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New Sustainable Processes Catalyzed by Acids with Interest in Pharmaceutical Chemistry

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In the present form, this document configures a dissertation proposed to accomplish the degree of Master in Industrial Pharmaceutical Chemistry by the Faculty of Pharmacy of the University of Coimbra.

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Contents

Resumo

O principal objectivo deste trabalho foi avaliar o poder catalítico de Starbon®400-SO_3H na Reacção de Ritter em moléculas esteróides.

A crescente importância da Química Verde e a aplicação dos seus princípios na produção e desenvolvimento de novos processos químicos mais eficientes, sustentáveis e ambientalmente aceitáveis têm sido tema de investigação, tanto no meio académico como na indústria farmacêutica. Entre as várias estratégias delineadas na procura de mais eficácia a via que mais se destaca é a Catálise. Neste contexto, Starbon®400-SO_3H posiciona-se como um catalisador ambientalmente aceitável por ser completamente orgânico e não tóxico.

Os compostos esteróides estão vastamente distribuídos na natureza e que são utilizados como substratos funcionalizáveis na síntese de muitas moléculas biologicamente activas, pelo que centenas de esteróides têm sido isolados a partir de fontes naturais e vários milhares foram obtidos sinteticamente, mantendo-se nos nossos dias, uma intensa investigação com o fim de isolar e identificar novos compostos esteróides com novas actividades biológicas.

Tendo em conta as considerações anteriores, foi estudada a actividade catalítica do Starbon®400-SO_3H em transformações envolvendo epóxidos esteróides como substratos, uma vez que esta função está presente num grande número de esteróides biologicamente activos.

A reacção de Ritter de 5β,6β-epóxidos de esteróides com nitrilos, catalisada por Starbon®400-SO_3H, permitiu a obtenção de derivados N-acilamino álcoois vicinais, com elevados rendimentos, e alta estereo- e regiosselectividade. Na reacção com 5α,6α-epóxidos esteróides há produção de estereoisómeros. Quando se usou 1,4-dioxano como solvente obteve-se o derivado oxazolínico como produto de reacção.

Abstract

The aim of this work was to evaluate the catalytic power of Starbon®400-SO_3H in the Ritter reaction in steroid molecules.

The growing importance of Green Chemistry and the application of its principles in the production and development of new, more efficient, sustainable and environmentally acceptable chemical processes have been the subject of research both in academia and in the pharmaceutical industry. Among the several strategies outlined in the pursuit of effectiveness, the one that stands out the most is Catalysis. In this context, Starbon®400-SO_3H appeared as an environmentally acceptable catalyst due to its entirely organic composition and non-toxicity.

Steroid compounds are widely distributed in nature and are used as functionalized substrates in the synthesis of many biologically active molecules. Hundreds of steroids have been isolated from natural sources and several thousands have been obtained synthetically. There is an intensive ongoing research to isolate and identify novel steroid compounds with new biological activities.

In view of the foregoing, we studied the catalytic activity of Starbon®400-SO_3H in transformations involving epoxysteroids as substrates, as this function is present in a large number of biologically active steroids.

The Ritter reaction in 5β,6β-epoxysteroids with nitriles catalyzed by Starbon®400-SO_3H afforded vic-N-acylamino-hydroxy products, in high yields and with high stereo- and regioselectivity. The reaction with 5α,6α-epoxysteroids afforded a mixture of stereoisomers. When 1,4-dioxane was used as a solvent, the reaction yielded the oxazoline derivative as the final product.

List of Abbreviations

δ – Chemical Shift (NMR)

AC – Activated Carbons

AcO - Acetoxyl

API – Active Pharmaceutical Ingredient

^{13}C CP MAS NMR - Cross-Polarization Magic Angle Spinning Carbon-**13** Nuclear Magnetic Resonance

$CDCl_3$ – Deuterated chloroform

CNS – Central Nervous System

Da - Daltons

d - doublet

dd – doublet of doublets

DEPT – Distortionless Enhancement by Polarization Transfer

DHEA - Dehydroepiandrosterone

DMAP – 4-(Dimethylamino)-pyridine

DoE - Design of Experiment

DRIFT - Diffuse Reflectance Infrared Fourier Transform Spectroscopy

E factor – Environmental factor, Sheldon's green chemistry metric

E_{DR} – Energy surface from the Dubinin-Radushkevich model

eq – equivalent(s)

ESI-MS – Electrospray Ionization Mass Spectroscopy

GMP - Good Manufacturing Practices

GSK – GlaxoSmithKline

h – hours

HMBC – 2D Heteronuclear Multiple Bond Correlation Spectroscopy

HMQC - 2D Heteronuclear Multiple Quantum Correlation Spectroscopy

HPLC – High Performance Liquid Chromatography

IUPAC – International Union for Pure and Applied Chemistry

IUB – International Union of Biochemistry

J – Coupling Constant

Kg – Kilogram(s)

LCT - Liquid Crystal Templating

m - multiplet

MCM - Mobil Composition of Matter

m-CPBA – *meta*-chloroperbenzoic acid

MeCN - Acetonitrile

NMR – Nuclear Magnetic Resonance Spectroscopy

nOe - Nuclear Overhauser Effect

NOESY - Nuclear Overhauser Effect Spectroscopy

PAT - Process Analytical Technology

PGC – Porous Glassy Carbons

PMA-SiO_2 – Phosphomolybdic acid supported in silica gel

PPDMs - Porous Polysaccharide-derived Materials

p-TSA – *p*-Toluenesulfonic Acid

R&D – Research and Development

Rf – Retention factor

r.t. – room temperature

s – singlet

SN_2 - bimolecular nucleophilic substitution

TGA - Thermogravimetric analysis

THF – Tetrahydrofuran

TLC – Thin Layer Chromatography

TOF - Turnover Frequency

TON - Turnover Number

vic - Vicinal

XPS - X-Ray photoelectron spectroscopy

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I. General Introduction

1. Green Chemistry in Pharmaceutical Chemistry

1.1. Green Chemistry

Over the past two centuries, fundamental theories in chemistry have been soundly established providing the foundations that allow us to achieve various medical wonders that save millions of lives and improve people's health, solve problems like world hunger and produce materials essential to the present and future needs of mankind.

However, despite such enormous achievements, we are facing great challenges in future chemical synthesis. The present state-of-the-art processes for synthesizing chemical products are highly inefficient because of their lack of resource conservation, and draws environmental and health concerns related to chemical wastes. Taking into account these concerns, the field of Green Chemistry was specifically design, providing a framework to build sustainability for the chemical industry as a whole.

The original definition of Green Chemistry was "the invention, design and application of chemical products and processes to reduce or eliminate the use and generation of hazardous substances"; sustainability was added shortly after as another fundamental concept(Poliakoff, M. 2002). Therefore the major rule of Green Chemistry is the design of environmentally benign products and processes, which is embodied in its 12 principles provided by Paul Anastas and John Warner(Anastas, P.T. 1998) (Table 1).

Table 1- The Twelve Principles of Green Chemistry*	
Prevention	It's better to prevent waste than to treat or clean up waste afterwards
Atom Economy	Design synthetic methods to maximize the incorporation of all materials used in the process into the final product
Less Hazardous Chemical Syntheses	Design synthetic methods to use and generate substances that minimize toxicity to human health and the environment
Designing Safer Chemicals	Design chemical products to affect their desired function while minimizing their toxicity
Safer Solvents and Auxiliaries	Minimize the use of auxiliary substances wherever possible make them innocuous when used
Design for Energy Efficiency	Minimize the energy requirements of chemical processes and conduct synthetic methods at ambient temperature and pressure if possible
Use of Renewable Feedstocks	Use renewable raw material or feedstock rather whenever practicable
Reduce Derivatives	Minimize or avoid unnecessary derivatization if possible, which requires additional reagents and generate waste
Catalysis	Catalytic reagents are superior to stoichiometric reagents
Design for Degradation	Design chemical products so they break down into innocuous products that do not persist in the environment
Real-time Analysis for Pollution Prevention	Develop analytical methodologies needed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances
Inherently Safer Chemistry for Accident Prevention	Choose substances and the form of a substance used in a chemical process to minimize the potential for chemical accidents, including releases, explosions, and fires

***Adapted from the literature(Anastas, P.T. 1998).**

1.2. Green Chemistry in Chemical Process

The lifecycle of chemical products generates waste and consumes resources which become extremely expensive to the industry. These cost problems, combined with the social pressure due to the poor image the chemical manufacturing has with the public and new environmental legislation are generating a need for cleaner and greener methods of chemical production.

Clark pointed out that these "*three cornerstones of sustainable development- economic, environmental and social benefit- provide the drivers of change that should help to push the application of green chemistry forward"* (Clark, J. H. 2006).

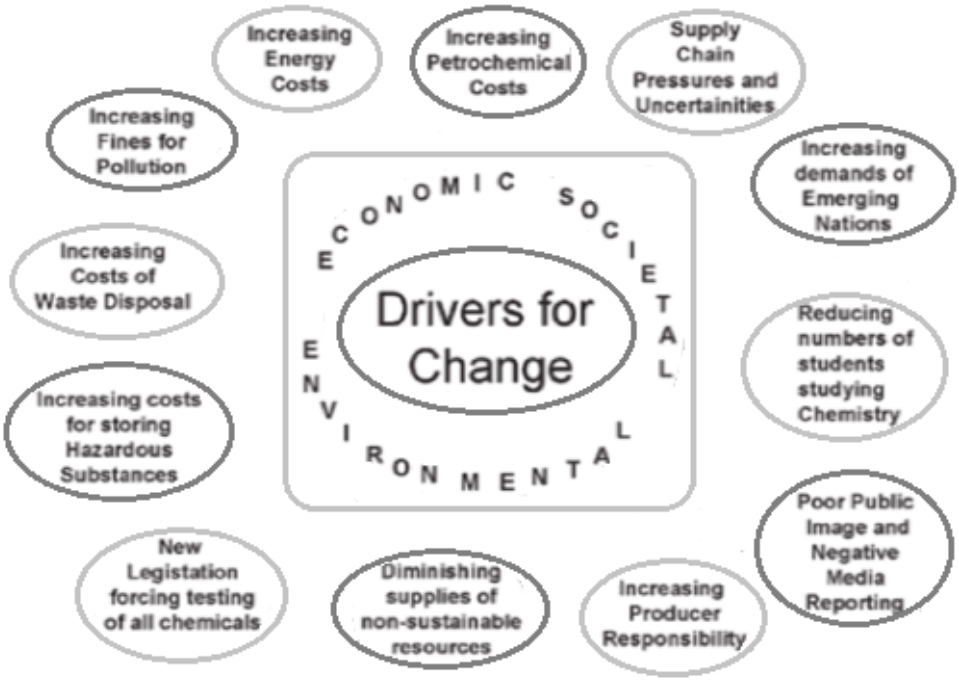


Figure 1- Drivers for change. Adapted from the literature(Clark, J. H. 2006).

To address these challenges, innovative and fundamentally novel chemistry is needed throughout the synthetic processes: feedstock, reactions, solvents and separations.

The main feedstock of chemical products come from nonrenewable petroleum that is being depleted rapidly both for chemical and energy needs(Li, C. J. 2008) and is getting more and more expensive each day(Clark, J. H. 2006). However, nature provides a vast amount of biomass in the renewable forms of carbohydrates, amino acids and triglycerides which are a sustainable alternative to obtain organic products(Argyropoulos, D. S. 2007).

The ideology of Green Chemistry is the development of new chemical reactivities and reaction conditions that can potentially provide benefits for chemical synthesis in terms of resource and energy efficiency, product selectivity, operational simplicity and health and environmental safety. For assessing the potential environmental acceptability of a chemical process different metrics have been developed, such as the Atom Economy(Trost, B. M. 1991; Trost, B. M. 1995) and the E factor(Sheldon, R. A. 1994).

Atom Economy is used to compare amounts of waste of alternative processes and is calculated by dividing the molecular weight of the product by the total sum of molecular

weights of all substances produced in the stoichiometric equation of the reaction(s) involved. The E factor (E for environmental) provides the quantity of waste that is produced for a given mass of product and is defined as the Kg of waste per Kg of product obtained.

To address these demands innovative reactions have been developed, such as isomerizations(Trost, B. M. 2002; Trost, B. M. 2006), addition reactions(Bower, J. F. 2008), direct conversion of C-H bonds(Chen, H. 2000; Chatani, N. 2001; Crabtree, R. H. 2001; Jia, C. 2004), synthesis without protections(Baran, P. S. 2007), catalysis(Reetz, M. T. 2000; Dalko, P. I. 2004) and innovative technologies, including photochemistry(Fagnoni, M. 2007), microwave irradiation(Nuchter, M. 2004; Strauss, C. R 2006).

Solvents play an important role in chemical production and synthesis because they facilitate mass transfer to modulate chemical reactions in terms of reaction rate, yield, conversions and selectivity. The ironic aspect of this process is that, after the reaction, the final product has to be separated from the solvent through energy-intensive means, that is why the largest amount of "auxiliary waste" in most chemical productions is associated with solvent usage(Sheldon, R.A. 2005).

The perfect green solvent should be natural, non-toxic, cheap, available and easy to separate. Water is the solvent that combines all these qualities, but in some chemical processes water is undesirable. For this reason, green solvents with different properties are needed(Webb, P. B. 2005; Tavener, S. J. 2003; Sheldon R. 2001; Bergbreiter, D. E 1998) and the use of solventless reactions(Cave, G. W. V 2001).

The achievement of greener chemical processes is a difficult task that can provide a world of opportunities for new studies and research. The challenges ahead for Green Chemistry are greater than ever!

1.3. Green Chemistry in Pharmaceutical Chemical Process

Advances in medical and pharmaceutical sciences improved the quality of human life and allowed to live longer and healthier.

Although our life is better, there are still a lot of diseases which are incurable and fatal or that can only be treated symptomatically like viral infections, cancer, autoimmune diseases or CNS disorders.

Therefore, academic researchers in Pharmaceutical sciences, particularly medicinal chemistry, and the pharmaceutical industry invest a lot of time and knowledge in the discovery of new

and better drugs that are more selective and active, with fewer side-effects and with less harmful contamination of the environment(Kourounakis, P.N. 1994).

According to the IUPAC definition(Wermuth, G.G. 1998) *medicinal chemistry is a chemistry-based discipline, also involving aspects of biological, medical and pharmaceutical sciences. It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the study of their metabolism, the interpretation of their mode of action at the molecular level and the construction of structure-activity relationships.*

Reduce costs and accelerate the discovery process are two main goals of drug discovery and the use of new computational methods(Parenti, M. D. 2012) and better identifying processes like parallel combinatorial chemical synthesis(Dexter, J. P. 2009), high-throughput screening(Aldib, I. 2012), virtual screening(Elsayed, M.S. 2012) and fragment-based drug discovery(Lee, K. 2013) could be very helpful in accomplishing these requirements.

Therefore, the synergy between computational techniques, biotechnology and medicinal chemistry will allow to find new reactions and optimized chemical processes, creating more efficient drug synthesis and assisting a faster delivery of new drugs on the market(Parenti, M. D. 2012).

The design and development of synthetic routes with the ultimate goal of manufacturing fine chemicals and, in particular pharmaceuticals, at a commercial scale are the foundation of process chemistry. To ensure that the best synthetic route is pursued, Process Research and Development (PR&D) requirements were created to each stage of drug development(Federsel H-J 2009).

For PR&D there are a number of tough criteria to be met like the development of safe and environmentally friendly procedures that offer cost competitiveness and allow operation on large scale in each step of the synthesis of complex molecules avoiding patent infringements and showing sustainability for long-term production(Federsel H-J 2010).

*Table 2- Process R&D requirements during drug development**

	Discovery	Early Development	Full Development	Launch
Amount of compound/batch	10mg - 10g	10g – 10Kg	10Kg – 100Kg	100Kg and more
Type of synthesis	Expedient	Practical	Efficient	Optimal
Site for preparation	Laboratory	Kilo Laboratory	Pilot plant	Plant
Number of batches	1 - 5	1 - 10	10 - 100	10 - 100

*Adapted from the literature(Pinto, R. M.. 2009).

As per in Table 2, expedient routes are used to prepare compounds in the early stages of drug development. It all starts with initial in vitro screenings in the laboratory with small quantities (10mg) where the best compound is chosen based on a series of criteria (toxicity, pharmacological properties, receptor affinity and physical properties). At this time, larger quantities of material (10g-10Kg) are required to define and develop the best synthetic route. The most cost-effective and efficient route is used to scale-up investigations and manufacture of bulk amounts (10Kg-100Kg). Lastly, the optimized process, validated and documented, is used in commercial production(Federsel H-J 2010).

In this context, and considering the importance of designing new biologically active molecules, a higher involvement and integration of chemical process R&D with drug discovery(Federsel H-J 2008) is necessary to allow the achievement of better final products, at a lower cost, supplied in a faster way and using more environmentally friendly synthetic approaches.

1.3.1. Pharmaceutical Industry: A Green Approach

Over the past century, the development of new pharmaceutical products has contributed to a revolution in medical care. Unfortunately this achievement has adversely impacted the environment.

Significant amounts of waste by-products and pollutants (contaminated solvents, depleted reagents and air pollutants) are generated by the manufacture of chemicals, and pharmaceuticals contribute greatly to this process. Table 3 reveals that the manufacture of drugs generates more waste and by-products when compared with other chemical industry sectors(Berkeley, W. 2009).

This must be taken in context, as the medical and regulatory requirements of pharmaceutical purity (high levels of chemo-, regio- and stereo-selectivity) will lead to more waste per Kilogram of product, as compared with the production of less sophisticated compounds, for which the purity requirements are less stringent.

The high E factor is also explained by the use of organic solvents, stoichiometric reagents, metal-based catalysts and purification and isolation procedures that use aqueous work-ups and chromatographic separations(Tucker J. L. 2006).

This issue is further elaborated by a report from GlaxoSmithKline (GSK)(Jimenez-Gonzalez, C. 2004; Curzons, A. D. 2007; Henderson, R. K. 2011) that estimates that 80% of their waste is solvent related and that addressing the selection, use, recovery and disposal of solvents will contribute dramatically to alleviating this problem.

*Table 3- Comparison of chemical industry sectors by quantity of byproduct per Kilogram of product**

	Product tonnage	E factor (Kg byproducts/Kg product)
Oil Refining	$10^6 – 10^8$	≅ 0.1
Bulk Chemicals	$10^4 – 10^6$	< 1 - 5
Fine Chemicals	$10^2 – 10^4$	5 - >50
Pharmaceuticals	$10 – 10^3$	25 - >100

*Adapted from the literature(Sheldon, R. A. 2000; Sheldon, R. A. 2008).

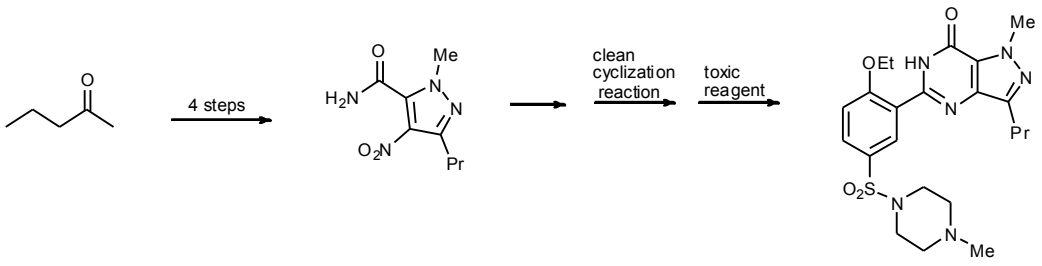
With the increasing emphasis on Green Chemistry, pharmaceutical process chemists have concentrated their focus and creative energies on minimizing the environmental impact of their craft. It is important to refer that due to the particular specificities of the pharmaceutical chemistry not all of the twelve principles can be applied. Therefore, Green Chemistry acts more as a guide for the pharmaceutical industry(Tucker J. L. 2006).

Tucker stated that Pharmaceutical Green Chemistry is a *"quest for benign synthetic processes that reduce the environmental burden within the context of enabling the delivery of our current standard of living"*.

So the incorporation of a green perspective in the Pharmaceutical Chemistry includes good corporate citizenship, reduced expense, lowered regulatory risk and a smaller environmental footprint(Fortunak, J. M. 2009), allowing drugs to be available rapidly and easily in the market, at a lower cost, with high standards of quality.

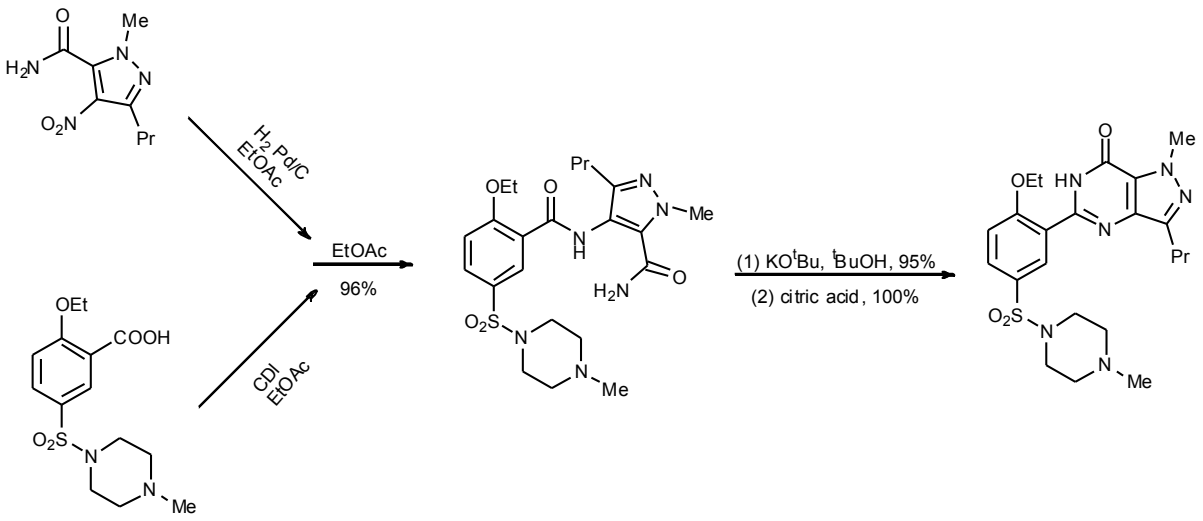
In the past few years there have been some success stories regarding the use of Green Chemistry principles to guide process design in the pharmaceutical industry(Berkeley, W. 2009).

One example is the synthesis of sildenafil citrate(Dale, D. J. 2000; Dale, D. J. 2002; Dunn, P. J. 2004; Dunn, P. J. 2008), also known as Viagra^R, where the first viable route was a linear eleven steps synthesis, which gave a 4.2% overall yield from 2-pentanone (scheme 1).



Scheme 1. First preparative route to sildenafil citrate(Berkeley, W. 2009)•

This synthesis was unsuitable for large scale manufacturing due to low yield and the use of noxious compounds. Pfizer optimized the process by minimizing solvent use, increasing solvent recovery, improving solvent selection, telescoping steps and using a convergent strategy in the synthesis (scheme 2).



Scheme 2. Convergent commercial preparation of sildenafil citrate(Berkeley, W. 2009)•

The new strategy lowered the ratio of solvent waste/Kg of product from 1300L/Kg to 7L/Kg and increased the overall yield significantly (average yield of the last three steps = 97%).

As the industry continues to come under pressure to hold down the cost of drugs, the adoption of an environmentally benign approach to drug candidate synthesis will contribute by reducing the increasing costs of reagent procurement and waste disposal.

2. *Catalysis in the Pharmaceutical Chemistry*

Catalysis plays a vital role in the welfare of humanity and is an important technology that supports the global economy. Many industries, including chemicals, petroleum, agriculture, polymer, electronics and pharmaceutical industries rely heavily on catalysis.

Over 90% of the chemicals are derived, in some way, from catalytic processes. The worldwide demand for catalysts in 2007 was approximately 850,000 tons and is estimated to have increased 3.5-4% per year until 2012, as the market value of the products generated by catalysis reached about 900 billion dollars per year(Armor, J. N 2011).

The use of catalysis provides a multitude of benefits to industries that include reducing costs, saving time and producing less waste. The increasing focus on the development of environmentally friendly manufacturing processes has led to the green chemistry movement and its 12 principles, which identify catalysis as the best technology(Burczyk, B. 2005).

The catalytic transformations avoid the waste of raw materials and increase the yield of the final product in less time and using less energy, as they allow the design of smarter synthesis, with shorter routes to the final products.

Therefore, scientific advances in catalytic processes have proven to be essential for solving many important problems concerning the chemopharmaceutical industry and society in general.

2.1. Definition of Catalysis

One of the valid definitions that exist today is due to Ostwald (1895) who recognized catalysis as a ubiquitous phenomenon explained by the laws of physics and chemistry, and stated that "*a catalyst accelerates a chemical reaction without affecting the position of balance.*"

Catalysts are, therefore, substances capable of directing and accelerating thermodynamically possible reactions (although without altering the thermodynamic equilibrium), remaining unchanged at the end of the reaction(Jens Hagen 2006). Therefore, the effect of the catalyst is purely

kinetic, accelerating the reaction to provide new reaction pathways with lowers activation energies, without affecting the free Gibbs energy of the total reaction (ΔG^0)(Figueiredo, J. L 1989).

Although it was assumed that the catalyst remained unchanged during the course of catalysis, it is known today that it binds to the reagent. Catalysis is a cyclic process in which the catalyst acts by combining with the reagents to generate intermediate compounds, thus facilitating the transformation into products. The intermediate catalyst is, in most cases, very reactive and therefore difficult to detect. After the formation of the final product the catalyst regenerates and is able to restart the catalytic cycle (Figure 7)(Jens Hagen 2006).

Theoretically, an ideal catalyst would not be consumed during the reaction, but because of side reactions the catalyst undergoes chemical changes and its activity decreases. Therefore, it must be regenerated or replaced. However, the lifetime of the active catalyst is always greater than the duration of the reaction cycle(Figueiredo, J. L 1989).

In addition to speeding up reactions, catalysts have the ability to influence the selectivity of the chemical reaction, which means that different products can be obtained from a common starting material using different catalytic systems; this means that by modifying the structure of the catalyst we can drive the reaction to a desired product(Jens Hagen 2006).

Reactions with industrial interest must be fast and clean, which is often achieved at the expense of a catalyst. Therefore, the use of catalysts can be regarded as a variable (in addition to temperature, pressure, composition and contact time) that allows controlling the speed and direction of a reaction(Figueiredo, J. L 1989).

The use of catalytic processes in industry has several advantages. The first and most important is the fact that thermodynamically favorable reactions in which the chemical equilibrium is not established at an economically acceptable time become viable. Furthermore, by using catalysts we can carry out reactions with less energy (lower temperature and pressure), which implies a considerable power gain and allows minor requirements of the manufacturing complex.

Additionally, when reactions are performed under lower pressures and temperatures, side or secondary reactions are reduced and therefore fewer by-products are formed, thus increasing the selectivity for the desired products. Another equally important aspect of the industrial application of catalysis is the great atom economy of many catalytic processes(Clark, J. H. 2002).

2.1.1. Characterization of Catalytic Processes

The properties to be considered when assessing the catalysts are selectivity, activity, stability, regenerability and mechanical and thermal properties(Jens Hagen 2006).

Selectivity is the ability to direct the conversion of the reagent to a specific pathway and is defined as the number of moles of a product on the total amount of products and can be of different types, namely, chemoselectivity, regioselectivity and stereoselectivity(Bhadury, S. 2000).

The activity of a catalyst is measured in accordance with the effect this has on the speed of a given reaction and can be determined, in practice, by the relative velocity of the catalytic chemical reaction (in comparison with the speed of the non-catalyzed reaction) or by other parameters, such as the temperature required to effect the conversion in a given period of time and under certain conditions. One way to compare the activities of various catalysts for a given reaction is to determine the speed of the reaction under the same conditions of temperature and concentration(Figueiredo, J. L 1989).

The catalytic activity can also be expressed as the Turnover Number (TON) and the Turnover Frequency (TOF), where TON is the number of product molecules produced by each molecule of catalyst and TOF is TON per unit of time and quantifies the specific activity of the catalytic center for a given reaction under defined conditions through the number of catalytic cycles that occur in the center per unit time(Jens Hagen 2006).

Stability (chemical, thermal and mechanical) is also very important, as a catalyst loses activity and selectivity with prolonged use, which can lead to its decomposition and contamination; the definition of stability is related to regenerability, which is the measure of the ability of the catalyst to have its activity and/or selectivity restored by some processes of regeneration(Jens Hagen 2006).

2.2. Catalysis in Industrial Synthesis of Biologically Active Compounds

The importance of catalysis in the pharmaceutical industry has increased steadily over the past two decades because of several interrelated factors: (i) the increasing demands in legislation, such as the policies of drugs with a single enantiomer and environmental protection (green

chemistry), (ii) the pressure to reduce costs and the time of drug development, and (iii) the rapid discovery of new catalysts. The interaction of these factors resulted in the frequent use of catalysis in research, development and production of pharmaceuticals(Jens Hagen 2006).

The increasing complexity of the targeted chemicals and the average number of manipulations required to synthesize an active pharmaceutical ingredient (API) makes necessary the use of catalysts to achieve more compact and economic synthesis. It turns out that the development of a viable catalytic process for the industrial scale is a difficult task that requires the cooperation of multiple disciplines, including organic chemistry, analytical chemistry, biochemistry, process safety, chemical engineering, and spectroscopy. The aim of this multidisciplinary effort is to develop a robust process that operates at the lowest cost, with less waste and for the shortest time possible, and to this end both scientific knowledge and regulatory knowledge are needed.

The process of drug development is highly regulated and controlled, and it requires the implementation of good manufacturing practices (GMP) in all phases of clinical development. Extensive studies should be conducted on the mechanism of reaction, the catalytic cycle, the formation of byproducts, the origin of selectivity, the limits of the process (DoE-design of experiment) and monitoring the reaction in real time by PAT (Process Analytical Technology)(Jens Hagen 2006).

Based on these ideals, industrial chemistries involved in catalysis must work creatively to achieve the objectives of safety, efficiency, economy, sustainability, freedom to operate and regulatory compliance in order to develop a catalytic process that provides viable APIs on a large scale.

2.3. Homogeneous Catalysis VS Heterogeneous Catalysis

Catalysts may be categorized according to various criteria: structure, composition, application area or aggregation state in which they operate and, according to the last categorization, catalysts can be divided essentially into two major groups: homogeneous catalysts and

heterogeneous catalysts(Jens Hagen 2006). However, the rapid development of biotechnology and forms of immobilization of homogeneous catalysts on supports makes it difficult to classify them adequately.

When catalytic processes occur in a uniform gas or liquid phase they are classified as homogeneous catalysis. The homogeneous catalysts are normally well defined chemical compounds or coordinated compounds which, together with the reactants, are molecularly dispersed in the reaction medium(Rinaldo, P. 2009). Examples of such catalysts are mineral acids and compounds with transition metals.

Heterogeneous catalysis occurs in several phases and the catalyst is usually solid and the reagents are gaseous or liquid(Petrov, L. A. 2011). Examples of heterogeneous catalysts are chains of Pt/Rh (Ostwald process), supported catalysts and amorphous or crystalline aluminosilicates(Jens Hagen 2006).

Whereas in heterogeneous catalysis the boundaries between the phases of the catalyst and the reagents are always present, in homogeneous catalysis the catalyst, the starting materials and the products are always in the same phase. As a result, this type of catalyst has a higher degree of dispersion, and, theoretically, each individual atom can be catalytically active; in heterogeneous catalysis only the surface atoms are active(Rinaldo, P. 2009; Petrov, L. A. 2011).

Due to their high degree of dispersion, homogeneous catalysts exhibit a greater activity per unit of mass than heterogeneous catalysts. The high mobility of the molecules in the reaction mixture results in more collisions with the substrates, i.e., the reagents can reach the active site of the catalyst from any direction, and a reaction in an active center does not block neighbor centers. This allows the use of lower concentrations of catalyst and smoother reaction conditions(Jens Hagen 2006).

In homogeneous catalysis the reaction site is well established and therefore it is relatively easy to understand the reaction mechanism by spectroscopic methods. In contrast, the processes occurring in heterogeneous catalysis are often obscure(Rinaldo, P. 2009; Petrov, L. A. 2011).

Both homogeneous and heterogeneous catalysts play an important role in the chemical industry, but about 85% of the total catalytic processes are based on heterogeneous catalysis(Jens Hagen 2006). This is not only due to its sphere of action and increased thermal stability but also because in homogeneous catalysis it is very difficult to separate the catalyst

from the product, which makes it necessary to use complex processes such as distillation, liquid-liquid extraction or ion exchange. In heterogeneous catalysis, the catalyst can be automatically removed in the process or by simple methods such as filtration or centrifugation(Rinaldo, P. 2009; Petrov, L. A. 2011).

It is noted that the comparison between homogeneous and heterogeneous catalysts is usually based on their activity and selectivity, and the ability to recover the catalyst, although there are other important characteristics to consider in this differentiation (Table 4).

Currently, there is a marked interest in research to obtain an ideal catalyst that has the advantages of homogeneous catalysts (high activity and selectivity and good reproducibility) and of heterogeneous catalysts (long lifetime and ease of recovery)(Lücke, B. 2004). Unfortunately, heterogenized homogeneous catalysts still present some problems, such as leaching and the relatively low stability and high sensitivity to poisoning.

Table 4- Comparison between homogeneous and heterogeneous catalysts.[*]

	Homogeneous	**Heterogeneous**

Active centers	All atoms	Only the surface atoms
Catalyst concentration	Low	High
Selectivity	High	Variable
Diffusion Problems	Low	Important
Reaction Conditions	Smooth	Harsh
Applicability	Limited	Wide
Poison Sensibility	Low	High
Structure	Defined	Less defined
Action Mechanism	Easy to determine	Difficult to determine
Possibility of modifying	High	Low
Thermal Stability	Low	High
Life Time	Variable	Long
Product Separation	Difficult	Easy
Recovery	Expensive	Affordable

*Adapted from the literature(Rinaldo, P. 2009; Petrov, L. A. 2011).

2.4. Supported Heterogeneous Catalysts

One way to achieve the heterogenization of homogeneous catalysts is via the immobilization of an active compound that is bound to an insoluble solid (support), which is typically a mesoporous solid (average pore diameter between 2 and 10 nm)(Lücke, B. 2004). In addition to improving the performance of the catalyst in terms of activity and / or selectivity, the main purpose of heterogeneity is to facilitate the separation, recovery and reuse of the catalyst allowing it to be more easily handled and less toxic than the homogeneous catalyst(Clark, J. H. 2000).

This support may be organic, a polymer (cross-linked polystyrene), or inorganic (silica, alumina, montmorillonite, zeolites and other aminossilicates)(Clark, J. H. 2000).

The interaction between the reactants and the surface of the catalyst can be achieved by binding non-covalently (ionic bonding, hydrogen bonding forces, van-der-Waals forces) or by covalent chemical bonding(Figueiredo, J. L 1989). Catalysts with the non-covalent bond are usually easier to prepare but, due to the fact that these bonds are generally weak interactions, they

have the great disadvantage of being partly destroyed by the leaching or during the separation and isolation of the products(Clark, J. H. 2000).

Much attention has been directed to the development of complex heterogenized compounds in which the active sites are chemically bound to the support. The immediate advantages of greater stability and less tendency to leach, which greatly facilitate the reuse of the material, are counter-balanced by the increased complexity of the synthetic material, the adverse effects provoked by the proximity between the support and the reactive species, and the fact that the compound chemically immobilized on a material support cannot be considered an exact equivalent of the "free" analog (typically in solution).

The use of spacer groups between the support and the functional group now permits a greater similarity between the immobilized and the free species. As a result, some of the main effects of the support become less significant and more of the reaction site is protruded into the solution phase. If the aim is for the immobilized species to behave similarly to the analogous free species, it is also important to maintain the structural integrity around the active centers(Clark, J. H. 2000).

The heterogeneous catalysts on solid supports can be prepared via various methods; the most common are impregnation, precipitation, ion-exchange, the sol-gel method and the multi-synthetic routes(Geus, J. W. 2001; Clark, J. H. 1998; Clark, J. H. 2000; Price, P. M. 2000; Campanati, M. 2003; Hoffmann, F. 2006).

3. Porous Materials

3.1. General Aspects

Previously, we referred that the supports for heterogeneous catalysts are usually mesoporous materials. This raises the questions: what are porous materials? Why mesoporous materials? In this section these matters will be elucidated.

By definition, a porous material is a solid matrix composed of an interconnected network of pores (voids) filled with a fluid (liquid or gas). Porous and highly dispersed materials represent a specific solid state and have numerous applications in research and industry and

their physical and chemical properties can be significantly changed by small alterations of the specific surface area and volume ratio.

According to IUPAC, there are three classes of porous materials: microporous, mesoporous and macroporous(Sing, K. S. 1985), each category being related to a specific pore size regime (Table 5).

Each pore size range corresponds to a characteristic nitrogen adsorption/desorption mechanism: in microporous materials, three-dimensional condensation of the adsorbate occurs inside a strong electromagnetic field induced by the narrow pore dimensions. Interphasic adsorbate–adsorbent interactions do not exist and the system properties are close to a single-phase. In mesoporous materials, adsorption proceeds via the consecutive formation of adsorbate layers which is completed by the phenomenon of capillary condensation. Due to their dimensions, macropores materials have porous properties similar in character to conventional flat surfaces and cannot be filled by capillary condensation(Greg, S. J. 1982).

*Table 5- IUPAC classification of pore size and adsorption mechanism.**

Pore Type	Size Regime	Condensation Mechanism
Micropore	< 2nm	Three-dimensional
Mesopore	≥ 2 ≤ 50nm	Capillary
Macropore	> 50nm	No condensation

*Adapted from literature(Sing, K. S. 1985).

The diversity of adsorption properties is responsible for the different applications of porous materials. Due to strong Van-der-Waals interactions, micropores are ideally suited to liquid and gas-phase adsorption. On the other hand, mesopores are more suitable for liquid phase applications such as heterogeneous catalysis or chromatographic separation because of their low potential energy escape surfaces and their pore sizes, which allow a high loading of

accessible active sites and, importantly, provide efficient diffusion/mass transfer of liquid phase analyte or substrate(Rodríguez-Reinoso, F. 1998). The presence of macropores significantly aids system filtering properties and enhances the flow/mass transfer/diffusion properties of the material. It is important to note that in ‘‘real’’ materials, pore networks are usually composed of all three types of pore sizes (Figure 2)(Rodríguez-Reinoso, F. 1998).

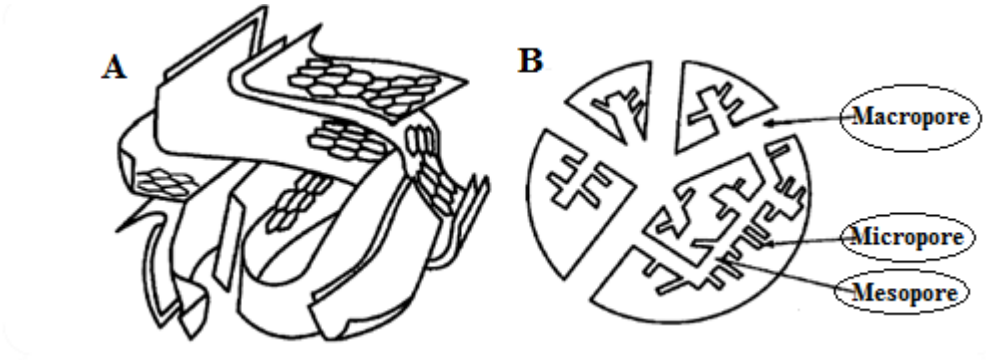


Figure 2. Schematic representation (a) three-dimensional and (b) two dimensional structure of an activated carbon. Adapted from literature(Rodriguez-Reinoso,, F. 1998).

Well-established technologies benefit from the coexistence of micro and mesopores since solid porous materials with an inhomogeneous pore distribution are usually inexpensive and easily prepared(Zhang, F. 2008). The search for more efficient porous materials to be used in tomorrow’s nanotechnologies is increasing, with the main goals of improved selectivity, efficiency, tuneability and ultimately economics and sustainability. To reach this “greener” sustainable nanotechnology, it is necessary to have the ability to control and manipulate the properties of material porous networks. Highly microporous (size selectivity) and mesoporous materials (surface functionality dependent selectivity) are two classes which are currently the focus of extensive research attention(Zhang, F. 2008).

3.2. Microporous Materials

Molecular Sieves are a major subclass of microporous materials, exemplified by the aluminosilicate (zeolite) family, which are structured regular arrays of uniformly-sized microporous channels, with the largest pore diameters so far synthesized in the 0.8–1.3 nm range(Beck, J. S. 1992 JACS).

Due to the narrow pore dimensions, these materials are used for shape/size selective catalytic transformations, and one of the classical applications of these materials is heterogeneous catalysis. On the other hand, the size selectivity advantage is one of the limitations of zeolites in catalytic processes involving molecules larger than the material pore dimensions (e.g. drug intermediates).

3.3. Mesoporous Materials

As per before, the demands for environmentally-friendly technologies, processes and products have led to an increase in research efforts regarding pore size manipulation, structuring and surface modification. Mesoporous materials arguably form the foundation for the generation of the future nanotechnologies needed in a sustainable society.

Beck *et al.* were responsible for the breakthrough in mesoporous materials' research, regarding the preparation of ordered mesoporous aluminosilicate molecular sieves (M41S or Mobil Composition of Matter (MCM) materials), in which they exploited, for the first time, the use of ''liquid crystal templating (LCT)'' to direct pore size into the mesoporous range, demonstrating the preparation of materials with ordered pore structures and pore sizes ranging from 2 to 10 nm (Figure 3)(Beck, J. S. 1992 Nat).

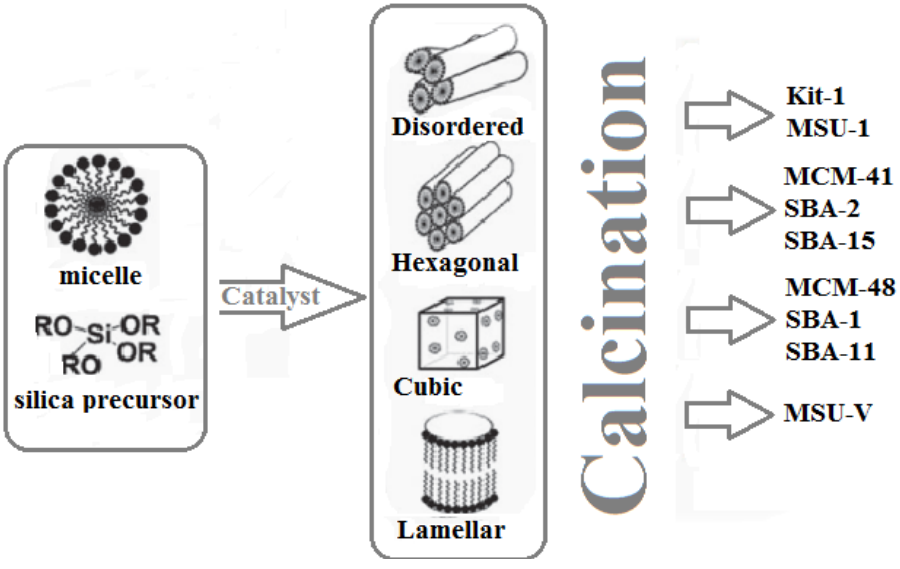


Figure 3. Mesoporous silicates produced by a templating method. Adapted from literature(Beck, J. S. 1992)

The LCT mechanism exploits micelle formation and solvent polarity to create inorganic walls between the surfactant liquid crystal structure and the growing inorganic species. The geometry of the materials may be controlled by the symmetry of the supramolecular assembly of surfactant molecules in the solvent–silicate solution.

This discovery has created significant attention around mesoporous materials, especially in areas where diffusion of the species within the pore network is essential (catalysis), as well as in separation and adsorption of large molecules such as proteins and enzymes (Beck, J. S. 1992 Nat).

Mesoporous materials easily functionalized (Mesoporous aluminosilicates, transition metal oxides (e.g., Ti, V, or Mn)(Taguchi, A. 2005), polymers(Johnson, S. 1999) and carbonaceous materials(Lu, A. 2005)), to give specific physical and chemical properties, have been of great interest and vastly explored in the past decade. Although these approaches allow extensive manipulation of textural properties, these inorganic materials are limited in terms of the surface chemistry available (e.g., for post functionalization), and in terms of applications where electron rich surfaces are desirable (e.g., graphitic carbon-chromatography).

4. Carbon-based Materials

4.1. General Introduction

Approximately 1 million tons/year of activated carbons (AC) are used in a wide range of applications (water treatment, gas purification, decolourisation and absorbency) and are typically derived from low cost renewable materials (e.g. coconut, wood and fruit stones). Classical AC preparation generates microporous materials with average pore diameters of less than 2 nm(White, R. J. 2009).

Although these materials have different applications, such as catalysis, electrochemistry, fuel cells, biomedical devices, hydrogen storage and automotive components, they present some limitations at an industrial level, partially due to the requirement for tuneable mesoporous carbon materials. Routes to such materials are resource and process intensive, not easily accessible and expensive from an industrial point of view.

Therefore, new synthetic approaches that are green, inexpensive, non-resource and process intensive are still required, allowing the production of tuneable materials with mainly mesoporous characteristics with chemically active surfaces, which may be produced in a facile manner(White, R. J. 2009; Knox, J. H. 1997).

4.2. Mesoporous Carbonaceous Materials

There are four major synthetic routes to porous carbon materials: (i) Hard Templating (use of porous inorganic templates); (ii) Soft Templating (direct carbonization of polymer blends consisting of carbonizable and pyrolyzable polymers); (iii) Polymer Aerogel Precursor Carbonization; (iv) Traditional Chemical and Physical Activation of Carbon.

One of the best examples of Hard Templating on an industrial scale was developed by Knox and Ross in 1979(Knox, J. H. 1997), where a highly porous HPLC silica gel is impregnated with a phenol-formaldehyde mixture to give a phenol-formaldehyde resin/silica hybrid.

This hybrid compound is then carbonized at temperatures > 1000ºC (under N_2 or Ar) and the siliceous component is removed using strong alkali solution. The product of this pyrolysis forms a glassy carbon, originating the name “porous glassy carbon (PGC)”.

PGC materials possess excellent textural properties, high mesoporosity, large pore volume ($\leq 0.85 cm^3 g^{-1}$) and low microporosity, which make them ideal stationary phase media for

chromatography. However, they present a disordered pore structure with limited scope for manipulation of pore network or post chemical functionalization(Knox, J. H. 1997).

This methodology inspired the use of different inorganic hard templates to generate novel ordered mesoporous carbon materials, typically at the expense of particle morphology and low micropore content(Kruk, M. 2000; Bazula, A. 2008). This approach includes the following steps: (i) Preparation of a silica gel with controlled pore structure (e.g., MCM-48). (ii) Impregnation/infiltration of template with monomer or polymer precursor. (iii) Cross-linking and carbonization of the organic precursors. (iv) Template removal via acidic or caustic dissolution of the inorganic matrix.

The interest in mesoporous carbons due to their chemical inertness, stability and inherent advantages over classical microporous AC originated many different synthetic approaches, with the primary goal of providing mesopore size extension.

Despite all their advantages, ordered mesoporous carbons do not have macropore character (i.e., pore diameters > 50 nm) and those features are advantageous because they enable the rapid transport of gases and liquids to the active sites within the smaller pore size range. A secondary macropore template is, therefore, necessary. Baumann and Satcher used polystyrene (latex) spheres to introduce 100 nm macropore character into a 6 nm pore diameter mesoporous carbons structure(Baumann, F. T. 2003).

In later works, soft templating techniques have been used (typically employed in the generation of mesoscopically ordered inorganic solids) to produce mesoporous carbons. A dilute aqueous route for the direct synthesis of mesoporous polymer (FDU-14) and carbon (C-FDU-14) materials via the self assembly of p123 triblock copolymer templates, using resols as carbon precursor has been reported by Zhang et al.(Zhang, F. 2005). Unfortunately, this technique suffers from the same limitations as hard template routes, materials present small mesopore size with diameter inferior to 10nm and developed microporosity.

Studies using soft templating of mesoporous carbons with renewable biomass precursors (e.g., sugars) are recent. Therefore, the preparation of useful materials using a simple efficient methodology that employs renewable sustainable carbon precursors would mark major progress in this field. In this Thesis a new type of mesoporous carbonaceous material called Starbon® will be reported.

5. Steroid Chemistry

5.1. Steroids in Organic and Medicinal Chemistry

Steroidal compounds are widely distributed in nature and have been extensively investigated in recent decades. They exist in different living beings, both animals and plants, and play an important role in their vital activity as physiological regulators(Noguchi, K. K. 2011), hormones(Mendre, K. A. 2011), pro-vitamins(Lal, A. 2010), among others.

In the pharmaceutical industry these compounds are used as functionalized substrates in the synthesis of many biologically active molecules, either via chemical routes or via microbiological routes.

The early research on steroids was somewhat complicated, because these compounds were isolated mainly from animal sources, which made it difficult to obtain large amounts(Goswami, A. 2003) of product. This was until Marker and his collaborators (in the 1940s) described the preparation of 16-dehydropregnenolone acetate via controlled degradation of the side chain of diosgenin, which was isolated from the Mexican wild yam root, *Dioscorea macrostachy* (plant of the *Dioscoriáceas*' family).

This process is called "Marker's degradation", and its discovery led to intense research into the chemistry of steroids in the following years, as 16-dehydropregnenolone acetate can be chemically modified to originate a large variety of steroids, such as progesterone(Goswami, A. 2003).

From that moment until now, hundreds of steroid compounds have been isolated from natural sources and several thousands have been obtained synthetically. There is ongoing intense research to isolate and identify new steroid compounds with new natural biological activities(Ivanchina, N. V. 2006).

Many synthetic steroids are presently used in the treatment of various diseases, such as hormone-dependent cancers(Hoffmann, J. 2005) (e.g., breast cancer and prostate cancer). Others are oral contraceptives(Paulos, P. 2010), anti-inflammatory corticosteroids(Liguori, A. 2006), anabolic

steroids(Sheri, A. H. 2010), neurosteroids(Chisari, M. 2010) and bile acids(Pathil, A. 2011). In addition, estrogens, progestagens are used as therapeutic drugs for various hormonal imbalances(Paulos, P. 2010).

Steroids have multiple chiral centers that allow, via relatively simple chemical reactions, to obtain isomerically enriched products, and, due to their complexity, stereoselective reactions are fundamental. Because these compounds have several sites that are susceptible to oxidative attack and other types of reactions, the study of regio and chemoselective transformations assumes great importance(Felix A. Carroll).

In summary, the pharmacobiological properties of steroids and the high cost of their synthesis justify the importance given to the development of new regio-, stereo- and chemoselective manufacturing processes with high yields, as well as the studies that have been carried out over the years.

In this context, steroid molecules have been chosen as substrates for the development of new chemical processes using Starbon^{R}400-SO_3H as a catalyst, which is the subject of this thesis. Therefore, a brief overview of their structure and basic nomenclature will be presented in the next pages.

5.2. Structure and Nomenclature of Steroids

A steroid molecule is an amphipathic organic compound (containing a hydrophobic and a hydrophilic region) that has a specific arrangement of four cycloalkanes rings linked together. Steroids have a chemical structure containing the core of gonane (Figure 4), or a skeleton derived from it, which is composed of three cyclohexane rings (A, B and C), the phenanthrene skeleton, and a cyclopentane ring (D). The entire structure is called cyclopentanoperhydrophenanthrene(Moss, G. P. 1989).

Steroids differ in the additional functional groups (alcohol, aldehyde, ketone, carboxylic acid) attached to the four rings of the core, in the configuration of the side chain, in the number of methyl groups and in the oxidation state of the rings. The four steroid rings are labeled and their carbon atoms are numbered according to the IUPAC-IUB Joint Commission on Biochemical Nomenclature(Moss, G. P. 1989).

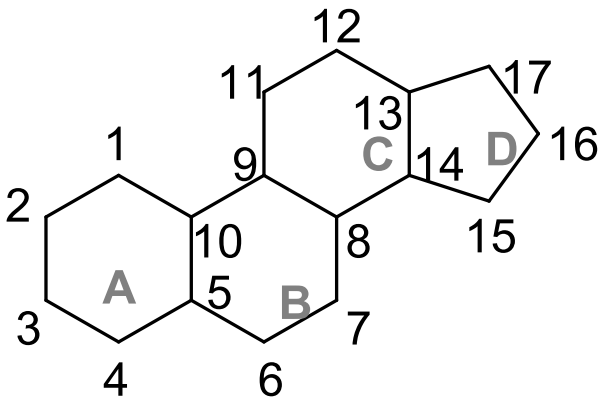


Figure 4. Cyclopentanoperhydrophenanthrene: the simplest steroid.

Steroids exist predominantly in eukaryotic cells, with cholesterol being the most abundant. It contains 27 carbons, one hydroxyl group at carbon C-3, methyl groups at carbons C-13 and C-10, designated as 18-CH_3 and 19-CH_3, respectively, and one branch of aliphatic hydrocarbons (8C) at carbon C-17 (Figure 5).

The methyl groups (18-CH_3 and 19-CH_3) are above the plane of the steroid skeleton and, by convention, have a β-configuration. All the other atoms located above the plane have β-configuration and the ones located below the plane have α-configuration(Brueggemeier, R. W. 2003; Hill, R. A. 1991).

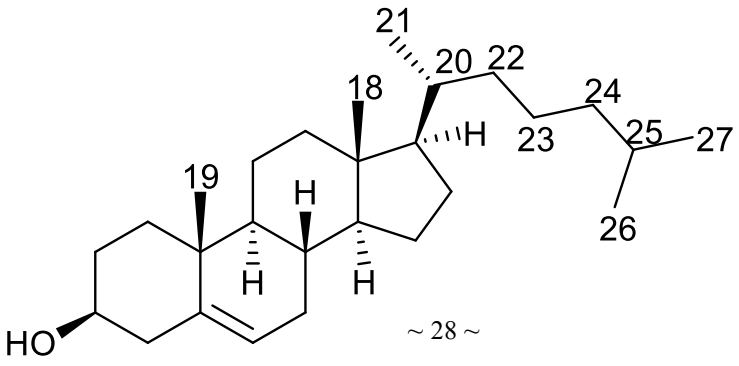


Figure 5. Structure of cholesterol. The angular methyl groups, the side-chain and the configuration of the hydrogen atoms at ring junctions are shown.

The three-dimensional structure of steroids is not planar; the cyclohexane rings exist in the preferred chair conformation to minimize strain. Consequently, its substituents can be located in the axial or equatorial positions. The cyclopentane ring exists in a half-chair or open-envelope conformation. The presence of substituents in the steroid core may force distortions or alternative conformations(Brueggemeier, R. W. 2003; Hill, R. A. 1991).

In most cases, naturally occurring steroids have B/C- and C/D-diequatorial *trans*-fused rings, with configurations at bridgehead positions C8, C9, C13 and C14, as shown in figure 4 for cholesterol: 8β-H, 9α-H, 13β-CH_3 and 14α-H(Brueggemeier, R. W. 2003; Hill, R. A. 1991).

The configuration at C5 is slightly different because it varies from steroid to steroid. Therefore, two possible ring fusions are observed between rings A and B. When the substituent at C5 possesses 5α-configuration, the steroid has A/B diequatorial (5α, 10β)- *trans* fused rings and is designated as 5α-steroid. In contrast, the C5 substituent of 5β-steroids is β-oriented and are characterized by A/B axial-equatorial (5β, 10β)- *cis* fused rings (Figure 5)(Brueggemeier, R. W. 2003; Hill, R. A. 1991).

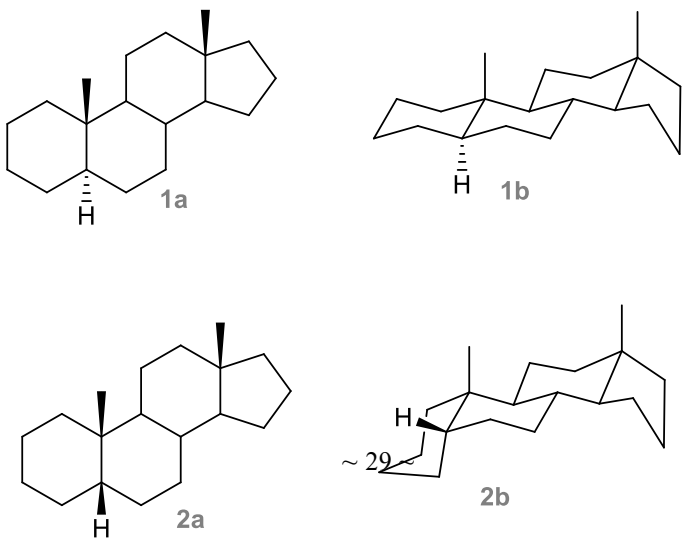


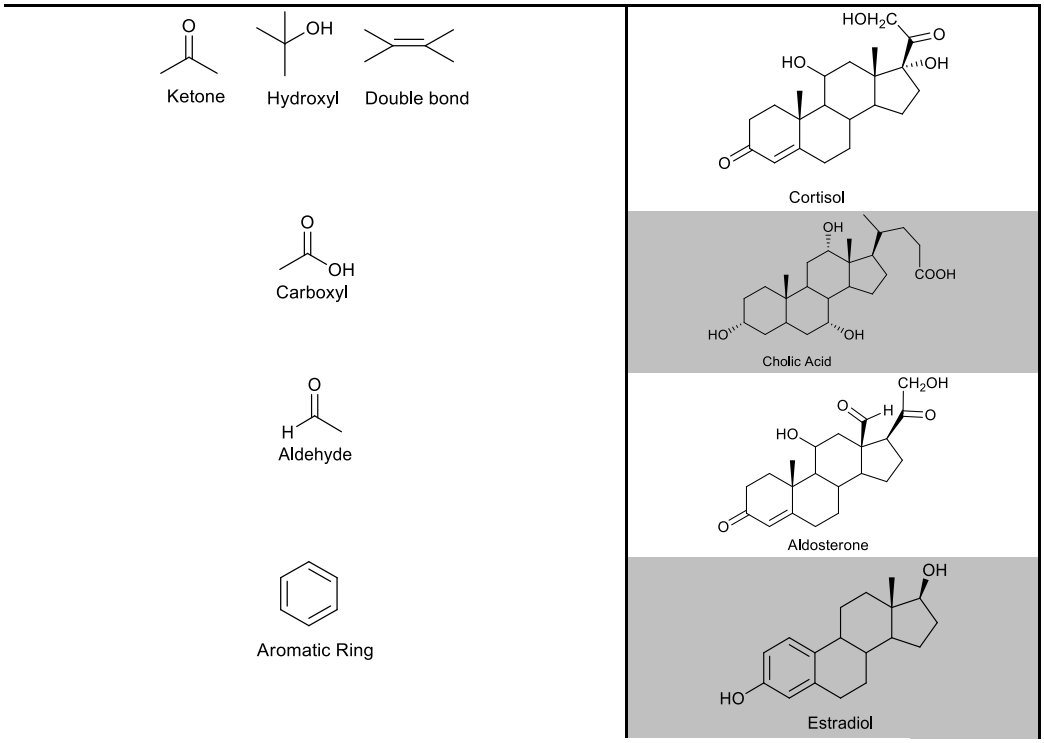
Figure 6. Representation of 5α-steroid (1a and 1b) and 5β-steroid (2a and 2b).

The systematic names of steroids have been developed by the IUPAC-IUB Joint Commission on Biochemical Nomenclature and are based on the hydrocarbon skeleton(Moss, G. P. 1989): 5α- or 5β- gonanes with 17 carbons and without methyl groups, 5α- or 5β- estranes with 18 carbons and one methyl group at carbon 13 (estrogen), 5α- or 5β- androstanes with 19 carbons and methyl groups at carbons 10 and 13 (androgens). When steroids have methyl groups at carbons 10 and 13 and a side chain at carbon 17 they can be 5α- or 5β- pregnanes with 21 carbons (progesterone and corticosteroids), 5α- or 5β- cholanes with 24 carbons (bile acids), 5α- or 5β- cholestanes with 27 carbons (cholesterol and other sterols), among others(Moss, G. P. 1989).

All steroids are derivatives of these parent hydrocarbons and their different characteristics are due to different functional groups present in the molecule. These may be ketone and hydroxyl groups or double bonds, as observed in the structure of cortisol, carboxyl or aldehyde groups like the ones present in bile acids and aldosterone, respectively, or aromatic rings like in estradiol and other steroids with 18 carbons (Table 6). Their nomenclature follows the general recommendations for the nomenclature of organic compounds(Moss, G. P. 1989).

Table 6- Different functional groups present in steroid molecules*

	Steroid



*Adapted from literature(Moss, G. P. 1989).

6. ***General Objectives***

The emphasis of science and technology is shifting towards environment-friendly and sustainable resources and processes. The recognition of the importance of the principles of Green Chemistry has identified catalysis as the key technology for the design and synthesis of new chemical entities.

The discovery of functional solid materials of high catalytic performance is crucial to most chemical processes as they allow the replacement of polluting homogeneous catalysts by reusable heterogeneous catalysts.

Starbon®400-SO_3H is a sulfonated mesoporous graphitizable carbon synthesized from mesoporous expanded starch without the need for a templating agent. It is proven to have catalytic activity in different acid-catalyzed reactions.

Steroid molecules have a wide range of applications in organic and pharmaceutical chemistry. For this reason, the development of chemical processes that can be used in steroid chemistry is of great interest.

Taking into account the above mentioned considerations, the main goal of this Thesis is the development of new chemical processes using Starbon®400-SO_3H as a catalyst in steroid chemistry.

In recent years, studies on the ring-opening of epoxides to create amides by nitriles have been published. Recent work by Salvador et al. reported that 5α,6α-epoxysteroids can be converted into the corresponding acylamino-hydroxy products under Ritter reaction conditions using bismuth salts as catalyst.

Based on that work, the one-step conversion of epoxysteroids into the acylamino-hydroxy compounds using Starbon®400-SO_3H as a catalyst under Ritter reaction conditions will be evaluated, with the aim of obtaining new steroid molecules of considerable biological interest.

The final goal of this work is to establish Starbon® materials as adequate catalysts for chemical processes within steroid chemistry.

II. New Sustainable Processes Catalyzed by Starbon®400-SO_3H under Ritter Reaction Conditions

1. Introduction

1.1. Starbons®: Starch-Derived Mesoporous Carbonaceous Materials

As exposed before, the outstanding potential of mesoporous carbonaceous materials requires a methodology that grants control over their surface area and distribution of pore sizes. The best method to achieve this is the templating route.

Nevertheless, the highly aggressive chemicals involved limit this approach to the production of stable graphitic carbons with inert hydrophobic surfaces(Jeong, S. 2005) and the functionalization requires difficult chemical modifications that reduce the availability of the mesopores(Li, Z. 2005).

Daniels *et al.* reported a new approach for the generation of mesoporous carbonaceous materials is reported. This method uses the degree of carbonization to control the range from hydrophilic to hydrophobic of the mesoporous carbonaceous materials' surfaces. The natural ability of the amylase and amylopectin polymer chains within the starch granules to assemble into an organized nanoscale lamellar structure that has crystalline and amorphous regions is used(Daniels, D. R 2004). Mesoporous carbons (Starbons) are synthesized by using mesoporous expanded starch(Milkowski, K. 2004; Budarin, V. 2005) as the precursor without the need for a templating agent.

This process allows the production of a whole range of mesoporous carbon-based materials from starch to activated carbon, including amorphous oxygen-containing carbons that, due to their varied surface area, have many applications, such as catalysis(Son, S. U. 2000; Toda, M. 2005), adsorption(Ko, D. C. 2000) and medicine(Orisakwe, O. E. 2001; Otero, M. 2004).

1.2. Porous Polysaccharide-derived Materials (PPDMs)

Nature provides a wide range of organic polymers (e.g., polysaccharides) with different structures and storage functions in higher plants (Table 7).

*Table 7- Examples of natural occurring organized biological structures.**

Structured biological material	Principal components	
	Mineral	Organic
Shells	Calcium carbonate	Chitin
Horns	Calcium phosphate	Keratin
Bones	Hydroxyapatite	Collagen
Teeth	Hydroxyapatite	-
Bird beaks	Calcium phosphate	Keratin
Insect cuticle	-	Chitin
Plants	-	Cellulose, Lignin, Hemicellulose
Spicules	Silica	-
Starch	-	Starch

*Adapted from literature(White, R.J. 2009).

These biomaterials are organized from the nanoscale to the macroscopic scale to give hierarchical materials that are sculpted with complex forms (spirals, spheroids and skeletons). The future of the research of mesoporous carbonaceous materials may lay on these available pre formed porous structures.

Clark *et al.* have reported the manipulation of polysaccharides in the aqueous phase to generate highly mesoscopic gels, the structure of which may be maintained in a final dried product via a solvent exchange/drying procedure(White, R.J. 2009). Gel ordering of the polysaccharide upon recrystallization from the gelation step promotes the formation of an amphiphilic network, in which hydrogen bond formation between associated polysaccharide chains is the key to phase separation and subsequent gel mesophase development.

Polysaccharides have thermal properties that present a short melting stage (e.g., hydrogen bond network breakdown) followed by a main sharp endothermic decomposition (ca. 280ºC). To avoid the destruction of the mesopore polysaccharide (e.g., starch) network, a strong

Bronsted acid catalysis (e.g. p-toluene sulfonic acid) was used to promote non-acidic polysaccharide dehydration at temperatures below the sharp endothermic decomposition (ca. 120-150ºC), to facilitate crosslinking and fixing of the mesoporous network(Daniels, D. R. 2004).

This technique worked very well for the generation of porous precursors from starch, which is composed of both linear α(1→4) amylose and branched α(1→4) and α(1→6) amylopectin that self assemble within the starch granule to generate a semi-crystalline polysaccharide composite, presenting crystalline dimensions in the mesopore size range (i.e. 4–5 nm)—attributed to spatial gaps in the crystalline amylopectin macromolecular structure, normally occupied by the amorphous amylose content of the granule(Daniels, D. R. 2004).

Systematic studies of synthetic amylose/amylopectin mixtures revealed that mesoporosity showed a strong negative linear dependence on the amylopectin content of the gel mixture (Figure 7)(White, R. J. 2008).

These results indicated that the key component to achieve highly mesoporous starches was a minimal amylose content, whilst materials with higher polymer ordering could be prepared by increasing the amylopectin content. Further subtle manipulation of the morphology and textural properties of high amylase starch based porous materials were performed, using (microwave) gelatinization temperature as a control vector, and pointed to the possibility of producing materials from the same polysaccharide with differing tuneable textural properties via the generation of differing metastable gel states(White, R. J. 2008).

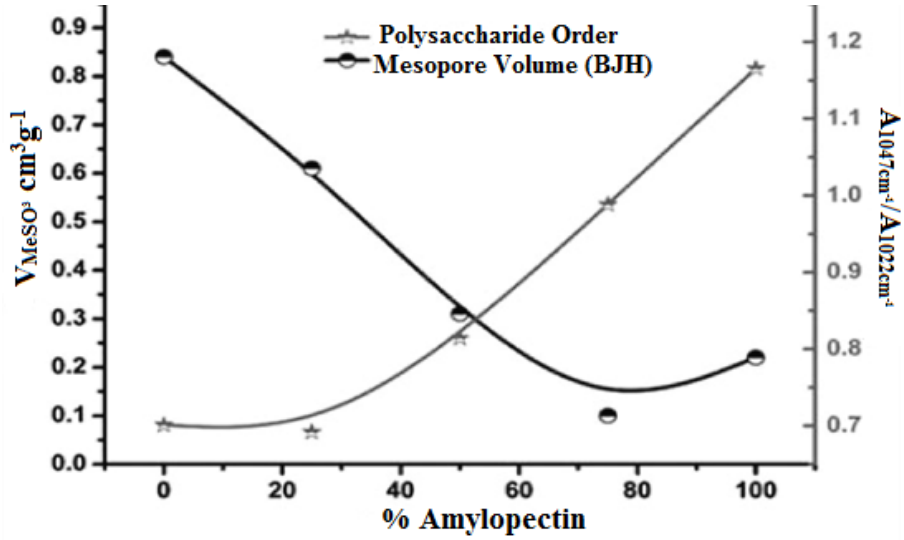


Figure 7. Relationship between mesopore volume, polymer ordering (A1047 cm^{-1}/A1022 cm^{-1}) and amylopectin (% mass) content in synthetic mixtures of the α-D-polysaccharides amylose and amylopectin. Adapted from the literature(White, R. J. 2008).

1.3. Starbon® Synthesis

Starbons® are a new family of highly mesoporous carbon-based materials with tuneable physico-chemical properties. Their low temperature generation is due to the initial acid-catalyzed thermal conversion of mesoporous starch-derived PPDMs into stable nanostructured porous carbonaceous materials(Budarin, V. L. 2007).

These materials reveal mesoporous textural properties, with pore sizes and volumes equal to those observed in materials prepared via hard template routes, and their flexibility in terms of carbonization temperature allows the possibility of tuneable surface chemistry. This is a feature that is not provided by the hard template route, as it needs a high temperature carbonization (> 700ºC), or by soft template based on the self-assembly and polymerization gel properties of aromatic compounds, as it chemically limits the post-processing surface functionality available(Atkin, N. 1998; Budarin, V. L. 2007).

Because no template is used, wasteful processing steps and harmful chemicals are avoided, and materials can be prepared at a temperature of choice (e.g., 200-1000ºC). This allows surface chemistry tuneability amenable to facile post-modification strategies. Consequently,

the hydrophilicity vs. hydrophobicity properties may be moderated, generating the possibility of designer material synthesis for specific applications(Budarin, V. L. 2007).

Starbons® synthesis comprises three main stages: Expansion, Drying and Pyrolysis (Figure 8)(Atkin, N. 1998; Shamai, K. 2004; White, R. J. 2008). In the final stage of the process, the mesoporous starch is doped with a catalytic amount of organic acid (e.g. p-toluenesulfonic acid) and heated under vaccum(Budarin, V. L. 2006). This enables fast carbonization and fixing of the mesoporous structure.

Heating at different temperatures (150-700ºC) has produced a variety of mesoporous materials from amorphous carbons to graphite-like activated carbons. As native starch granules do not produced mesoporous materials when carbonized, the formation of expanded starch as a precursor to starbon® is crucial.

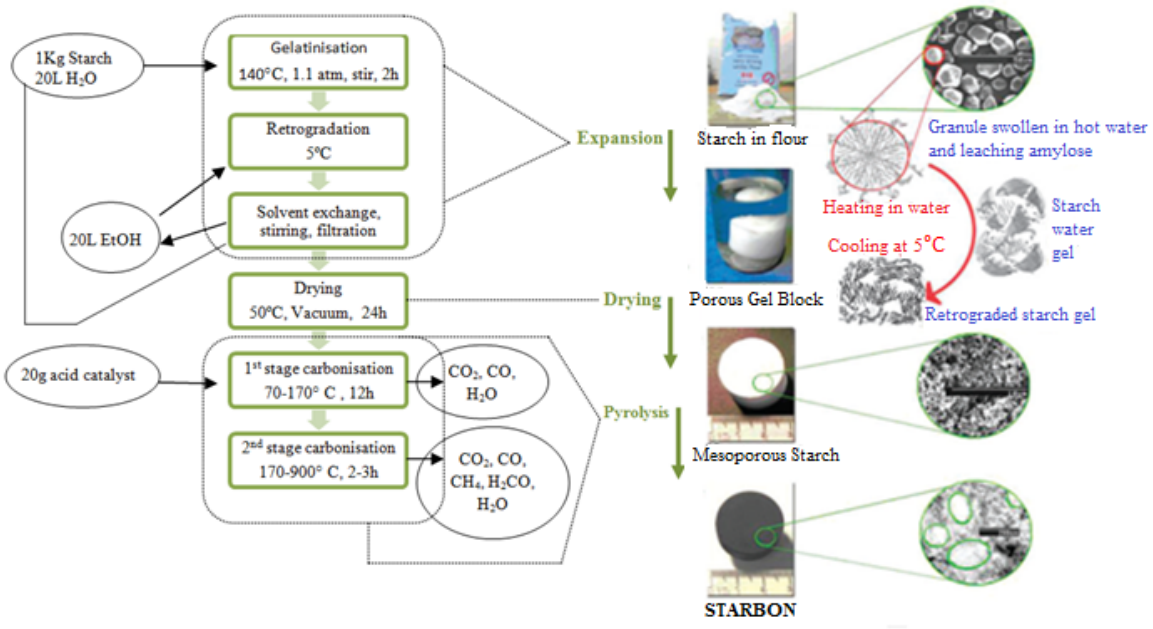


Figure 8. Diagrammatic representation of the main processing steps in the production of starch-derived Starbon® materials. Adapted from the literature(Budarin, V. L. 2007).

1.4. Starbon® Properties and Characterization

The porous structure of the starch precursor is preserved in the Starbon® product, which prevents the problem of micelle collapse that occurs in micelle templated polymer methods for the synthesis of mesoporous carbons(Li, Z. 2004) and removes the need for synthesis of mesoporous templates, such as silica, to define the structure.

The granular morphology of mesoporous starch can be maintained during pyrolysis with only minor changes due to shrinkage. The porous/textural properties of starch-derived Starbons® were characterized via N_2 sorption studies (Table 8).

*Table 8- Physical analysis of starch and starch-derived Starbon® materials**

Material	Surface area (m^2g^{-1})		Pore Volume (cm^3g^{-1})		C/O atomic ratio		Surface energy (E_{DR} $kJmol^{-1}$)	Pore diameter (nm)
	S_{BET}	*Mesoporous*	*Total*	*Mesoporous*	*EA*	*XPS*		
Mesoporous Starch	184	160	0.62	0.61	1.20	1.10	7.4	7.6
Acid/doped Mesoporous starch	230	170	0.67	0.66	1.20	1.30	8.2	8.6
Starbon®100ºC	179	171	0.67	0.61	1.26	1.34	6.9	10.5
Starbon®150ºC	172	137	0.68	0.58	1.55	1.99	6.5	10.4
Starbon®220ºC	151	90	0.57	0.42	2.71	2.73	10.5	16
Starbon®300ºC	293	60	0.53	0.37	3.43	3.79	17.7	17.2
Starbon®350ºC	332	65	0.56	0.38	5.00	5.10	18.2	16.8
Starbon®450ºC	475	70	0.52	0.32	6.01	6.04	20.6	14.5
Starbon®600ºC	528	153	0.62	0.43	7.53	7.55	24.4	12.1
Starbon®700ºC	538	158	0.73	0.55	8.54	8.50	26.6	10.6
Starbon®800ºC	600	167	0.63	0.43	8.60	8.60	25.8	7.0

*Adapted from the literature(White, R. J. 2009).

The total pore volume (0.4-0.6 cm^3g^{-1}) and the average pore diameter (8-16a nm) indicate a predominance of mesopores throughout the carbonization process. This diameters are greater than 5 nm, which is the typical pore size for carbons prepared by the templating method(Lu, A. 2005). Although there is a substantial increase in the contribution of microporous region to the

total surface area, the actual volume that this corresponds to is small in comparison to the total mesoporous volume.

Table 8 also indicates that Starbon® combines both carbon and starch-like properties at lower preparation temperatures, and progressively gains more carbon-like characteristics with increasing temperatures. This is demonstrated by the surface energy, E_{DR}, (from the Dubinin–Radushkevich model) that has an overall increase as the carbonization preparation temperature rises. More starch-like properties are retained up to a temperature of 150 ºC, above which there is gradual change towards increasingly carbon-like properties(Budarin, V. L. 2006).

The properties obtained from TGA, ^{13}C CP MAS NMR and XPS spectroscopy and DRIFT are summarized in Figure 9. It can be seen that there is a progressive increase in the hydrophobicity of the functional groups present, from a starch to graphite-like structure above 700ºC.

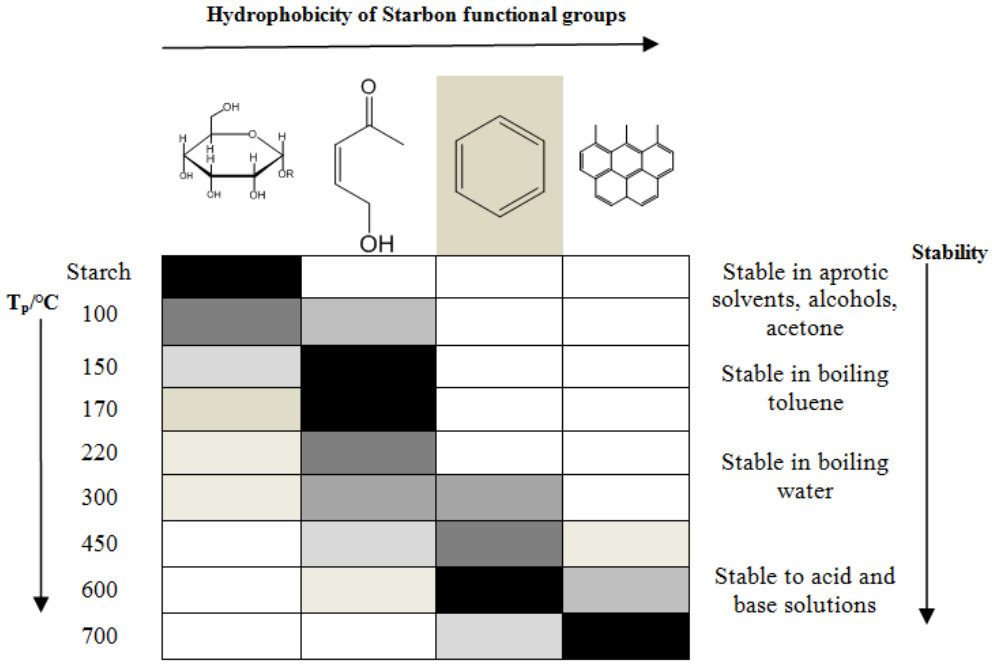


Figure 9. Distribution of functional groups on starbons prepared at different temperatures: color scale to indicate relative amounts of different groups (black represent highest). Tp=temperature of starbon preparation. Adapted from the literature(Budarin, V. L. 2006).

In the first step (100–200ºC), the CH_2OH groups in the starch condensed to carbonyl groups conjugated with olefinic groups. The second step (150–450ºC) involves the progressive degradation of these groups to form aliphatic and alkene/aromatic functions. In the penultimate step (300–600ºC), these aliphatic groups are almost completely converted to aromatic π systems. Finally, as the temperature increases, these aromatic compounds condense to form graphite-type structures(Budarin, V. L. 2006; White, R. J. 2008).

Starbons® prepared at the temperature range of 100–700 ºC present accessible surface functionality that is ideal for chemical modification (e.g., silylation, alkylation, esterification, etherification, amination), allowing post functionalization strategies to further manipulate their physical and chemical properties; arguably features not typical of the synthesis of other porous carbons. Information about the mechanism of starch decomposition is useful to predict the temperature of Starbon® preparation for certain applications(Budarin, V. L. 2006).

1.5. Starbon®400-SO_3H: Preparation and Applications in Organic Chemistry

1.5.1. Heterogeneous Catalysis

Solid acid catalysts are present in a wide variety of industrially important chemical transformations. Starch-derived Starbon® materials, treated with sulfuric acid, provided a series of porous solid Brønsted acids that were shown to be efficient catalysts in a wide range of acid catalyzed processes including esterifications of organic acids in aqueous medium, acylations of alcohols and amines, and alkylations of aromatics(White, R. J. 2010).

We will focus on Starbon®400-SO_3H, the sulfonated Starbon® that we used in the experimental work of this Thesis. This catalyst is produced via a carbonization at 400ºC and a subsequent functionalization. The material is suspended in H_2SO_4 (99.999% purity, 10mL acid per g material) and is heated for 4h at 80ºC. After sulfonation, the solid acid is washed with distilled water until washings became neutral, conditioned in boiling toluene (150ºC, 4h) and water (100ºC, 3h) and finally oven dried over night (100ºC).

The catalytic power of Starbon®400-SO_3H was tested in the esterification(Budarin, V. L. 2007) reaction in aqueous ethanol of four different substrates (succinic, fumaric, levulinic and itaconic acids), yielding a very high conversion and selectivity to their respective esters. The rates of esterification of diacids (succinic, fumaric and itaconic) for Starbon® were between 5 and 10 times higher than for any commercial alternative solid acid catalyst (zeolites, sulfated zirconias, acidic clays, etc.). A diester selectivity improvement (from 35–50% range for the majority of the solid acids to >90% for Starbon® at conversion levels of ca. 90%) was also obtained(Budarin, V. L. 2007).

Acylation(Budarin, V. L. 2007) reaction catalyzed by Starbon®400-SO_3H performed under microwave conditions provided very good results, in terms of conversion and selectivity, for a wide range of substrates, in a very short period of time (less than 10 min). Starbon® catalyzed benzyl alcohol acylation reaction was between 5 and 10 times higher than that of any of the reactions using commercial catalysts [microporous beta-25zeolite (SiO2/Al2O3 ratio 25), mesoporous Al-MCM-41 and acidic Montmorillonite KSF] and 2 times higher than that of reactions using similar sulfonated microporous carbonaceous materials(Budarin, V. L. 2007; Constable, D. J. 2007).

The acylation of amines(White, R. J. 2009) to prepare amides is an important transformation in organic chemistry. The efficient and atom economic preparation of aromatic amides via N-acylation of amines was successfully carried out using Starbon®400-SO_3H as heterogeneous catalyst under microwave irradiation. Quantitative conversions of starting material were typically achieved in 5–15 min with very high selectivities to the target product, applicable to a wide range of compounds (including aromatic and aliphatic amines), substituents and acids. Starbon® acids provided starkly improved activities compared with other acid catalysts, including zeolites, Al-MCM-41 and acidic clays(Constable, D. J. 2007).

Starbon®400-SO_3H was tested in the liquid-phase alkylation(Budarin, V. L. 2007) of different aromatic substrates with benzyl chloride under microwave conditions. Sulfonated Starbon® afforded the alkylated compounds in very good yields and selectivity, where only either the *o*- or *p*- monoalkylated products were found, except for the m-xylene alkylation, in which a *p*-/*o*- 3:2 ratio mixture was found. The rate of Starbon®400-SO_3H catalyzed reaction was at least comparable with that of beta-25 zeolite catalyzed reaction, and it was much higher than the

rate afforded by the other catalysts tested, including sulfated zirconias (SZr) and mesoporous Al-MCM-41 materials(Budarin, V. L. 2007).

Other studies using the catalytic aspect of Starbon® were published, such as redox catalysis using supported metal nanoparticles. Pd-starbon® materials converted phenols, napthols and dihydroxybenzenes into cyclohexanones, tetrahydronaphtols and hexanones, respectively(Makowski, P. 2008), and Ru-starbon® materials catalysed the hydrogenation of a variety of biomass platform molecules, including succinic, fumaric, itaconic, levulinic and pyruvic acids in aqueous solutions under mild reaction conditions(Luque, R. 2010).

In summary, it was demonstrated that it is possible to harness the natural ability of plant starch to form nanochannelled structures without the need for a templating agent. This provides an entirely new, simpler, and less wasteful route to the mesoporous graphitizable carbons named Starbons®. Because of the diversity of surface functional groups, Starbons® can easily be modified. This, in conjunction with a high surface area in the mesoporous region and mechanical stability, makes Starbons® particularly suitable for applications such as catalysis and chromatography.

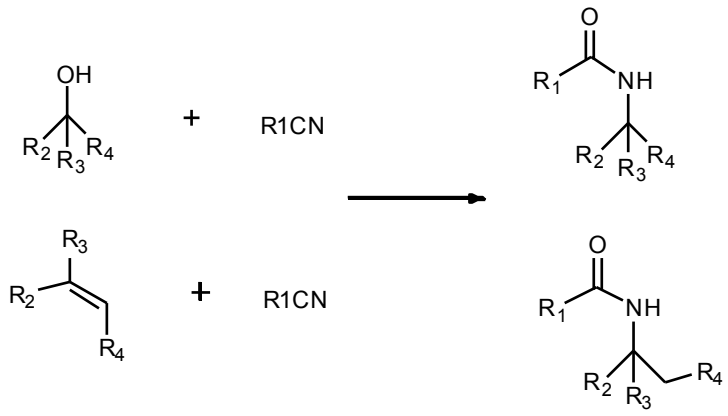
2. *New Sustainable Processes Catalyzed by Acids with Interest in Pharmaceutical Chemistry*

On Chapter I, we presented the importance of the Green Chemistry Principles applied to process chemistry to obtain new chemical procedures more efficient and clean. In this section we describe the use of acid catalysis in new environmentally friendly processes, providing suitable and greener alternatives as well as relevant biological molecules for the pharmaceutical chemistry.

One of the most fundamental processes in organic chemistry is the formation of a carbon-nitrogen bond, being the synthesis of amides an important reaction in pharmaceutical chemistry(Ritter, J. J. 1948; Constable, D. J. C. 2007). For these reasons, we focused our attention in the Ritter Reaction.

2.1. The Ritter Reaction

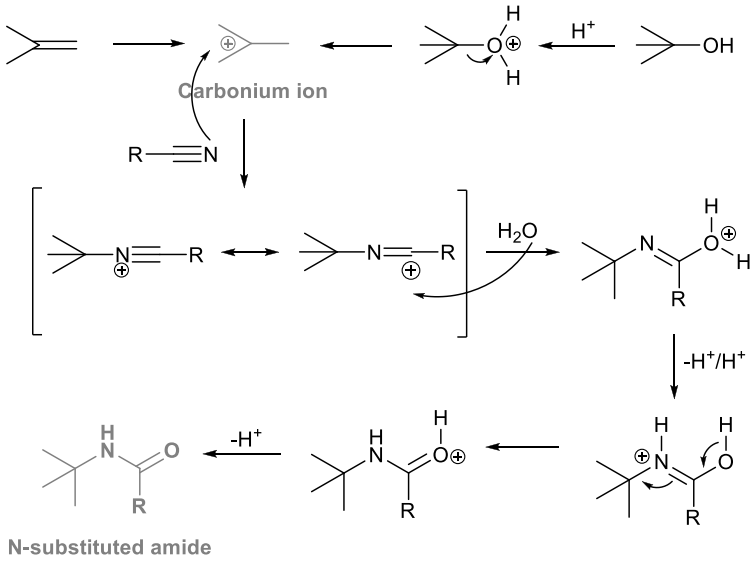
The Ritter reaction is named after John J. Ritter, an American Chemist who, in collaboration with his student P. Paul Mineri, described the formation of an N-substituted amide via the addition of nitriles to alkenes in the presence of concentrated sulphuric acid (scheme 3)(Ritter, J. J. 1948; Constable, D. J. C. 2007).



Scheme 3. Ritter Reaction of alkenes and alcohols: synthesis of N-substituted amides(Guérinot, A. 2012).

Since this discovery in 1948, Ritter and co-workers have preformed the Ritter reaction with a wide range of nitriles(Krimen, L. I. 1969), including dinitriles(Benson, F. R. 1951) and unsaturated nitriles(Plaut, H. 1951), which were added to a variety of compounds capable of forming carbonium ions, such as alcohols(Ritter, J. J. 1948) (scheme 3), carboxylic acids and esters(Ritter, J. J. 1956).

The known reaction mechanism involves protonation of an alcohol or alkene, to generate a carbonium ion that adds to the nitrile to afford a nitrilium ion that is subsequently trapped with water resulting in N-substituted amides (scheme 4)(Hathaway, B. A. 1989; Colombo, M. I. 2002; Li, J. J 2006).



Scheme 4. Mechanism for the Ritter reaction of alkenes and alcohols(Hathaway, B. A. 1989; Colombo, M. I. 2002; Li, J. J 2006).

A recent review about this matter was published by Guérinot and co-workers, were new applications and progress in the field of Ritter-type and multicomponent reactions is summarize(Guérinot, A. 2012).

Green approaches have been used recently in the Ritter Reaction. In 2008, Yadav and co-workers used phosphomolybdic acid supported in Silica gel (PMA-SiO_2) to convert alcohols into their corresponding amides in excellent yields(Yadav, J. S. 2008). This method offered high conversions, short reaction times, cleaner reaction times and the use of inexpensive and readily available PMA-SiO_2.

Ma'mani *et al.* also used the Ritter Reaction to obtain amides from alcohols in high yields(Ma'mani, L. 2010). They used $HClO_4$-functionalized silica-coated magnetic nanoparticles as a catalyst, since it can be simply removed using an external magnetic device, enhancing product purity, and recycled, promising economic. Ionic liquids had been used by Kalkhambkar *et al.* for the high yield synthesis of amides under Ritter Reaction conditions using alcohols and nitriles(Kalkhambkar, R. G. 2011).

In addition, epoxides were used as substrates for the Ritter reaction. Norman *et al.* reported that epoxides could be efficiently converted into 1,2-hydroxycarboxamides under Ritter

reaction conditions(Johansen, S. K. 1999). Higher yields were obtained in the presence of $BF_3.OEt_2$ than with H_2SO_4 and $SnCl_4$.

In 2005, the formation of (*2R,3S*) and (*2S,3S*)-1,3-diaminoalkan-2-ols from the opening of the ring of the enantiopure (*2R,1'S*) and (*2S,1'S*)-2-(1aminoalkyl)epoxides under Ritter Reaction conditions, with total selectivity and high yields, was described by Concéllon and co-workers(Concéllon, J.M. 2005).

A new and easy method to obtain trifluoromethyl diamino alcohols by the ring opening of a trifluoromethyl amino epoxide by the Ritter Reaction with nitriles in the presence of $BF_3.OEt_2$ and trifluoroethanol was described by Dos Santos et al.(Dos Santos, M. 2009).

2.2. Ritter Reaction in Epoxysteroids

The Ritter reaction has been accomplished using epoxysteroids as substrates for the synthesis of *vic*-N-acylamino-hydroxy steroids. $BF_3.OEt_2$ was used in the treatment of a solution of 5α,6α-epoxycholestan-3β-yl in acetonitrile, affording the 6β-acetamido-5α-hydroxy derivative with an yield of 85% (Ducker, J. W 1970). The 5α-acetamido-6β-hydroxy product was also obtained in high yields from the 5β,6β-epoxide in acetonitrile using gaseous BF_3 instead of $BF_3.OEt_2$.

Julia and co-workers used $BF_3.OEt_2$ and $HClO_4$ to promote the *trans*-dial ring opening of 5α,6α, 5β,6β- and 2β,3β-epoxysteroids to give the corresponding *vic*-N-acylamino-hydroxysteroid using acetonitrile as a nucleophile(Bourgery, G. 1972).

The same research team reported that under Ritter reaction conditions, and in the presence of $HClO_4$, the 4α,5α- and 4β,5β-epoxycholestanes with a hydroxyl or an acetoxyl group at C3 consistently afforded the product that results from the addition of the nitrogen atom at C5(Ryan, R. J. 1973). However, Wadia et al. showed that when there is no substituent groups at C3 in the epoxycholestanes, the products obtained have an acetamide at C4 due to a "true SN_2 attack"(Narayana, C. R 1974).

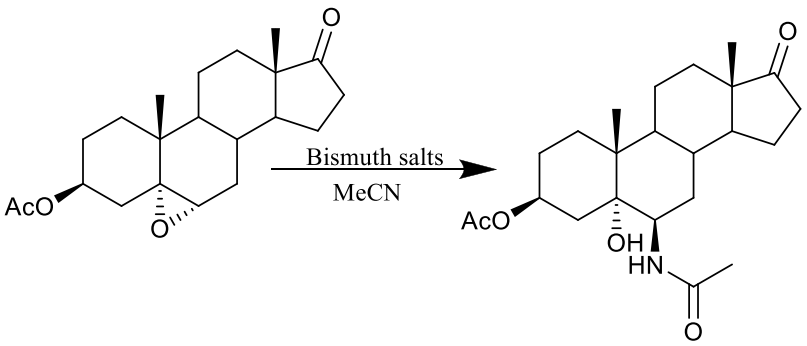
Teutsch et al. reported that $SnCl_4$ acts as a Lewis acid to promote the *trans*-dial ring opening of 6α,7α-epoxysteroids by acetonitrile and the one-pot synthesis of the 6β-acetamido-7α-acetoxy derivative using *p*-Toluenesulfonic Acid (*p*-TSA) in CH_3CN/Ac_2O(Teutsh, G. 1970). 6β-

benzamido-6α-hydroxysteroids were obtained in good yields in an analogous work by Narayana and co-workers. The 5α,6α-epoxysteroids reacted with benzonitrile, in the presence of $HClO_4$(Narayana, C. R. 1977).

Reactions of epoxides in the five-membered ring D, under Ritter conditions, have several side reactions. In the presence of $BF_3.OEt_2$ and various nitriles, 16β,17β-epoxysteroids give the corresponding *vic*-*N*-acylamino-hydroxysteroid, but in the 16α,17α-epoxysteroids a Wagner-Meerwein-type rearrangement occurred(Schneider, G. 1982). The substrates 14α,15α-, 14β,15β- and 15β,16β-epoxysteroids did not produce *vic*-N-acylamino-hydroxy compounds under Ritter reaction conditions, and the products that were obtained were the result of various types of rearrangements(Schubert, G. 1982).

Vincze *et al*. reported the preparation of *vic*-*N*-acylamino-hydroxysteroids from 5α,6α-epoxy-20-oxopregnan-3β-yl acetate and chloroacetonitrile. Aminoacid residues were incorporated in the final product and screened for immunological and antiarrythmic activities(Vincze, I. 1996).

Recently, Salvador *et al.* reported that epoxysteroids can be converted into the corresponding acylamino-hydroxy product under Ritter reaction conditions using bismuth salts as catalyst (squeme 5)(Pinto, R. M. A. 2006). In this process, different nitriles and reaction conditions were used to produce the *vic*-*N*-acylamino-hydroxysteroids and, in all cases, good yields were obtained. This experimental work was the foundation for this Thesis.



Scheme 5. Ritter reaction promoted by Bismuth salts in 5α,6α- and 5β,6β-epoxysteroids(Pinto, R. M. A. 2006).

The Ritter reaction in epoxysteroids is an important route for the stereoselective introduction of the *vic*-trans-N-acylamino-hydroxy moiety to obtain products that may be considered as *N*-protected aminoalcohols, which are very important from a medicinal point a view(Pinto, R. M. A.

2006). Several 2β-amino-3α-hydroxyandrostanes have potent properties as neuromuscular blocking agents(Bergmeier, S. C. 2000; Tuba, Z. 2002; Gyermerk, L. 2005), and similar compounds have been shown to inhibit the proliferation of leukemia cells(He, Q.2001; Zlotos, D. P. 2005).

3. Results and Discussion

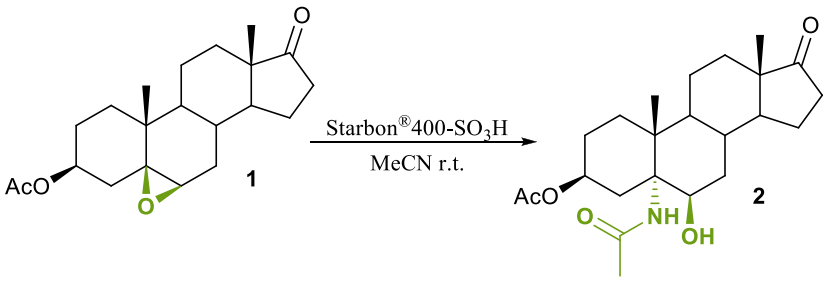
Although the described processes that use the Ritter reaction in epoxysteroids presented good yields, the use of corrosive and toxic acids or metal reagents and the difficult work-up make these procedures unsuitable on a commercial scale production. Thus, a more eco-friendly process that uses non-toxic reagents and catalytic amounts of the interveners would be of major interest.

Bismuth salts presented a cleaner and safer approach to this reaction; unfortunately, this catalyst is a metal, which is not ideal for the use in Green Chemistry reactions.

In this chapter we reported the use of an organic catalyst, Starbon®400- SO_3H, to promote the one-step conversion of epoxysteroids into the corresponding acylamino-hydroxy compounds, under Ritter reaction conditions.

3.1. Starbon®400-SO_3H Catalyzed Ritter Reaction in Epoxysteroids

Several of the procedures described before used acetonitrile as a reagent. We decided to screen the reactivity of Starbon®400-SO_3H with the same reagent, under Ritter reaction conditions using 5β,6β-epoxy-17-oxoandrostan-3β-yl acetate **1** as a substrate. After a few hours, TLC analysis revealed that the reaction was completed and that a highly polar product had been formed (**Scheme 6** and **Table 9**). In the absence of Starbon®400-SO_3H, the reaction did not occur (**Table 9**, entry 1).



Scheme 6. Ritter Reaction catalyzed by Starbon®400-SO_3H using 5β,6β-epoxy-17-oxoandrostan-3β-yl acetate 1 as a substrate.

Table 9- Ritter Reaction catalyzed by Starbon®400-SO_3H using 5β,6β-epoxy-17-oxoandrostan-3β-yl acetate 1 as a substrate and acetonitrile as a reagent/solvent*

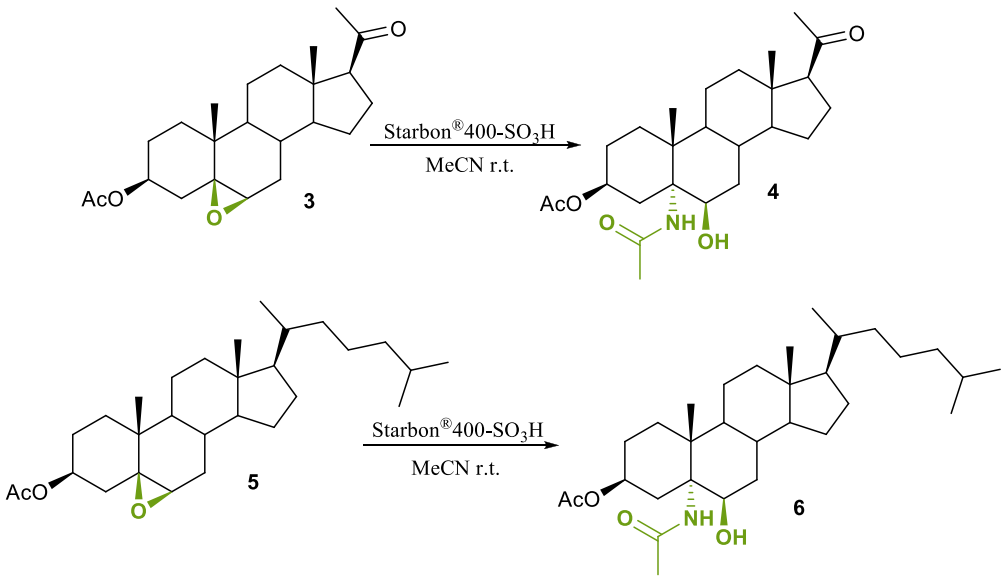
Entry	Substrate	Product	Reagent (mmol)	Solvent	Starbon®400-SO_3H (mol%)	Reaction time (h)	Yields (%)
1	1 (0.08)	2	MeCN (57.5)	-	-	10	-
2	1 (0.08)	2	MeCN (57.5)	-	5	6	95
3	1 (0.14)	2	MeCN (57.5)	-	10	6	95

*Reactions were performed at room temperature under magnetic stirring. Traces of a by-product were visible on the TLC plates after work-up procedures, but not detectable in ^{1}H-NMR spectrum (300 MHz).

The Ritter reaction in the 5β,6β-epoxy-17-oxoandrostan-3β-yl acetate **1** resulted in a stereoselective *trans*-dial nucleophilic attack occurring at C5 by the α-face to obtain the corresponding acylamino-hydroxy products, in high yields(**Scheme 6** and **Table 9**).

These observations led to the study of other 5β,6β-epoxysteroids [5β,6β-epoxy-20-oxopregnan-3β-yl acetate **3**, 5β,6β-epoxycholestan-3β-yl acetate **5**], as well as the nature of the polar products (**Scheme 7** and **Table 10**).

Just as it happened before, after a few hours the reaction was completed (by TLC control) with the formation of highly polar products, in high yields (90-98%). The acylamino-hydroxy products were obtained by the same *trans*-dial nucleophilic attack as compound **1.**



Scheme 7. Ritter Reaction Catalyzed by Starbon®400-SO_3H in 5β,6β-epoxysteroids.

*Table 10- Ritter Reaction catalyzed by Starbon®400-SO_3H using acetonitrile**

Entry	Substrate	Product	Reagent (mmol)	Solvent	Starbon®400-SO_3H (mol%)	Reaction time (h)	Yields (%)
1	3 (0.07)	4	MeCN (32.6)	-	5	20	98
2	3 (0.07)	4	MeCN (32.6)	-	10	6	98
3	3 (0.07)	4	MeCN (32.6)	-	10 (recycled)	200	90
4	5 (0.06)	6	MeCN (32.6)	-	5	6	95
5	5 (0.11)	6	MeCN (65.2)	-	5	8	98
6	5 (0.11)	6	MeCN (65.2)	-	5	$46_{(a)}$	-
7	5 (0.06)	6	MeCN (65.2)	-	5 (recycled)	100	93
8	5 (0.06)	6	MeCN (65.2)	-	5 (2x recycled)	$180_{(b)}$	90

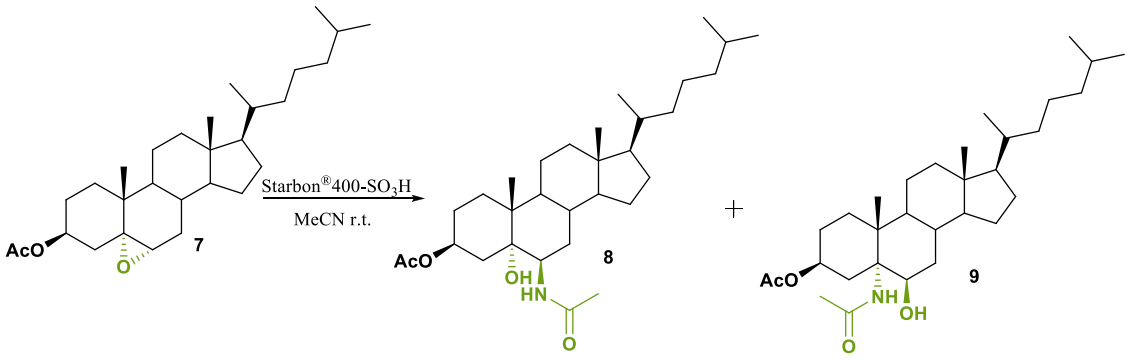
* Reactions were performed at room temperature under magnetic stirring. Traces of a by-product were visible on the TLC plates after work-up procedures, but not detectable in ^{1}H-NMR spectrum (300 MHz). (a) Under nitrogen; according to TLC plates the reaction was not completed. (b) The catalyst was recycled from a previous reaction performed with recycled catalyst.

To evaluate if the catalytic activity of the catalyst changed after it had been used, reactions with recycled catalyst were performed, and the results were somewhat disappointing. Although the reaction occurred, the reaction time increased significantly (entries 3, 7 and 8, **Table 10**) when compared with the reactions performed with normal catalyst.

We also observed an increase in the reaction time when the catalyst was reused for the second time in comparison with the reaction time when the catalyst was reused for the first time (entries 7 and 8, **Table 10**), which means that Starbon®400-SO_3H loses activity in each new use.

Considering that water has a predominant role in the Ritter reaction, we decided to study the influence of humidity in this reaction, so we performed it under a nitrogenous dry atmosphere (entries 6, **Table 10**). When the water was removed of the reaction's environment (under nitrogen), the substrate was not completely converted into the product **6** (observed on TLC plates, entry 7, **Table 10**). In this case, the reaction occurred while some water was in the system and was unable to proceed in its absence. These data confirm the relevance of water in the Ritter reaction.

To explore further the Ritter reaction, the opening of 5α,6α-epoxysteroids was studied (**Scheme 8** and **Table 11**). There is an increase in the reaction times when compared with the results presented in **Tables 9** and **10.**



Scheme 8. Ritter Reaction Catalyzed by Starbon®400-SO_3H in 5α,6α-epoxysteroids.

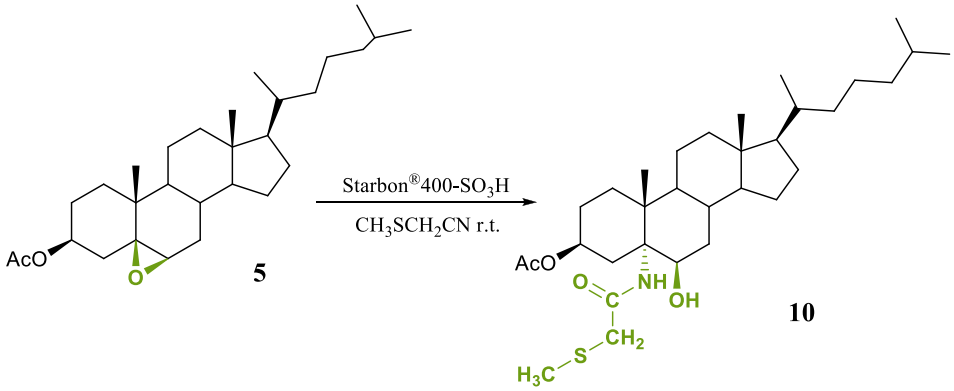
*Table 11- Ritter Reaction catalyzed by Starbon®400-SO_3H using 5α,6α-epoxycholestan-3β-yl acetate 7 as a substrate and acetonitrile as a reagent**

Entry	Substrate	Product	Reagent (mmol)	Starbon®400-SO_3H (mol%)	Reaction time (h)	Yields (%)
1	7 (0.06)	8/9	MeCN (32.6)	5	45	90
2	7 (0.06)	8/9	MeCN (32.6)	10	16	94

*Reactions were performed at room temperature under magnetic stirring.

When the reaction was carried out using 5α,6α-epoxycholestan-3β-yl acetate **7** as a substrate, the *trans*-diaxial ring opening with MeCN, with the nucleophilic attack occurred at C6 by the β-face, afforded the product 6β-Acetamido-5α-hydroxycholestan-3β-yl acetate **8** (**Scheme 8**, **Table 11**). Contrarily to what happens in the literature, there is also a nucleophilic attack at C5 by the α-face, creating the stereoisomer **9**.

As the results obtained showed the ability of Starbon®400-SO_3H to catalyzed the Ritter reaction in epoxysteroids, we studied this new process using other nitriles. The first we used was methylthioacetonitrile (**Table 12, scheme 9**).



Scheme 9. Ritter Reaction Catalyzed by Starbon®400-SO_3H in 5β,6β-epoxysteroids, using methylthioacetonitrile as a reagent.

*Table 12- Ritter Reaction catalyzed by Starbon®400-SO_3H using methylthioacetonitrile**

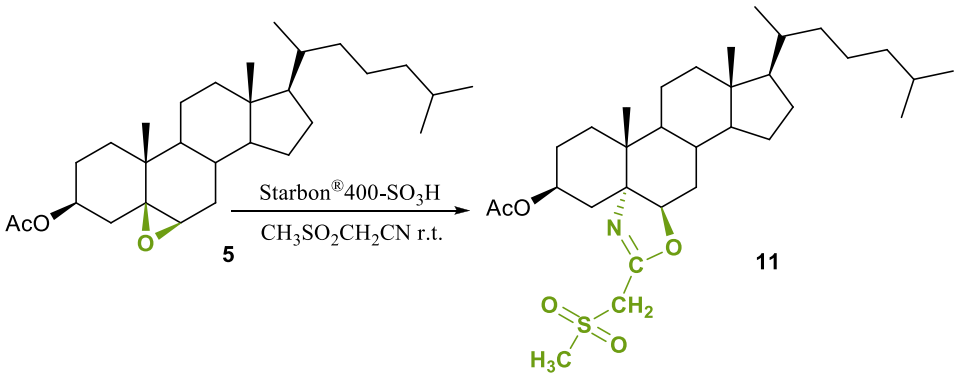
Entry	Substrate	Product	Reagent (mmol)	Solvent	Starbon®400-SO_3H (mol%)	Reaction time (h)	Yields (%)
1	5 (0.06)	10	Methylthioacetonitrile (CH_3SCH_2CN) (6.0)	-	5	$6_{(a)}$	-
2	5 (0.06)	10	Methylthioacetonitrile (CH_3SCH_2CN) (6.0)	-	10	5	90

*Reactions were performed at room temperature under magnetic stirring. Traces of a by-product were visible on the TLC plates after work-up procedures, but not detectable in the ^{1}H-NMR spectrum (300 MHz). (a) According to the TLC plates the reaction was not completed.

Methylthioacetonitrile is a liquid nitrile like acetonitrile. When it was used in the Ritter reaction with 5mol% of catalyst, the substrate was not completely converted into the product **10** (observed on TLC plates, entry 1, **Table 12**). The reaction ceased to proceed two hours after it started, and we decided to stop it after six hours and repeat it with 10 mol% of catalyst.

The reaction occurred smoothly and the final product was recovered in high yields (entry 2, **Table 12**). The need for a higher percentage of catalyst may be explained by the use of a smaller quantity of reagent (6.0 mmol), when compared with the acetonitrile (32.6 mmol), which complicated the progress of the reaction.

The second nitrile used was methylsulfonylacetonitrile, a solid nitrile (**Table 13**, **Scheme 10**). The reaction occurred with 5 mol% of catalyst, but the reaction time was higher (1, **Table 13**) than that obtained in the experiments with acetonitrile. This may also be explained by the smaller quantity of nitrile (4.20 mmol) used in this case and by the larger size of the acetamide moiety introduced into the molecule.



Scheme 10. Ritter Reaction Catalyzed by Starbon®400-SO_3H in 5β,6β-epoxysteroids, using Methylsulfonylacetonitrile as a reagent.

*Table 13- Ritter Reaction catalyzed by Starbon^{R}400-SO_3H using Methylsulfonylacetonitrile**

Entry	Substrate	Product	Reagent (mmol)	Solvent	Starbon®400-SO_3H (mol%)	Reaction time (h)	Yields (%)
1	5 (0.06)	11	Methylsulfonylacetonitrile ($CH_3SO_2CH_2CN$) (4.20)	1,4-dioxane (2)	5	34	80

*Reactions were performed at room temperature under magnetic stirring. Traces of a by-product were visible on the TLC plates after work-up procedures, but not detectable in the ^{1}H-NMR spectrum (300 MHz).

In this reaction we obtained an oxazoline instead of an acetamide. This particular result raised the question: why was the outcome of this reaction different from the others?

In searching for the answer, we were faced with an experimental work by John R. and co-workers where, in 1975, they converted several epoxides into Δ^2-oxazolines via the reaction

with acetonitrile and benzonitrile in the presence of boron trifluoride-ether complex(Smith, J. R. L. 1975).

More recent work from Brent D. Feske and Jon D. Stewart(Feske, B. D. 2005 JOC; Feske, B. D. 2005 Tetrah.), in which they used the Ritter reaction with benzonitrile to directly convert epoxides into the oxazoline form of the target molecules also contributed for the elucidation of the compound **11.** In these experiments the oxazoline form was obtained directly from the Ritter reaction and was hydrolyzed under acidic conditions to give the acetamide form(Feske, B. D. 2005 JOC).

In view of these data, we thought of the possibility of generating an oxazoline as an intermediate of the Ritter reaction.

Because methylsulfonylacetonitrile is a solid nitrile we needed to add a solvent, 1,4-dioxane, to the reaction, which is less acidic than the liquid nitriles (acetonitrile and methylthioacetonitrile) used before. Possibly, this caused the reaction to stop in the intermediate form (oxazoline form) because the reaction medium was not acidic enough to promote the formation of the acetamide form. This conclusion is purely based on the literature presented(Feske, B. D. 2005 JOC; Feske, B. D. 2005 Tetrah. Smith, J. R. L. 1975) and to confirm the results it is necessary to perform more studies with different acidic mediums.

Considering the importance of the oxazolines in the pharmaceutical Industry as appetite suppressants, as stimulant drugs or even as intermediates in various reactions(Feske, B. D. 2005 JOC; Feske, B. D. 2005 Tetrah. Smith, J. R. L. 1975), the previous result was very rewarding in terms of future research and potential biological activity.

3.2. Considerations of structural elucidation of the obtained products

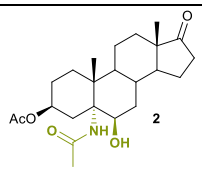
The Acylamino-hydroxy steroids **2**, **4**, **6** and **8** were previously obtained in prior studies(Pinto, R. M. A. 2009) in the FFUC (Faculty of Pharmacy of the University of Coimbra) and were published by Salvador et al(Pinto, R. M. A. 2006). These data were used as comparison elements for the characterization of these compounds.

The formation of the *vic*-*N*-acylamino hydroxyl products by the epoxysteroids was followed by TLC control, and the reactions were stopped after full substrate consumption. After work-up, the obtained compounds were characterized by ^{1}H-NMR (**Table 14**) and ^{13}C-NMR.

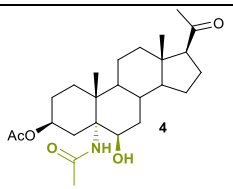
Accordingly to ^{1}H-NMR spectroscopy, the opening of the epoxide ring was efficient since no signal at 3.07-3.14ppm(Silvestre, S. M. 2004) was found in the spectra of the *vic-N*-acylamino hydroxyl compounds **2**, **4**, **6** and **8.**

The ^{1}H-NMR analytical data of the compounds **2, 4 and 6** synthesized in **Table 14** were in accordance with the ones published (Pinto, R. M. A. 2006, Pinto, R. M. A. 2009) proving the authenticity of our results.

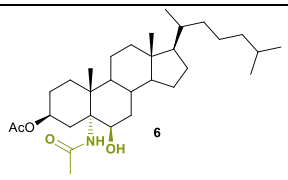
*Table 14- ^{1}H-NMR data of the vic-N-acylaminohydroxyl compounds 2, 4 and 6**



2



4



6

Position	δH	Position	δH	Position	δH
3	4.82, m, 3α-H	3	4.83, m, 3α-H	3	4.83, m, 3α-H
3β-OCOCH$_3$	1.99, s, CO<u>CH$_3$</u>	3β-OCOCH$_3$	2.00, s, CO<u>CH$_3$</u>	3β-OCOCH$_3$	2.00, s, CO<u>CH$_3$</u>
5	-	5	-	5	-
5β-NHCOCH$_3$	NH: 5.21, s CO<u>CH$_3$</u>: 2.00, s	5β-NHCOCH$_3$	NH: 5.15, s CO<u>CH$_3$</u>: 2.00, s	5β-NHCOCH$_3$	NH: 5.15, s CO<u>CH$_3$</u>: 2.00, s
6	4.75, s, 6α-H	6	4.69, s, 6α-H	6	4.69, s, 6α-H
17	-	17	2.52, t, 8.64Hz, 17α-H	17	-
18	0.88, s, 18-H$_3$	18	0.64, s, 18-H$_3$	18	0.68, s, 18-H$_3$
19	1.34, s, 19-H$_3$	19	1.31, s, 19-H$_3$	19	1.30, s, 19-H$_3$
-	-	21	2.12, s, 21-H$_3$	21	0.09, d, 4.99Hz, 21-H$_3$
-	-	-	-	26 and 27	0.85 and 0.86, d, 5.74Hz, 26-H$_3$ and 27-H$_3$

*^{1}H-NMR: δ ppm, multiplicity, J in Hz; NMR samples prepared in CDCl$_3$. Signals for 3β-OCO<u>CH$_3$</u> and 5β-NHCO<u>CH$_3$</u> may be reversed, attribution made based on literature values(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009). Signals for 26-H$_3$ and 27-H$_3$ may be reversed.

After a thorough analysis of the ^{1}H-NMR spectrum of the compound **8** (**Figure 10**), we realized that, although the reaction occurred and the peaks were accordingly to the ones in literature(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009), the integration values presented in the spectrum were higher than what they suppose to be.

For instance, the peaks corresponding to the 18-H$_3$ and 19-H$_3$ (0.69 and 1.31 respectively) should have values of integration similar to 3 but their values are 6.10 and 6.07, correspondingly. The same happens in the other peaks suggesting the existence of another compound with similar structure to the final product.

The double doublet signal at 5.66ppm corresponds to the 6α-H of compound **9** and due to its integration value we could verify that the ratio between compound **8 and 9** is 65:35.

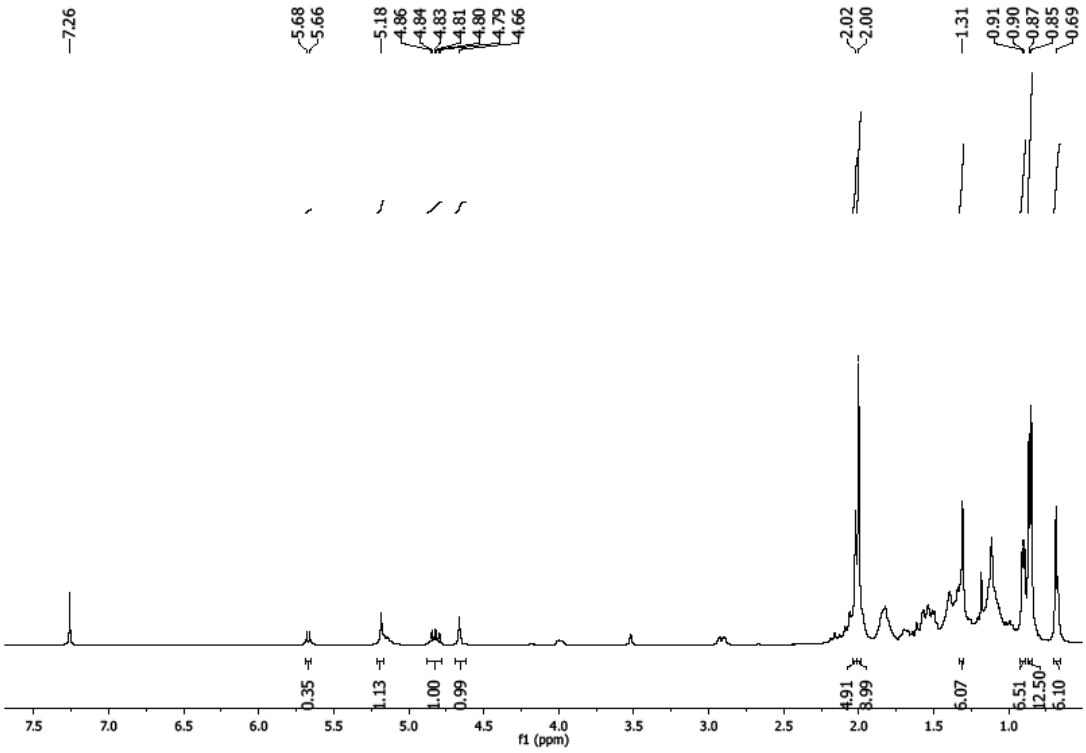


Figure 10. ^{1}H-NMR Spectrum (400MHz) of compound 8. Peak values on top of the spectrum and the integration values on the bottom.

Compound **10** was synthesized for the first time in the course of this work. We did not find in the literature any reference to this compound. Therefore, a meticulous characterization by different spectroscopic techniques was performed with the aim of verifying the results.

The 1H-NMR spectrum (**Figure 11**) of compound **10** showed signals at 0.69ppm (3H, s, 18-H_3), 0.85ppm and 0.86ppm (3H each, 2d, J=5.16 Hz, 26-H_3, 27-H_3), 0.90ppm (3H, d, J= 6.48 Hz, 21-H_3), 1.32ppm (3H, s, 19-H_3), corresponding to the protons of the methyl groups in the methylene envelope.

The signals at 1.99ppm (3H, s), 4.64ppm (1H, s) and 4.81ppm (1H, m) represented the methyl group of the acetoxy group at C_3, the 6α-H and the 3α-H, respectively. The signals corresponding to the protons of the methyl group of the acetamide moiety were visible at 2.20ppm (3H, s), and the signal of the H of the secondary amide group was present at 7.16ppm (1H, s). Signals for 3β-OCO$\underline{CH_3}$ and 5β-NHCO$\underline{CH_3}$ may be reversed since its attribution was made based on literature values(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009).

The ^{13}C-NMR spectrum (**Figure 12**) showed the presence of 32 carbons in the molecule and, according to the literature(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009), the signals at 170.65ppm and 167.09ppm correspond to the carbonyl carbons of the acetoxy group in C_3 and to the acetamide moiety. The absence, in the DEPT spectrum (**Figure 13**), of these signals confirmed the previous correspondence, since signals from carbons without attached protons are nonexistent in this kind of spectrum.

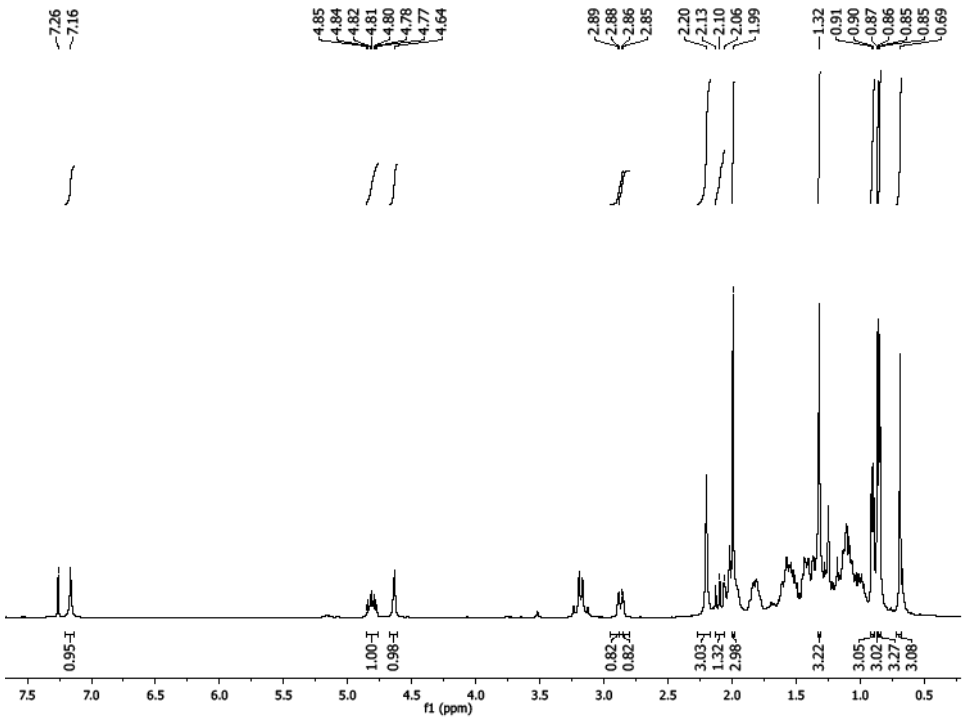


Figure 11. ^{1}H-NMR Spectrum (400MHz) of compound 10. Peak values on top of the spectrum and the integration values on the bottom. Sample with traces of ethyl acetate, presenting a quartet at 3.20ppm ($\underline{CH_2}CH_3$), a singlet at 2.02ppm ($\underline{CH_3}CO$) and a triplet at 1.25ppm ($CH_2\underline{CH_3}$)(Gottlieb, H. E. 1997).

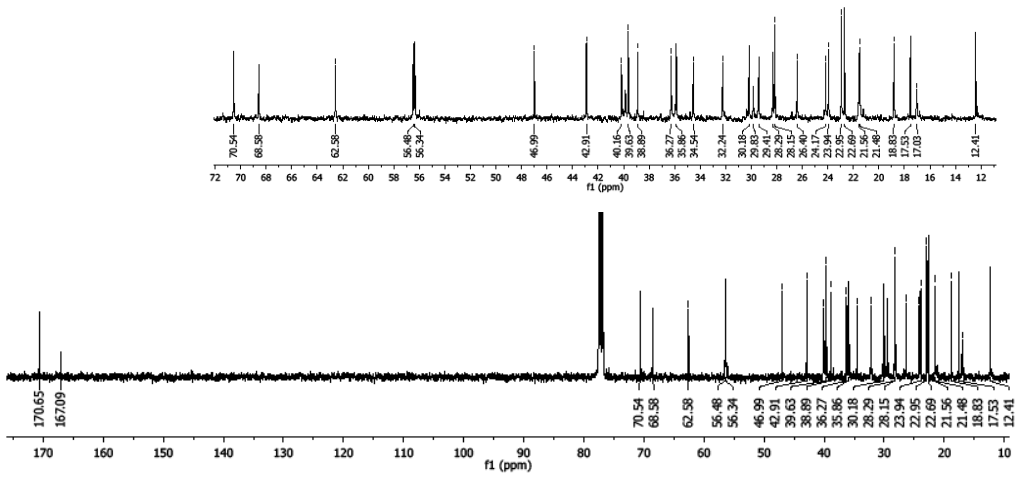


Figure 12. ^{13}C-NMR Spectrum (100MHz) of compound 10. Peak values on the bottom of the spectrum.

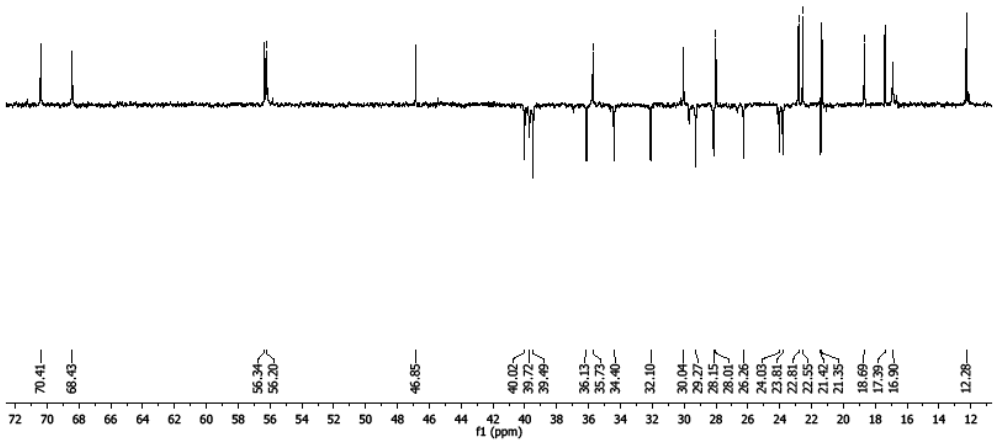


Figure 13. DEPT Spectrum (100MHz) of compound 10. Peak values on the bottom of the spectrum.

The analysis of the NOESY spectrum (**Figure 12**) allowed us to assign the signals at 2.87ppm (dd) and at 2.10ppm (t). A nOe interaction was found between 3α-H (4.81ppm) and the signal at 2.87ppm and at 2.10ppm. In addition, the signal at 2.87ppm showed another cross-peak with the signal at 2.10ppm.

In **Figure 13** we can verify that the 3α-H has nOe interactions with 2α-H (yellow arrow), 4α-H and the protons of the NH_2 group of the acetamide moiety (blue arrows). One the other hand, the protons in the NH_2 group have a nOe correlation with 4α-H.

These findings, together with the integration values of the ^{1}H-NMR spectrum, enabled us to assign the signals at 2.87ppm (dd, 2H) and at 2.10ppm (t, 1H) to the protons of the CH_2 of the acetamide moiety and to the 4α-H, respectively.

ESI-MS spectrum (**Figure 16**) of compound **10** showed a molecular ion peak at 550.37m/z corresponding to the molecular weight of the compound plus one proton (M+1).

The information provided by the spectral analysis confirmed that the structure of compound **10** was in accordance with the one presented in **scheme 9**.

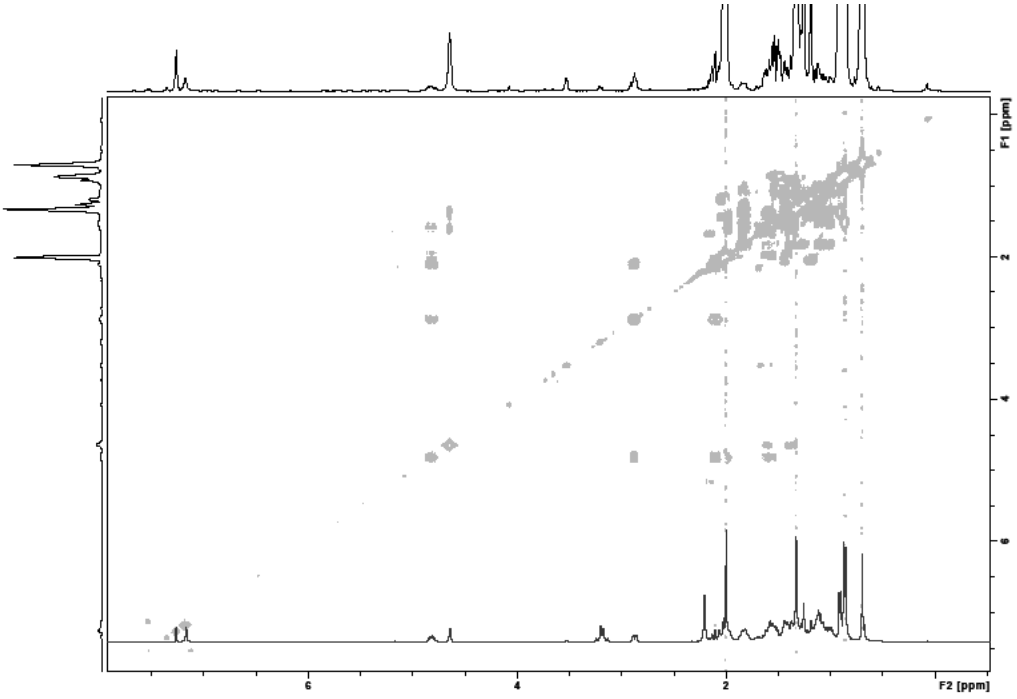


Figure 14. NOESY Spectrum of compound 10.

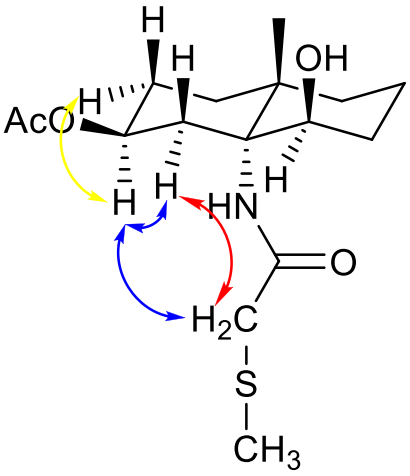


Figure 15. Selected nOe correlations for the 3*α*-H (4.81ppm) of compound 10.

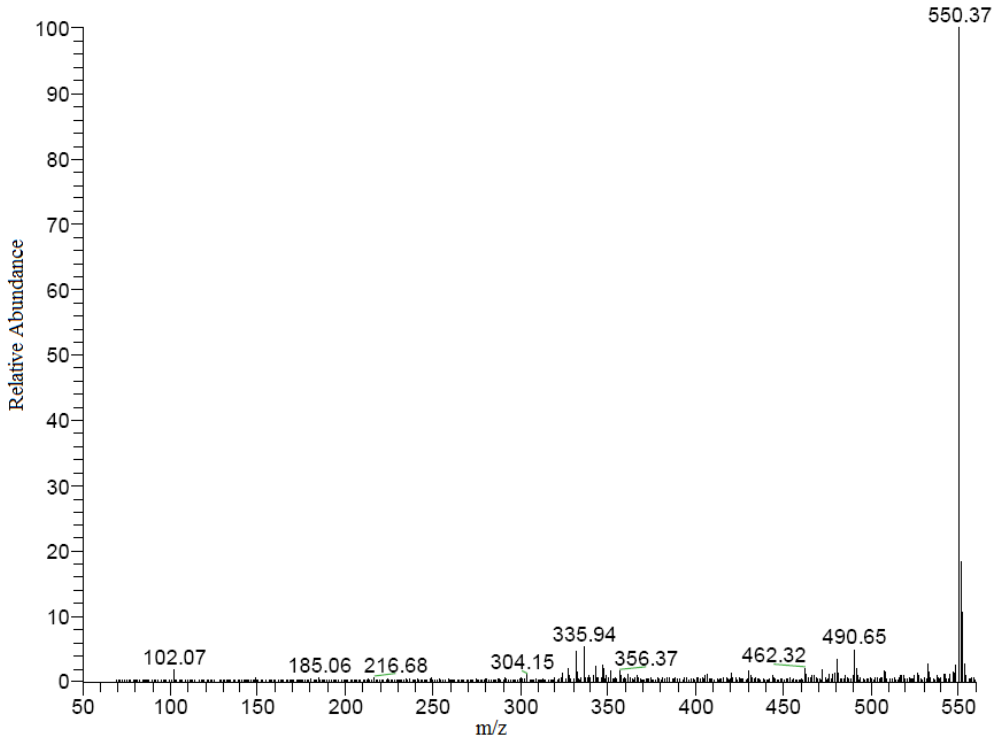
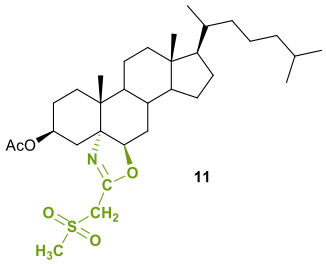


Figure 16. ESI-MS Spectrum of compound 10 ($C_{32}H_{55}NO_4S$, MW= 549.34).

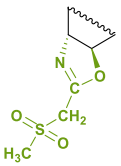
Compound **11** is also new, so a detailed analysis was made to prove its authenticity. In this case an oxazoline was obtained instead of an acetamide. In the ^{1}H-NMR spectrum (**Figure 17**) there was not a signal for the NH group of the acetamide moiety, but the signals for the CH_2 (4.03ppm) and the CH_3 (3.21ppm) were present.

In the ^{13}C-NMR spectrum (**Figure 18**) there were 32 signals corresponding to the 32 carbons of the molecule and the DEPT spectrum **(Figure 19)** confirmed the nature of the different carbon. The HMQC experiment (**Figure 20**) allowed us to correlate between the protons and the carbons in the different spectra and to elaborate Table **15**.

*Table 15- Selected ^{1}H-NMR and ^{13}C-NMR data of compound 11 based on the analysis of the HMQC experiment**



11

Position	δH	δC
3	5.14, m, 3α-H	-
3β-OCOCH$_3$	2.02, s, CO<u>CH$_3$</u>	CO<u>C</u>H$_3$: 21.59
4	2.14, t, 24.10Hz, 4α-H	37.08
5	-	-
6	3.52, s, 6α-H	76.29
18	0.67, s, 18-H$_3$	12.27
19	1.18, s, 19-H$_3$	16.84
21	0.90, d, 6.23Hz, 21-H$_3$	18.79
26 and 27	0.85 and 0.86, d, 6.02Hz, 26-H$_3$ and 27-H$_3$	-
	CH_2: 4.03, s CH_3: 3.21, s	CH_2: 44.02 CH_3: 41.06

*^{1}H-NMR: δ ppm, multiplicity, J in Hz; ^{13}C-NMR: δ ppm; DEPT: δ ppm; NMR samples prepared in $CDCl_3$. Signals for 26-H$_3$ and 27-H$_3$ may be reversed.

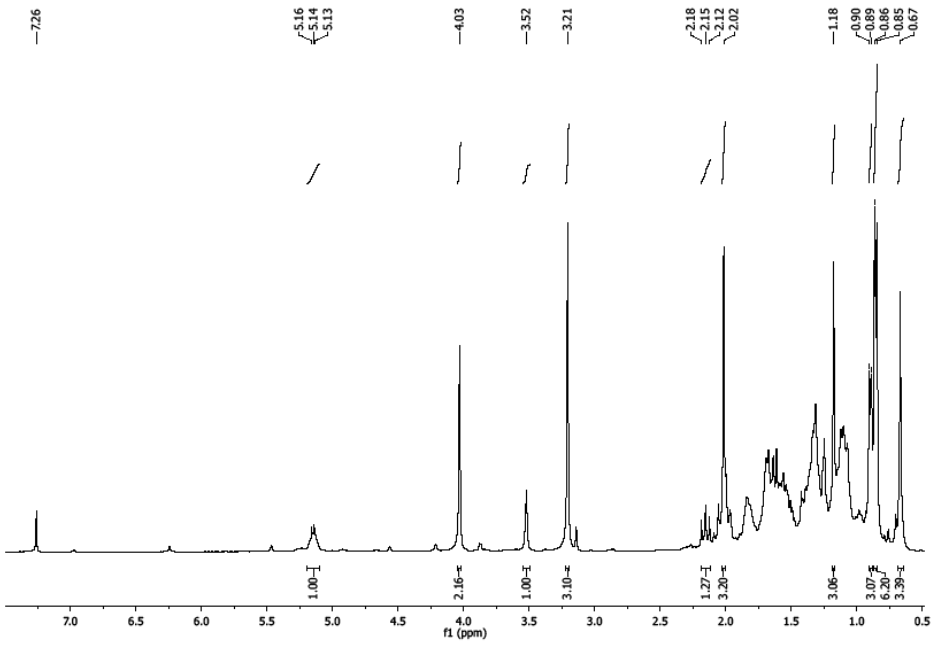


Figure 17. [1]H-NMR Spectrum (400MHz) of compound 11. Peak values on top of the spectrum and the integration values on the bottom.

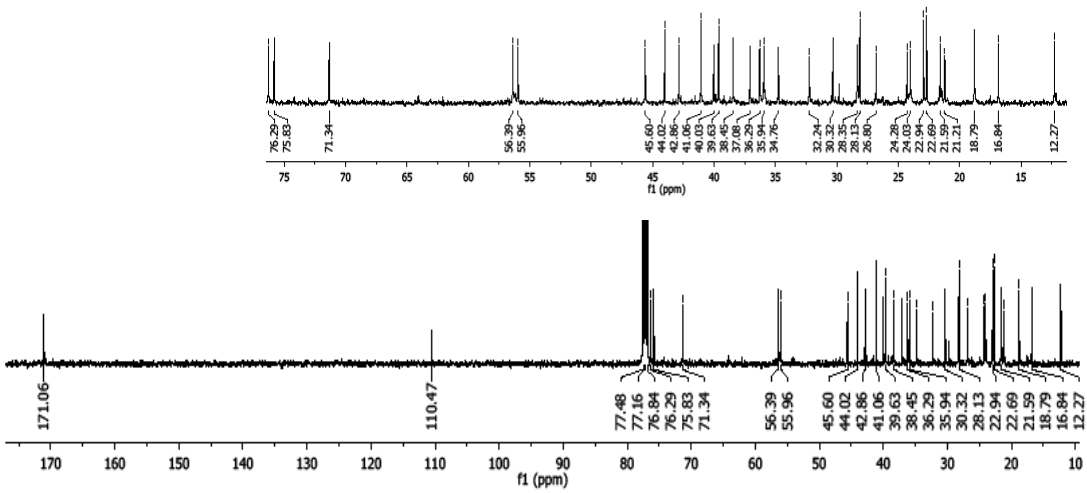


Figure 18. [13]C-NMR Spectrum (100MHz) of compound 11. Peak values on the bottom of the spectrum.

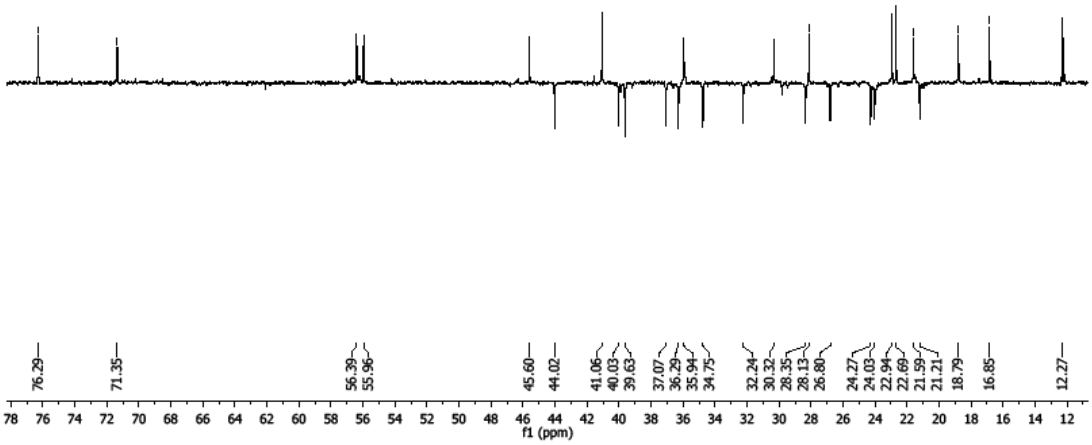


Figure 19. DEPT Spectrum (100MHz) of compound 11. Peak values on the bottom of the spectrum.

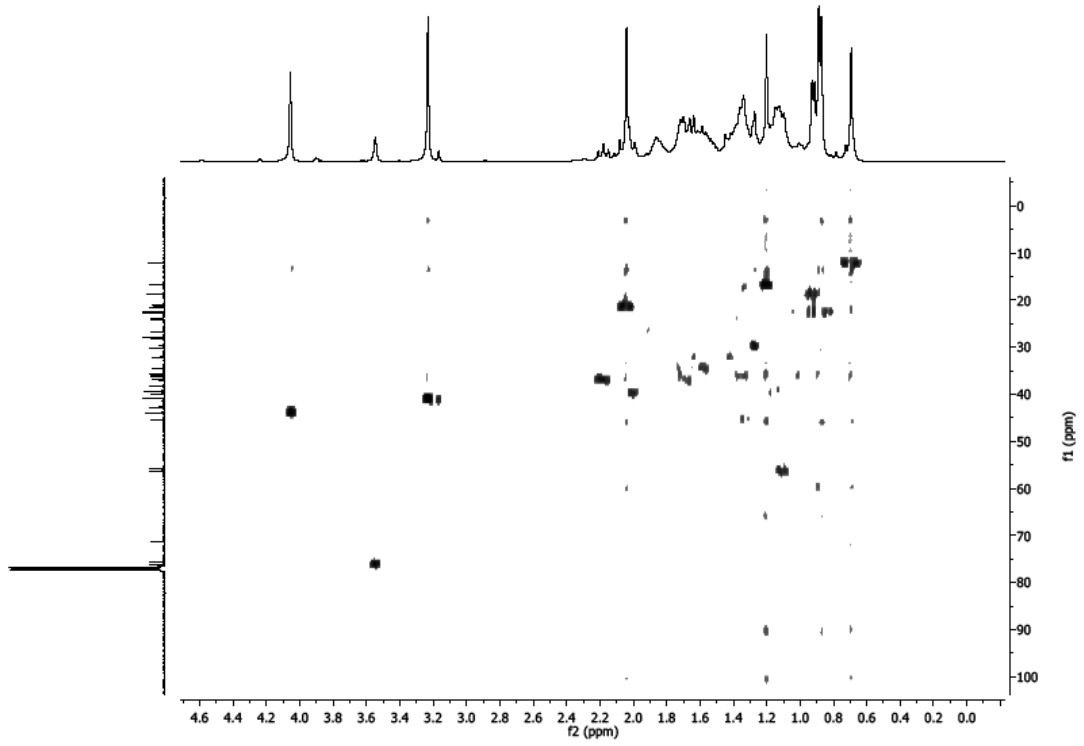


Figure 20. HMQC Spectrum of compound 11. Horizontal axis represent the ^{1}H-NMR spectrum and the vertical axis represent the ^{13}C-NMR spectrum.

The imine carbon of the oxazoline moiety was attributed to the signal at 110.47ppm in the ^{13}C-NMR due to a cross-peak between this carbon and the protons of the CH_2 group (4.03ppm) observed in the HMBC experiment (**Figure 21,** red). The cross-peak between the protons of the methyl group at 2.02ppm and the carbon at 171.06ppm confirmed that this signal corresponded to the carbonyl carbon of the acetoxy group in C_3 (violet). The absence of these signals in the DEPT spectrum also confirmed these attributions.

The analysis of the cross-peaks between the 19-H_3 and the carbons at 32.24ppm, 38.45ppm and 45.60ppm and the data in the DEPT spectrum allowed us to assign these signals to the C_1, C_{10} and C_9, respectively (blue). Because 19-H_3 and 6α-H both have a cross-peak with the carbon at 76.84ppm, we could confirm that this carbon is the quaternary carbon C_5 (blue and green). The cross-peak between the 4α-H and the carbon at 31.75ppm confirmed that this signal corresponds to C_3 (orange).

The carbons C_{12}, C_{13} and C_{14} were attribute to the signals at 39.63ppm, 42.86ppm and 55.96ppm, respectively, due to the interaction between 18-H_3 and the carbons at these signals (black) and the data in the DEPT spectrum. C_{17} was assigned to the signal at 56.39ppm because both 18-H_3 and 21-H_3 interact with the carbon at this signal (black and yellow). The cross-peak between 21-H_3 and the carbon at 35.94ppm, along with the data from the DEPT experiment, allowed us to attribute this signal to the carbon C_{20}.

The results obtained from the NMR Techniques were in agreement with what was expected for compound **11** and confirmed the structure presented in **Scheme 10**.

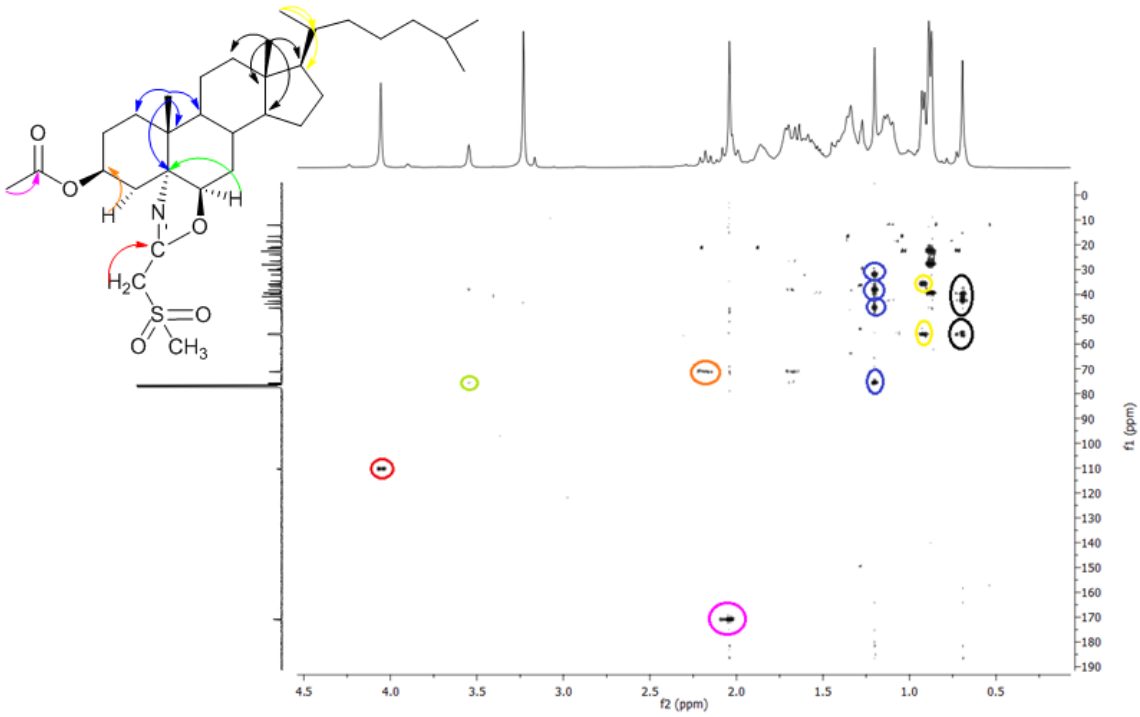


Figure 21. HMBC Spectrum of compound 11. Horizontal axis represent the ^{1}H-NMR spectrum and the vertical axis represent the ^{13}C-NMR spectrum.

3.2.1. General Conclusions

Based on the previous information, we were able to conclude that Starbon®400-SO_3H catalyzes the conversion of epoxysteroids into *vic*-*N*-acylamino-hydroxysteroids under Ritter reaction conditions, in high yields and with low reaction times.

Although the reaction occurred with a recycled catalyst, it was necessary to increase the percentage of catalyst and/or the reaction time. Water has a major role in the Ritter reaction, as its absence was able to increase the reaction time or even prevent the progression of the reaction.

The catalyst was more effective in the ring opening of 5β,6β-epoxysteroids than in that of 5α,6α-epoxysteroids, as in the first case there was only one reaction product and in the second one there was a mixture of stereoisomers.

The reactions proceeded well in the presence of other functional groups in the substrate, such as esters and ketones, proving the chemo-, regio- and stereoselectivity of this reaction. The nucleophilic attack invariably occurred at C6 in 5α,6α-epoxysteroids and at C5 in 5β,6β-epoxysteroids.

When 1,4-Dioxane was used as a solvent, the final product was in the oxazoline form.

3.2.2. Future challenges

To make this experimental work more precise, it would be of interest to replace the 1,4-dioxane by a greener solvent, as this solvent does not fulfill the Green Chemistry parameters. The GSK Solvent Selection Guide(Henderson, R. K. 2011) will provide suitable alternatives.

Another pertinent approach would be testing the catalytic power of Starbon®400-SO_3H in steroid compounds with functional groups other than epoxides, such as alcohols or aldehydes, under Ritter reaction conditions.

Furthermore, Starbon®400-SO_3H could also be used in compounds other than steroids for which the Ritter reaction is suitable, or in other acid catalysis reactions.

Considering the pharmaceutical relevance of the *vic*-*N*-acylamino-hydroxysteroids and of the oxazoline compounds it would be of significance to test the biological activity of the compounds produced.

4. Concluding Remarks

Our future challenges regarding resource, environmental, economic and societal sustainability demand the development of new scientific technologies for working with chemical processes and products. Green Chemistry addresses such challenges by providing the guidelines for novel reactions that can maximize the production of the desire products and minimize by-products, with the aim of designing new synthetic routes and instrumentation that can simplify operations in chemical production, as well as seeking greener solvents that are environmentally and ecologically benign.

Catalysis is an important tool for drug discovery and for the development of industrial synthesis, as it is a powerful instrument for the design of environmentally friendly, cheap and competitive processes. The use of the 12 principles for process design is a standard approach in the pharmaceutical industry and catalysis is the most efficient and green technology available. Therefore, the scientific efforts for the discovery of new reactions, catalysts or ligands are critical for the development of efficient and competitive green processes.

Steroid chemistry has been the focus of research in the past years. Every day there are reports of the isolation, characterization and synthesis of new compounds and of new biological evaluations. The attention given to steroid molecules is justified by their biological properties that make them useful in medicine and pharmacy.

Although there is an abundance of classical chemical reactions with wide applicability in steroid chemistry, these reactions often suffer from disadvantages, such as handling toxic, sensitive and/or expensive reagents, difficult work-ups, low yields, weak selectivities and lack of catalytic methods. For these reasons, the development of new catalytic chemical processes in steroid chemistry that use environmentally friendly, cheap and easily available reactants, as well as mild reaction conditions would be of great interest.

Taking into account the previous considerations, the work presented on this Thesis was based in the development of new environmentally friendly chemical processes using steroids as substrates. Starbon®400-SO_3H was chosen as catalyst due to the following features: Starbon®400-SO_3H has shown good catalytic power in many organic chemical reactions; it is a safe, non-toxic, organic catalyst; there is no record of its use in steroid chemistry.

Epoxysteroids are readily available substrates obtained by one-step procedures from the corresponding olefinic steroids. Various selective epoxidation procedures have been reported to obtain diastereomerically pure epoxides, in high yields. These substrates were used to study the reactivity of Starbon®400-SO_3H to catalyze the nucleophilic ring opening of epoxides.

In Chapter II we described the formation of *vic*-*N*-acyloamino-hydroxy compounds via the *trans*-dial ring opening of epoxysteroids catalyzed by Starbon®400-SO_3H under Ritter reaction conditions. These transformations presented high yields and good reaction times.

Even though the catalyst lost some of its activity when recycled, the reaction still occurred but with a longer reaction time. Water had a preponderant role in the Ritter reaction and the solvent used was important for the outcome of the reaction.

In summary, the results presented in this Thesis highlight the potential of Starbon®400-SO_3H as a renewable, sustainable and ecofriendly catalyst in transformations involving steroids as substrates. Acid Starbon® materials have proven to be very effective in acid catalyzed reactions.

III. Experimental

1. General Methods

1.1. Reagents and Solvents

Starbon®400-SO_3H was provided by Prof. James H. Clark from the Green Chemistry Centre of Excellence in The University of York.

General reagents, such as acetonitrile (MeCN), methylthioacetonitrile (CH_3SCH_2CN), and methysulfonylacetonitrile ($CH_3SO_2CH_2CN$) were obtained from Sigma-Aldrich Co.

Solvents were obtain from VWR Portugal; when necessary, solvents were purified according to standard procedures(Perrin, D. D. 1988).

Steroid compounds that were used in the preparation of epoxysteroids, namely cholesterol, dehydroepiandrosterone (DHEA) and pregnenolone were purchased from Sigma-Aldrich Co.

5α,6α-Epoxysteroids were prepared from the corresponding Δ^5-steroid via epoxidation with *m*-chloroperbenzoic acid (*m*CPBA)(Matthews, G. J. 1972). 5β,6β-Epoxysteroids were obtained by β-selective epoxidation of Δ^5-steroids using a method developed by Salvador et al.(Salvador, J. A. R. 1996). Acetylations were carried out at r.t., with Ac_2O (1.5eq), in dry THF, using 2.5 mol % of DMAP as a catalyst.

1.2. Chromatography and Purification Techniques

1.2.1. TLC

Thin Layer Chromatography (TLC) was done in commercial Kieselgel 60 F_{254}/Kieselgel 60G Merck TLC plates, purchased from VWR Portugal. The development was done with ethyl acetate/ petroleum ether in a v/v proportion suitable for each product, followed by heating at 150ºC in an appropriate heating plate. When pertinent, the TLC plates were observed at UV light (254nm).

1.2.2. Flash Column Chromatography

Silica gel 60 (230-400 mesh) was used for flash column chromatography. The crude mixture was immobilized in silica gel, and then transferred to the column, previously filled with stacked silica gel 60 (230-400 mesh). Analytical grade solvents were used for the preparation of the eluents. The eluent is indicated as v/v proportions. The control was done by TLC.

1.2.3. Recrystallizations

Recrystallizations were made following the standard procedures. Analytical grade solvents were used to afford crystals at r.t.(Furniss, B. S. 1989).

1.2.4. Recycling of the Catalyst

After filtration of the final solution to separate the catalyst, Starbon®400-SO_3H was washed with acetone and diethyl ether and dried, during two days, in a high vacuum stove. The recycled catalyst was then used in the same reaction than before to evaluate its catalytic power.

2. Analytical Instrumentation

2.1 Nuclear Magnetic Resonance

^{1}H, ^{13}C NMR, NOESY, HMBC and HMQC experiments were performed on a Bruker Avance 300 MHz equipped with a BBO-ATMA 5mm probe.

NMR samples were prepared in $CDCl_3$ solution and calibration of chemical shift scale was made using the $CDCl_3$ signal at 7.26 ppm (^{1}H NMR) or 77.0 ppm (^{13}C NMR). Chemical shifts (δ) are given in ppm and coupling constants (J) are presented in Hz.

2.2 Mass Spectroscopy

Electrospray ionization mass spectroscopy ESI-MS was made on a Quadrupole *Ion Trap* Mass Spectrometer (QIT-MS) (LCQ Advantage MAX, THERMO FINNINGAN). The samples were dissolved in MeOH- $CHCl_3$ (80-20).

2.3 Melting Poits

Melting points were determined on a Buchi Melting Point B-540.

3. Ritter Reaction Catalyzed by Starbon®400-SO_3H

3.1 *5α-Acetamido-6β-hydroxy-17-oxoandrostan-3β-yl acetate 2*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5β,6β-epoxy-17-oxoandrostan-3β-yl acetate 1 in dry MeCN. The reaction was maintained under magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated under reduced pressure in a rotative evaporator to give compound 2, as a white solid. TLC [ethyl acetate/petroleum ether (3:1 v/v)] revealed two products, the major one appearing at R_f = 0.18; The by-product was not detectable by NMR spectroscopy; Mp (ºC): 209.5-210.5; ^1H NMR (400MHz, $CDCl_3$) δ (ppm): 0.88 (3H, s, 18-H_3), 1.34 (3H, s, 19-H_3), 1.99 (3H, s, CH_3COO), 2.00 (3H, s, CH_3CONH), 4.75 (1H, m, 6α-H), 4,82 (1H, m, 3α-H), 5.21 (1H, s, NH) (Pinto, R. M. A. 2006, Pinto, R. M. A. 2009).

3.2 *5α-Acetamido-6β-hydroxy-20-oxopregnan-3β-yl acetate 4*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5β,6β-epoxy-20-oxopregnan-3β-yl acetate 3 in dry MeCN. The reaction was maintained under magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated

under reduced pressure in a rotative evaporator to give compound 4, as a white solid. TLC [petroleum ether/ethyl acetate (4:3 v/v)] revealed two products, the major one appearing at R_f = 0.27; The by-product was not detectable by NMR spectroscopy; Mp (ºC): 136.1-138.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.64 (3H, s, 18-H_3), 1,31 (3H, s, 19-H_3), 2.00 (3H, s, CH_3COO), 2.00 (3H, s, CH_3CONH), 2.12 (3H, s, CH_3CO), 2.52 (1H, t, J= 8.64Hz, 17α-H) 4.69 (1H, m, 6α-H), 4,83 (1H, m, 3α-H), 5.15 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 209.48, 170.75, 169.37, 70.63, 68.29, 63.77, 62.70, 56.44, 46.76, 44.38, 39.03, 38.55, 34.46, 32.11, 31.60, 30.23, 29.32, 26.33, 25.06, 24.38, 23.04, 21.51, 21.51, 17.51, 13.74(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009).

3.3 *5α-Acetamido-6β-hydroxycholestan-3β-yl acetate 6*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5β,6β-epoxycholestan-3β-yl acetate (5) in dry MeCN. The reaction was maintained under magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated under reduced pressure in a rotative evaporator to give compound 6, as a white solid. TLC [petroleum ether/ethyl acetate (2:1 v/v)] revealed two products, the major one apearing at R_f = 0.20; The by-product was not detectable by NMR spectroscopy; Mp (ºC): 109.0-110.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.68 (3H, s, 18-H_3), 0.85 and 0.86 (3H each, 2d, J=5.74 Hz, 26-H_3, 27-H_3), 0.90 (3H, d, J= 4.99 Hz, 21-H_3), 1,30 (3H, s, 19-H_3), 2.00 (3H, s, CH_3COO), 2.00 (3H, s, CH_3CONH), 4.66 (1H, s, 6α-H), 4,83 (1H, m, 3α-H), 5.16 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 170.72, 169.31, 70.76, 68.57, 62.64, 56.40, 56.34 46.91, 42.89, 40.04, 39.63, 38.48, 36.27, 35.88, 34.51, 32.11, 30.17, 29.35, 28.31, 28.16, 26.39, 25.07, 24.16, 23.94, 22.94, 22.70, 21.56, 21.52, 18.84, 17.53, 12.40(Pinto, R. M. A. 2006, Pinto, R. M. A. 2009).

3.4 *6β-Acetamido-5α-hydroxycholestan-3β-yl acetate 8*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5α,6α-epoxycholestan-3β-yl acetate (7) in dry MeCN. The reaction was maintained under

magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated under reduced pressure in a rotative evaporator to give compound 8, as a white solid. TLC [petroleum ether/ethyl acetate (2:1 v/v)] revealed two products, the major one appearing at R_f = 0.38; The by-product was detectable by NMR spectroscopy, the stereoisomer 9; Mp (ºC): 107.4-114.0; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.69 (3H, s, 18-H_3), 0.85 and 0.87 (3H each, 2d, J= 6.4 Hz, 26-H_3, 27-H_3), 0.90 (3H, d, J= 5.29 Hz, 21-H_3), 1,31 (3H, s, 19-H_3), 2.00 (3H, s, CH_3COO), 2.02 (3H, s, CH_3CONH), 4.66 (1H, m, 6α-H of compound 8), 4,83 (1H, m, 3α-H), 5.18 (1H, s, NH), 5.67 (1H, dd, J= 4.74Hz and 9.24Hz, 6α-H of compound 9) (Pinto, R. M. A. 2006, Pinto, R. M. A. 2009);

3.5 *5α-methylthioAcetamido-6β-hydroxycholestan-3β-yl acetate 10*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5β,6β-epoxycholestan-3β-yl acetate (5) in CH_3SCH_2CN. The reaction was maintained under magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated under reduced pressure in a rotative evaporator to give compound 10, but the reagent did not evaporate so it dried over time in a high vacuum stove. TLC [petroleum ether/ethyl acetate (2:1 v/v)] revealed two products, the major one appearing at R_f = 0.18; The by-product was not detectable by NMR spectroscopy; Mp (ºC): 161.9-164.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.69 (3H, s, 18-H_3), 0.85 and 0.85 (3H each, 2d, J=5.16 Hz, 26-H_3, 27-H_3), 0.90 (3H, d, J= 6.48 Hz, 21-H_3), 1,32 (3H, s, 19-H_3), 1.99 (3H, s, CH_3COO), 2.10 (1H, t, J= 24.90 Hz, 4-αH), 2.20 (3H, s, CH_3SCH_2OCNH), 2.87 (2H, dd, J_1= 7.69 and J_2= 4.71, CH_3SCH_2OCNH), 4.64 (1H, m, 6α-H), 4,81 (1H, m, 3α-H), 7.16 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 170.65, 70.54, 68.58, 62.58, 56.48, 56.34, 45.99, 42.91, 40.16, 39.85, 39.63, 38.89, 36.27, 35.85, 34.54, 32.24, 30.18, 29.83, 29.41, 28.29, 28.15, 26.40, 24.17, 23.94, 22.95, 22.69, 21.56, 21.48, 18.83, 17.53, 17.03, 12.41; ESI-MS m/z (%): 550 (100) M+1, 490 (5), 462 (2), (356) (2), 335 (5), 304 (2), 216 (1), 185 (1), 102 (2).

3.6 *5α,6β –methylsulfonylmethylene oxazole-cholestan-3β-yl acetate 11*

Starbon®400-SO_3H (HSO_3 loading 0,5mmol g^{-1}) was added and suspended into a solution of 5β,6β-epoxycholestan-3β-yl acetate (5) with $CH_3SO_2CH_2CN$ (solid nitrile) in 1,4-dioxane. The reaction was maintained under magnetic stirring, at r.t., until it was completed. This was verified by TLC control. The solution was then filtered to separate the catalyst. The remaining solution was concentrated under reduced pressure in a rotative evaporator to give compound 11 and the excess solid nitrile. The solid material was diluted in diethyl ether (max 50mL) and washed two times with 30mL of distilled water. The organic phase was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure in a rotative evaporator to give compound 1.10, as a white solid; TLC [petroleum ether/ethyl acetate (2:1 v/v)] revealed two products, the major one appearing at R_f = 0.43; The by-product was not detectable by NMR spectroscopy; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.67 (3H, s, 18-H_3), 0.85 and 0.85 (3H each, 2d, J=6.02 Hz, 26-H_3, 27-H_3), 0.90 (3H, d, J= 6.23 Hz, 21-H_3), 1,18 (3H, s, 19-H_3), 2.02 (3H, s, CH_3COO), 2.15 (1H, t, J= 24.10 Hz, 4-αH), 3.21 (3H, s, $CH_3O_2SCH_2CNO$), 3.52 (1H, m, 6α-H), 4.03 (2H, s, $CH_3O_2SCH_2CNO$), 5.14 (1H, m, 3α-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 171.05, 110.47, 76.29, 75.83, 71.34, 56.39, 55.95, 45.60, 44.02, 42.85,41.05, 40.03, 39.63, 38.45, 37.08, 36.29, 35.94, 34.76, 32.24, 30.32, 28.35, 28.13, 26.80, 24.28, 24.03, 22.94, 22.69, 21.59, 21.21, 18.79, 16.84, 12.27; Further analytical data is being acquired.

IV References

Aldib, I.; Soubhye, J., Boudjeltia, K. Z. *et al*. (2012) Evaluation of new scaffolds of Myeloperoxidase inhibitors by rational design combined with High-Throughput Virtual screening. J. Med. Chem.; 55: 7208-7218;

Anastas, P.T. & Warner, J.C., (1998). *Green Chemistry, Theory and Practice*. *Oxford University Press*, NY, USA, pages: 1-19;

Anastas, P.T.; Williamson, T.C., Eds.; Green Chemistry: Frontiers in Benign Chemical Syntheses and Processes; *Oxford University Press:* Oxford, UK, 1998;

Anderson, N., (2000). Practical process research and development. *Academic Press*: New York;

Argyropoulos, D.S.; et al (2007). Materials, Chemicals, and Energy from Forest Biomass. ACS Symposium Series 954, *American Chemical Society*, Washington DC;

Armor, J.N., (2011). A history of industrial catalysis. *Catalysis Today,* 163: 3-9;

Atkin, N.; Abeysekera, R. & Robards, A.W., (1998). The events leading to the formation of ghost remnants from the starch granule surface and the contribution of the granule surface to the gelatinization endotherm. *Carbohydr. Polym*., 36: 193-204;

Atkin, N.; Abeysekera, R.; Cheng, S.L.; *et al*., (1998). An experimentally-based predictive model for the separation of amylopectin subunits during starch gelatinization. *Carbohydr. Polym*., 36: 173-192;

B. D. Feske, J. D. Stewart; (2005) Chemoenzymatic formal total synthesis of (-)-bestatin. Tetrahedron: Asymmetry, 16, 3124–3127;

Baran, P.S.; Maimone, T.J. & Richter, J.M., (2007). Total synthesis of marine natural products without using protecting groups. *Nature*, 446: 404–408;

Baumann, F.T.; Satcher, J.H.; (2003) Homogeneous Incorporation of Metal Nanoparticles into Ordered Macroporous Carbons *Chem. Mater*.; 15, 3745–3747;

Baxendale, I.R. ; Deeley, J.; Griffiths-Jones, C.M.; *et al.*, (2006). A flow process for the multi-step synthesis of the alkaloid natural product oxomaritidine: a new paradigm for molecular assembly. *Chem Commun,* 24: 2566–2668;

Bazula, A.; Lu, A.; Nitz J.; *et al*., (2008). Surface and pore structure modification of ordered mesoporous carbons via a chemical oxidation approach. *Microporous Mesoporous Mater*, 108: 266–275;

Beck, J.S.; Vartuli, J.C.; Roth, W.J.; *et al*., (1992). A new family of mesoporous molecular sieves prepared with liquid crystal templates. *J. Am. Chem. Soc.,* 114: 10834–10843;

Beck, J.S.; Vartuli, J.C.; Roth, W.J.; et al., (1992). Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *Nature,* 359: 710–712;

Beckman, E.J., (2003). Oxidation reactions in CO2: Academic exercise or future green processes? *Environ Sci Technol,* 37: 5289–5296;

Benson, F.R.; Ritter, J.J; (1951) A new reaction of nitriles. 3. Amides from dinitriles. *J. Am.Chem.Soc*; 73; 4076-4077;

Bergbreiter, D.E., (1998). The use of soluble polymers to effect homogeneous catalyst separation and reuse. *Catal Today*, 42: 389-397;

Bergmeier, S.C., (2000). The synthesis of vicinal amino alcohols. *Tetrahedron*, 56: 2561-2576;

Bergmeier, S.C.; (2000) The synthesis of vicinal amino alcohols. *Tetrahedron*; 56, 2561-2576;

Berkeley W. Cue; Ji Zhang; (2009) Green process in the pharmaceutical industry. *Green Chemistry Letters and Review;* vol.2, no.4, 193-211;

Bhadury, S & Mukesh, D. *Homogeneous Catalysis: Mechanisms and Industrial Applications*. *John Wiley & Sons*: New York, Chichester, Weinheim, Brisbane, Singapore, Toronto, 2000. Chapters 1 and 2.

Bourgery, G.; Frankel, J.J.; Ryans, R.J.; Julia, S.; (1972) Applications of ritter reaction in steroid series. 1. Preparation of trans-acetamido-alcohols from 5,6-epoxides: conformation of amide group. *Tetrahedron*; 28, 1377-1390;

Bower, J.F.; Patman, R.L. & Krische, M.J., (2008). Iridium-catalyzed C-C coupling via transfer hydrogenation: Carbonyl addition from the alcohol or aldehyde oxidation level employing 1,3-cyclohexadiene. *Org Lett.,* 10: 1033–1035;

Britz, D.A.; Khlobystov, A.N. ; Wang, J.; *et al.*, (2004). Selective host-guest interaction of single-walled carbon nanotubes with functionalised fullerenes. *Chem Commun*., 2: 176–177;

Brueggemeier, R.W. & Li, P.K., (2003). *Burger's Medicinal Chemistry and Drug Discovery: Cardiovascular agents and endocrines,* 6th ed. Vol:3; *John Wiley & Sons, Inc*: New York, pages: 594-627;

Brueggemeier, R.W.; Li, P.-K.; (2003) Fundamentals of steroid chemistry and biochemistry. *Burger's Medicinal Chemistry and Drug Discovery, Vol:3 cardiovascular agents and endocrines,* 6th ed.; Abraham, D.J.; Ed. John Wiley & Sons, Inc: New York, 594-627;

Budarin, V., Clark, J.H., Deswarte, F.E.I., Hardy, J.J.E., Hunt, A.J. & Kerton, F.M. (2005). Delicious not siliceous: expanded carbohydrates as renewable separation media for column chromatography. Chem Comm, 2903-2905.

Budarin, V.; Clark, J.H.; Deswarte F.E.I.; *et al*., (2005). Delicious not siliceous: expanded carbohydrates as renewable separation media for column chromatography. *Chem. Commun*., 23: 2903-2905;

Budarin, V.L., Luque, R.A., Macquarrie, D.J. & Clark, J.H. (2007). Towards a bio-based industry: benign catalytic esterifications of succinic acid in the presence of water. Chemistry-A European Journal, 13, 6914-6919.;

Budarin, V.L.; Clark, J.H.; Hardy, J.J.E. *et al*., (2006). *Starbons: New Starch-derived Mesoporous Carbonaceous Materials with Tunable Properties*. *Angew. Chem., Int. Ed*., 45: 3782–3786;

Budarin, V.L.; Clark, J.H.; Luque, R.; *et al*., (2007). Versatile mesoporous carbonaceous materials for acid catalysis. *Chem. Commun*., 6: 634–636;

Budarin, V.L.; Clark, J.H.; R. Luque, Macquarrie, D.J.; (2007) versatile mesoporous carbonaceous materials for acid catalysis. *Chem. Commun*.; 634–636;

Budarin, V.L.; Clark, J.H.; R. Luque, Macquarrie, D.J.; Milkowski, K.; White, R.J.; Hardy, J.J.E.; Tavener, S.J.; Wilson, A.J.; (2006) Starbons: new starch-derived mesoporous carbonaceous materials with tunable properties. *Angew. Chem., Int. Ed*.; 45, 3782–3786;

Burczyk, B., (2005). Starbons: new starch-derived mesoporous carbonaceous materials with tunable properties. *Przemysl Chemiczny*, 84: 162-166;

Campanati, M.; Fornasari, G. & Vaccari, A., (2003). Fundamentals in the preparation of heterogeneous catalysts. *Catal. Today*, 77: 299-314;

Campanati, M.; Fornasari, G.; Vaccari, A.; (2003) Fundamentals in the preparation of heterogeneous catalysts *Catal. Today*; *77*, 299-314;

Cave, G.W.V.; Raston, C.L.; Scott, J.L.; (2001) Recent advances in solventless organic reactions: towards benign synthesis with remarkable versatility. *Chem. Commun.*; 2159-2169;

Chatani, N.; Asaumi, T.; Yorimitsu, S.; *et al.,* (2001). $Ru_3(CO)_{12}$ -catalyzed coupling reaction of sp^3 C-H bonds adjacent to a nitrogen atom in alkylamines with alkenes. *J Am Chem Soc.,* 123: 10935–19041;

Chen, H.; Schlecht, S.; Semple, T.C.; *et al*., (2000). Thermal, catalytic, regiospecific functionalization of alkanes. *Science*, 287: 1995-1997;

Chisari, M.; Eisenman Lawrence, N.; Covey Douglas, F.; *et al*., (2010). The sticky issue of neurosteroids and GABA(A) receptors. *Trends in Neurosciences*, 33: 299-306;

Clark, J. H. & Rhodes, C. N., (2000). *Clean synthesis using porous inorganic solid catalysts and supported reagents. RSC Clean Technology Monographs*: Cambridge, Chapter 1 and 4;

Clark, J. H.; Macquarrie, D. J.; (1998) Catalysis of liquid phase organic reactions using chemically modified mesoporous inorganic solids. *Chem. Commun.*; 853-860;

Clark, J. H.; Macquarrie, D. J.; Handbook of Green Chemistry & Technology; Blackwell Science Ltd.: Oxford, 2002.

Clark, J. H.; Rhodes, C. N. *Clean synthesis using porous inorganic solid catalysts and supported reagents*; RSC Clean Technology Monographs: Cambridge, 2000. Capítulos 1 e 4;

Clark, J.H., (2005). Green chemistry today (and tomorrow). *Green Chem*., 8: 17-21;

Clark, J.H.; (2006) Green Chemistry: today (and tomorrow) *Green Chem*., 8, 17–21;

Colombo, M.I.; Bohn, M.L.; Ruveda, E.A.; (2002) The mechanism of the ritter reaction in combination with wagner-meerwein rearrangements: A cooperative learning experience. *J. Chem. Educ.*; 79, 484-485;

Concéllon, J.M.; Suarez, J.R.; del Solar, V.; (2005) Regiosselective ring opening of amino epoxides with nitriles. An easy synthesis of (2r,3S)- and (2S,3S)-1,3-diaminoalkan-2-ols with differently protected amine functions. *J. Org. Chem.*; 70, 7447-7450;

Constable, D.J.; Dunne, P.J.; Haysler, J.D.; *et al*., (2007). Key green chemistry research areas – a perspective from pharmaceutical manufacturers. *Green Chem*., 9: 411–420;

Crabtree, R.H., (2004). Organometallic alkane CH activation. *J Organomet Chem*., 689: 4083–4091;

Cue, B.W. & Zhang, J., (2009). Green process chemistry in the pharmaceutical industry. *Green Chemistry Letters and Review.,* 4: 193-211;

Curzons, A.D.; Jimenez-Gonzalez, C.; Duncan, A.L.; *et al*., (2007). Fast Life Cycle Assessment of Synthetic Chemistry (FLASCTM) Tool. *J. LCA*, 12: 272-280;

Dale, D.J.; Draper, J.; Dunn, P.J.; *et al*., (2002). The process development of a scaleable route to the PDE5 inhibitor UK-357,903. *Org. Proc. Res. Dev*., 6: 767-772;

Dale, D.J.; Dunn, P.J.; Golightly, C.; *et al*., (2000). The chemical development of the commercial route to sildenafil: A case history. *Org. Proc. Res. Dev*., 4: 17-22;

Dalko, P.I.; Moisan, L.; (2004) In the golden age of organocatalysis. *Angew. Chem., Int. Ed. Engl.*; 43; 5138-5175;

Daniels, D.R. & Donald, A.M., (2004). Soft Material Characterization of the Lamellar Properties of Starch: Smectic Side-Chain Liquid-Crystalline Polymeric Approach. *Macromolecules*, 37: 1312-1318;

Dexter, J.P.; Parker, W.; (2009) Parallel combinatorial chemical synthesis using single-layer poly(dimethylsiloxane) microfluids devices. Biomicrofluidics 3, 034106;

Dos Santos, M.; Crousse, B. & Bonnet-Delpon, D., (2009). Improved Ritter reaction with CF_3 – containing oxirane for an access to central units of protease inhibitors. *Tetrahedron Letters*, 50: 857-859.

Dunn, P.J. Gadamasetti, K.G., Braish, T., Eds.; In Process Chemistry in the Pharmaceutical Industry. *CRC Press: Boca Raton, FL*, 2008; 267-277;

Dunn, P.J.; Galvin, S. & Hettenbach, K., (2004). The development of an environmentally benign synthesis of sidenafil (Viagra^{TM}) and its assessment by green chemistry metrics. *Green Chem.*, 6: 43-48;

Earle, M.J.; Katdare, S.P. & Seddon, K.R., (2004). Paradigm confirmed: The first use of ionic liquids to dramatically influence the outcome of chemical reactions. *Org Lett*., 6: 707-710;

Elsayed, M.S.; El-Araby, M.E. *et al.*(2012) Virtual screening and synthesis of new chemical scaffolds as VEGRFR-2 linase inhibitors. Arzneimittelforschung; 62: 554–560;

Fagnoni, M.; Dondi, D.; Ravelli, D.; Albini, A.; (2007) Photocatalysis for the formation of c-c bond. *Chem. Rev.*; 107, 2725-2756;

Federsel, H.J.; (2008) Handing over the baton: Connecting medicinal chemistry with process R&D. *Drugs News and Perspectives;* 21: 193-199;

Federsel, H.J.; (2009) Chemical Process Research and development of the 21st century: Challenges, strategies and Solutions from a Pharmaceutical Industry perspective. *Accounts of Chemical Research*; 42, no 5, 193-199;

Federsel, H-J.; (2010) Process R&D under the magnifying glass: Organization, business model, challenges and scientific context. Bioorganic & Medicinal Chemistry 18: 5775–5794;

Felix A. Carroll; Perspectives on Structure and Mechanism in Organic Chemistry, 2nd edition.

Feske, B. D. & Stewart, J. D., (2005). Chemoenzymatic Formal Total Synthesis of (-)-Bestatin. *Tetrahedron: Asymmetry*, 16: 3124–3127;

Feske, B.D. Kaluzna, I.A. and Stewart, J.D.; (2005) Enantiodivergent, Biocatalytic Routes to Both Taxol Side Chain Antipodes. *J. Org. Chem. 70*, 9654-9657;

Feske, B.D.; Kaluzna, I.A. & Stewart, J.D., (2005). Enantiodivergent, Biocatalytic Routes to Both Taxol Side Chain Antipodes. *J. Org. Chem.*, 70: 9654-9657

Figueiredo, J. L & Ramôa Ribeiro, F. (1989) Catálise Heterogénea. Fundação Calouste Gulbenkian: Lisboa.

Fortunak, J. M., (2009). Current and future impact of green chemistry on the pharmaceutical industry. *Future Med. Chem.*, 1: 571-575;

Fortunak, J. M.; (2009) Current and future impact of green chemistry on the pharmaceutical industry. *Future Med. Chem.*; 1(4), 571-575;

Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; *et al*., (1989). *Vogel's textbook of practical organic chemistry*. 5th ed.; *John Wiley & Sons, Inc:* New York.

Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; Tatchell, A.R.; *Vogel's textbook of practical organic chemistry*, 5th ed.; John Wiley & Sons, Inc: New York, 1989.

Geus, J. W. & van Dillen, A. J. Preparation of Solid Catalysts, In Sheldon, R. A.; van Bekkum, H. Fine Chemicals through Heterogeneous Catalysis; Wiley-VCH: Weinheim, New York, Chichester, Brisbane, Singapore, Toronto, 2001; 26-34;

Geus, J. W.; van Dillen, A. J. Preparation of Solid Catalysts, *In* Sheldon, R. A.; van Bekkum, H. *Fine Chemicals through Heterogeneous Catalysis*; Wiley-VCH: Weinheim, New York, Chichester, Brisbane, Singapore, Toronto, 2001, 26-34;

Gladysz, J.A.; Curran, D.P. & Horvath, I.T. *Handbook of Fluorous Chemistry. Wiley-VCH*, Weinheim, Germany: 2004.

Goswami, A.; Kotoky, R.; Rastogi, R. C.; *et al*., (2003). A one-pot efficient process for 16-dehydropregnenolone acetate. *Org. Proc. Res. Dev*., 7: 306-308;

Gottlieb, H.E.; Kotlyar, V. and Nudelman, A.; (1997) NMR chemical shifts of common laboratory solvents as trace impurities. *J. Org. Chem*; 62, 7512-7515;

Greg, S.J.; K. S. Sing, K.S.; Adsorption, Surface area and Porosity, Academic press, London, New York, Paris, Tokio, 1982;

Guérinot, A., reymond, S.; Cossy, J. (2009) Ritter Reaction: Recent Catalytic Developments. Eur. J. Org. Chem. 19-28

Gyermerk, L.; (2005) Development of ultra short-acting muscle relaxant agents: history, research strategies, and challenges. *Med. Res. Rev.;* 25, 610-654;

Hathaway, B.A.; (1989) an investigation into the mechanism of the Ritter reaction. *J. Chem. Educ.*; 66, 776;

He, Q.; Jiang, D.; (1999) A novel aminosteroid is active for proliferation inhibition and differentiation induction of human acute myeloid leukemia HL-60 cella. *Leuk. Res.*; 23, 369-372;

He, Q.; Na, X.; (2001) the effects and mechenisms of a novel 2-aminosteroid on murine WEHI-3B leukimia cells in vitro and in vivo. *Leuk. Res.*; 25, 455-461;

Henderson, R.K.; Jimenez-Gonzalez, C.; Constable, D.J.C.; *et al*., (2011). Expanding GSK's solvent selection guide – embedding sustainability into solvent selection starting at medicinal chemistry. *Green Chem.*, 13: 854-862;

Henderson, R.K.; Jimenez-Gonzalez, C.; Constable, D.J.C.; et al.; (2011) Expanding GSK's solvent selection guide – embedding sustainability into solvent selection starting at medicinal chemistry. *Green Chem.*; 13, 854;

Hill, R.A.; Kirk, D.N.; Makin, H.L.J.; Murphy, G.M.; (1991) Description of main steroids. *Dictionary of Steroids*; Eds. Chapman & Hill: London, XIV-XXIX;

Hoffmann, F.; Cornelius, M.; Morell, J.; Fröba, M.; (2006) Silica-Based Mesoporous Organic-Inorganic Hybrid Materials. *Angew. Chem. Int. Ed.*, *45*, 3216-3251;

Hoffmann, J. & Sommer, A., (2005). Steroidhormone receptors as targets for the therapy of breast and prostate cancer-recent advances, mechanisms of resistance, and new approaches. *J. Steroid Biochem. Mol. Biol*., 93: 191-200;

Houston, J.G.; Banks, M.N., (2003). *Burger's Medicinal Chemistry and Drug Discovery, Vol.2: Drug Discovery and Drug Development*, 6thed. Abraham, D.J.; Ed. *John Wiley & Sons, Inc*, Vol.2, pages37-70;

IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). The nomenclature of steroids. Recommendations 1989.

Ivanchina, N. V.; Kicha, A. A.; Kalinovsky, A. I.; *et al*., (2006). Polar steroidal compounds from the far eastern starfish henricia leviuscula. *J. Nat. Prod*., 69: 224-228;

Janzen, N; Sander, S; Terhardt, M; *et al*., (2008). Fast and direct quantification of adrenal steroids by tandem mass spectrometry in serum and dried blood spots. *Journal of Chromatography b-analytical Technologies in the Biomedical and Life Sciences*, 861: 117-122;

Jens Hagen. (2006) Industrial Catalysis - A Practical Approach, Second, Completely Revised and Extended Edition.

Jeong, S. & Werth, C., (2005). Evaluation of methods to obtain geosorbent fractions enriched in carbonaceous materials that affect hydrophobic organic chemical sorption. *Environ. Sci. Technol*., 39: 3279-3288;

Jia, C.; Kitamura, T. & Fujiwara, Y., (2001). Catalytic functionalization of arenes and alkanes via C-H bond activation. *Acc Chem Res*., 34: 633–639;

Jimenez-Gonzalez, C.; Curzons, A.D.; Constable, D.J.C.; *et al*., (2004). Cycle Inventory and Assessment of Pharmaceutical Compounds. *Int. J. LCA*, 9: 115-121;

Johnson, S.; Ollivier, P.; Mallouk, T., (1999). Ordered mesoporous polymers of tunable pore size from colloidal silica templates. *Science*, 283, 963;

Kalkhambkar, R.G.; Waters, S.N. & Laali K.K., (2011). Highly efficient synthesis of amides via Ritter chemistry with ionic liquids. *Tetrahedron Letters*, 52: 867-871;

Knox, J.H. & Ross, P., (1997). Carbon-based packing material for liquid chromatography: application. *Adv. Chromatography*, 37: 73-119;

Ko, D.C.; Porter, J.F. & MaKay, G., (2000). Optimised correlations for the fixed bed sorption of metal ions on bone char. *Chem. Eng. Sci*., 55: 5819-5829;

Kourounakis, P.N.; Rekka, E. A.; (1994) Introduction. In Advanced drug design and development. A medicinal chemistry approach, *Kourounakis, P.N.; Rekka, E. A., Eds. Ellis Horwood Limited: New York*, 1-4;

Kourounakis, P.N.; Rekka, E. A.; Kourounakis, P.N.; Rekka, E. A.; *Eds. Ellis Horwood Limited: New York*, 1994; 1-4;

Krimen, L.I.; Cota, D.J.; (1969) The Ritter reaction. *Org. React.*; 17, 213-313;

Kruk, M.; Jaroniec, M.; Ryoo, R.; *et al*., (2000). Characterization of ordered mesoporous carbons synthesized using MCM-48 silicas as templates. *Phys. Chem. B*, 104: 7960-7968;

Lal A; Micaily, I; Foote, D; *et al*., (2010). A Simple Regimen to Correct Vitamin D Deficiency In Transfusion-Dependent Thalassemia with High-Dose Ergocalciferol. *Blood*, 116: 1729-1729;

Lee, K.; Pham, V. C.; *et al*. (2013) Fragment-based discovery of novel and selective mPGES-1 inhibitors Part 1: Identification of sulfonamide-1,2,3,-triazole-4,5-dicarboxylic acid. Bioorganic & Medicinal Chemistry Letters 23: 75–80;

Leitner, W., (2002). Supercritical carbon dioxide as a green reaction medium for catalysis. *Acc Chem Re*., 35: 746-756;

Li, C.J. & Trost, B.M., (2008). Green chemistry for chemical synthesis. *PNAS*, 105: 13197–13202;

Li, C.J.; Trost, B.M.; Green chemistry for chemical synthesis (2008) *PNAS* 105, no. 36, 13197–13202;

Li, J.J., (2006). *Name reactions. A collection of detailed reaction mechanisms.* Springer-Verlag: Berlim, 501-502;

Li, J.J.; *Name reactions. A collection of detailed reaction mechanisms.* Springer-Verlag: Berlim, 2006, 501-502;

Li, Z. & Dai, S., (2005). Surface functionalization and pore size manipulation for carbons of ordered structure. *Chem. Mater*., 17: 1717-1721;

Li, Z.; Yan, W. & Dai, S., (2004). A novel vesicular carbon synthesized using amphiphilic carbonaceous material and micelle templating approach. *Carbon*, 42: 767-770;

Liguori, A.; Perri, F. & Siciliano, C., (2006). D-homoannulation of 17 alpha, 21-dihydroxy-20-keto steroids (corticosteroids). *Steroids*, 71: 1091-1096;

Lu, A.; Li, W.; Schmidt, W.; *et al*., (2005). Template synthesis of large pore ordered mesoporous carbon. *Microporous Mesoporous Mater*, 80: 117-128;

Lücke, B.; Köckritz, A.; Vorlop, K.-D.; Bischoff, S.; Kant, M.; (2004) Heterogenization of homogeneous catalysts by encapsulation-stiff cage versus flexible skin. *Topics in Catalysis*; Vol. 29, Nos. 3–4;

Luque, R. & Clark, J.H., (2010). Water-tolerant Ru-Starbon® materials for the hydrogenation of organic acids in aqueous ethanol. *Catalysis Communications*, 11: 928-931;

Luque, R.; Clark, J.H.; (2010) Water-tolerant ru-starbon materials for the hydrogenation of organic acids in aqueous ethanol. *Catalysis Communications*; 11, 928-931;

Ma'mani, L.; Heydari, A. & Sheykhan, M., (2010). The Ritter reaction under incredibly green protocol : Nano magnetically silica-supported BrØnsted acid catalyst. Applied Catalysis A : General, 384: 122-127.

Makowski, P.; Cakan, R.D.; Antonietti, M. *et al*. Selective partial hydrogenation of hydroxy aromatic derivatives with palladium nanoparticles supported on hydrophilic carbon. *Chem. Commun*., 2008 ; 999-1001.

Mason, B.P.; Price, K.E.; Steinbacher, J.L.; *et al*., (2007). *Chem. Rev.*, 107: 2300-2318;

Matthews, G.J. & Hassner, A. *Organic Reactions in Steroid Chemistry.* Vol.2, Fied, J.; Edwards, J.A.; Eds. *Van Nostrand Reinhold Company*: New York, 1972; 1-52;

Matthews, G.J.; Hassner, A.; (1972) Synthesis of oxiranes, aziridines and episulfides. *Organic Reactions in Steroid Chemistry.* Vol.2, Fied, J.; Edwards, J.A.; Eds. Van Nostrand Reinhold Company: New York, 1-52;

Mendre K, A; Lakis, N & Jimenez, J., (2011). Associations of sex steroid hormones with cerebral activations during mental rotation in men and women with schizophrenia. *Psychoneuroendocrinology,* 36: 1422-6;

Milkowski, K., Clark, J.H. & Doi, S. (2004). New materials based on renewable resources: chemically modified highly porous starches and their composites with synthetic monomers. Green Chemistry, 6, 189-190.;

Mitscher, L.A. & Dutta, A. *Burger's Medicinal Chemistry and Drug Discovery, Vol.2: Drug Discovery and Drug Development*, 6th ed. Abraham, D.J.; Ed. *John Wiley & Sons, Inc*:2003. 36;

Moss, G.P.; (1989) Nomenclature of steroids (recommendations 1980) *Pure Appl. Chem.*; 61, 1783-1822;

Muegge, I.; Enyedy, I.; *Burger's Medicinal Chemistry and Drug Discovery, Vol.1: Drug Discovery*, 6thed. Abraham, D.J.; Ed. *John Wiley & Sons, Inc:* 2003, 243-279;

Narayana, C.R.; Kulkarny, A.K.; Landage, A.B.; Wadia, M.S.; (1974) Novel cleavage of a steroid epoxide on ritter reaction. *Indian J. Chem*; 12, 992-993;

Narayana, C.R.; Kulkarny, A.K.; Landage, A.B.; Wadia, M.S.; (1977) One-step conversion of epoxides into vic-acylamino-hydroxy compounds. *Synthesis*; 35-36;

Noguchi, K.K; Lau, K; Smith D.J; *et al*., (2011). Glucocorticoid receptor stimulation and the regulation of neonatal cerebellar neural progenitor cell apoptosis. *Neurobiology of disease*, 43: 356-363;

Nuchter, M.; Ondruschka, B.; Bonrath, W.; Gum, A.; Microwave assisted synthesis: A critical technology overview. (2004) *Green Chem.*; 6, 128-141;

Orisakwe, O.E., Oluboyo, A.; Ofoefule, S., Obi, E.; Ilondu, N.; Afonne, O.A.; Agbasi, A.; Chiroma, D.H.; Absorption studies of artesunate: Evaluation of saline cathartics as additive in management of artesunate poisoning. *J. Health Sci*.; 2001, 47, 491 – 494;

Otero, M.; Grande, C.A.; Rodrigues, A.E.; Adsorption of salicylic acid onto polymeric adsorbents and activated charcoal. *React. Funct. Polym*.; 2004, 60, 203 – 213;

Parenti, M.D.; Rastelli, G.; (2012) Advances and applications of binding affinity prediction methods in drug discovery. Biotechnology Advances 30: 244–250;

Pathil, A.; Warth, A.; Chamulitrat, W.; *et al*., (2011). The synthetic bile acid-phospholipid conjugate ursodeoxycholyl lysophosphatidylethanolamide suppresses TNF alpha-induced liver injury. *Journal of Hepatology,* 54: 674-684;

Paulos, P.; Tamsin J.R.; Nallani G.; *et al*., (2010). Reproductive responses in fathead minnow and Japanese medaka following exposure to a synthetic progestin. Norethindrone. *Aquatic toxicology*, 99: 256-262;

Perrin, D.D.; Armarego, W.L.F.; *Purification of Laboratory Chemicals.* 3rd ed.; Pergamon Press plc: Oxford, 1988;

Perrin, D.D.; Armarego, W.L.F.; *Purification of Laboratory Chemicals.* 3rd ed.; Pergamon Press plc: Oxford, 1988;

Petrov, L. A; Alhamed, Y; Al-Zahrani, A; *et al*., (2011). Role of Chemical Kinetics in the Heterogeneous Catalysis Studies. *Chinese Journal of Catalysis*, 32: 1085-1112;

Pinto, R.M.A. et al; (2006) ritter reaction mediated by Bismuth(III) salts: one-step conversion of epoxides into vic-acylamino-hydroxy compounds. *Syntlet*, No:13, 2047-2050;

Pinto, R.M.A., (2009). *New processes with Bismuth(III) Reagents and Catalysts using Steroids as Substrates. PhD Thesis;* Faculdade de Farmácia da Universidade de Coimbra;

Pinto, R.M.A.; *New processes with Bismuth(III) Reagents and Catalysts using Steroids as Substrates- PhD Thesis;* 2009 Faculdade de Farmácia da Universidade de Coimbra;

Pinto, R.M.A.; Salvador, J.A.R. & Le Roux, C., (2006). Ritter Reaction Mediated by Bismuth (III) Salts: One-Step Conversion of Epoxides into *vic*-Acylamino-Hydroxi Compounds. *Syntlet*, 13: 2047-2050;

Plaut, H.; Ritter, J.J.; (1951) A new reaction of nitriles. 6. Unsaturated amides. *J. Am. Chem. Soc.*; 73, 4076-4077;

Poliakoff, M.; Fitzpatrick, M.J.; Farren, T.R.; Anastas, P.T.; Green Chemistry: Science and Politics of Change. *Science* 297, 807-810, 2002;

Price, P. M.; Clark, J. H.; Macquarrie, D. J.; (2000) Modified silicas for clean technology. *J. Chem. Soc., Dalton Trans.*, 101-110;

Ralet, M.C.; Dronnet, V.; Buchholt H.C; et al.; Enzymatically and chemically de-esterified lime pectins: Characterization, polyelectrolyte behaviour and calcium binding properties. *Carbohydr. Res*.; 2001, 336, 117–125;

Reetz, M.T.; Jaeger, K.E.; Enantioselective enzymes for organic synthesis created by directed evolution. *Chem Eur J*; 2000; 6: 407–412;

Rinaldo, P., (2009). The elucidation of mechanisms in homogeneous catalysis Comments on Inorganic Chemistry, 30: 177-228;

Ritter, J.J. & Minieri, P.P.; A new reaction of nitriles; amides from alkenes and mononitriles. *Journal of the American Chemical Society;* 1948, 70 (12): 4045–8;

Ritter, J.J.; (1952) N-acyl-β-phenethylamines, ans a new isoquinoline synthesis. *J. Am. Chem. Soc.*; 74, 763-765;

Ritter, J.J.; Kalish, J.; (1948) A new reaction of nitriles. 2. Synthesis of t-carbanamines. *J. Am. Chem. Soc.*; 70, 4048-4050;

Rodríguez-Reinoso, F.; The role of carbon materials in heterogeneous catalysis. *Carbon*; 1998, 36, 159–175;

Ryan, R.J.; Julia, S.; (1973) Applications of ritter reaction in steroid series. 3. Cleavage of 4,5β-epoxy cholestanes by acetonitrile. Conformation of amide group. *Tetrahedron*; 29, 3649-3654;

Salvador, J.A.R.; Sá e Melo, M.L. & Campos Neves, A.S., (1996). Oxidations with potassium Permanganate – Metal sulphates and nitrates. β-Selective epoxidation of Δ^5- unsaturated steroids. *Tetrahedon letters*, 37: 687-690.

Salvador, J.A.R.; Sá e Melo, M.L.; Neves, A.S.C.; (1996) Oxidations with potassium permanganate/metal suphates and nitrates. B-selective epoxidation of Δ^5-unsaturated steroids- *Tetrahedron Lett.*; 37, 687-690;

Schneider, G.; Schonecker, B.; (1982) Ritter reaction on steroids. 1. Cleavage of epimeric 16,17-epoxides of 3-methoxyoestra-1,3,5-(10)-triene with nitriles. *Acta Chim. Hung.;* 95, 321-331.

Schubert, G.; Schneider, G.; Schade, W.; Dombi, G.; (1982) Ritter reaction on steroids.2. Reaction of the 14,15-epoxide epimers and 15β,16β-epoxides of ostratriene series with acetonitrile and BF_3-etherate. *Acta Chim. Hung.;* 111, 173-187;

Shamai, K.; Shimoni, E. & Bianco-Peled, H.; Small Angle X-Ray Scattering of Resistant Starch Type III (RSIII). Biomacromolecules. *Biomacromolecules*; 2004, 5, 219 – 223;

Sheldon, R.; (2001) Catalytic Reactions in Ionic Liquids. Chem. Commun, 2399-2407;

Sheldon, R.A.; (2000) Atom efficiency and catalysis in organis synthesis. *Pure Appl. Chem.;* 72, 1233-1246;

Sheldon, R.A.; (2008) *E* factors, green chemistry and catalysis: an odyssey. *Chem. Commun.*; 2008, 3352-3365;

Sheldon, R.A.; Consider the environmental quotient. *Chem Tech;* 1994; 38–47;

Sheldon, R.A.; Green solvents for sustainable organic synthesis: State of the art. *Green Chem*; 2005; 7: 267–278.

Sheri, A.; Attardi, B.J.; Koduri, S.; *et al*., (2010). Effects of Synthetic Androgens on Liver Function Using the Rabbit as a Model. *Journal of Andrology*, 31: 472-481;

Silvestre, S.M.; Salvador, J.A.R.; Clark, J.H.;(2004) β-selective epoxidation of Δ^5-steroids by O_2 using surface functionalized silica supported cobalt catalysts. Journal of Molecular Catalysis A: Chemical 219: 143-147;

Sing, K. S. W.; Everett, D. H.; Haul, R. A. W.; Moscou, L., Pierotti, R. A.; Rouquerol, J.; Siemieniewska, T.; Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity (Recommendations 1984). *Pure Appl. Chem*.; 1985, 57(4), 603–619;

Smith, J.R.; Norman, R.O.C.; Stillings, M.R.; (1975) Synthesis of oxazolines from epoxides. *J. Chem. Soc., Perkin Trans. 1*; 1200-1202;

Smith, J.R.L; Norman, R.C. and Stillings, M.R.; (1975) Synthesis of Oxazolines from Epoxides. J.C.S. Perkin, 1200-1202;

Son, S.U.; Lee, S.I.; Chung, Y.K.; Cobalt auf Holzkohle: ein bequemer und preiswerter heterogener Pauson-Khand-Katalysator. *Angew. Chem*.; 2000, 112, 4318 – 4320;

Strauss, C.R.; Varma, R.S.; (2006) Microwaves in green and sustainable chemistry. *Top Curr. Chem.*; 266, 199-231;

Taguchi, A.; Schüth, F.; Ordered mesoporous materials in catalysis. *Microporous Mesoporous Mater*.; 2005, 77, 1–45;

Tavener, S.J.; Clark, J.H.; (2003) Can fluorine chemistru be green chemistry? *J. Fluorine Chem.*; 123, 31-36;

Teutsh, G.; Shapiro, E.L.; Herzog, H.L; (1970) Preparation of 6-acetamido-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate. *J. Med. Chem.*; 13, 750-751;

Toda, M.; Takagaki, A.; Okamura, M.; Kondo, J.N.; Hayashi, S; Domen, K.; Hara, M.; Green chemistry: biodiesel made with sugar catalyst. *Nature;* 2005, 438, 178;

Trost, B.M.; Atom Economy. A Challenge for Organic Synthesis *Angew Chem Int;* 1995*;* 34:259–281;

Trost, B.M.; On inventing reactions for atom economy. *Acc Chem Res;* 2002; 35:695–705;

Trost, B.M.; The atom economy--a search for synthetic efficiency. *Science,* 1991, 254:1471–1477;

Trost, B.M.; Weiss, A.H.; Catalytic enantioselective synthesis of adociacetylene. *Org Lett;* 2006; 8:4461–4464;

Tuba, Z.; Maho, S; Vizi, E.S.; (2002) Synthesis and structure-activity relationships of neuromuscular blocking agents. *Curr. Med. Chem.*; 9, 1507-1536;

Tucker, J.L.; (2006) Green Chemistry, a Pharmaceutical Perspective. Organic Process Research & Development 10: 315-319.

Tucker, J.L.; (2006) Green Chemistry, a Pharmaceutical perspective. *Org. Process Res. Dev.*; 10, 315-319;

Vilotijevic, I.; Jamison, T.F.; Epoxide-opening cascades promoted by water. *Science*; 2007; 317: 1189–1192;

Vincze, I.; Hackler, L.; Szendi, Z; Schneider, G.; (1996) Steroids 54. Amino acylamidosteroids. *Steroids*; 61, 697-702;

Webb, P.B.; Kunene, T.E.; Cole-Hamilton, D.J.; Continuous flow homogeneous hydroformylation of alkenes using supercritical fluids. *Green Chem;* 2005; 7:373–379;

Wermuth, G.G.; Ganellin, C.R.; Lindberg, P.; Mitscher, L.A.; (1998) Glossary of terms used in medicinal chemistry (IUPAC recommendations 1998) *Pure Appl. Chem.*, 70, 1129-1143;

White, R.J.; Antonio, C.; Budarin, V.L., et al., (2010). Polysaccharide-Derived carbons for polar analyte separations. *Adv. Funct. Mater.*, 20: 1834-1841;

White, R.J.; Antonio, C.; Budarin, V.L.; Bergström, E.; Thomas-Oates, J.; Clark, J.H.; (2010) Polysaccharide-derived carbons for polar analyte separations. *Adv. Funct. Mater.*; 20, 1834-1841;

White, R.J.; Budarin, V.; Luque, R.; Clark, J.H.; Macquarrie, D.J.; (2009) Tuneable porous carbonaceous materials from renewable resources. *Chem. Soc. Rev.*; 38, 3401-3418;

White, R.J.; Budarin, V.; Luque, R.; *et al*., (2009). Tuneable porous carbonaceous materials from renewable resources. *Chem. Soc. Rev*., 38: 3401-3418;

White, R.J.; Budarin, V.L. & Clark, J.H., (2010). Pectin-Derived Porous Materials. *Chem. Eur. J.*, 16: 1326-1335;

White, R.J.; Budarin, V.L.; Clark, J.H.; (2010) Pectin-derived porous materials. *Chem. Eur. J.*; 16, 1326-1335;

White, R.J.; Budarin, V.L.; Clark, J.H.; *Tuneable Mesoporous Materials from a-d-Polysaccharides. ChemSusChem*; 2008, 1, 408-411;

Yadav, J.S.; Reddy, B.V.; Pandurangam, T.; *et al*., (2007). PMA/SiO_2 catalyzed amidation of alcohols with nitriles: A simple, cost-effective and recyclable catalytic system for Ritter reaction. Catalysis Commun., 9: 1297-1301.

Yatime, L; Laursen, M; Morth, J. P; *et al*., (2011). Structural insights into the high affinity binding of cardiotonic steroids to the Na(+),K(+)-ATPase. *Journal of Structural Biology*, 174: 296-306;

Zhang, F.; Ma, H.; Chen, J.; *et al*., (2008). Preparation and gas storage of high surface area microporous carbon derived from biomass source cornstalks. *Bioresour. Technol.,* 99: 4803–4808;

Zhang, F.; Meng, Y.; Gu, D.; *et al*., (2005). A facile aqueous route to synthesize highly ordered mesoporous polymers and carbon frameworks with Ia3d bicontinuous cubic structure. *J. Am. Chem. Soc.*, 127: 13508-13509;

Zlotos, D.P.; (2005) Recent advances in neuromuscular bloking agents. *Mini-Rev. Med. Chem.* 5, 595-606;