCM (1 ml). Then, DCC (1.1 Equiv. 29mg 0.143 mmol) and DMAP (one crystal) were added. After 4h the reaction mixture was checked through TLC(AcOEt:Hexan 3:7) observing the complete conversion of the starting material. The solvent was evaporated by vacuum obtaining the crude product. This crude was purified by liquid chromatography (3:7 AcOEt:Hexane as eluent) to afford the product 4b (yield = 66%).

MS (HESI): 611.45 ($M+H^+$).

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.94 (2H, d, J=8.40 Hz); 7.67-7.18 (12H, m); 6.35-6.08 (8H, m); 4.12 (2H, s); 3.31 (2H, s); 1.43-1.18 (3 BH3 typically signals bs)

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 165.18; 146.45; 135.62; 133.89; 133.67; 132.99; 132.81; 131.36; 130.75; 129.74; 128.66; 128.45; 128.33; 124.01; 116.048; 42.21; 36.41; 29.47; 28.75;

Synthesis of compound 6a

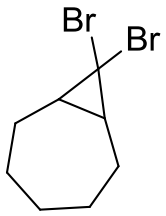


Chemical Formula: $C_{31}H_{27}Cl_2OPSTi$
Exact Mass: 596,04

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 5a (1.0 Equiv., 20 mg, 0.04 mmol) was added and dissolved in dry Toluene (1 ml). Then, DABCO (1.2 Equiv. 5mg 0.048 mmol) was added and, after 6h the reaction mixture was checked through TLC(AcOEt:Hexan 2:8) observing the complete conversion of the starting material. The solvent was evaporated by vacuum and filtered through a plug of silica gel obtaining the crude product. This crude product was used without further purification to avoid the oxidation of the posphine.

MS (HESI): 597.65 ($M+H^+$).

Synthesis of compound 7:



Chemical Formula: $C_8H_{12}Br_2$
Exact Mass: 265,93

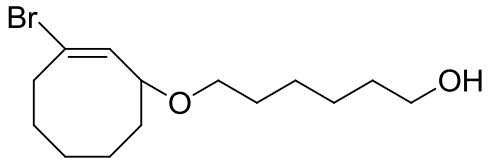
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, cycloheptene (1.0 Equiv., 500 mg, 5.20 mmol) was added and dissolved in dry pentane (0.1 M). The reaction mixture was cooled to 0 °C and Bromoform (0.466 ml, 5.2 mmol) was added in 6 h. the reaction mixture allowed to warm to R.t. and react overnight.Reaction was monitored with TLC (Hexan as eluent). The reaction mixture was quenched with H_2O and HCl conc. until neutralization checked with pH indicator strip. Pentane (15 ml) was added and layers were separated in a separatory funnel. The aqueous layer was extracted three times with pentane (3x20 ml). Combined organic layers were washed with brine (20 ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (Pure Hexane as eluent) to afford the product 7 (yield = 68%).

MS (EI)= three peaks 67.80,265.80,269.80

1H-NMR (CDCl3) 300 MHz: δ (ppm)= 2.31-2.25(m, 2H), 1.93-1.82(m, 3H), 1.77-1.69(m, 2H), 1.45-1.34(m, 2H), 1.27-1.14(m, 3H);

13C-NMR (CDCl3) 75 MHz: δ (ppm)= 40.7, 34.7, 32.2, 28.9, 28.0;

Synthesis of compound 8



Chemical Formula: $C_{14}H_{25}BrO_2$
Exact Mass: 304,10

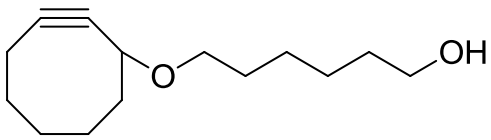
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 7 (1.5g, 5,61mmol) was added and dissolved in dry acetone (30 ml); At The reaction mixture 1,6-hexandiol (20 EQ, 112 mmol) and $AgClO_4$ (3 EQ, 16.8 mmol) were added. The resulting mixture was covered with an alu foil and allowed to warm to R.t. Reaction was monitored with TLC (Hexane as eluent) and was completed in 18 hours.The reaction mixture was filtered on celite and quenched with HCl (1N, 2ml). The silver salts were filtered and layers were separated in a separatory funnel. The aqueous layer was extracted three times with AcOEt (3x30 ml). Combined organic layers were washed with HCl (1x5ml), H_2O (1x10ml), Brine (1x10ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (2:8 AcOEt/Hexane, $Rf_{product}$: 0.17) to afford the product 8 (yield = 62%).

MS (HESI): 305.30 ($M+H^+$), 327.30 ($M+Na^+$)

1H-NMR (CDCl3) 300 MHz: δ (ppm)= 4.15-4.11(m, 1H), 3.91-3,82(m, 1H), 3.55-3.48(m, 1H), 3.29(t, J=6.9 Hz,3H), 2.33-2.09(m, 3H), 1.92-1.78(m, 6H), 172-1.38(m, 9H);

13C-NMR (CDCl3) 75 MHz: δ (ppm)= 98.4, 94.5,74.3, 68.9, 43.6, 36.5, 34.8, 31.3, 31.0, 30.6, 27.6, 22.5, 9.3;

Synthesis of compound 9



Chemical Formula: $C_{14}H_{24}O_2$
Exact Mass: 224,18

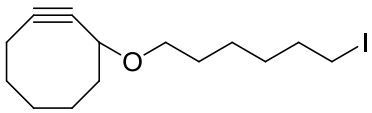
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 8 (460mg, 1.52mmol) was added and dissolved in dry DMSO (15 ml). The reaction mixture was heated at 80 °C and DBU (10 EQ, 15.2 mmol) was added dropwise. The solution was stirred for 18 h at 80°C. Reaction was monitored with TLC to observe the complete conversion of the starting material. The reaction mixture was quenched with a saturated solution of NH_4Cl and layers were separated in a separatory funnel and the aqueous layer was extracted three times with Et_2O (3x30 ml).Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (AcOEt/esano (3:7) $Rf_{product}$: 0.35) to afford the product 9 (Y=95%).

MS (HESI): 225.36 ($M+H^+$), 247.36 ($M+Na^+$)

1H-NMR (CDCl3)300 MHz: δ (ppm)= 4.15-4.09 (m, 1H), 3.57-3.48(m, 1H), 3.29(t, J=6.8 Hz,3H), 2.25-2.09(m, 3H), 1.92-1.78(m, 6H), 172-1.38(m, 9H);

13C-NMR (CDCl3) 75 MHz: δ (ppm)= 97.9, 94.6, 73.3, 66.8, 43.6, 37.5, 32.8, 31.6, 31.2, 29.6, 27.6, 21.5, 7.3;

Synthesis of compound 10



Chemical Formula: $C_{14}H_{23}IO$
Exact Mass: 334,08

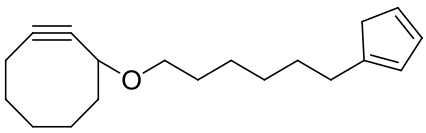
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 9 (1.0 Equiv., 200 mg, 0.9mmol) was added and dissolved in dry THF (2.5 ml). Then, imidazole (1.2 Equiv., 73mg, 1.08 mmol) and PPh_3 (1.3 Equiv., 306mg, 1.17 mmol) were added to the reaction vessel. The reaction was cooled to 0 °C and I_2 (1.1 Equiv., 251mg, 0.99 mmol) was added in 3 portions; the resulting mixture was covered with an alu foil and allowed to warm to R.t. Reaction was monitored with TLC (8:2 Hexane:Acetate as eluents) and was completed in 4 hours. The reaction mixture was quenched with a saturated solution of di $Na_2S_2O_3$ (5ml) and diluited with Et_2O (5ml). Layers were separated in a separatory funnel and the acqueous layer was ecxtracted three times with Et_2O(3*25ml).Combined organic layers were washed with brine (10ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (98:2 Hexane:MTBE as eluents) to afford the product 10.

MS (HESI): 335.56 ($M+H^+$), 347.56 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$)300 MHz: δ (ppm)= 4.15-4.11(m, 1H), 3.55-3.48(m, 1H), 3.29(t, J=6.9 Hz,3H), 2.33-2.09(m, 3H), 1.92-1.78(m, 6H), 172-1.38(m, 9H);

^{13}C-NMR ($CDCl_3$)75 MHz: δ (ppm)= 99.9, 94.6,73.3, 69.9, 43.6, 35.5, 34.8, 31.3, 31.0, 30.6, 27.6, 21.5, 8.3;

Synthesis of compound 11



Chemical Formula: $C_{19}H_{28}O$
Exact Mass: 272,21

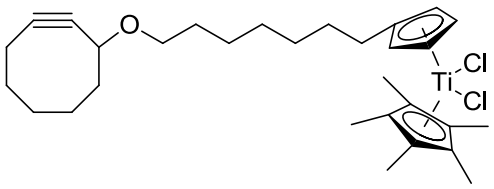
In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 10 (260 mg, 0.72 mmol) was added and dissolved in dry THF (1.8 ml). Then, DMPU (2 EQ, 1.44 mmol) was added to the reaction vessel. The reaction was cooled to -78 °C and CpNa (1.05 EQ, 0.82 mmol) in THF (1 ml) was cannulated. The resulting mixture was covered with an alu foil and allowed to warm to -30°C. Reaction was monitored with TLC after the complete conversion of the starting material. The reaction mixture was quenched with a saturated solution of NH_4Cl and H_2O (2 ml). Layers were separated in a separatory funnel and the aqueous layer was extracted three times with a mixture of MTBE/Hexane 1:1 (3x20 ml). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by flash chromatography (AcOEt/Hexane (2:98) $Rf_{product}$:0.37) to afford the product 11 (yield = 68%).

MS (HESI): 273.24 ($M+H^+$)

^{1}H-NMR ($CDCl_3$):300 MHz δ (ppm)= 6.16-6.13(m, 2H), 6.09-6.08(m,2H), 4.15-4.10(m, 1H), 3.54-3.46(m, 1H), 3.30-3.25(m, 1H), 2.65(t, J=7.55, 2H), 2.26-2.07(m, 2H), 2.00-1.75(m, 4H), 1.65-1.53(m, 6H), 1.48-1.39 (m, 6H);

^{13}C-NMR ($CDCl_3$):75 MHz δ (ppm)=139.4, 133.2, 124.4, 113.0, 96.9, 92.7, 75.2, 70.0, 45.5, 35.4, 31.7, 31.2, 29.9, 29.7, 29.4, 27.5, 27.1, 21.3, 13.9;

Synthesis of compound 12b



Chemical Formula: $C_{29}H_{42}Cl_2OTi$
Exact Mass: 524,21

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 11 (255 mg, 1 mmol) was added and dissolved in dry THF (2 ml). The reaction was cooled to -78 °C and *t*-BuLi (1.1 EQ, 0.9 mmol) was added dropwise.The reaction mixture was stirred for 1 h to obtain a deep yellow solution (anion formation).TiCp*Cl_3 (0.9 EQ, 0.9 mmol) was cannulated (-78°C) into the reaction vessel and the solution was stirred for 2 h at -30 °C. Reaction was monitored with TLC (AcOEt/esano 15:85) to observe the product formation.Then, the solvent was evaporated under vacuum obtaining the crude product. This crude was purified by flash chromatography (AcOEt/Hexane (15:85) $Rf_{product}$: 0.45) to afford the product 12b (yield = 52%).

MS (HESI): 525.34 ($M+H^+$)

^{1}H-NMR ($CDCl_3$):300 MHz δ (ppm)= 6.17-6.15(m, 2H), 6.09-6.08(m,2H), 4.14-4.10(m, 1H), 3.54-3.46(m, 1H), 3.30-3.25(m, 1H), 2.65(t, J=7.55, 2H), 2.25-2.07(m, 2H), 2.04(s, 15H), 2.00-1.75(m, 4H), 1.64-1.53(m, 6H), 1.48-1.39 (m, 6H);

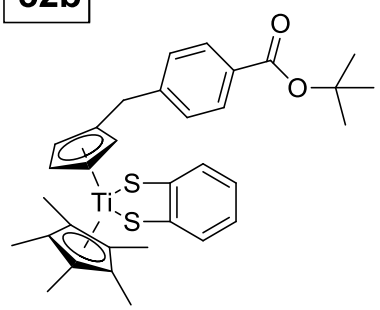
^{13}C-NMR ($CDCl_3$):75 MHz δ (ppm)=139.4, 130.2, 123.4, 117.0, 99.9, 94.7, 73.2, 70.0, 43.5, 35.4, 31.7, 31.2, 30.9, 30.7, 30.5, 30.3, 30.2, 29.9, 29.7, 29.4, 27.5, 27.1, 21.3, 13.9;

General procedure for Chlorine-Thiolic exchange.

Synthesis of compounds: 32,33, 36a and 36b

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound **X** (1 Equiv.) was added and dissolved in dry THF (resulting solution 0.125 M). Then Et_3N (2 Equiv.) and 1,2 benzendithiole (1.1 Equiv.). The reaction mixture was stirred for 16h and was checked through TLC, and was observed the complete conversion of starting material (the colour of the resulting solution turned from red dark to a dark green). The resulting reaction mixture was evaporated and purified through a silica gel column affording the corresponding dithiolic derivative.

32b



Chemical Formula: $C_{33}H_{38}O_2S_2Ti$
Exact Mass: 578,18

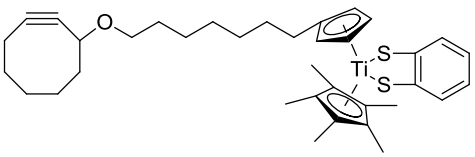
Column: 95:5 DCM: AcOEt as eluents; Y= 70%

MS (HESI): 579.24 ($M+H^+$), 601.24 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.40-7.10(6H, m); 6.15-6.08 (4H, m); 4.14 (2H, s), 2.11 (15H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 164.90; 145.25; 135.32; 133.24; 130.25; 129.44; 129.11; 128.25;123.51; 112.68; 81.12; 42.21; 37.47; 30.17; 28.25; 14.02.

33



Chemical Formula: $C_{35}H_{46}OS_2Ti$
Exact Mass: 594,25

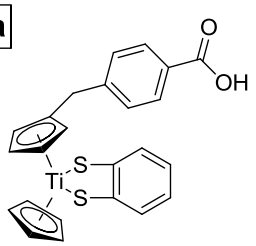
Column: 7:3 Hexane: AcOEt as eluents; Y= 85%

MS (HESI): 595.61 ($M+H^+$); 617.61 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$):300 MHz δ (ppm)= 7.25 (2H, d, J=8.10 Hz); 7.05-(2H, dd J=7.80 Hz) 6.17-6.15(m, 2H), 6.09-6.08(m,2H), 4.14-4.10(m, 1H), 3.54-3.46(m, 1H), 3.30-3.25(m, 1H), 2.65(t, J=7.55, 2H), 2.25-2.07(m, 2H), 2.04(s, 15H), 2.00-1.75(m, 4H), 1.64-1.53(m, 6H), 1.48-1.39 (m, 6H);

^{13}C-NMR ($CDCl_3$):75 MHz δ (ppm)=139.4, 132.44; 131.20; 130.12; 125.66 123.4, 117.0, 99.9, 94.7, 73.2, 70.0, 43.5, 35.4, 31.7, 31.2, 30.9, 30.7, 30.5, 30.3, 30.2, 29.9, 29.7, 29.4, 27.5, 27.1, 21.3, 13.9;

36a



Chemical Formula: $C_{24}H_{20}O_2S_2Ti$
Exact Mass: 452,04

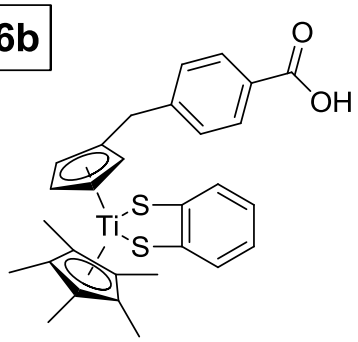
Column: 9:1 DCM: MeOH as eluents; Y= 60%

MS (HESI): 451.22 ($M-H^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.93 (2H, d, J=8.15 Hz); 7.39-7.06(6H, m); 6.30-6.11 (8H, m); 4.09 (2H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 164.94; 145.45; 134.23; 133.24; 129.45; 129.44; 129.11; 128.25;124.51; 114.68; 44.41; 37.80; 31.37.

36b



Chemical Formula: $C_{29}H_{30}O_2S_2Ti$
Exact Mass: 522,12

Column: 95:5 DCM:MeOH as eluents; Y= 90%

MS (HESI): 521.44 ($M\text{-}H^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.40-7.10(6H, m); 6.20-6.13 (4H, m); 4.24 (2H, s), 2.10 (15H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 164.90; 145.25; 135.32; 132.24; 130.25; 129.44; 129.81; 128.25;123.51; 113.68; 43.21; 37.17; 30.27; 27.55;

General procedure for Chlorine Fluorine exchange.

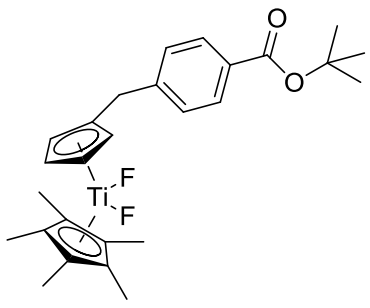
In a one-necked round bottom flask equipped with a magnetic stir-bar, the desired dichloro-compound (1 Equiv.) was added and dissolved in a 1:1 mixture of $CHCl_3$:H_2O (resulting solution 0.05 M).Then KF (2.3 Equiv.) was added and the conversion of the reagent was checked through TLC every five minutes. When the complete conversion of starting material was observed, the reaction mixture was transferred in to a separatory funnel and layers were separated and the acqueous layer was exctracted with $CHCl_3$ (6ml*3). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product that was filtered on a plug of silica gel (DCM as eluent) in order to obtain the pure difluoro compound of interest.

General procedure for Dithiolic-Fluorine exchange.

Synthesis of compounds 34, 35, 37a and 37b.

In a polhyethylene test tube equipped with a magnetic stir-bar, the dithiolic derivative (1 Equiv.) was added and dissolved in CH_3CN (resulting solution 0.1 M).Then HF (H_2O solution at 48% .10 Equiv.) was added and the conversion of the reagent was checked through TLC every five minutes. When the complete conversion of starting material was observed, CuF_2 (1 Equiv.) was added. The reaction mixture was dried under vacuum and the crude obtained was filtered on a plug of silica gel (DCM as eluent) in order to obtain the pure difluoro compound of interest.

34



Chemical Formula: $C_{27}H_{34}F_2O_2Ti$
Exact Mass: 476,20

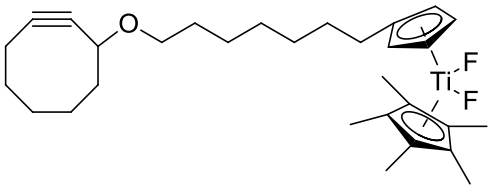
Y= 90%

MS (HESI): 477.57 ($M+H^+$); 498.95 ($M+Na^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.91 (2H, d, J=8.20 Hz); 7.29 (2H, d, J=8.12 Hz); 6.35-6.05 (4H, m); 4.12 (2H, s), 2.11 (15H, s); 1.73 (9H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 166.73; 146.19; 135.72; 130.85; 130.12; 125.01; 116.048; 80.75; 42.91; 36.97; 31.17; 28.14; 13.92.

35



Chemical Formula: $C_{29}H_{42}F_2OTi$
Exact Mass: 492,27

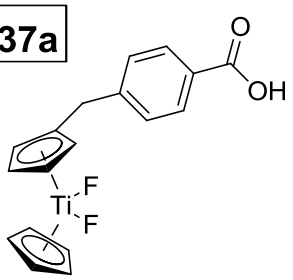
Yield = 96%

MS (HESI): 492.98($M+H^+$); 514.98 ($M+Na^+$);

^{1}H-NMR ($CDCl_3$):300 MHz δ (ppm)= 6.17-6.15(m, 2H), 6.09-6.08(m,2H), 4.14-4.10(m, 1H), 3.54-3.36(m, 2H), 2.64(t, J=7.55, 2H), 2.25-2.02(m, 2H), 2.14(s, 15H), 2.00-1.75(m, 4H), 1.64-1.53(m, 6H), 1.48-1.39 (m, 6H);

^{13}C-NMR ($CDCl_3$):75 MHz δ (ppm)=138.14, 131.25, 124.45, 118.5, 100.12, 93.17, 71.25, 70.56, 43.55, 35.24, 31.17, 31.89, 30.12, 30.05, 29.96, 29.85, 29.75 28.75, 28.47, 28.14, 27.55, 25.14 21.63, 13.69;

37a



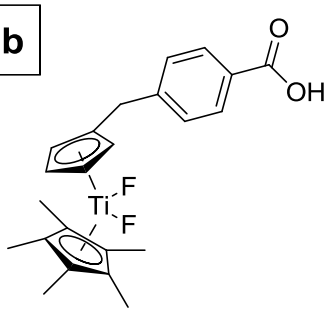
Chemical Formula: $C_{18}H_{16}F_2O_2Ti$
Exact Mass: 350,06

MS (HESI): 349.15 ($M\text{-}H^+$).

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.93 (2H, d, J=7.90 Hz); 7.34 (2H, d, J=8.11 Hz); 6.45-6.28 (8H, m); 4.22 (2H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 166.18; 144.95; 134.54; 133.11; 131.91 130.25; 127.96; 124.42; 116.33; 40.97; 36.41; 28.47.

37b



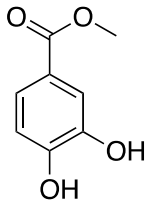
Chemical Formula: $C_{23}H_{26}F_2O_2Ti$
Exact Mass: 420,14

MS (HESI):419.11($M\text{-}H^+$)

^{1}H-NMR ($CDCl_3$) 300 MHz: δ (ppm): 7.96 (2H, d, J=8.20 Hz); 7.36 (2H, d, J=8.12 Hz); 6.40-6.21 (4H, m); 4.24 (2H, s), 2.18 (15H, s).

^{13}C-NMR ($CDCl_3$) 75 MHz: δ (ppm): 165.23; 147.29; 134.62; 132.05; 128.74; 124.39; 112.41; 43.841; 37.47; 30.17; 13.82.

Synthesis of compound 38



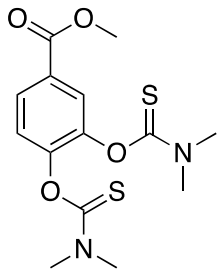
Chemical Formula: $C_8H_8O_4$
Molecular Weight: 168,15

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, the 3,4-Dihidroxibenzoic acid (5 g, 32.46 mmol) was added and dissolved in MeOH (200 ml). HCl (1ml) was added at the reaction mixture and the solution was heated at 80 °C for 18 h. Reaction was monitored with TLC after the complete conversion of the starting material.The reaction mixture was quenched with H_2O (500 ml). Layers were separated in a separatory funnel and the acqueous layer was ecxtracted three times with a mixture of EtOAc/Hexane 60:40 (3x30ml). Combined organic layers were washed with brine (10ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the pure product.Yield: 81%.

Spectroscopic data matched those reported in literature[92].

[92] *Y. Mahendran, A. Vuong, D. Aebisher, Y. Gong, R. Bittman, G. Arthur, A. Kawamura, A. Greer, J. Org. Chem. 2010, 75, 5549–5557*.

Synthesis of compound 39



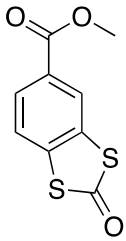
Chemical Formula: $C_{14}H_{18}N_2O_4S_2$
Molecular Weight: 342,43

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, the methyl-3,4-Dihidroxibenzoate (2 g, 11.9mmol), DABCO (4 EQ, 47.6mmol), N,N-dimethylthio- carbamoyl chloride (4 EQ, 47.6mmol) were added and dissolved in DMF(25 ml). Reaction was monitored with TLC (8:2 Hexane:Acetate as eluents) and was completed in 30 min. The reaction was quenched with H_2O (100ml). Layers were separated in a separatory funnel and the acqueous layer was ecxtracted three times with EtOAc (3x30ml). Combined organic layers were washed with brine (10ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by liquid chromatography (EtOAc/Hexane, 4:6) R_f = 0.4 to afford the product 39 (yield = 78%).

Spectroscopic data matched those reported in literature[93].

[93] *Y. Mahendran, A. Vuong, D. Aebisher, Y. Gong, R. Bittman, G. Arthur, A. Kawamura, A. Greer, J. Org. Chem. 2010, 75, 5549–5557*.

Synthesis of compound 40



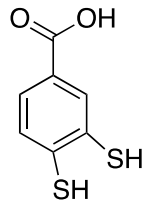
Chemical Formula: $C_9H_6O_3S_2$
Molecular Weight: 226,26

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 39 (2.5 g, 11 mmol), was added and dissolved in Ph_2O (50 ml). The reaction mixture was heated at 240 °C for 90 min. Reaction was monitored with TLC (EtOAc/Hexan, 4:6) to observe the complete conversion of the starting material. The crude product was purified from the Ph_2O by liquid chromatography EtOAc/Hexane (from 2:98 to 20:80) to afford the product 40 (yield = 44%).

Spectroscopic data matched those reported in literature[94].

[94] *Y. Mahendran, A. Vuong, D. Aebisher, Y. Gong, R. Bittman, G. Arthur, A. Kawamura, A. Greer, J. Org. Chem. 2010, 75, 5549–5557*.

Synthesis of compound 41



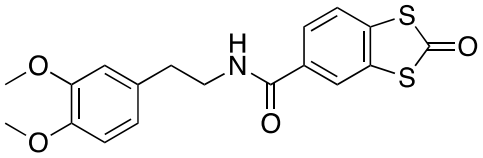
Chemical Formula: $C_7H_6O_2S_2$
Molecular Weight: 186,24

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 40 (800 mg, 3.5mmol) was added and dissolved in NaOH (1N in H_2O, 7ml). The reaction mixture was heated to 70 °C for 4 h. Reaction was monitored with TLC (EtOAc/Hexan, 4:6) to observe the complete conversion of the starting material. The solution was allowed to r.t. and was quenched with HCl 1N (2 ml). Layers were separated in a separatory funnel and the acqueous layer was ecxtracted three times with EtOAc (3x25 ml). Combined organic layers were washed with brine (10ml), dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. This crude was purified by water recrystallization to afford the product 41 (yield = 78%).

Spectroscopic data matched those reported in literature[95].

[95] *Y. Mahendran, A. Vuong, D. Aebisher, Y. Gong, R. Bittman, G. Arthur, A. Kawamura, A. Greer, J. Org. Chem. 2010, 75, 5549–5557*.

Synthesis of compound 42



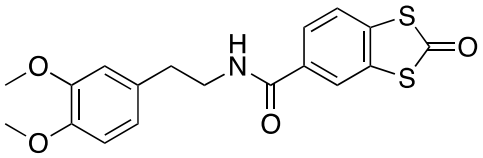
Chemical Formula: $C_{18}H_{17}NO_4S_2$
Molecular Weight: 375,46

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 41 (200mg, 1.07mmol) N-methylmorfoline (3 EQ, 2.14 mmol) were added and dissolved DME (10 ml). The reaction was cooled to 0 °C and Methylcloroformate (3 EQ, 2.14 mmol) was added dropwise. Solution was stirred for 3 h at room temperature. The morfolinium salts were filtered and the solution was concentrated under vacuum obtaining the active carbonate. 2-(3,4-dimetoxyphenil)etan-1-amine (1.3 EQ, 1.4 mmol) was added dropwise and the solution was dissolved in DME (10 ml) and stirred for 2 h. The reaction mixture was heated to 70 °C for 4 h. Reaction was monitored with TLC (EtOAc/Hexane, 3:7) to observe the complete conversion of the starting material. The solution was concentrated under vacuum obtaining a white solid that was dissolved in a mixture of $H_2O/CHCl_3$(1:1). The reaction mixture was quenched with KOH (2N, 1ml) and layers were separated in a separatory funnel, the acqueous layer was ecxtracted three times with $CHCl_3$ (3x20 ml). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. The crude product was purified by liquid chromatography EtOAc/Hexane (3:7) to afford the product 42 (yield = 50%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.8(t, 2H), 3.75 (t, 2H), 3.8 (s, 6H), 6.23 (s, 1H), 6.9 (m, 3H), 8 (m,3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 35.01, 41.27, 54.83, 55.78, 55.83, 111.33, 111.37, 111.80, 120.58, 121.77, 122.84, 124.72, 128.58, 133.22, 136.06, 136.65, 149.07, 165.59.

Synthesis of compound 42



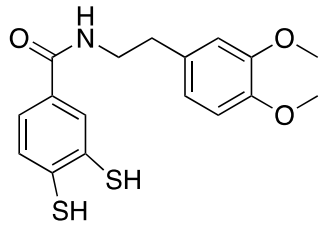
Chemical Formula: $C_{18}H_{17}NO_4S_2$
Molecular Weight: 375,46

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, 41 (200mg, 1.07mmol) N-methylmorfoline (3 EQ, 2.14 mmol) were added and dissolved DME (10 ml). The reaction was cooled to 0 °C and Methylcloroformate (3 EQ, 2.14 mmol) was added dropwise. Solution was stirred for 3 h at room temperature. The morfolinium salts were filtered and the solution was concentrated under vacuum obtaining the active carbonate.2-(3,4-dimetoxyphenil)etan-1-amine (1.3 EQ, 1.4 mmol) was added dropwise and the solution was dissolved in CH_2Cl_2 (10 ml) and stirred for 2 h. The reaction mixture was heated to 70 °C for 4 h. Reaction was monitored with TLC (EtOAc/Hexane, 3:7) to observe the complete conversion of the starting material. The solution was concentrated under vacuum obtaining a white solid that was dissolved in a mixture of $H_2O/CHCl_3$(1:1). The reaction mixture was quenched with KOH (2N, 1ml) and layers were separated in a separatory funnel, the acqueous layer was ecxtracted three times with $CHCl_3$ (3x20 ml). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum obtaining the crude product. The crude product was purified by liquid chromatography EtOAc/Hexan (3:7) to afford the product 42 (yield = 57%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.8(t, 2H), 3.75 (t, 2H), 3.8 (s, 6H), 6.23 (s, 1H), 6.9 (m, 3H), 8 (m,3H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 35.01, 41.27, 54.83, 55.78, 55.83, 111.33, 111.37, 111.80, 120.58, 121.77, 122.84, 124.72, 128.58, 133.22, 136.06, 136.65, 149.07, 165.59.

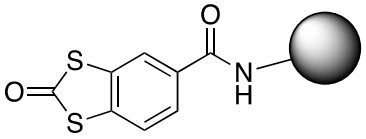
Synthesis of compound 43



Chemical Formula: $C_{17}H_{19}NO_3S_2$
Molecular Weight: 349,46

In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compounf 42 (30mg, 0.08 mmol) was added and dissolved in THF (10 ml).Then, *t*-BuOK (2 EQ, 0.16 mmol) was added and the solution was stirred for 1 h at r.t.Reaction was monitored with TLC (EtOAc/Hexan, 3:7) and quenched with MeI (2 EQ, 0.16 mmol). The reaction mixture was filtered and concentrated under vacuum obtaining the crude product that was used with any further purification.

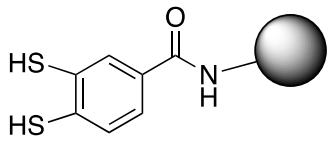
Synthesis of compound 44



In a two-necked round bottom flask under Ar atmosphere and equipped with a magnetic stir-bar, compound 41 (200mg, 1.07mmol) N-methylmorfoline (3 EQ, 2.14 mmol) were added and dissolved DME (10 ml). The reaction was cooled to 0 °C and Methylcloroformate (3 EQ, 2.14 mmol) was added dropwise. Solution was stirred for 3 h at room temperature. The morfolinium salts were filtered and the solution was concentrated under vacuum obtaining the active carbonate that was dissolved in CH_2Cl_2 (10 ml). The solution was cannulated in the reactor for solid phase synthesis containing Tentagel-NH_2 resin (previously swelled) (0.8 EQ, loading: 0.4 mmol/g). The heterogeneous mixture was left under N_2 flux for 2 h at r.t.Then the reaction solvent was eliminated and the solid phase was washed with CH_2Cl_2(x3), DMF (x3), CH_2Cl_2(x3) and dried under N_2 flux.

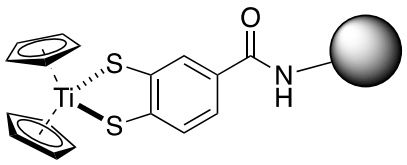
Yield: 85%.

Synthesis of compound 45



In a dry solid phase synthesis reactor under N_2 dry atmosphere compound 44 (500 mg) was added and swelled in THF. At the reaction mixture *t*-BuOK (2 EQ, 1.2 mmol) was added and the solution was stirred for 18 h at r.t. Then the reaction solvent was eliminated and the solid phase was washed with THF(x3), DMF (x3), CH_2Cl_2(x3) and dried under N_2 flux.

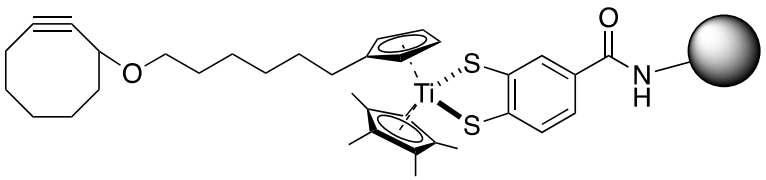
Synthesis of compound 46



In a dry solid phase synthesis reactor under N_2 dry atmosphere 45 (500 mg) was added and swelled in THF. At the reaction mixture $(Cp)_2TiCl_2$ (1.5 EQ 0.6mmol) and Et_3N (2.2 EQ, 0.88 mmol) were added dropwise and the reaction mixture was stirred for 18 h at r.t. Then the reaction solvent was eliminated and the solid phase was washed with THF(x3), DMF (x3), CH_2Cl_2(x3) and dried under N_2 flux.

Yield: 50%

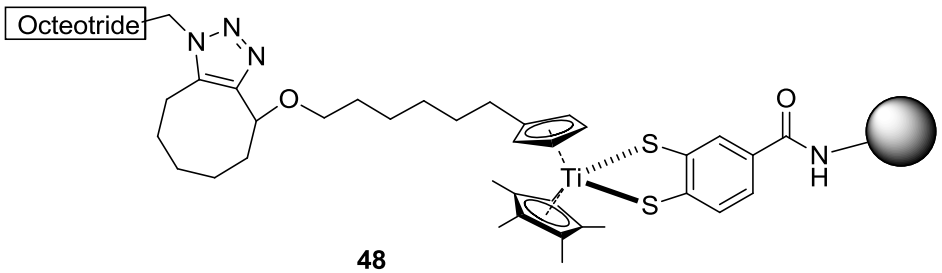
Synthesis of compound 47



In a dry solid phase synthesis reactor under N_2 dry atmosphere resin 45 (220 mg) was added and swelled in THF. At the reaction mixture compound 12b (1.5 EQ 0.39mmol) and Et_3N (2.2 EQ, 0.88 mmol) were added dropwise and the reaction mixture was stirred for 18 h at r.t.Then the reaction solvent was eliminated and the solid phase was washed with THF(x3), DMF (x3), CH_2Cl_2(x3) and dried under N_2 flux.

Yield: 47%

Synthesis of compound 48



48

In a dry solid phase synthesis reactor under N_2 dry atmosphere resin 47 (80 mg) was added and swelled in DMF.Then, Octoetride-N3 (15 mg 1.Equiv) was added and the reaction mixture was stirred for 18 h at r.t.Then the reaction solvent was eliminated and the solid phase was washed with DMF (x3), CH_2Cl_2(x3) and dried under N_2 flux.