New Synthetic Methodologies for pharmacological active compounds

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Abstract: In this work, were studied synthetic methodologies of building blocks with potential pharmacological activity, namely carbocyclic moieties (topic I) and dirhodium(II) compounds (topic II).

The first topic was based on the study of **Prins reaction** between cyclopentadiene and para-formaldehyde. For the first time, a catalyst screening was done in order to improve the reaction selectivity. Thus, the reaction catalysed by $LaCl_3$ yields the new Prins products **8** and **11a** or **11b**. These products were not easily isolated by usual techniques and products´ derivatizations do not allowed the purification as well. The combination of nanofiltration followed by chromatography allowed the isolation of the products mixture in satisfactory purity grade.

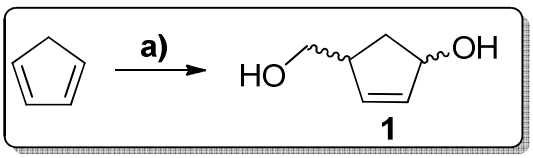
The second topic was based on the reaction between $Rh_2(OAc)_4$ and (*L*)-PhAla in water above room temperature, which provides a new structural motif **$Rh_2(OAc)_2$((*L*)-PhAla)$_2$** with catalytic and anticancer activity. The influence of several factors was studied, such as the temperature, microwave radiation, the presence of an inorganic base and the presence of an electron donating group in the aromatic ring of phenylalanine. This reaction provides several products, from which the two major ones are proposed to be geometric isomers. These products may be isolated by semi-preparative HPLC and applied in catalysis of diazo compounds decomposition and biological activity studies.

Keywords: Prins reaction, $LaCl_3$, nanofiltration, carbocyclic nucleosides, Rh, HPLC, pharmacological activity.

Introduction

The **Prins reaction** between cyclopentadiene and formaldehyde using formic acid - **Scheme 1** - was reported for the first time by Bajorek *et al.* in 1974[1]. The major product is 4-(hydroxymethyl)cyclopent-2-en-ol **1**.

This reaction has been studied by some research groups in order to improve the selectivity, screening acid source and reaction conditions[2,3,4].



Scheme 1 - Prins reaction between cyclopentadiene and para-formaldehyde in formic acid[1]

a) 1. HCOH, HCO_2H (η=65%); 2. NaOH (aq.), EtOH

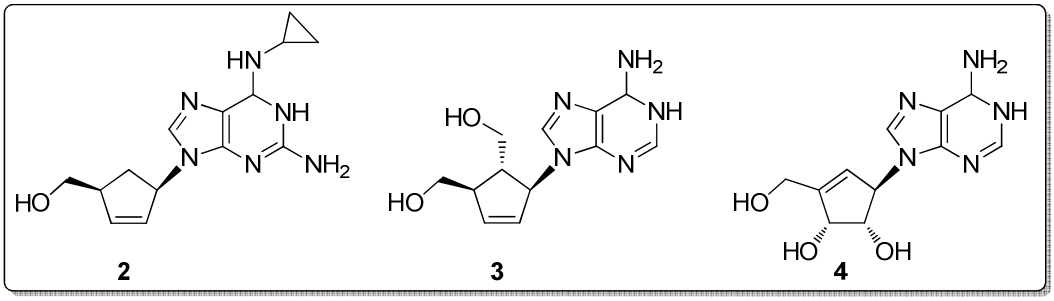
The compound **1** is used as pseudo-sugar building block in carbocyclic nucleosides, which are current drugs for the control of viral diseases and cancer.

For instance, Abacavir **2** and BCA **3** are used for the treatment of AIDS and Neplanocin A **4** for Hepatitis C - **Scheme 2**[3,5,6,7]. Carbocyclic nucleosides assume an important role in pharmaceutical industry due to high frequency of population infected with viral diseases.Thus, it is of great interest to achieve robust and cheaper synthetic pathways to produce these antiviral drugs.

The major drawback in carbocyclic nucleosides production is the synthesis of the carbocyclic block[8]. In the literature there are different synthetic aproachs to obtain this type of moieties in both racemic and enantiomeric forms. However, there is no efficient method for the synthesis of these important buildng blocks.

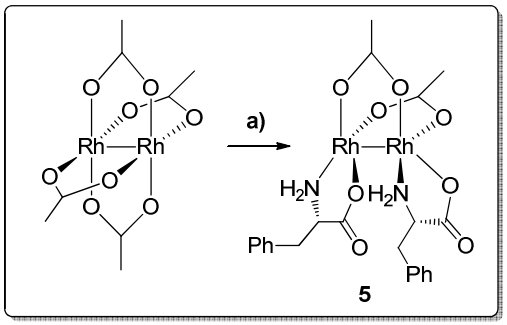
The Prins reaction between cyclopentadiene and para-formaldehyde is an efficient methodology but gives rise to many side products. So far, there are no catalytic system applied in this transformation and, thus, the search for a catalyst which may improve the reaction selectivity seems a challenge with high potentiality.

Rhodium is the rarest and the most expensive transition metal used in catalysis. Nevertheless, dirhodium compounds are well known for its catalytic activity in several organic reactions[9] and anticancer activity[10,11].



Scheme 2 - Carbocyclic nucleosides structures

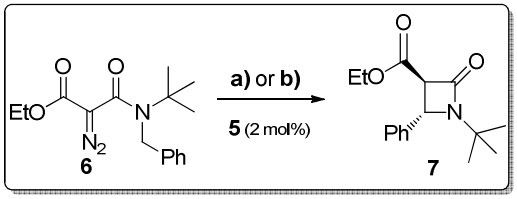
The reaction between $Rh_2(OAc)_4$ and (*L*)-PhAla in water (**Scheme 3**) yields $Rh_2(OAc)_2((L)\text{-PhAla})_2$ **5**, a known complex by its catalytic and anticancer activity[12,13].



Scheme 3 - Synthesis of $Rh_2(OAc)_2((L)\text{-PhAla})_2$ **5**[12]

a) (*L*)-PhAla (5 eq.), H_2O, 80ºC, 56 h, η=29%

The complex **5** does not have the usual lantern structure and has an alternative partially open structure, resulting from the exchange of two acetate ligands by two molecules of amino acid, where each amino acid is exclusively linked to one of the Rh(II) metal centers[12].
This complex was tested as catalyst in C-H insertion of diazo compounds[12] (**Scheme 4**).



Scheme 4 - $Rh_2(OAc)_2((L)\text{-PhAla})_2$ catalysed intramolecular C-H insertion of α-diazo **6**[12]

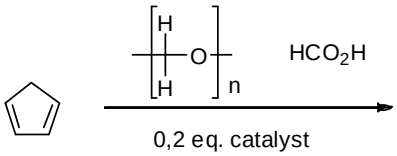
a) H_2O, 60ºC, 6 h, η=85%, e.e.=66%, (3*R*,4*S*)-**7**
b) CH_2Cl_2, 20ºC, 27 h, η=72%, e.e.=73%, (3*R*,4*S*)-**7**

Dirhodium carboxylate compounds are well known by its anticancer activity and the mechanism of action is quite studied[10,11]. Therefore, the compound **5** was submitted to biological activity tests[13] and this complex shows strong activity towards human colon adenocarcinoma cells in a more efficient way than cisplatine. Moreover, its effect was not accompanied by generation of reactive oxygen species neither by activation of caspase-3, contrary to the well known cisplatine, sugesting different mechanisms of action.

Results and Discussions

Prins reaction

The Prins reaction was submitted to a catalyst screening of several Lewis acids in order to enhance the reaction selectivity (**Scheme 5**). After work-up, the samples were analysed by TLC and GC. The tests A-R (**Table 1**) allowed the identification of the three standard Prins products (PP) **PP1**, **PP2** and **PP3** and four new Prins products (NPP) **NPP1**, **NPP2**, **NPP3** and **NPP4**.



Scheme 5 - Catalyst screening in Prins reaction

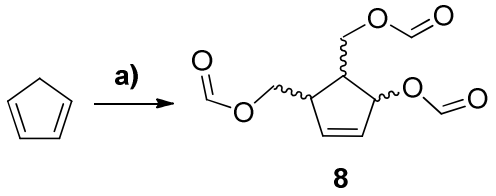
The tested Lewis acid displayed different catalytic behaviour in the course of Prins reaction. Some catalysts, such as *p*-TsOH, BF_3OEt_2 and $CuCl_2{\cdot}2H_2O$ (entries **B**, **E** and **H**) inhibited the course of the reaction. On the other hand, $FeCl_3$, $LaCl_3$, $RuCl_3{\cdot}xH_2O$ and $AlCl_3$ (entries **D**, **I**, **P** and **R** of **Table 1**) inhibited (almost completely) the formation of **PP** and provided the **NPP**. From this class, **$LaCl_3$** seems to be the catalyst with highest selectivity and, thus, subject to further study.
In a first approach, this reaction was performed with 0.07 eq. of **$LaCl_3$** and the reaction crude was purified by column chromatography followed by preparative thin-layer chromatography plate, which furnish two fractions. The first fraction was identified as

Table 1 - Percentage of Prins products and new Prins products in tests A-R[a), b)]

Entry	Catalyst	%NPP1	%NPP2	%NPP3	%NPP4	%PP1[d)]	%PP2[d)]	%PP3[d)]
rt(min)		11.9	39.5	40.2	44.6	28.0	29.5	29.9
A	Blank 1[c)]	0.0	0.0	0.0	0.0	12.4	2.6	45.5
B	*p*-TsOH	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	$ZnCl_2$	1.4	0.9	0.0	2.9	9.4	2.0	35.2
D	$FeCl_3$	0.0	0.0	0.0	4.9	0.0	0.0	0.0
E	BF_3OEt_2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	$CaCl_2{\cdot}2H_2O$	2.2	2.4	0.0	3.3	7.9	1.6	29.9
G	MgO	6.8	0.0	2.3	0.0	10.2	2.0	37.6
H	$CuCl_2{\cdot}2H_2O$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I	**$LaCl_3{\cdot}7H_2O$**	**0.0**	**6.5**	**0.0**	**11.9**	**0.0**	**0.0**	**3.2**
J	Blank 2[c)]	0.0	0.0	0.0	0.0	9.6	1.9	36.8
K	*Amberlyst*	0.0	0.0	0.0	16.8	7.0	1.9	29.2
L	Molecular Sieves	3.5	0.0	1.4	0.0	10.0	2.0	39.1
M	$CoCl_2{\cdot}6H_2O$	1.7	2.7	0.0	2.7	6.3	1.3	24.7
N	$AuClPPh_3$	1.6	0.7	0.5	1.2	9.4	1.8	36.5
O	$PdCl_2$	2.0	0.0	0.0	0.0	6.8	1.4	23.7
P	$RuCl_3{\cdot}xH_2O$	0.0	1.7	0.0	5.1	0.0	0.0	0.4
Q	$SnCl_2{\cdot}2H_2O$	1.7	2.6	0.0	5.7	4.9	1.0	18.7
R	$AlCl_3$	0.0	1.9	0.0	8.2	0.0	0.0	0.0

a) The percentages of products were calculated considering that **PP** and **NPP** have equal responses to GC detector;
b) Tests A–I: cyclopentadiene (264µL), HCHO (0.48 g), HCO_2H (2 mL), 0.2 eq. of catalyst, 60 h, -10ºC to rt;
Tests J–R: cyclopentadiene (132µL), HCHO (0.24 g), HCO_2H (1 mL), 0.2 eq. of catalyst, except for N test (0.02 eq.), 60 h, -10ºC to rt;
c) Blank 1 is related to tests A-I and Blank 2 is related to tests J-R;
d) The Prins products PP1, PP2 and PP3 were analysed by comparison with authentic sample isolated of the products mixture.

(5-(formyloxy)cyclopent-3-ene-1,2-diyl)bis(methylene) diformate **8** (analysed by ^{1}H NMR, ^{13}C NMR and MS) in a satisfactory purity grade and the second as a mixture of **8** and another similar product (analysed by ^{1}H NMR and ^{13}C NMR).



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Scheme 6 - Prins reaction catalysed by $LaCl_3$

a) Experimental conditions: cyclopentadiene (6.45 g; 6.59 mL; 98 mmol), HCHO (12 g; 0.4 mol), HCO_2H (50 mL; 1.3 mol), $LaCl_3$ (2.508 g; 0.07 eq.), 60 h, -10ºC-rt.

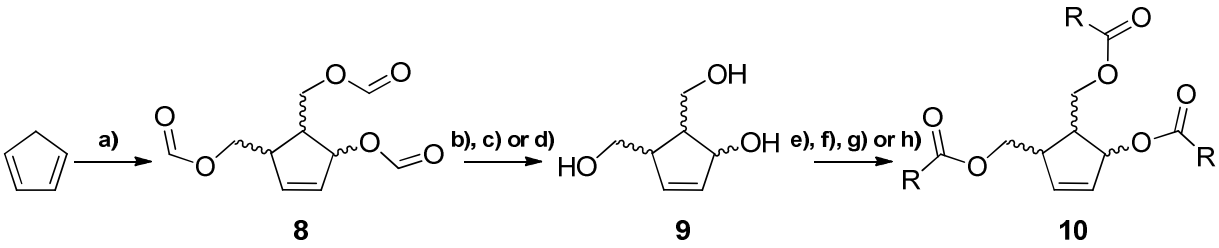
The second approach of isolation of the new Prins products was based on distillation, which was also fruitless for structure elucidation.

In a third approach, several derivatizations of reaction´s products were done, in order to facilitate its isolation and identification - **Scheme 7**. However, products´ decomposition and/or separation issues did not allow the pretended structure elucidation.

In an attempt to improve the Prins reaction catalysed by $LaCl_3$, the sources of protic acid and formaldehyde were screening. The reactions with 1) formic acid and aqueous formaldehyde (37%); 2) acetic acid and aqueous formaldehyde (37%); 3) acetic acid and para-formaldehyde and 4) without Brönsted acid using aqueous formaldehyde (37%) in THF were not better than previous with formic acid and para-formaldehyde. However, these negative results are contributors for the mechanism elucidation. The reaction 4) show no formation of any type of products (PP, NPP or other), indicating the key function of Brönsted acid in the reaction outcome.

The major issue in the isolation of the Prins products (PP and NPP) is the polymeric material yielded by reagents monomers, with distinct chain size. Some of these oligomers keep the same chromatography behaviour as the Prins products, making its isolation difficult.

Previous unsuccessful results force a new approach for the isolation of the **NPP** and **nanofiltration** was chosen as a recourse technique for purification. In a preliminary test, three membranes were chosen with distinct Molecular Weight Cut-off (MWCO) and composition - **Table 2**.



Scheme 7 – Derivatizations approaches for Prins reaction catalysed by $LaCl_3$

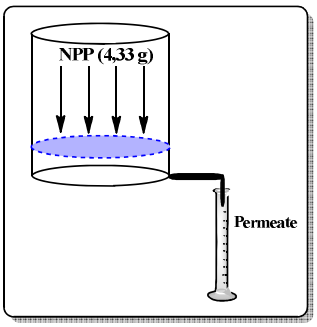
a) Cyclopentadiene, para-formaldehyde, HCO_2H, $LaCl_3$ (0.07 eq.),60 h, -10ºC to rt;
b) MeONa, MeOH, 6h (rt) + 24 h (45ºC);
c) NaOH, EtOH, 20 h, rt;
d) NH_3, MeOH, high pressure reactor, -40ºC to rt, 24 h;
e) **9** from **b)**, *p*-nitrobenzoyl chloride, $N(Et)_3$, CH_2Cl_2, 21h (rt) + 27 h (40ºC); (**10**, R=$C_6H_4NO_2$);
f) **9** from **c)**, acetyl chloride, $N(Et)_3$, CH_2Cl_2, 0ºC to rt (**10**, R=Me);
g) **9** from **d)**, acetic anhydride, $N(Et)_3$, DMAP (cat.), THF, 0ºC to rt, 3 days (**10**, R=Me);
h) **9** from **d)**, phenyl acetyl chloride $N(Et)_3$, DMAP (cat.), THF, 0ºC to rt, 24 h (**10**, R=CH_2Ph)

Table 2 - Conditions for nanofiltration tests 1-3[a)]

Entry	Membrane	Pressure (bar)	MWCO (Da)	Stirring (rpm)	M_{sample} (mg)	$m_{retentate}$ (mg)	$m_{permeate}$ (mg)
1	DURAMEM^{TM} 300	30	300	500	509.7	375.2	89.3
2	STARMEM^{TM} 122	22	220	300	502.0	293.1	45.7
3	DURAMEM^{TM} 200	22	200	300	371.5	190.3	109.3

[a)] Introduction of the sample dissolved in 8 mL of AcOH and collection of 4 mL of permeate.

The three tests indicate an enrichment of the NPP in permeate, however polymeric material was still present, requiring the use of chromatography as a complementary technique. Permeates were purified by preparative thin-layer chromatography plate and the best result was obtained with DURAMEM^{TM} 300 (**1**). Therefore a scale-up process - **Scheme 8** - was done using this membrane, with reposition of solvent and the collected permeates were analysed and selected according to its purity pattern by ^{1}H RMN ($\eta_{nanofiltration}$=88.7%).



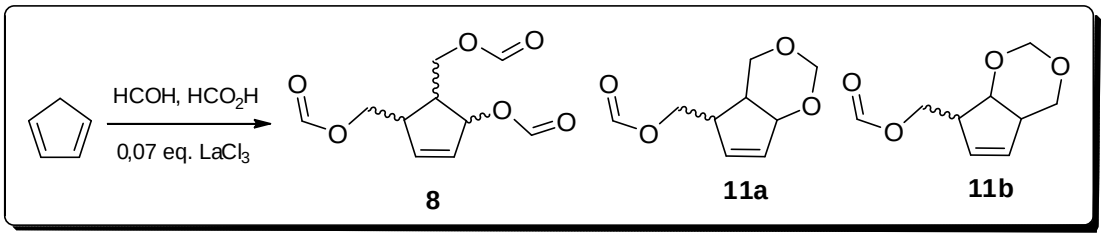
Scheme 8 - Nanofiltration approach[a)]

[a)] Introduction of the sample dissolved in 30 mL of AcOEt and collection of 15 mL of permeate (12 cycles), P=20 bar, 300 rpm.

The five chosen permeates (3.84 g) were purified in 23 preparative thin-layer chromatography plates, giving rise to a sample (394.2 mg) in higher purity grade than previously ($\eta_{chromatography}$=10.7%). However the two NPP were present in equal quantities and structures elucidation was still not possible. This sample was submitted to a second preparative thin-layer chromatography plate and a sample rich in different percentage of the NPP was identified by ^{13}C NMR. This spectra and DEPT 135 allows the proposed of structure **11a** or **11b** as the second NPP.
In order to explain the formation of these NPP **8** and **11a** or **11b**, an intermediary with structure **9** is proposed, which may derive from two consecutive Prins addition reactions followed by hydrolysis and HCO_2H elimination. The introduction of formate groups gives rise to NPP **8** and the incorporation of formaldehyde, followed by incorporation of formate in the free hydroxyl group gives rise to NPP **11a** or NPP **11b** (**Scheme 9**).

Dirhodium(II) complexes

Previous work in topic II by this laboratory suggested that complex **5** was not in high purity level, indicating the formation of similar compounds which may affect biological studies reproducibility and, probably, with catalysis results.



Scheme 9 – Proposed Prins reaction behaviour catalysed by $LaCl_3$

Therefore a methodology for analysis and isolation of different products formed in exchange ligands reactions was required HPLC was chosen as a method to perform kinetic studies and also products´ isolation. The analytical conditions for the reaction of **Scheme 3** were not easily achieved. After screening several conditions for stationary phase and eluents additives, an appropriate system with peaks´ resolution and reproducibility was found.

The repetition of the standard reaction (**Scheme 3**) yielded two major products **Rh_2D** (**5**) and **Rh_2E** after 52 hours - **Figure 1**.

These products **Rh_2D** (**5**) and **Rh_2E** are, probably, geometric isomers and may have similar UV detector responses (i.e. similar ε) and if so, they are formed in similar yields, which can explain the observed low reaction yield (**Scheme 3**).

Microwave radiation[14,15] has been used to induce reaction selectivity although its mechanism of action is still controversy and a combination of thermal and non-thermal with high polarization effects has been proposed. Therefore the standard reaction (**Scheme 3**) was submitted to microwave radiation in order to get 80ºC (P=1 W). **Graphic 1** allows the comparison between thermal (T) and microwave (M) reaction: for the first 3.75 h the microwave reaction show higher consumption of both reagents than thermal, however after 3.75 h the kinetics proceed slower. This fact may be indicative of a competition between the decoordenation of acetate ligand and (*L*)-PhAla coordination.

Standard synthesis of dirhodium compounds[16] uses a Soxhlet extraction to trap AcOH with Na_2CO_3. However this approach to neutralize AcOH can not be used in this aqueous approach.

In an attempt to trap the AcOH formed, the reaction (**Scheme 3**) was performed with Na_2CO_3 in the solution. This reaction gives rise to different dirhodium products whose isolation and characterization has not been done until this moment, due to time constrains. Those new products may be of type $Rh_2(OAc)_{4-n-m}((L)\text{-PhAla})_n(CO_3)_m$, $m+n \leq 4$, suggesting carbonate coordination. Moreover, one of the products has similar chromatographic behaviour (rt) as previous **Rh_2 E**, however this association must be proved.

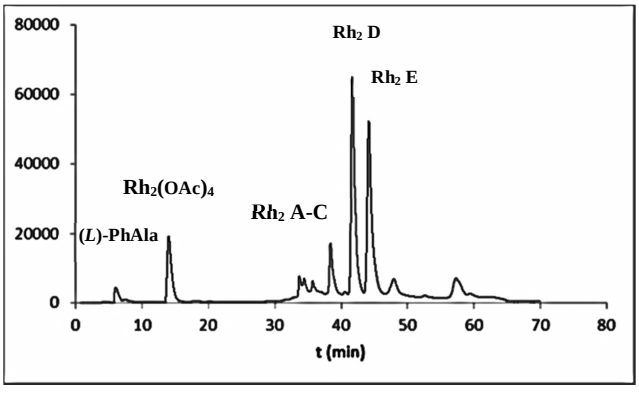
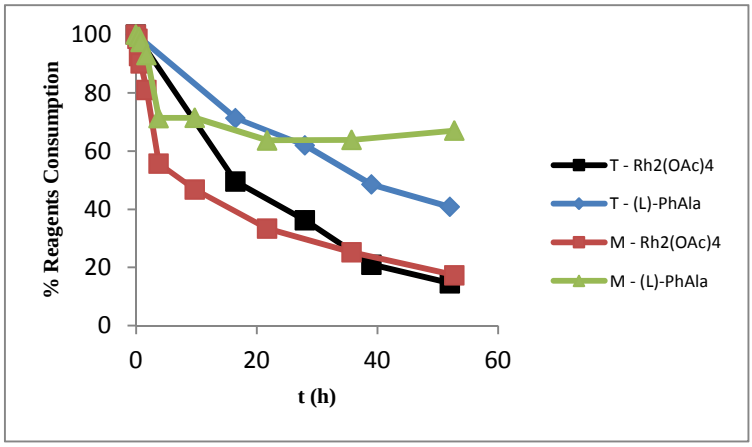


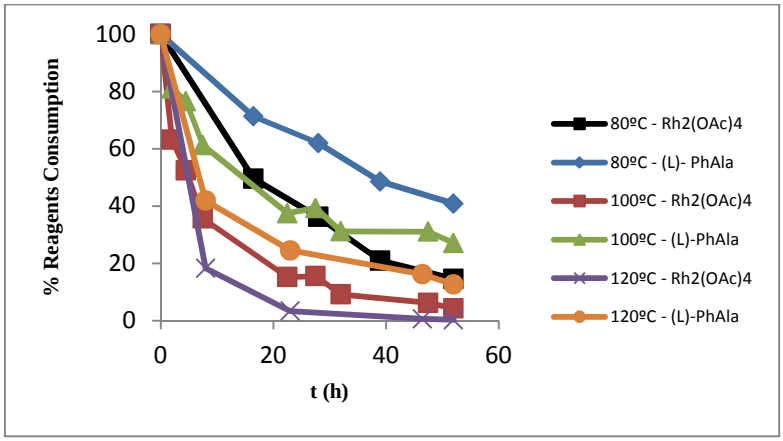
Figure 1 - Chromatogram for reaction between $Rh_2(OAc)_4$ e (*L*)-PhAla in water at 80ºC after 52 h[a,b]

[a] - Chromatographic conditions: gradient program: 90:10 H_2O:CH_3CN (t=0-25 min); 78:22 H_2O:CH_3CN (t=30-60 min) (both solvents with 0.01% TFA); 1 mL/min; λ=220 nm

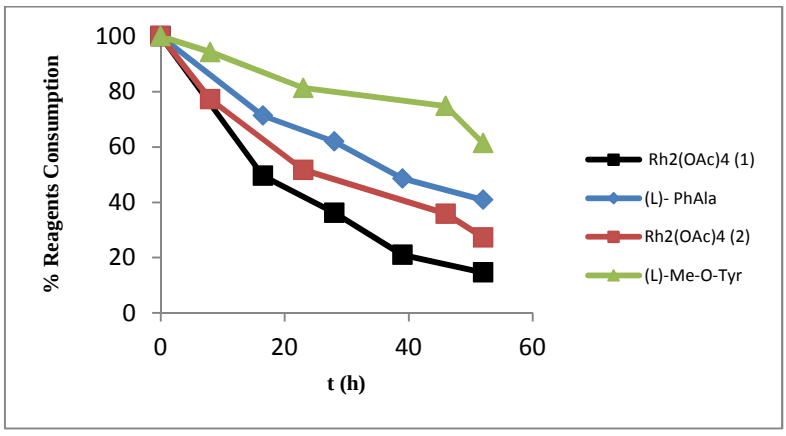
[b] - Retention time (min): (*L*)-PhAla (6,0), $Rh_2(OAc)_4$ (14,0), Rh_2 A (33,7), Rh_2 B (35,7), Rh_2 C (38,4), Rh_2 D (41,7) and Rh_2 E (44,1).



Graphic 1 - Percentage of reagents consumption in thermal (T) and microwave (M) reactions in function of reaction time



Graphic 2 - Percentage of reagents consumption in thermal reactions at 80ºC, 120ºC and 120ºC in function of reaction time



Graphic 3 - Percentage of reagents consumption in thermal reactions at 80ºC with amino acids (*L*)-PhAla (1) and (*L*)-Me-*O*-Tyr (2) in function of reaction time

The temperature effect on reaction outcome was studied at 80ºC, 100ºC and 120ºC following standard conditions (**Scheme 3**).

Graphic 2 allows the comparison between thermal reactions at 80ºC, 100ºC and 120ºC. The reaction outcome after 8 h at 120ºC is

comparable to the outcome after 22.5 h at 100ºC and after 52 h at 80ºC. After 8 h at 120ºC and 52 h at 100ºC, a proportion inversion of products **Rh_2D** (**5**) and **Rh_2E** was observed, in relation to 52 h at 80ºC (**Figure 1**). Only at 120ºC was observed the total consumption of $Rh_2(OAc)_4$ after 23 h of reaction. Even so, the reaction was kept at 120ºC and, after 52 h, the peak of compound **Rh_2D** (**5**) decreases and **Rh_2A** peak increases, suggesting conversion between these rhodium species.

The reaction (**Scheme 3**) was performed using (*L*)-Me-*O*-Tyr as amino acid instead of (*L*)-PhAla. **Graphic 3** allows the comparison between reactions with these amino acids, where index (1) is related to (*L*)-PhAla and index (2) to (*L*)-Me-*O*-Tyr.

The presence of an electron donating group in aromatic ring of PhAla in (*L*)-Me-*O*-Tyr was expected to increase ligand coordinating/donor potential, however reaction kinetics with (*L*)-Me-*O*-Tyr as amino acid seems slower than with (*L*)-PhAla. This may be explained by the combination of electronic and stereo factors. Further studies with distinct substituents in PhAla aromatic ring will allow an appropriate correlation.

The semi-preparative HPLC isolation of products **Rh_2D** (**5**) and **Rh_2E** is already in course and in a preliminary test chromatographic conditions were found at a satisfactory resolution level - **Figure 2**, which open the opportunity for the isolation and full structure elucidation of each Rh(II) complex.

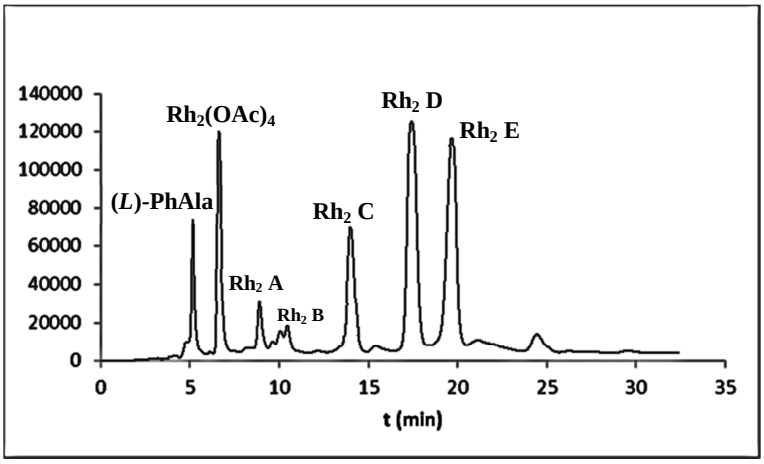


Figure 2 - Chromatogram for reaction between $Rh_2(OAc)_4$ e (*L*)-PhAla in water at 80ºC after 52 h in semi-preparative column Phenomenex Luna 10µ C18(2), UV detection at 220 nm using 75:25 of H_2O:CH_3CN (both with 0,01% TFA) as eluent.

Conclusions

The Prins reaction was, for the first time, submitted to a catalyst screening and the catalysis mediated by $LaCl_3$ provided new Prins products. Although chromatography, distillation and derivatizations efforts were fruitless, the combination of nanofiltration followed by preparative thin-layer chromatography plate allowed the isolation of the products mixture in satisfactory purity grade. The identification of NPP point to compounds **8** and **11a** or **11b**, however structure elucidation is still not completed. After confirmation of NPP structures, the carbocyclic moiety will be couple to DNA bases and the carbocyclic nucleosides obtained will be tested as antivirals.

The discovery of new dirhodium(II) complexes is a topic of high potential due to its ambivalent applications as catalysts and anticancer drugs. In this work, a methodology for the analysis of dirhodium compounds by HPLC was developed, which allows kinetic studies of ligand exchange reactions. The influence of several factors in the reaction between $Rh_2(OAc)_4$ and (*L*)-PhAla was studied, such as the temperature, microwave radiation, the presence of an inorganic base and the presence of an electron donating group in the aromatic ring of phenylalanine. Furthermore, efforts to isolate compounds **Rh_2D** (**5**) and **Rh_2E** are in course and each pure sample will be applied in C-H insertion of diazo compounds (**Scheme 4**) and in biological activity studies.

Experimental Section

General Information

All reagents were obtained from commercial sources. Anhydrous solvents - CH_2Cl_2, $N(Et)_3$ and THF - were freshly distilled over calcium hydride prior to use.

Flash chromatography was prepared with silica from Merck (ref 109385) or from Sharlau (230-400 mesh ASTM). Preparative thin-layer chromatography plate was prepared with silica gel 60 GF254 Merck (ref 107730).

NMR spectra were recorded at room temperature in a Bruker AMX 300 or 400 using $CDCl_3$ as solvent and TMS as internal standard.

GC analysis was performed on a Shimadzu 2014 gas chromatograph equipped with a TRB-50, TR-500233 column (30 m × 0.32 mm × 0.25 µm); Injector: 280ºC; detector (FID): 300ºC; split ratio = 50, column flow (He): 2.03mL/min; oven 70ºC for 10 minutes, ramp 2ºC/min to 100ºC for 5 minutes and ramp 5ºC/min to 200ºC. Dodecane was used as internal standard.

Nanofiltration membranes DURAMEM and STARMEM were provided by Membrane Extraction Technology Ltd, UK (MET); DURAMEMTM is a mark of MET; STARMEMTM is a mark of W.R. Grace & Co., USA.

HPLC analysis was performed using a pump Shimadzu LC-20AT, a detector Merck UV-Vis Gilson 118 (λ=220 nm) and analytical column KROMASIL 100 C18 02671.

Microwave reactions were performed in a CEM apparatus, Discover model.

HPLC solvents were Millipore water, which was filtrated in a cellulose acetate membrane (0.45 µm, 47 MM) and CH_3CN, commercially obtained to Sigma-Aldrich and Panreac. Solvents preparation required addition of 0.01% of TFA filtrated and then, eluents were degassed in ultra-sound bath for 30 minutes.

Reactions between $Rh_2(OAc)_4$ and (*L*)-PhAla were analysed using a gradient program of 90:10 H_2O:CH_3CN, t=0-25 min; 78:22 de H_2O:CH_3CN, t=30-60 min, 1.0 mL/min. the reaction between $Rh_2(OAc)_4$ and (*L*)-Me-*O*-Tyr was analysed using a gradient program of 90:10 H_2O:CH_3CN, t=0-20 min; 74:26 de H_2O:CH_3CN, t=25-60 min, 1.0 mL/min.

Semi-preparative conditions were obtained using a semi-preparative column Phenomenex Luna 10µ C18(2), UV detection at 220 nm and an isocratic eluent of 75:25 of H_2O:CH_3CN (both with 0,01% TFA).

Catalyst screening in Prins reaction

The reaction was performed as reported by Bajorek *et al.*[1] with the addition of 0.2 eq. of each catalyst, except for N test (0.02 eq.). For tests A-I and J-R the reaction scales were 1/25 and 1/50 of the reported, respectively.

Prins reaction catalysed by $LaCl_3$

The reaction was performed as reported by Bajorek *et al.*[1] (reported scale) with the addition of 0.07 eq. of $LaCl_3$. After work-up (neutralization with Na_2CO_3; filtration; extractions with $(C_2H_5)O$, CH_2Cl_2 and 2 × AcOEt; drying of organic layer with anhydrous Na_2SO_3; solvent´s evaporation), an orange oil (R_f (silica, Hex/AcOEt 7:3)=0.09 and 0.30) was obtained (5.042 g).

Attempt for the preparation of (5-((4-nitrobenzoyl)oxy)cyclopent-3-ene-1,2-diyl)bis(methylene) bis(4-nitrobenzoate) (**10**, R= $C_6H_4NO_2$)

To a solution of **8** (2,287 g) in MeOH (10 mL), MeONa (0.5925 g) was added at rt under Argon atmosphere and, after 6 h at rt, the reaction was heated at 45ºC for 24 h. After evaporation of solvent, the triol **9** was obtained (R_f (silica, Hex/AcOEt 7:3)=0.11). To a solution of **9** and $N(Et)_3$ (5.56 mL; 4 eq.) in CH_2Cl_2 under Argon atmosphere at 0ºC, a solution of *p*-nitrobenzoyl chloride was added. The reaction proceeds at rt for 21 h and at 40ºC for further 27 h. After evaporation of solvent (R_f (silica, Hex/AcOEt 7:3)=0.83), the crude was purified by preparative thin-layer chromatography plate (Hex/AcOEt 7:3) but ^{1}H NMR and ^{13}C NMR show products´ degradation because aromatic and olefinic integrations did not correspond properly.

Attempt for the preparation of (5-acetoxycyclopent-3-ene-1,2-diyl)bis(methylene) diacetate (**10**, R= Me)

To a solution of **8** (1.07 g) in EtOH (10 mL), NaOH (0.58 g; 3 eq.) was added at 0ºC and, after 20 h at rt, the triol **9** was obtained (R_f (silica, Hex/AcOEt 7:3)=0.11), after filtration and solvent´s evaporation. To a solution of **9** and $N(Et)_3$ (10 mL) in CH_2Cl_2 (25 mL) under Argon atmosphere at 0ºC, a solution of acetyl chloride (3.2 mL; 5 eq.) in CH_2Cl_2 was slowly added. TLC analysis did not appear promising and this product was not further purified.

Attempt for the preparation of (5-acetoxycyclopent-3-ene-1,2-diyl)bis(methylene) diacetate (**10**, R= Me)

To a solution of **8** (2.064 g) in MeOH (reactor height=4 cm) in a high pressure reactor at -40ºC, NH_3 was bubbled until reactor height achieves 5.5 cm. Then, the reactor was sealed and the reaction remains at rt for 24 h. After the removing NH_3 excess, the solvent was evaporated and the sample of triol **9** was divided in 2 portions. To a solution of **9** (one of the portions) and $N(Et)_3$ (4 mL; 4 eq.) in THF (22 mL) under Argon atmosphere at 0ºC, acetic anhydride (2 mL; 3 eq.) was slowly added. After 26 h, a catalytic amount of DMAP was added and the reaction proceeds for 3 days. After evaporation of solvent, a dark orange oil was obtained (R_f (silica, Hex/AcOEt 7:3)=0.34) which, upon column chromatography, yields a mixture of 2 similar products whose identification was not allowed by NMR.

Attempt for the preparation of (5-(2-phenylacetoxy)cyclopent-3-ene-1,2-diyl)bis(methylene) bis(2-phenylacetate) (**10**, R= CH_2Ph)

To a solution of **9** (0.7627 g; second portion) and $N(Et)_3$ (4 mL; 4 eq.) in THF (10 mL) under Argon atmosphere at 0ºC, a solution of phenyl acetyl chloride (3.7 mL; 4.0 eq.) in THF (15 mL) was slowly added. After 6 h, a catalytic amount of DMAP was added and the reaction proceeds for further 24 h. After extraction with THF and CH_2Cl_2, the organic layer was dried with anhydrous Na_2SO_3 and the solvent was evaporated. The crude was purified by preparative thin-layer chromatography plate, which was re-chromatographed. However, NMR analysis indicates product´s decomposition since there were no olefinic signals in ^{1}H NMR.

Attempts to improve Prins reaction catalysed by $LaCl_3$

The four reactions **1-4** were performed as reported[1] (1/10 scale), with the following changes:

1 - Aqueous formaldehyde (37%) (3.3 ml) instead of para-formaldehyde;

2 - Acetic acid (5 mL) and aqueous formaldehyde (37%) (3.2 mL) instead of formic acid and para-formaldehyde;

3 - Acetic acid (5 mL) instead of formic acid;

4 - Without Brönsted acid, with aqueous formaldehyde (37%) (3.2 mL) in THF (5 mL).

Purification by nanofiltration of the Prins reaction catalysed by $LaCl_3$

After washing PEG from membrane, the crude was dissolved in AcOEt and this solution was added to the cell. Application of the target Pressure (N_2) allowed the collection of permeates (half of solution volume) and reposition of solvent was done. The solvent of permeates and retentate were evaporated and NMR analyses were performed.

After two preparative thin layer chromatography plate sessions, the ^{13}C NMR allowed the following identification of products **8** and **11a** or **11b** (**Figure 3**).

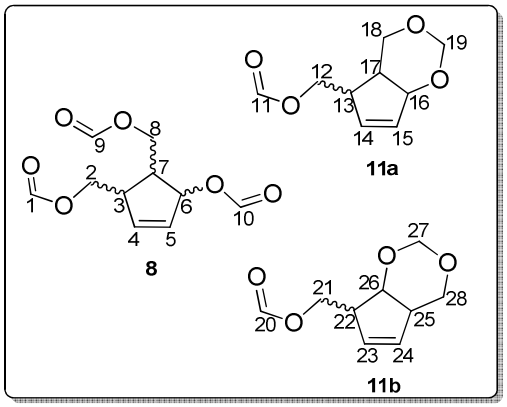


Figure 3 - NPP **8** and **11a** or **11b** numbered structures for ^{13}C NMR

^{13}C NMR (300 MHz, $CDCl_3$, 25ºC, TMS): δ 40,0 (C_{17} or C_{25}), 44,8 (C_{13} or C_{22}), 46,3 (C_3), 46,9 (C_7), 63,8 (C_8), 64,2 (C_{18} or C_{21}), 65,4 (C_2), 65,9 (C_{12} or C_{28}), 79,0 (C_{16} or C_{26}), 80,8 (C_6), 91,3 (C_{19} or C_{27}), 130,4 (C_4), 131,9 (C_{14} or C_{24}), 136,2 (C_2), 139,3 (C_{15} or C_{23}), 160,6 (C_{10}), 160,7 (C_1, C_9), 160,9 (C_{11} or C_{20}).

Reaction between $Rh_2(OAc)_4$ and (*L*)-PhAla

To a solution of $Rh_2(OAc)_4$ (5.0 mg) in water (5.75 mL), (*L*)-PhAla (9.4 mg) was added and the reaction mixture was kept stirring at rt for 80 minutes, where ´0 h' sample was taken (to ensure complete reagents solubility). Then, the reaction was heated in a bath at 80ºC and the reaction was followed by HPLC until 52 h. The reaction colour progressively changes from $Rh_2(OAc)_4$ blue to green of known $Rh_2(OAc)_2$((*L*)-PhAla)$_2$.

The reaction under microwave radiation was performed at 80ºC induced by 1 W.

The reaction in the presence of an inorganic base was performed by adding Na_2CO_3 (31.2 mg; 5.2 eq). After 52 h the reaction was yellow.

Thermal reaction at 120ºC required the use of a high pressure reactor from Aldrich and precipitation of a green solid was observed.

Reaction between $Rh_2(OAc)_4$ and (*L*)-PhAla

The procedure was similar to previous reaction with (*L*)-PhAla, using (L)-Me-*O*-Tyr (11.0 mg). The reaction colour progressively changes from $Rh_2(OAc)_4$ blue to green of known $Rh_2(OAc)_2$((*L*)-Me-*O*-Tyr)$_2$.

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Supporting Information Available: Detailed information of experimental procedures and results is available in Master thesis of M. Carolina Carias, IST, 2010.

References

(1) Bajorek, J. J. S.; Battagli.R; Pratt, G.; Sutherla.Jk, *J. Chem. Soc. Perk. T. 1*, **1974**, 1243.

(2) Paulsen, H.; Maass, U., *Chemische Berichte-Recueil*, **1981**, *114*, 346.

(3) Savillestones, E. A.; Lindell, S. D.; Jennings, N. S.; Head, J. C.; Ford, M. J., *J. Chem. Soc. Perk. T. 1*, **1991**, 2603.

(4) Lackey, J. W.; Mook, R. A.; Partridge, J. J.; Glaxo Group Ltd (Glax) Glaxo Inc (Glax).

(5) Kurteva, V. B.; Afonso, C. A. M., *Chem. Rev.*, **2009**, *109*, 6809.

(6) Banerjee, S.; Ghosh, S.; Sinha, S.; Ghosh, S., *J. Org. Chem.*, **2005**, *70*, 4199.
(7) Crimmins, M. T., *Tetrahedron*, **1998**, *54*, 9229.
(8) Pinheiro, E. D.; Antunes, O. A. C.; Fortunak, J. M. D., *Antiviral Res.*, **2008**, *79*, 143.
(9) Doyle, M. P., *J. Org. Chem.*, **2006**, *71*, 9253.
(10) (a) Erck, A.; Sherwood, E.; Bear, J. L.; Kimball, A. P., *Cancer Res.*, **1976**, *36*, 2204; (b) Howard, R. A.; Kimball, A. P.; Bear, J. L., *Cancer Res.*, **1979**, *39*, 2568.
(11) (a) Asara, J. M.; Hess, J. S.; Lozada, E.; Dunbar, K. R.; Allison, J., *J. Am. Chem. Soc.*, **2000**, *122*, 8; (b) Dunham, S. U.; Chifotides, H. T.; Mikulski, S.; Burr, A. E.; Dunbar, K. R., *Biochemistry*, **2005**, *44*, 996; (c) Kang, M.; Chifotides, H. T.; Dunbar, K. R., *Biochemistry*, **2008**, *47*, 2265; (d) Chifotides, H. T.; Koshlap, K. M.; Perez, L. M.; Dunbar, K. R., *J. Am. Chem. Soc.*, **2003**, *125*, 10714.
(12) Candeias, N. R., Ph. D. Thesis, *Intramolecular C-H Insertion of α-Diazoacetamides in Water*, Ph. D. Thesis, Universidade Técnica de Lisboa, **2008**.
(13) Frade, R. F. M.; Candeias, N. R.; Duarte, C. M. M.; Andre, V.; Duarte, M. T.; Gois, P. M. P.; Afonso, C. A. M., *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 3413.
(14) de la Hoz, A.; Diaz-Ortiz, A.; Moreno, A., *Chem. Soc. Rev.*, **2005**, *34*, 164.
(15) Strauss, C. R.; Rooney, D. W., *Green Chem.*, **2010**, *12*, 1340.
(16) Doyle, M. P.; Bagheri, V.; Wandless, T. J.; Harn, N. K.; Brinker, D. A.; Eagle, C. T.; Loh, K. L., *J. Am. Chem. Soc.*, **1990**, *112*, 1906.