Università degli studi del Piemonte Orientale
"Amedeo Avogadro"

Department of Pharmaceutical Sciences

Ph.D. in Chemistry & Biology

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DEVELOPMENT OF INNOVATIVE SYNTHETIC PROCESSES FOR THE INDUSTRIAL PRODUCTION OF APIs

PhD student: Michele Castaldi

Supervisor: Giovanni B. Giovenzana

PhD coordinator: Luigi Panza

SSD CHIM/03

Chemical methodologies for
new molecule and nanomaterials

UNIVERSITÀ DEL PIEMONTE ORIENTALE
DOTTORATO DI RICERCA
IN CHEMISTRY & BIOLOGY

Via Duomo, 6
13100 – Vercelli (ITALY)

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Summary

1. APIs

1.1 Introduction

> *"Any substance used in a finished pharmaceutical product (FPP), intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings."*
> Definition of API by the World Health Organization[1.1]

APIs (Active Pharmaceutical Ingredients) are a very important class of chemicals for their fundamental role in the human health. They are the key compound in pharmaceutical formulations, due to their bioactivity that can be exploited to cure several diseases. For this reason, APIs have an immense market value related to their industrial production and commercialisation.

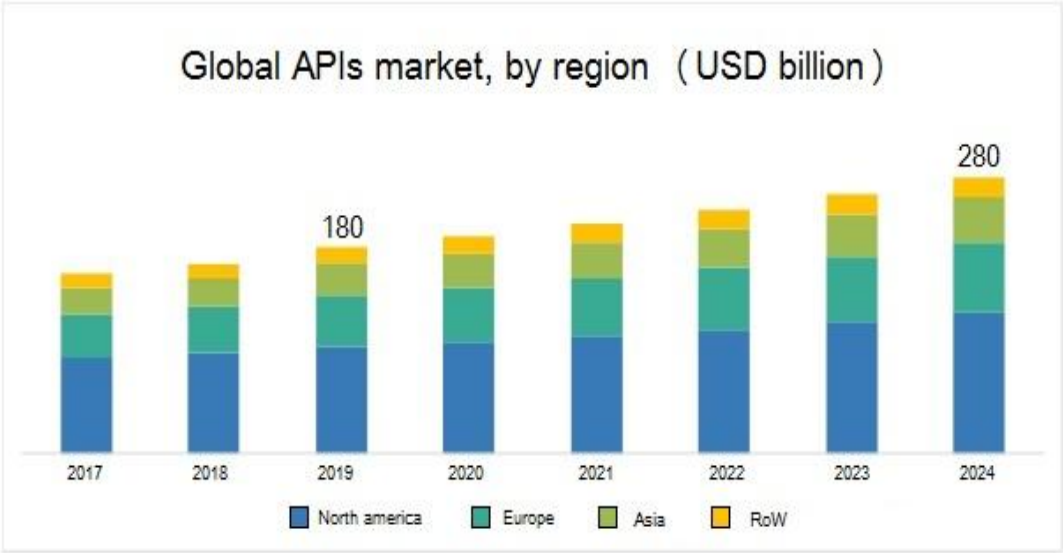


Figure 1.1 - Global market of APIs

Over the last few decades, the global market of Active Pharmaceutical Ingredients has undergone to an exponential growth (*Figure 1.1*), thanks to the increased use of drugs for the treatment of diseases. The major factors

that govern the market are the rising prevalence of diseases like cardiovascular condition, diabetes and lifestyle diseases, cancer and infections.[2.1]

The global API market is anticipated to exhibit an annual growth rate of 6.8 % in the forecast period (2018 – 2024), with the surpass of US $ 274.9 billion value in 2024.[3.1]

The pharmaceutical industries play an important and complex competition in this fields, either for the discovery of new API or for the research and development of new syntheses for existing drugs, improving the process design, reducing the costs of the production and increasing the quality standard. To keep up with this competition, the pharmaceutical industries are expanding by establishing most of their productions in the Asian and Pacific region. Lower production costs in China and India have stimulated the increase of the manufacturing of APIs in these regions.

Another important field is represented by High Potency APIs, active ingredients with therapeutic daily dose much smaller than traditional APIs,[4.1] which are in rising demand by pharmaceutical manufacturers. Research and development departments are focusing heavily on the field of oncology. The IMS institute of healthcare informatics estimated that in 2016 more than 550 drugs were under development for cancer treatment. Moreover, 70 new oncology treatments reached in the market between 2010 and 2015.[3.1]

1.2 Intellectual property

APIs are almost always protected by strong intellectual property (IP), allowing the owners of the newly invented drug to have a complete but temporary exclusivity in terms of use, production and commercialization. The patent, as it is a technical-legal document, must be written in one of the languages recognized by the jurisdiction to which it was deposited.

In addition to individual states, the patent application can be filed under international conventions, like the Patent Cooperation Treaty (PCT, *Figure 2.1*) or the European Patent Convention (EPC, *Figure 3.1*).[5.1]

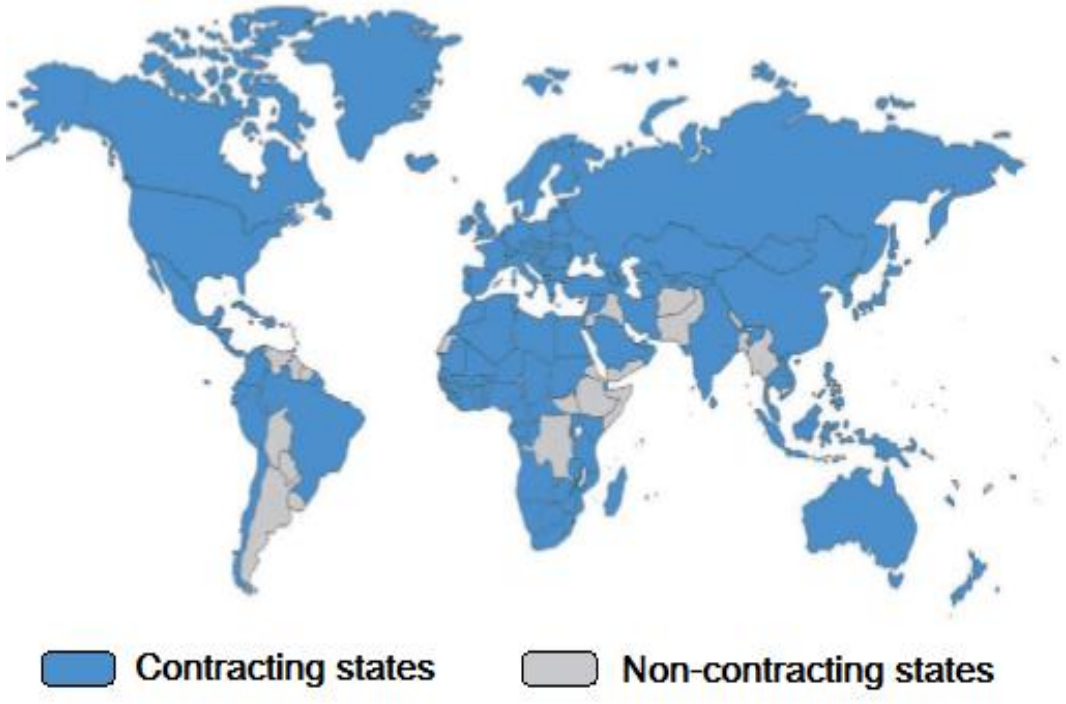


Figure 2.1 - Patent Cooperation Treaty

After the initial filing of the patent there is a period, generally 18 months, in which the patent application is secreted by the receiving office, the latter operating a thorough examination of the novelty of the invention. At the end of this period, a publication number is assigned together with the publication date.

Another crucial step is represented by the concession of the patent which, in most cases, does not take place before 3 years from the filing of the patent application.

The inventors and the owner are two legally distinct entities that only sometimes coincide. While the inventors are necessarily real persons, as those who have achieved the invention object of the patent, the owners are very often juridical persons, mostly employers of the inventors.

The first part of a patent is represented by the abstract, a brief summary consisting in a synthetic description of the invention. It focuses on the advantages that the inventive solution achieves.

An important part of a patent are the claims, defining concisely the scope of protection required by the patent owner. In the case of chemical and chemical-pharmaceutical fields, the patents first report, in the main claim, the starting materials and the synthetic steps strictly necessary to reach the product. In the subsequent claims, the optimal reaction conditions (*e.g.* temperature, nature of solvents, ratio of the chemicals, the eventual presence of a catalyst) are specified.[4.1]

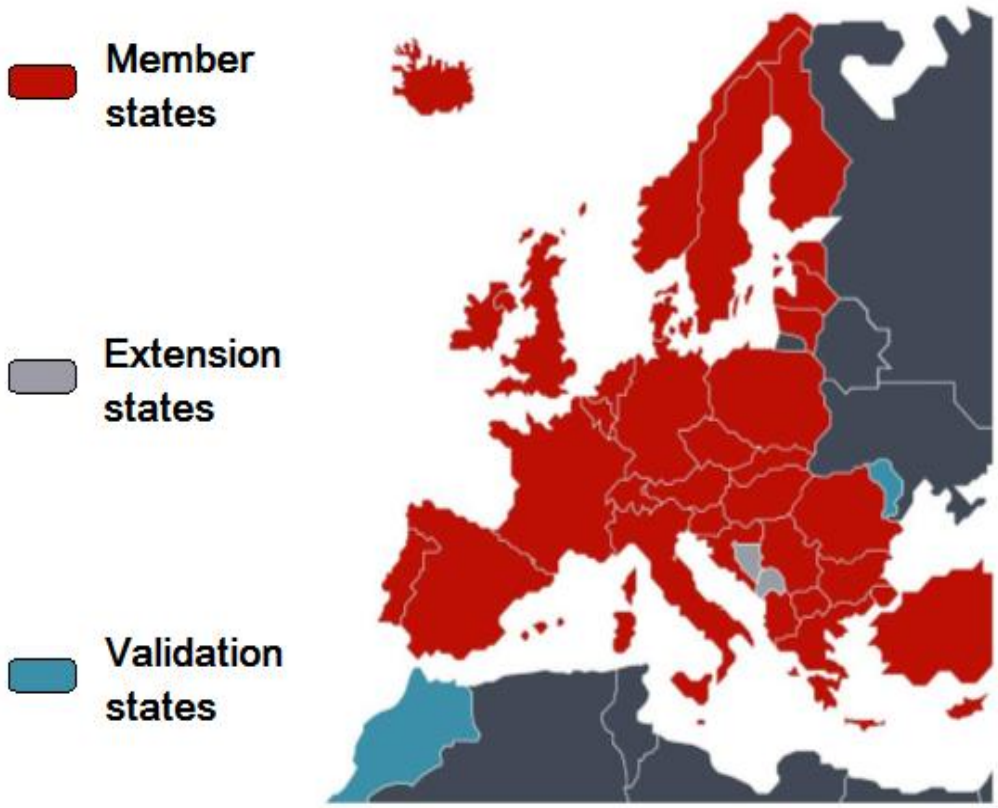


Figure 3.1 - European Patent Convention

The patent for an invention has a duration of 20 years, starting from the date of patent application filing. Extension of the duration of the patent is provided for those inventions in which the placing on the market is significantly delayed by administrative procedure, as in the case of Active Pharmaceutical Ingredients. The extension of the duration of the patents is

permitted by a SPC (Supplementary Protection Certificate), which extends the validity to a maximum of 5 years.[6.1]

Patents in chemical and chemical-pharmaceutical fields may be classified as product patents and process patents.

1.2.1 Product patents

Product patents are the first to be registered because they cover the structure of the new molecule, for which the biological activity must be demonstrated. Moreover, the molecule, object of the patent, must be synthesized or isolated by man. These patents are applied to any form of the drug, whether it is formulated as a tablet, capsule, cream or other final dosage forms.

The new compound must be defined by using the general structure formulas. These include all the possible equivalent substituents, in a specific position on a basic chemical structure (the “skeleton” of the molecule). In order to create a valid patent for a product it is necessary, in addition to the structural formula, also to describe the synthesis, isolation or the purification of the aforementioned product.[6.1]

Product patents have 20 years of validity and cannot be subject to renewal when their expiry dates. During this period only the inventor of the new molecule, except for concessions to third parts, can use, produce, commercialize and import the API.

After the expiration date, the other competitors can start using and selling the molecule object of the patent.

1.2.2 Process patent

Process patents are related to the other type of patents but refer to the synthesis/production process employed for the preparation of the commercially available speciality, even if the Active Pharmaceutical Ingredient is not patentable because it is already known.[6.1]

These documents follow the same rules as the product patent: 20 years of validity and exclusivity of the inventor, no renewability by the SPC.

When the product patent expires, the API can be produced freely but if a process patent is still operating on the original synthesis, the latter cannot be pursued. This leads to a very active research of alternative synthetic routes for the preparation of the API.

1.3 References

[1.1] *Market Research Report - API Market* **2019**.

[2.1] World Health Organization, *Working Documents* **2011**, QAS/11.426/ Rev.1.

[3.1] Rees V., *Eur. Pharm. Review* **2019**.

[4.1] Van Arnum P., *Pharm. Technol.* **2009**, 33(7).

[5.1] Giammarioli I., Primiceri M.V., *La Chimica e L'Industria Web* **2016**, 3(7).

[6.1] Merli S., *Pharmastar.it - I Quaderni di Pharmastar*.

2. Outline of the thesis

The PhD activity was funded by Chemelectiva S.r.l., a pharmaceutical company located in Novara. The activity of Chemelectiva is focused on the research of alternative and efficient synthesis of APIs, for which product/process patents are no longer valid or they are about to expire.

These alternative syntheses must satisfy requirements:

- Efficiency: related to the global yield of the entire protocol of synthesis;

- Innovation: the synthetic route must have some synthetic strategy not used in the prior art;

- Practicality: all the steps must be executed with easy operations and mild conditions;

- Industrial scalability: all the operations and results must be the same obtained in laboratory scale, without any significant modification of the process;

- Use of commercially available and cheap starting materials: for not having difficulty in finding raw materials and to lower costs of the final API.

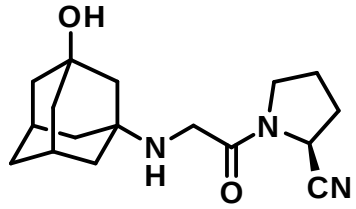
During the PhD, the work was based on APIs selected according to these parameters. In detail, the molecules selected for the research of alternative syntheses was:

- vildagliptin, an important drug for the treatment of type 2 diabetes mellitus, object of the first year of the PhD;

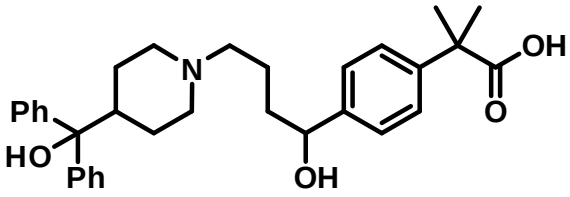
- fexofenadine hydrochloride, used on a large scale as a therapy for allergic rhinitis (second year of the PhD);

- ravidasvir hydrochloride, a novel API, not yet approved, for the treatment of chronic hepatitis C, studied during the last year of the PhD.

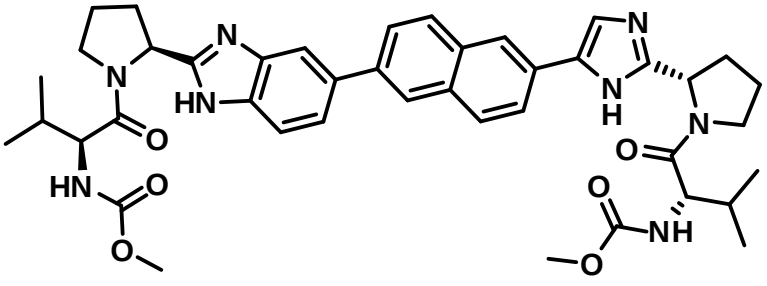
The aim of this work is to designing alternative preparations for these selected APIs (*Figure 1.2*), optimising all the synthetic steps by adjusting reactions conditions and maximizing the yield of the processes and finally scaling up the whole syntheses to hundred-grams scale, for introduction of the processes to the industrial scale.



vildagliptin



fexofenadine



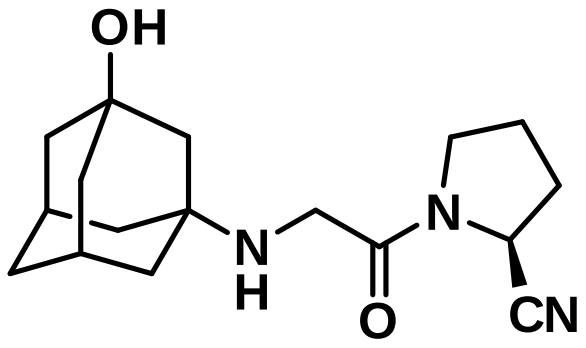
ravidasvir

Figure 1.2 - Selected APIs for the PhD work

3. Vildagliptin

Vildagliptin **1.3** is an important Active Pharmaceutical Ingredient for the treatment of type 2 diabetes mellitus (T2DM).

During the first year of the PhD, the work was focused on the research & development of a new synthetic strategy for the preparation of vildagliptin (*Figure 1.3*) and subsequent scale up of the process.



1.3

Figure 1.3 - Vildagliptin

3.1 Type 2 diabetes mellitus

Diabetes mellitus (DM) is a syndrome of disordered metabolism leading to a very high blood levels of glucose (hyperglycemia). There are two most common forms of diabetes: type 1 diabetes mellitus and type 2 diabetes mellitus (T1DM, T2DM). T1DM is characterized by a diminished production of insulin (mediated by pancreatic β-cells), while T2DM arises subsequently to a reduction of insulin secretion from β-cells and an impaired response to insulin by peripheral tissues, such as muscles adipose and liver.[1.3]

In 2012 DM affects about 370 million people worldwide with more than 90% of these cases diagnosed as T2DM. Due to the graving burden of diabetes in developing counties, the number of affected people is estimated to increase to approximately 550 million adults by 2030.[2.3]

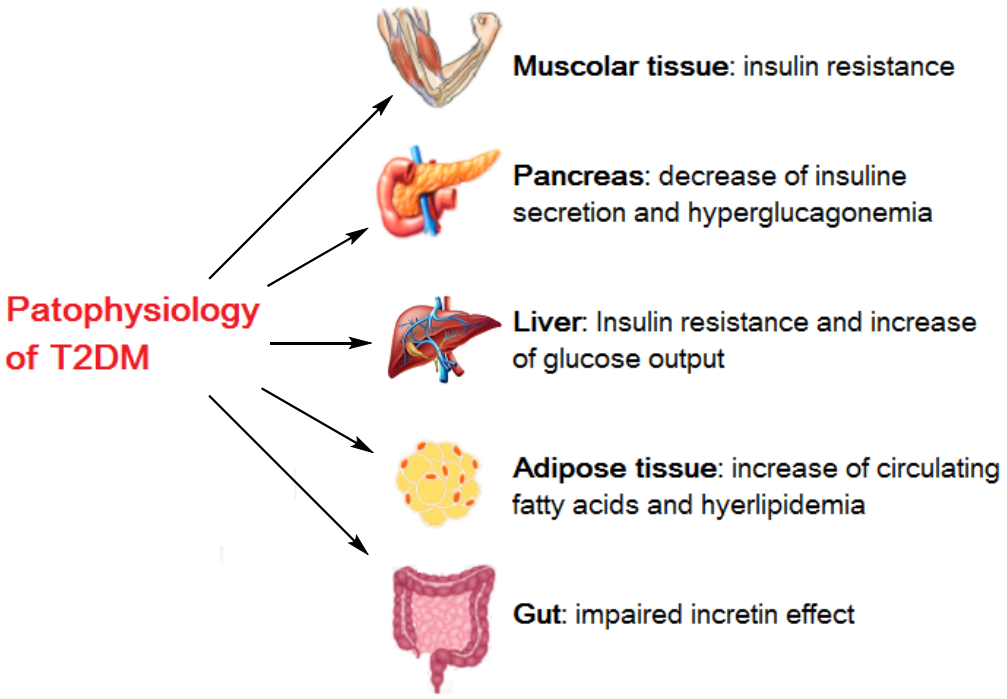


Figure 2.3 - Pathophysiology of T2DM

Diabetes mellitus leads to hyperglycemia, excessive urine production, increased fluid intake, compensatory thirst, unexplained weight loss, letargy and changes of energy metabolism.[1.3]

In addition, an important role is played by the hormones glucagon-like peptide 1 (GLP-1) and glucose-depedent insulinotropic peptide (GIP), responsible for the incretin effect. It is a phenomenon whereby insulin secretion increase in response to the ingestion of food and governing blood glucose levels.[2.3]

3.2 Gliptins - Drugs for the treatment of T2DM

A new class of oral antidiabetic drugs, for the treatment of type 2 diabetes mellitus, is represented by gliptins *(Figure 3.3)*. These oral hypoglycemic agent are inhibitors of the enzyme dipeptidyl peptidase-IV (DPP-IV). This enzyme inhibits the incretin peptides GLP-1 and GIP, obtaining a diminution

of the insulin release and an increase of glycemia. By inhibiting DPP-IV, gliptins can stimulate the quantity of insulin released and decrease the glucose blood level.[3.3]

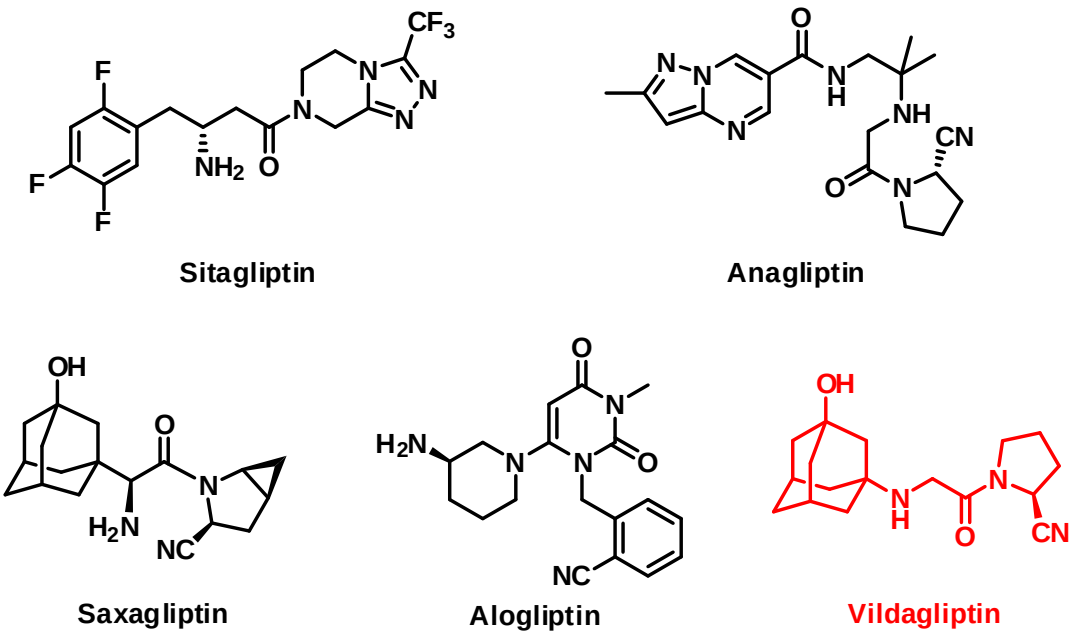


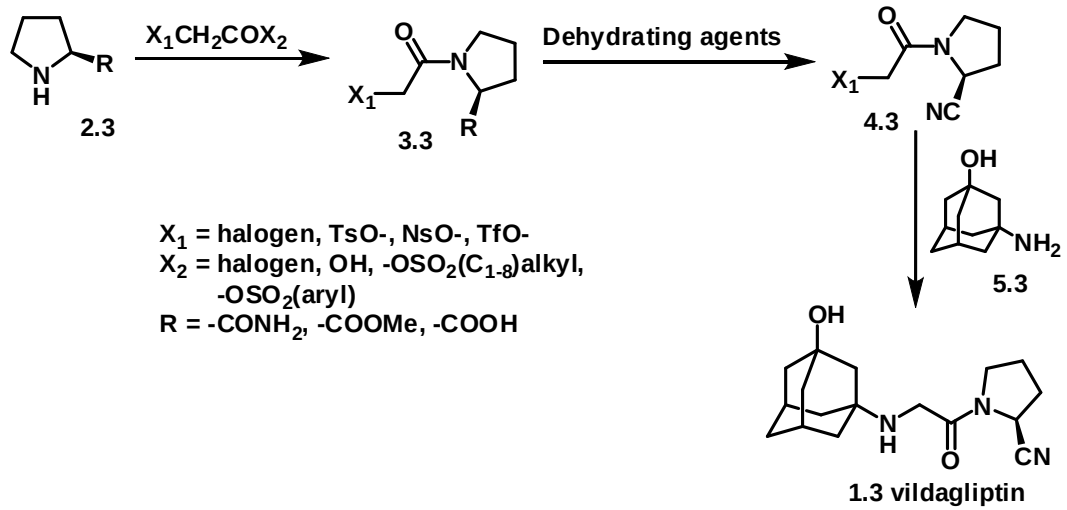
Figure 3.3 - Gliptins

Vildagliptin is a reversible inhibitor of dipeptidyl peptidase-IV and it is widely used for its high selectivity. The inhibition of the enzyme is attributable to the presence of the (2*S*)-cyanopyrrolidine moiety, known to be a mimic of the proline contained in the aminoacidic sequence of the incretin hormones.[4.3,5.3,6.3] This proline mimic group is shared with other gliptins like saxagliptin and anagliptin. The nitrile group is very important for the chemical stability, allowing oral administration of these drugs, and for the nanomolar-level inhibition of the DPP-IV.

A portion of the patients affected by type 2 diabetes mellitus (~9%) are intolerant to the other classes of drugs used in a large scale (metformin, glibenclamide). The treatment with vildagliptin implies slightly higher costs than those using metformin or sulfonilureas, but provides an important economic impact in the treatment of the adverse effects (heart failure and severe hypoglicemic attacks).[7.3]

3.3 Results and discussion

The molecule of vildagliptin **1.3** (*Scheme 1.3*) includes an 1-hydroxyadamantane substructure bearing in position 3 a secondary amino group. The latter is connected to a (2*S*)-cyanopyrrolidine by a C_2-linker moiety (acetyl). Retrosynthetic approaches to vildagliptin are based on a common strategy, starting with proline derivatives **2.3**. By acylation of the nitrogen atom of the proline residue, the C_2-linker was introduced (**3.3**) and, after dehydration of the prolinamide moiety (to give the compound **4.3**), 3-amino-1-adamantanol **5.3** was connected to give the final API. The last reaction relies on the presence a suitable leaving group placed on the distal carbon of the C_2-linker moiety.



Scheme 1.3 - previous syntheses of vildagliptin

The reported syntheses differ in the specific proline derivative used as starting materials. L-Prolinamide (R = -$CONH_2$) is the substrate of choice in the first reaction (acylation step) because it is commercially available in the desired enantiomerically pure form.[8.3,9.3,10.3] L-Proline and its methyl ester (R = -COOH, -COOMe) are valid alternative raw materials, provided that, after the acylation step, the carboxylate/carboxylic group is converted to the primary amide and then to the nitrile.[11.3,12.3] The preferred compound may be prolinonitrile (R = -CN) because it does not require a dehydration step. Since this compound is quite expensive and it is commercially available

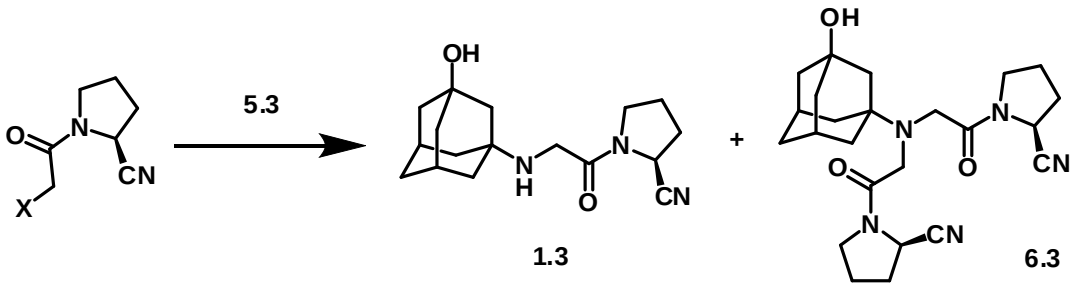
only as the Boc-protected derivative (additional deprotection is required), it is not convenient as a starting material the preparation of vildagliptin.

Existing synthetic protocols for the acylation of the chosen proline derivatives rely on the use of 2-chloroacetyl chloride,[8.3,13.3] or alternative acylating agents (X_1 = -Br, -I, -OTs, -ONs, -OTf and X_2 = -Br, -I, -OH, -$OSO_2(C_{1-8})$alkyl, -OSO_2(aryl)).[9.3,10.3]

The second step is the dehydration of the carboxamide group, leading the corresponding nitrile. This transformation may be delayed to a later stage, keeping the prolinamide residue through the following step.[14.3] In the prior art trifluoroacetic anhydride,[8.3,11.3,13.3,15.3] 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride),[11.3] Vilsmeier's reagent[9.3,16.3] or phosphoryl chloride[13.3] were used as dehydrating agents.

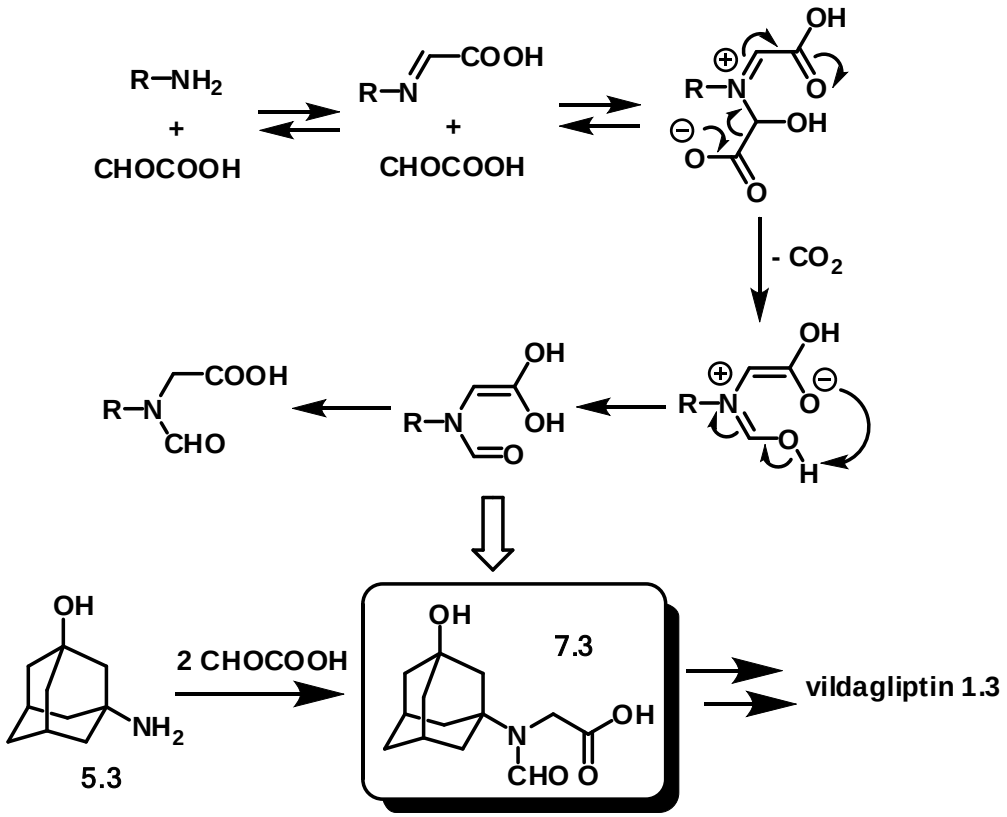
The obtained haloacetyl-derivative is then used as alkylating agent for the reaction with 3-amino-1-adamantanol **5.3**. Compound **5.3** is commercially available or can be prepared by direct oxidation of 1-aminoadamantane (HNO_3/H_2SO_4).[12.3,15.3]

The *N*-alkylation of the compound **5.3** is generally performed by using K_2CO_3 as base.[8.3] Nevertheless, this reaction is often plagued by the unwanted formation of the bis-alkylated byproduct **6.3**, which is very difficult to remove from vildagliptin **1.3** and determines a drop of the overall process yield (*Scheme 2.3*).[12.3] This bis-alkylated byproduct may be formed even if 3-amino-1-adamantanol **5.3** is alkylated in the early steps.[12.3] Reductive amination with glyoxylic acid is reported and this approach can partially circumvent the problem of the bis-alkylation.[17.3]



Scheme 2.3 - Formation of the bis-alkylation byproduct

Problems related to the previous synthetic strategies led us to study a new route for the preparation of vildagliptin, to avoid these issues and to prevent the formation of byproducts. Our alternative synthesis starts from the observation that glyoxylic acid is known to react with primary amines in a one-pot formylation and carboxymethylation of the nitrogen atom.[18.3] This represent the best way for the simultaneous introduction of the C_2-linker moiety on the amino group of the 3-amino-1-adamantanol **5.3**, leading to a key intermediate **7.3** avoiding the formation of the bis-alkylated compound (*Scheme 3.3*).



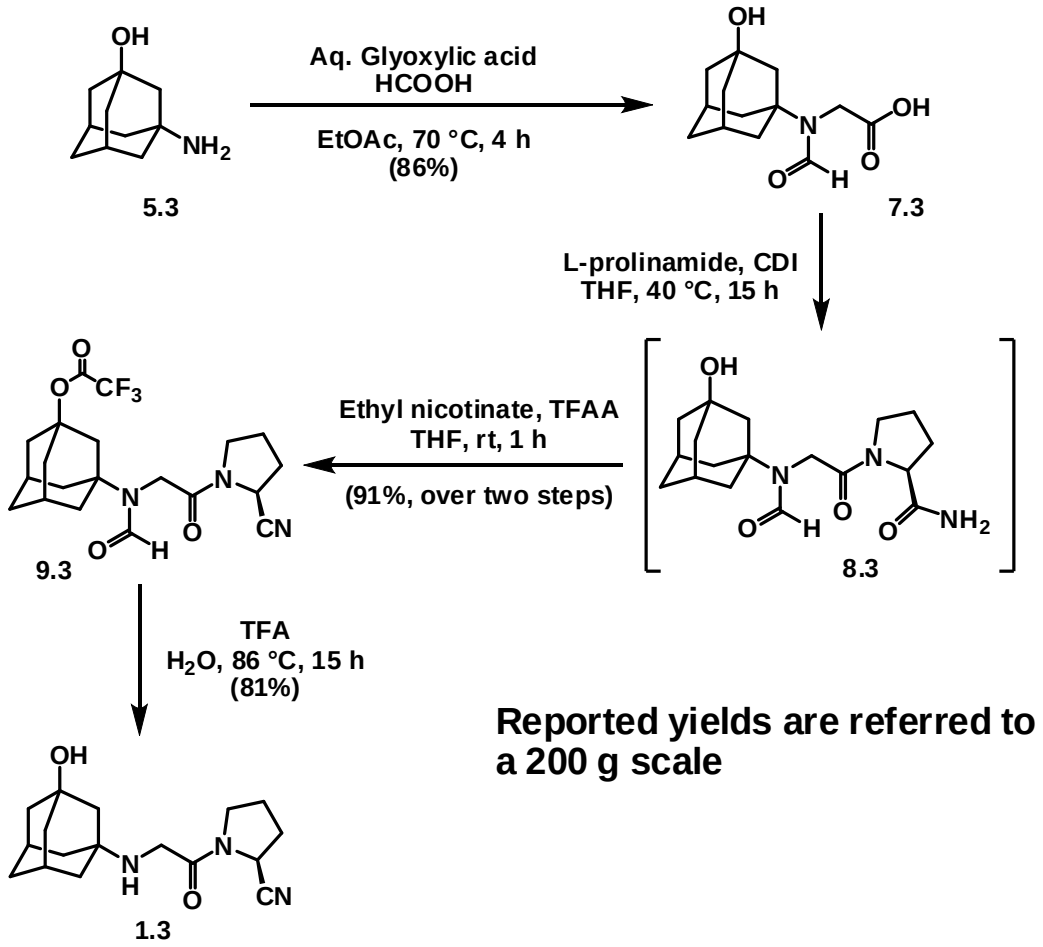
Scheme 3.3 - new approach to vildagliptin

With this key intermediate we developed a novel and original 4-step synthesis for the preparation of vildagliptin (*Scheme 4.3*).[19.3, 20.3]

Initially, the key intermediate **7.3** was generated by reacting 3-amino-1-adamantanol **5.3** with 3 equivalents of glyoxylic acid (50%$_{w/w}$ aq. solution) at 70°C for 4 hours, performing the one-pot *N*-formylation/*N*-

carboxymethylation. At the end of the reaction, the obtained mixture was slowly cooled at room temperature and the product was collected by simple filtration in good yield (86%).

To complete the assembly of the entire backbone of the final product, the key intermediate **7.3** was directly reacted, in the second step of this synthesis, with the selected raw material L-prolinamide in a dehydrative coupling. Initially the carboxylic function of **7.3** was activated by using *N,N'*-carbonyldiimidazole as condensing agent and tetrahydrofuran as solvent. Subsequently, the desired amide **8.3** was obtained by adding L-prolinamide and maintaining the mixture at 40°C for 15 hours. Since the purification of compound **8.3** was complex, due to its high water-solubility, it was used directly in the next step without purification.



Scheme 4.3 - new route of synthesis of vildagliptin

By using the combination ethyl nicotinate/trifluoroacetic anhydride[21.3], the primary amide of intermediate **8.3** (tetrahydrofuran solution) was transformed in the corresponding nitrile (room temperature, 1 hour, 91% yield). During the reaction, the hydroxyl group of the adamantane moiety was also protected as the corresponding trifluoroacetyl to give the compound **9.3**, isolated after a simple work-up.

The last step of the reported synthesis consisted in a cleavage of the protecting groups (*O*-trifluoroacetyl and *N*-formyl), leading the final API vildagliptin **1.3**. The product was achieved by reacting **9.3** with aqueous trifluoroacetic acid (5.4%$_{w/w}$) at 86°C for 15 hours, followed by the crystallization of the crude product from 2-butanone. Highly pure (>99.5%) vildagliptin **1.3** was obtained in a 81% yield as a white crystalline powder.[19.3, 20.3]

3.4 Conclusion and future developments

We report an innovative and convenient synthetic approach for the preparation of the important DPP-IV inhibitor vildagliptin **1.3**. This 4-step synthesis, consisting in a one-pot *N*-formylation-*N*-carboxymethylation of the amino group of the commercially available adamantane derivative **5.3**, leading to vildagliptin in a 63% overall yield. Previously reported syntheses ranged from 18%[15.3] to 55%[12.3] overall yield, worse than the way reported in this work. Moreover, the final product and all intermediate do not require chromatographic separation during the work-up, allowing the entire protocol to be adapted and performed on a large scale and easily transferable to the industrial production. In addition to this work, a patent[20.3] was filed by Chemelectiva s.r.l., claiming the key intermediates **7.3**, **8.3** and **9.3**.

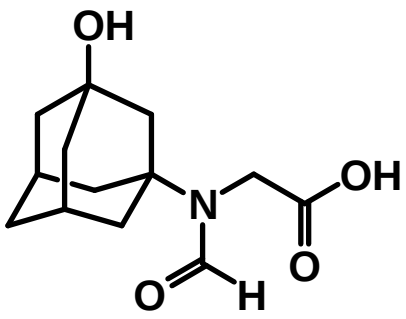
3.5 Experimental section

3.5.1 Materials and methods

The chemicals used for the experiments were of the highest analytical grade. Nuclear magnetic resonance spectra (^{1}H, ^{13}C-NMR) were recorded on a JEOL ECP300 (7.04 T) spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (*J*) are quoted in Hertz (Hz). Mass spectra (MS) were recorded on a ThermoFinnigan TSQ700 triple-quadrupole instrument equipped with an electrospray ionization source. DSC analysis were performed on a Nietzsch DSC 3500 Sirius instrument; the sample (~2 mg) was placed in an aluminum pierced pan and heated from 35°C to 400°C (heating rate 10°C/min) in a dry nitrogen atmosphere.

3.5.2 Synthesis of *N*-formyl-*N*-(3-hydroxy-1-adamantyl)aminoacetic acid (7.3)

3-Amino-1-adamantanol (**5.3**, 50.00 g, 0.299 mol) was suspended in ethyl acetate (350 mL) at room temperature. Formic acid 95% (15.80 mL, 0.419 mol) was added dropwise in about 10 minutes (slightly exothermic reaction). Aqueous glyoxylic acid (50%$_{w/w}$, 79.15 mL, 0.717 mol) was added and the suspension was heated at 70°C and stirred for 6 hours (formation of CO_2 was observed). After this period the reaction was cooled to room temperature in about 30 minutes and maintained at this temperature for further 30 minutes allow the complete precipitation of the product. The suspension was filtered and the solid was washed twice with acetone/water 2:1 (50 mL). The solid was dried in a vacuum oven at 45±5°C until constant weight to give *N*-formyl-*N*-(3-hydroxy-1-adamantyl)aminoacetic acid (**7.3**, 65.40 g, 86% yield) as a yellowish solid.



7.3

^{1}H-NMR (300 MHz, D_2O): δ (ppm) = 8.52 (s, 1H), 4.29 (s, 2H), 2.48 (m, 2H), 2.00 (m, 6H), 1.86 (m, 4H), 1.69 (m, 2H).

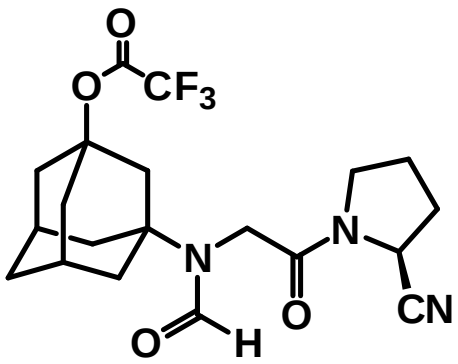
^{13}C-NMR (75.6 MHz, D_2O): δ (ppm) = 173.24 (C), 162.68 (CH), 69.83 (C), 59.88 (C), 47.54 (CH_2), 42.46 (CH_2), 42.13 (CH_2), 39.39 (CH_2), 33.83 (CH_2), 30.36 (CH).

MP = 218.6°C (DSC).

ESI-MS m/z 254.2 $[M+H]^+$ (calcd for $C_{13}H_{19}NO_4$, 253.1).

3.5.3 Synthesis of 1-[*N*-formyl-*N*-(3-trifluoracetyloxy-1-adamantyl)aminoacetyl]pyrrolidine-2-carbonitrile (9.3)

N,N'-Carbonyldiimidazole (121.20 g, 0.747 mol) was suspended in tetrahydrofuran (1030 mL) then the suspension was heated to 30±5°C to obtain complete dissolution. The reaction was cooled to room temperature and *N*-formyl-*N*-(3-hydroxy-1-adamantyl)aminoacetic acid (**7.3**, 159.00 g, 0.628 mol) was added in 4 portions in about 30 minutes. The resulting suspension was stirred at room temperature until complete dissolution (~1 hour). During this time the evolution of CO_2 was observed. *L*-Prolinamide (76.66 g, 0.672 mol) was then added and the reaction mixture was stirred and heated to 40±2°C for 15 hours. Complete solution was observed followed by incipient precipitation of 1-[*N*-formyl-*N*-(3-hydroxy-1-adamantyl)aminoacetyl]pyrrolidine-2-carboxamide (**8.3**). The suspension was cooled to room temperature. In a separate flask ethyl nicotinate (332.25 g, 2.198 mol) was dissolved in tetrahydrofuran (890 mL) and the reaction was cooled to 0±5°C. Trifluoroacetic anhydride (461.91 g, 2.198 mol) was added dropwise in about 30 minutes. The resulting solution was added dropwise to the stirred suspension of **8.3** in about 30 minutes, maintaining the temperature at 25±3°C. The reaction mixture then was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure. Toluene (1900 mL) and water (750 mL), cooled at 10°C, was added to the oily residue. The addition of water is slightly exothermic. The mixture was stirred until complete dissolution of the oily residue. The two phases were separated, and the organic layer was washed with 1N aqueous HCl (1x850 mL), water (3x850 mL) and saturated aqueous NaCl (1x750 mL). The organic phase was filtered on a Celite®/charcoal pad and the filter was washed with toluene (150 mL). The solvent was removed under reduced pressure to obtain 1-[*N*-formyl-*N*-(3-trifluoracetyloxy-1-adamantyl)aminoacetyl]pyrrolidine-2-carbonitrile (**9.3**, 244.61 g, 91% yield) as a yellow oily product, directly used in the following step.



9.3

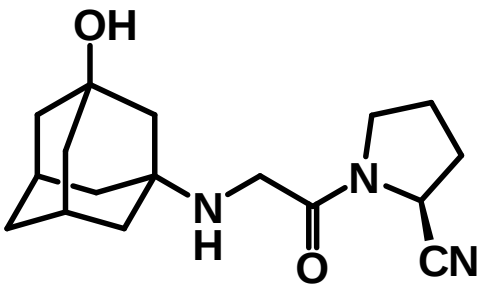
^{1}H-NMR (300 MHz, $CDCl_3$, mixture of rotamers in approx. ratio 80:20): δ (ppm, major rotamer) = 8.48 (s, 1H), 4.76 (dd, 1H, J_1 = 6.8 Hz, J_2 = 2.3 Hz), 4.21 (d, 1H, J = 16.3 Hz), 3.84 (d, 1H, J = 16.2 Hz), 3.65 (m, 2H), 2.47-1.90 (m, 16H), 1.65 (d, 1H, J = 14.2 Hz), 1.63 (d, 1H, J = 14.4 Hz). δ (ppm, minor rotamer) = 8.47 (s, 1H), 4.98 (dd, 1H, J_1 = 7.1 Hz, J_2 = 3.1 Hz), 4.32 (d, 1H, J = 15.9 Hz), 3.91 (d, 1H, J = 15.9 Hz), 3.54 (m, 2H), 2.47-1.59 (m, 18H).

^{13}C-NMR (75.6 MHz, $CDCl_3$, mixture of rotamers in approx. ratio 80:20): δ (ppm, major rotamer) = 167.0 (C), 161.4 (CH), 155.9 q(C) J_{C-F} = 41.6 Hz, 118.4 (C), 114.3 q(C) J_{C-F} = 287.1 Hz, 85.9 (C), 58.5 (C), 46.8 (CH), 46.1 (CH_2), 45.4 (CH_2), 42.3 (CH_2), 40.7 (CH_2), 39.3 (CH_2), 34.2 (CH_2), 30.6 (CH), 29.9 (CH_2), 25.4 (CH_2). δ (ppm, minor rotamer, selected signals) = 167.2 (C), 161.6 (CH), 155.9 q(C) J_{C-F} = 41.8 Hz, 118.8 (C), 85.8 (C), 58.7 (C), 47.1 (CH), 46.8 (CH_2), 45.6 (CH_2), 42.7 (CH_2), 40.8 (CH_2), 39.4 (CH_2), 32.5 (CH_2), 30.6 (CH), 23.1 (CH_2).

ESI-MS m/z 428.3 $[M+H]^+$ (calcd for $C_{20}H_{24}F_3N_3O_4$, 427.2).

3.5.4 Synthesis of vildagliptin (1.3)

1-[*N*-formyl-*N*-(3-trifluoracetyloxy-1-adamantyl)aminoacetyl]pyrrolidine-2-carbonitrile (**9.3**, 244.61 g, 0.572 mol) was suspended in water (680 mL). The oily suspension was vigorously stirred and trifluoroacetic acid (39.15 g, 0.343 mol) was added, then the reaction mixture was heated at 86±2°C for 15 hours. The clear solution was cooled to room temperature and NaCl (250.00 g) was added. Stirring was maintained until complete dissolution of the inorganic salt. Aqueous NaOH (30%$_{w/w}$, 147.80 mL) was added dropwise in about 15 minutes. Dichloromethane (670 mL) was added and the reaction mixture was stirred for 30 minutes. The organic phase was separated, and the aqueous layer was washed twice with dichloromethane (180 mL). The organic extract and washings were combined and washed with a solution of NaCl (60.00 g), $NaHCO_3$ (6.10 g) and water (300 mL). The solvent was removed under reduced pressure to obtain the crude **1.3**. 2-Butanone (500 mL) was added and the suspension was heated at reflux until complete dissolution. The solution was filtered hot through a Celite®/charcoal pad and the filter was washed with 2-butanone (60 mL, 60°C). The solution was cooled to 20±2°C, under stirring, in about 2 hours. Crystallization of the product was observed, and the suspension was maintained under stirring for about 2 hours to complete the precipitation process. The suspension was filtered and the solid was washed twice with 2-butanone (60 mL). The purified wet product was dried in a vacuum oven at 45°C to give vildagliptin (**1.3**, 140.6 g, 81% yield) as a colorless solid.



1.3

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.85 (dd, 0.2H, J_1 = 7.6 Hz, J_2 = 2.0 Hz), 4.74 (m, 0.8H), 3.68-3.34 (m, 0.8H+3.2H), 2.35-1.80 (1.6H+6.4H), 1.68-1.42 (m, 2.4H+9.6H).

^{13}C-NMR (75.6 MHz, $CDCl_3$): δ (ppm) = 170.64 (C), 118.33 (C), 69.64 (C), 53.64 (C), 49.93 (CH_2), 46.64 (CH), 45.58 (CH_2), 44.46 (2xCH_2), 43.49 (CH_2), 41.37 (CH_2), 41.20 (CH_2), 35.21 (CH_2), 30.78 (CH), 30.00 (CH_2), 25.15 (CH_2).

MP = 153.3°C (DSC).

ESI-MS m/z 304.3 $[M+H]^+$ (calcd for $C_{17}H_{25}N_3O_2$, 303.2).

3.6 References

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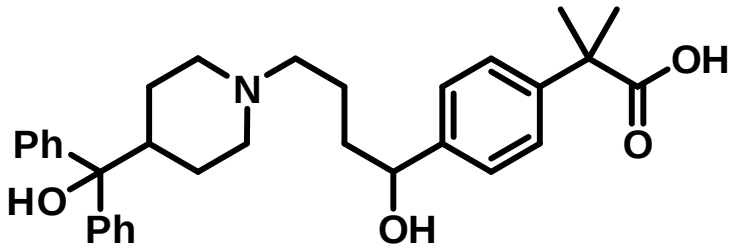
4. Fexofenadine

The API fexofenadine **1.4** (*Figure 1.4*) is a second-generation oral H_1-antihistamine, used worldwide for the treatment of allergic rhinitis (AR). It was selected as the target of the second year of the PhD, with the aim to achieve the same goals as the first year (R&D of a new route of synthesis and the corresponding scale up).



1.4

Figure 1.4 - Fexofenadine

4.1 Allergic rhinitis

Allergic rhinitis (AR) is a common inflammatory disease of the nose, induced by allergen exposure, which includes pollens, animal danders, airborne dust mite fecal particles and molds.[1.4]

AR affects 10% to 25% of people worldwide and up to 40% of the population of some countries (about 31 million people annually in the United States).[1.4,2.4] This pathology is characterized by 4 main symptoms like rhinorrea, sneezing, itching and nasal congestion. The latter is the predominant manifestation of AR and has a severe impact on the quality/duration of sleep, quality of life, ability to perform daily activities and school/work performances.[2.4] Nasal congestion is associated with sleep-disordered breathing, a condition that can have important effects on mental health, including psychiatric disorders.[3.4]

Inhalation of pollen allergens and their subsequent contact with the epithelial layer of the respiratory tract (upper airway and, possibly, lower

airway mucosa) causes an interaction with antigen presenting cells, in particular with histocompatibility complex molecules. This interaction triggers a development of specific classes of TH_2-cells, followed by the production of allergen-specific IgE (*Figure 2.4*). The IgE response mediates degranulation of basophils and mast-cells which release different substances (*e.g.* histamine, leukotrienes, tryptase) playing the role of mediators of AR symptoms.[3.4,4.4]

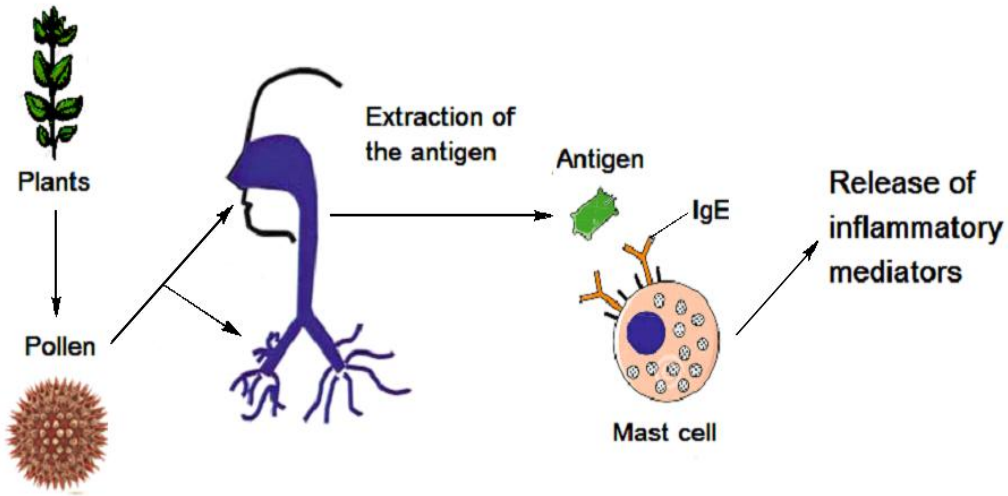


Figure 2.4 - Pathophysiology of allergic rhinitis[4.4]

4.2 Oral antihistamines for the AR treatment

Histamine is the major mediator of the early-phase reaction, stimulating sensory nerves and causing the AR symptoms, by interaction with its receptor H_1. This receptor mediate an increased vascular and epithelial permeability in the nasal mucosa, with the consequent vasodilatation and plasma extravasion.[2.4,4.4]

With the aim to block this cascade of events, a class of oral H_1-antihistamines was developed (*Figure 3.4*). These molecules, which are not structurally similar to histamine, are not receptor antagonists but bind H_1-receptor in different sites, to produce the opposite effect (inverse agonist).[5.4]

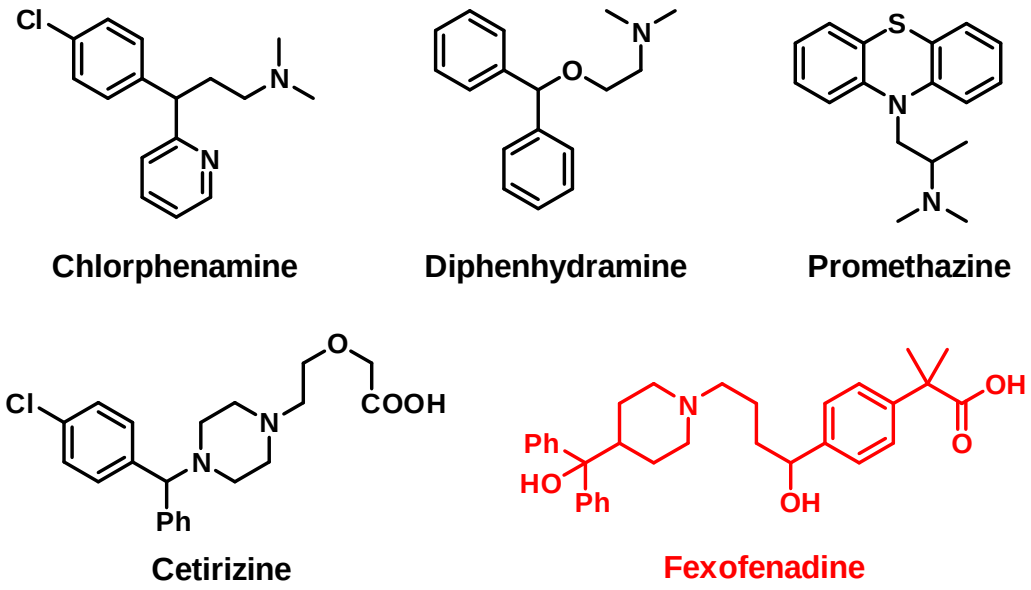


Figure 3.4 - H_1-Antihistamines

There are two classes of oral H_1-antihistamines (*Figure 3.4*):

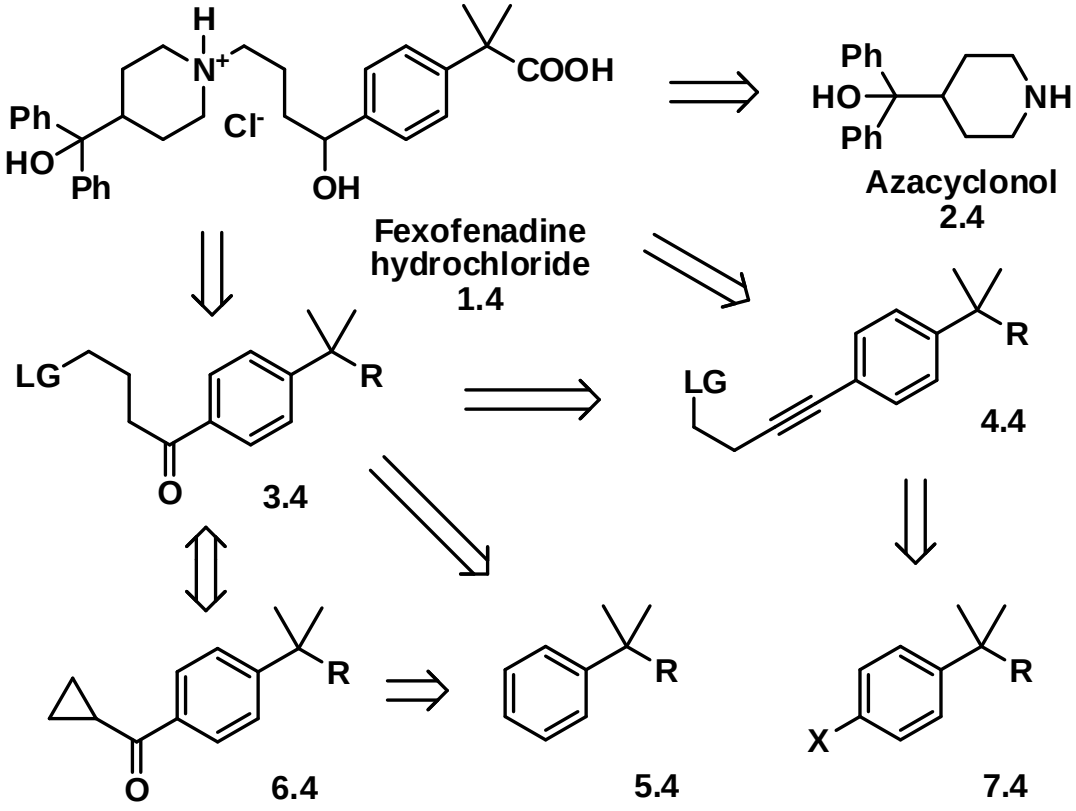
- 1st generation antihistamines (*e.g.* chlorphenamine, diphenhydramine, promethazine);
- 2nd generation antihistamines (*e.g.* cetirizine, fexofenadine).

The formers are no longer the treatment of choice for allergic rhinitis because of their adverse-effect profile, including poor selectivity (anticholinergic effects), cardiovascular effects, somnolence and sedation. Second generation H_1-antihistamines have become the preferred therapy for AR because their minimal or reduced sedative effects, high potency, longer duration of action compared with 1st generation drugs and pharmacodynamic properties that allow once-daily dosing.[2.4,3.4]

Fexofenadine hydrochloride is a potent, non-sedating and selective H_1-antihistamine and has a favourable effect on nasal congestion. Results of comparative trials showed that fexofenadine hydrochloride might offer many advantages compared with some others H_1-antihistamines. It is also selective for peripheral H_1-receptors and does not cross the blood-brain barrier. In addition fexofenadine hydrochloride is well tolerated in children aged 2-5 years with allergic rhinitis.[3.4]

4.3 Results and discussion

The API fexofenadine hydrochloride **1.4** (*Scheme 1.4*) includes an aromatic ring (α,α-dimethylphenylacetic acid) bearing in *para* position a C_4-aliphatic linker, terminating with azacyclonol (**2.4**, α,α-diphenyl-4-piperidinemethanol). The carbon atom directly connected to the aromatic moiety carries a secondary alcohol. This stereocenter configuration is racemic in the commercial product.



Scheme 1.4 - Retrosynthetic approaches to fexofenadine hydrochloride

Retrosynthetic approaches for the preparation of fexofenadine hydrochloride **1.4** rely on a common disconnection of the azacyclonol **2.4** (commercially available and cheap). The latter was introduced as such by a standard S_N2 nucleophilic displacement exploiting the presence of a leaving group (= LG) on the other precursor.

The alkylating agents used in this cross-coupling are intermediates **3.4** and **4.4**, consisting of the aralkyl portion and bearing a terminal leaving group, reactive in the S_N2 with azacyclonol **2.4**. Both the intermediates contain a group playing the role of precursor of the final secondary alcohol (keto group and alkyne moiety, respectively for intermediates **3.4** and **4.4**). Classical leaving groups employed in the alkylation step are represented by sulfonate esters (LG = OMs[6]) and all halogen atoms (LG = Cl,[7] Br, I[8]).

The C≡C triple bond may be formally hydrated to convert **4.4** to intermediate **3.4**, or later after the alkylation of azacyclonol.

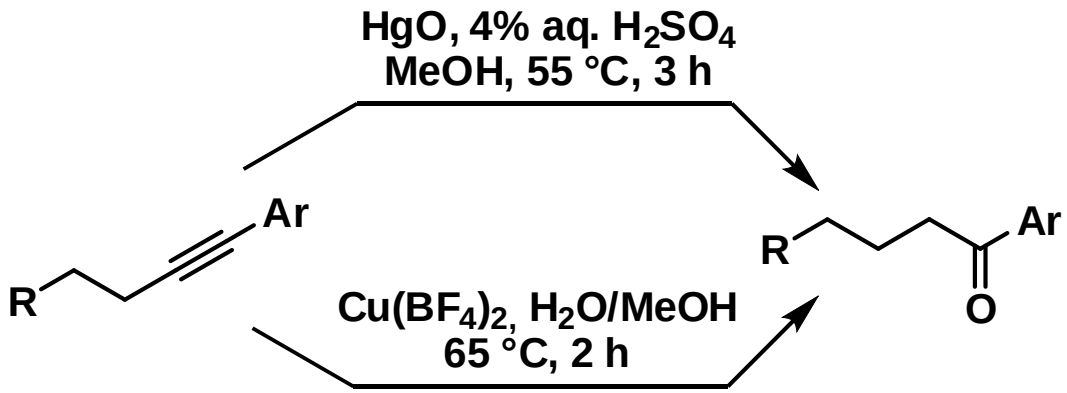
The key intermediates **3.4** and **4.4** share the common precursor **5.4**, represented by α,α-dimethylphenylacetic acid (R = COOH), precursors such as the corresponding nitrile (R = CN[9]), primary alcohol (R = CH_2OH[7]), the oxazoline[8] (converted to the carboxylic function during the multistep route of synthesis) and derivatives as the esters (R = COOMe, COOEt).

The γ-LG-butanoyl side chain of the compound **3.4** may be directly introduced with a Friedel-Crafts acylation of 4-chlorobutanoyl halides on intermediate **5.4**.[10] An alternative strategy consists in the introduction of a cyclopropanecarbonyl residue as a synthetic equivalent (compound **6.4**),[11] whose strained 3-membered rings could be easily opened to the linear-chain counterpart.[7] In a different approach, the alkynyl side chain of intermediate **4.4** is almost exclusively linked to the aromatic ring by a Sonogashira coupling[6] on the cheap and commercially available α,α-dimethylphenylacetic acid or esters thereof (**7.4**).

All the starting materials, used in these approaches, are commercially available at low price and most of the reactions are easy and practical. For these reasons our synthetic approach started from methyl 2-(4-bromophenyl)-2-methylpropanoate and take advantage on its proclivity to react efficiently with alkynes in the Sonogashira coupling.[12]

Nevertheless, the hydration of the C≡C triple bond of the intermediate arising from the Sonogashira reaction requires harsh conditions and toxic reagent, hardly compatible with large scale production. In a first example (*Scheme 2.4*), HgO in aqueous H_2SO_4 was used for the transformation of

the alkyne moiety in a keto group,[6] while in another patent the use of copper tetrafluoborate in refluxing aqueous methanol was described.[8] Another big problem for the industrial application of these hydration of the C≡C triple bond is the generation of toxic reaction wastes, difficult to dispose.

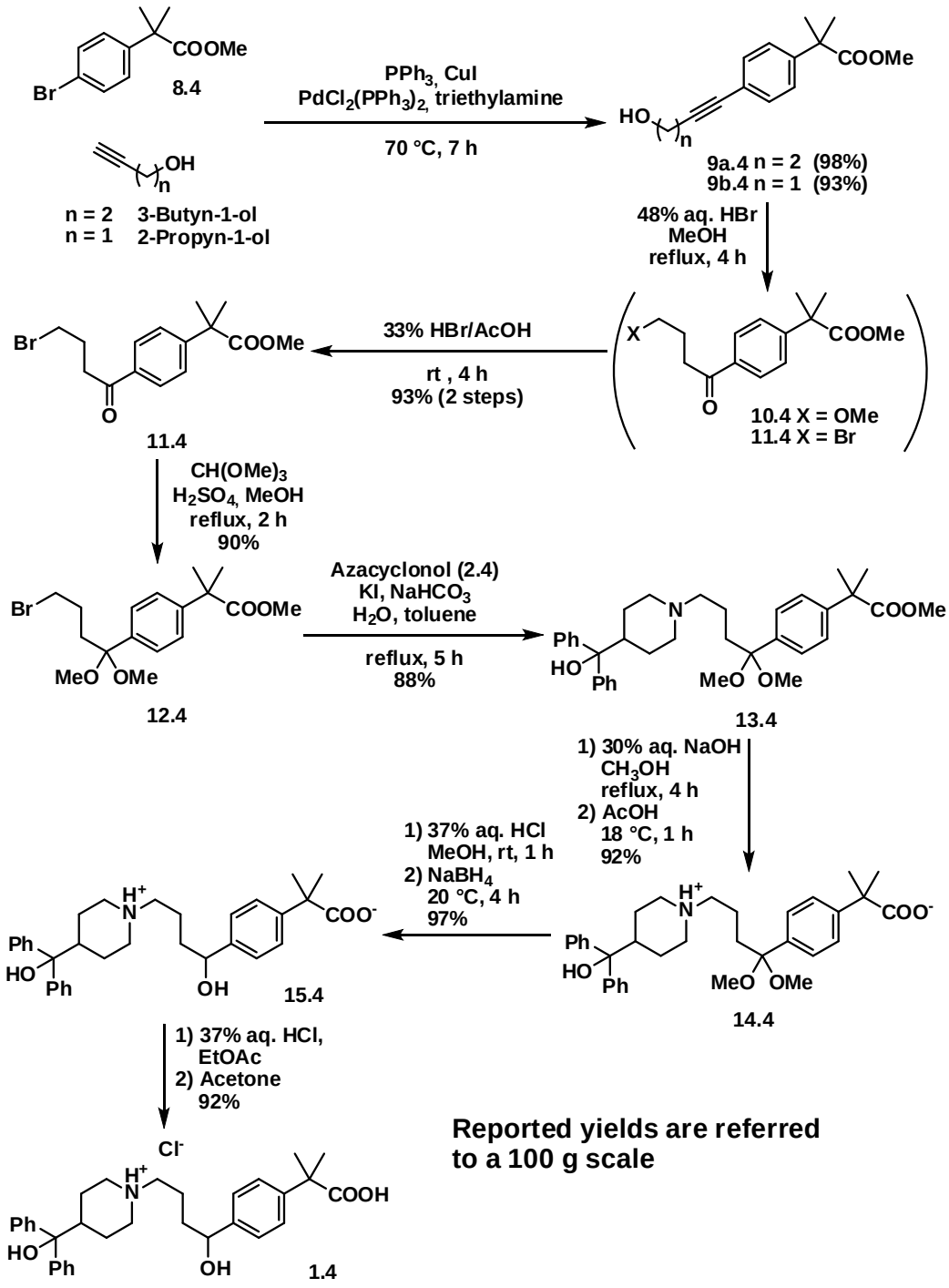


Scheme 2.4 - Existing protocols for the C≡C triple bond hydration

With the aim to eliminate the use of metal ions (either difficult to remove and toxic), we started to study new and alternative protocols for the alkyne hydration.

We found that the presence of a hydroxyl group, at the correct distance from the triple bond, gave an anchimeric assistance and increase the alkynyl group reactivity, for example in a reduction mediated by $LiAlH_4$.[13,14] For these reason we used 3-butyn-1-ol as the alkyne partner in the Sonogashira reaction. The hydroxyl group plays the double role of intramolecular assistance[15] and may finally converted to a leaving group for the subsequent attack of the azacyclonol residue.

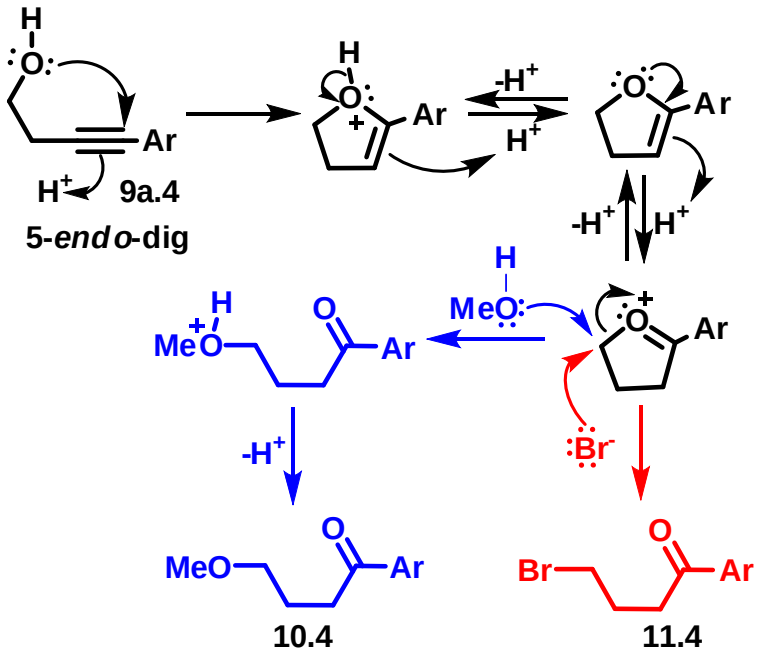
By using appropriate conditions (CuI, PPh_3, $PdCl_2(PPh_3)_2$ as catalyst and triethylamine, 70 °C for 7 h) we obtained the desired β-hydroxyalkyne **9a.4** in 98% yield (*Scheme 3.4*).



*Scheme 3.4 - Improved synthetic protocol to fexofenadine hydrochloride. No reaction was observed for compound **9b.4***

Treatment of this intermediate with 48%$_{w/w}$ aqueous HBr in refluxing methanol, give after 4 hours of reaction a mixture of two products, initially separated by a silica gel column cromatography and identified a methyl 2-(4-(4-methoxybutanoyl)phenyl)-2-methylpropanoate **10.4** and methyl 2-(4-(4-bromobutanoyl)phenyl)-2-methylpropanoate **11.4** (ratio **10.4/11.4**: 20:80).

The structure of both these compounds confirms the successful addition to the C≡C triple bond. The formation of these two molecules, γ-methoxyketone **10.4** and γ-bromoketones **11.4**, suggests the important anchimeric assistance of the remote hydroxyl group. A mechanistic hypothesis (*Scheme 4.4*) suggests that the formation of the γ-substituted ketones **10.4** and **11.4** arise from a 5-*endo*-dig cyclization (intramolecular attack of the OH-group to the triple bond), allowed according to the original Baldwin rules.[17] After the initial formation of a protonated 2-aryl-4,5-dihydrofurane in prototropic equilibrium with the isomeric 5-aryl-3,4-dihydro-2H-furan-1-ium ion, the latter undergoes nucleophilic attack (ring-opening) either by the bromide anion to give compound **11.4**, or of methanol leading to the generation of the γ-methoxyketone **10.4** as a by-product.



Scheme 4.4 - Proposed mechanism

To gain additional information about this unexpected cyclisation process, we synthesised the α-hydroxyalkyne **9b.4**. This lower homologue of **9a.4** was also prepared via Sonogashira coupling in a 93% yield, by using 2-propyn-1-ol. Intermediate **9b.4** was treated in the same conditions (reaction **9a.4** → **10.4**+**11.4**). No conversion was observed even after prolonged reaction times (*Scheme 3.4*), with quantitative recovery of the starting material unchanged.

Even if the transformation of **9b.4** involves a formally allowed 4-*endo*-dig cyclization, according to Baldwin rules, it is likely that the high strain of the incoming 4-membered 4-aryl-2H-oxete hinders its formation in the used conditions. A new revision of the original Baldwin rules showed the generally unfavourable outcome of these cyclization process.[17] This further supports that the hydroxyl group must be located in the correct position to give assistance and exploit its beneficial role.

The formation of a mixture of γ-bromoketone **11.4** and γ-methoxyketone **10.4** is not suitable for a future scale-up of the process, so we tried to direct the reaction toward a single product. We explored the possibility to convert the by-product **10.4** in the desired intermediate **11.4**, as reported in literature (cleavage of alkyl ethers),[18] by treating the raw mixture with 33%$_{w/w}$ HBr/AcOH (rt, 4 hours). Gratifyingly, in these conditions, the mixture of the two compounds converged to a single product, represented by the γ-bromoketone **11.4** in a 93% overall yield on two steps.

An attempt to perform a one-pot hydration/cleavage of ether (**9a.4**→**11.4**) was made by refluxing the substrate in 33%$_{w/w}$ HBr/AcOH. This reaction gave a complex reaction mixture including **11.4**, along with an elevate number of unidentified by-products.

The γ-bromoketone **11.4** paves the way for the preparation of fexofenadine hydrochloride **1.4**, representing a key intermediate important for the introduction of the azacyclonol residue. However, the ketone group needs to be suitably protected, prior to the S_N2 reaction with azacyclonol **2.4**. This protection was exploited with trimethylorthoformate and catalytic sulphuric acid in methanol (reflux temperature, 2 hours, 90% yield), leading the *O,O*-dimethylacetal **12.4**.

The γ-bromoketal reacts cleanly with azacyclonol using, in a biphasic mixture of toluene and water, sodium bicarbonate as the base and potassium iodide as the catalyst. After 5 hours at reflux temperature the advanced intermediate **13.4** was obtained in high yield (88%).

30%$_{w/w}$ Aqueous NaOH in methanol (4 hours, reflux temperature) was used to hydrolyse the ester moiety of the intermediate **13.4**, isolated after acidification with acetic acid in the corresponding zwitterionic form **14.4** in an excellent 97% yield.

The subsequent step consisted in a conversion of the ketal group in the secondary alcohol, representing the correct functional group of fexofenadine hydrochloride **1.4** (racemic). Initially, by using 37%$_{w/w}$ aqueous HCl, the ketal moiety was hydrolysed to regenerate the ketone group. Without further isolation of the keto-acid, addition of $NaBH_4$ concluded this one-pot hydrolysis/reduction, obtaining fexofenadine base **15.4** in very high yield (97%).

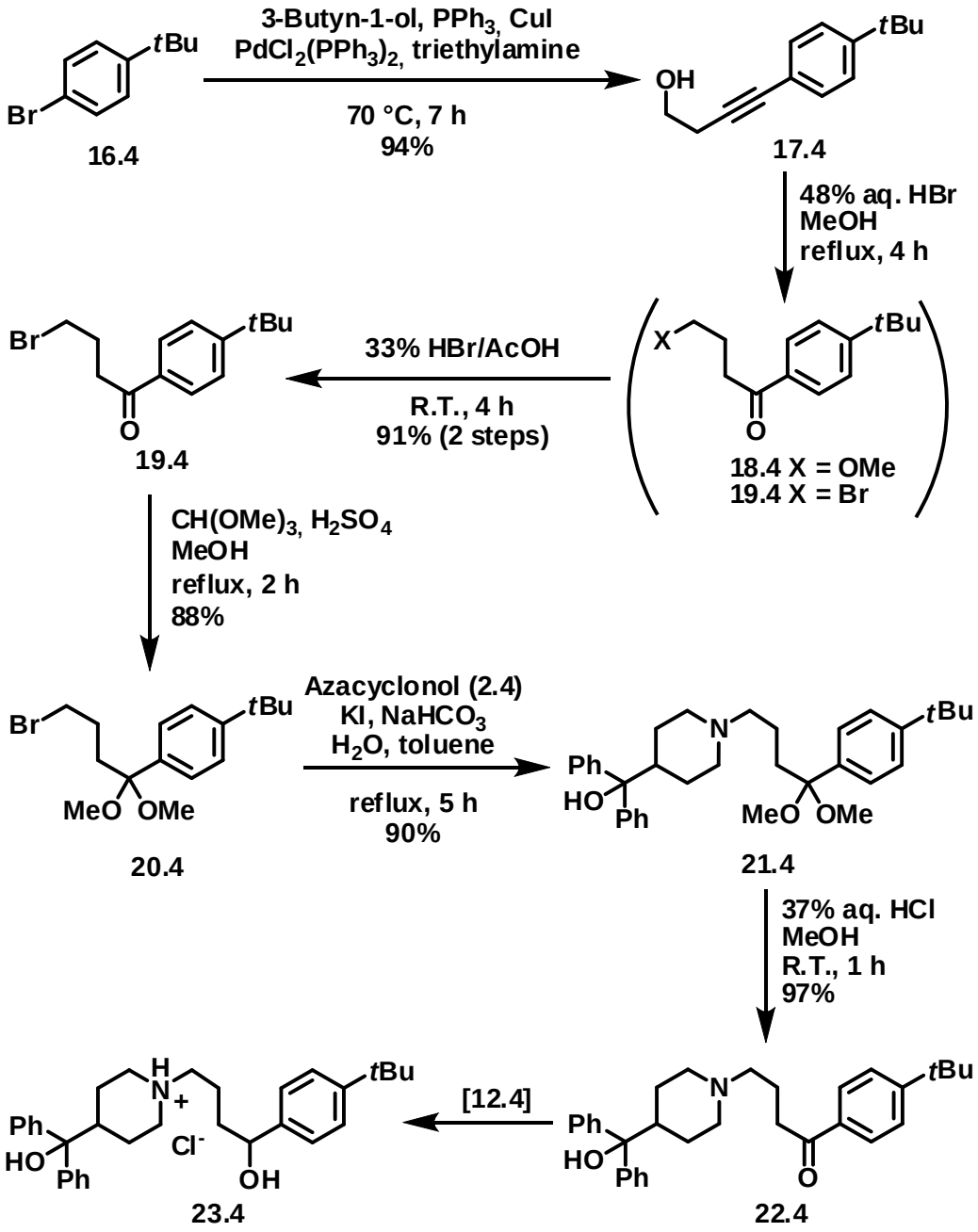
By the addition of 37%$_{w/w}$ aqueous HCl to a solution of fexofenadine base **15.4** in ethyl acetate, the last synthetic step was completed. After a short induction time, fexofenadine hydrochloride **1.4** starts to precipitate and was collected by filtration after 2 hours. After an additional digestion in acetone, analytically pure (> 99.5%) **1** was obtained as a white crystalline powder.

This 8-steps approach led in to the final API in an overall 59% yield. Moreover, the whole described process can be performed on a large scale (reported yields are referred to a 100 grams scale) and easily scaled to the industrial production,[19] also thanks to the fact that chromatographic separation was avoided in the isolation and purification of fexofenadine hydrochloride **1.4** and all the intermediates.

The entire protocol for the preparation of fexofenadine hydrochloride was applied for the synthesis of a similar API, terfenadine hydrochloride **23.4**. The aim of this work was to test the generality of all the steps used in our novel synthetic approach, first of all the formal alkyne hydration.

To this purpose, 1-bromo-4-*t*-butylbenzene **16.4** (*Scheme 5.4*) was used as the starting material. By using a Sonogashira reaction, with the same

conditions reported for fexofenadine, the β-hydroxyalkyne **17.4** was obtained in 94% yield.



Scheme 5.4 - Adapted route of synthesis for terfenadine hydrochloride

As expected, the treatment of the latter with 48%$_{w/w}$ aqueous HBr in methanol at reflux temperature for 4 hours gave a mixture of two compounds identified as the γ-bromoketone **19.4** and the γ-methoxyketone **18.4**. Application of the same strategy for the raw mixture of **10.4**/**11.4**, allowed a total conversion of **18.4**+**19.4** into **19.4** (33%$_{w/w}$ HBr/AcOH, 91% yield of the γ-bromoketone).

Protection of the carbonyl group was achieved by reaction with trimethylorthoformate and catalytic sulphuric acid in methanol. After 2 hours at reflux temperature, the corresponding γ-bromoketal **20.4** was isolated in high yield (88%). This protection was followed by the alkylation of the nitrogen atom of the azacyclonol **2.4** ($NaHCO_3$, KI as catalyst, biphasic mixture of water/toluene, 5 h, 90% yield). Hydrolysis of the ketal group of the obtained product **21.4**, led to the aminoketone **22.4**, converted in the last step of this synthesis into terfenadine hydrochloride **23.4**, according to an existing protocol known in literature.[12] This preparation was characterised by a 50% overall yield (7 steps).

4.4 Conclusion and future developments

In this work we reported an innovative and scalable synthetic strategy for the preparation of fexofenadine hydrochloride **1.4**, an important H_1-antihistamine. The synthesis consists in 8 steps, relying on a intramolecular cyclization which allows to perform a facile hydration of the C≡C triple bond. This approach led to fexofenadine hydrochloride **1.4** in a good overall yield (59%) and can be adapted for the preparation of similar molecules, *e.g.* terfenadine hydrochloride **23.4** (50% overall yield). No chromatographic separations are required, during work up, to isolate intermediates and final product, allowing the process to be performed on an industrial scale.

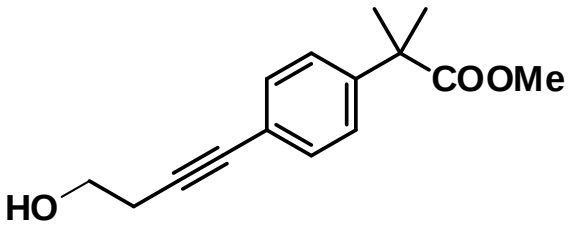
4.5 Experimental section

4.5.1 Materials and methods

The chemicals used for the experiments were of the highest analytical grade. Nuclear magnetic resonance spectra (^{1}H, ^{13}C-NMR) were recorded on a JEOL ECP300 (7.04 T) spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (*J*) are quoted in Hertz (Hz). Mass spectra (MS) were recorded on a ThermoFinnigan TSQ700 triple-quadrupole instrument equipped with an electrospray ionization source. DSC analysis were performed on a Nietzsch DSC 3500 Sirius instrument; the sample (~2 mg) was placed in an aluminum pierced pan and heated from 35°C to 400°C (heating rate 10°C/min) in a dry nitrogen atmosphere.

4.5.2 Synthesis of methyl 2-(4-(4-hydroxybut-1-ynyl)phenyl)-2-methylpropanoate (9a.4)

A solution of methyl 2-(4-bromophenyl)-2-methylpropanoate (**8.4**, 0.416 mol, 107.0 g) in triethylamine (1.040 mol, 144.2 mL) was stirred under nitrogen flux for 15 minutes. Triphenylphosphine (0.003 mol, 0.66 g) and CuI (0.002 mol, 0.31 g) was added and the reaction mixture was stirred until complete dissolution was obtained. To the resulting solution dichlorobis(triphenylphosphine)palladium(II) (0.001 mol, 0.58 g) was added and the reaction was heated to 70°C. 3-Butyn-1-ol (0.458 mol, 34.5 mL) was added dropwise over a period of 4 hours. At the end of the addition the resulting reaction mixture was stirred at 70°C for 7 hours. The reaction mixture was allowed to warm to room temperature and toluene (300 mL) was added. Aqueous HCl (3.5M, 180 mL) was added dropwise, maintaining the temperature under 30°C (slightly exothermic). At the end of the addition the phases were separated, and the organic layer was washed with water (3x150 mL). Charcoal (5 g) was added and the mixture was stirred for 30 minutes then was filtered off on a Celite® pad. The filter was washed with toluene (2x20 mL) and the solvent was removed under reduced pressure to give methyl 2-(4-(4-hydroxybut-1-ynyl)phenyl)-2-methylpropanoate (**9a.4**, 100.0 g, 98% yield) as an orange oil.



9a.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.35 (2H, d, *J* = 8.3 Hz), 7.27 (2H, d, *J* = 8.6 Hz), 4.88 (1H, t, *J* = 5.5 Hz), 3.59 (5H, m), 2.55 (2H, t, *J* = 6.9 Hz), 1.48 (6H, s).

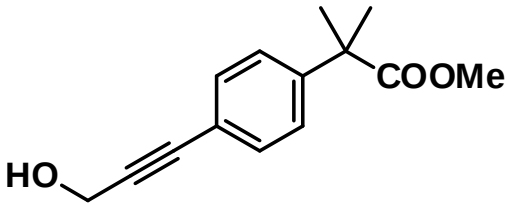
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 176.0 (C), 144.3 (C), 131.3 (CH), 125.7 (CH), 121.6 (C), 88.5 (C), 80.7 (C), 59.8 (CH_2), 52.1 (CH_3), 46.1 (C), 26.1 (CH_3), 23.3 (CH_2).

FT-IR (KBr): ṽ = 3413, 2978, 2950, 2885, 1729, 1508, 1467, 1435, 1255, 1147, 1098, 1046, 838 cm^{-1}.

ESI-MS m/z 247.1 $[M+H]^+$ (calcd for $C_{15}H_{18}O_3$, 246.1).

4.5.3 Synthesis of methyl 2-(4-(3-hydroxyprop-1-ynyl)phenyl)-2-methylpropanoate (9b.4)

A solution of methyl 2-(4-bromophenyl)-2-methylpropanoate (**8.4**, 0.019 mol, 5.0 g) in triethylamine (0.049 mol, 6.7 mL) was stirred under nitrogen flux for 15 minutes. Triphenylphosphine (0.12 mmol, 31 mg) and CuI (0.10 mmol, 19 mg) was added and the reaction mixture was stirred until complete dissolution was obtained. To the resulting solution dichlorobis(triphenylphosphine)palladium(II) (0.04 mmol, 27 mg) was added and the reaction was heated to 70°C. 2-Propyn-1-ol (0.021 mol, 1.3 mL) was added dropwise over a period of 4 hours. At the end of the addition the resulting reaction mixture was stirred at 70°C for 7 hours. The reaction mixture was allowed to warm to room temperature and toluene (15 mL) was added. Aqueous HCl (3.5M, 9 mL) was added dropwise, maintaining the temperature under 30°C (slightly exothermic). At the end of the addition the phases were separated, and the organic layer was washed with water (3x9 mL). Charcoal (0.25 g) was added and the mixture was stirred for 30 minutes then was filtered off on a Celite® pad. The filter was washed with toluene (2x1 mL) and the solvent was removed under reduced pressure to give methyl 2-(4-(3-hydroxyprop-1-ynyl)phenyl)-2-methylpropanoate (**9b.4**, 4.2 g, 93% yield) as an orange oil.



9b.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.39 (d, 2H, *J* = 8.6 Hz), 7.30 (d, 2H, *J* = 8.3 Hz), 5.35 (t, 1H, *J* = 6.0 Hz), 4.32 (d, 2H, *J* = 5.5 Hz), 3.58 (s, 3H), 1.49 (s, 6H).

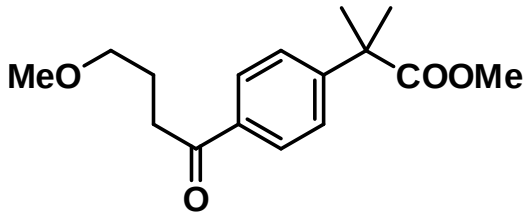
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 176.1 (C), 144.9 (C), 131.3 (CH), 125.9 (CH), 120.9 (C), 89.9 (C), 83.3 (C), 52.1 (CH_3), 49.6 (CH_2), 46.2 (C), 26.1 (CH_3).

FT-IR (KBr): ṽ = 3432, 2978, 2943, 2870, 1729, 1507, 1467, 1435, 1256, 1148, 1098, 1024, 838 cm^{-1}.

ESI-MS m/z 233.2 $[M+H]^+$ (calcd for $C_{15}H_{18}O_3$, 232.1).

4.5.4 Synthesis of methyl 2-(4-(4-bromobutanoyl)phenyl)-2-methylpropanoate (11.4)

A solution of 2-(4-(4-hydroxybut-1-ynyl)phenyl)-2-methylpropanoate (**9a.4**, 0.406 mol, 100.0 g) in methanol (200 mL) was cooled down to 10°C and aqueous HBr (48%$_{w/w}$, 0.406 mol, 45.9 mL) was added dropwise, maintaining the temperature below 25°C. At the end of the addition, the reaction mixture was heated to reflux temperature for 4 hours. The solution was then cooled down to room temperature and the solvent was removed under reduced pressure to give a mixture of methyl 2-(4-(4-methoxybutanoyl)phenyl)-2-methylpropanoate **10.4** and methyl 2-(4-(4-bromobutanoyl)phenyl)-2-methylpropanoate **11.4** as a brown oil. A small aliquot of the mixture was subjected to column chromatography (eluant hexane-ethyl acetate 95:5), obtaining an analytical sample of **10.4**. The whole residue was cooled down to 5°C and acetic HBr (33%$_{w/w}$, 100 mL) was added dropwise, maintaining the temperature below 20°C. At the end of the addition the resulting mixture was stirred at room temperature for 4 hours. At the end of the reaction toluene (250 mL) and water (100 mL) were added. The phases were separated and the product was extracted with toluene (30 mL). The combined organic phases were washed with aqueous $NaHCO_3$ saturated solution (150 mL) and the solvent was removed under reduced pressure to give methyl 2-(4-(4-bromobutanoyl)phenyl)-2-methylpropanoate (**11.4**, 123.0 g, 93% yield) as a brown oil.



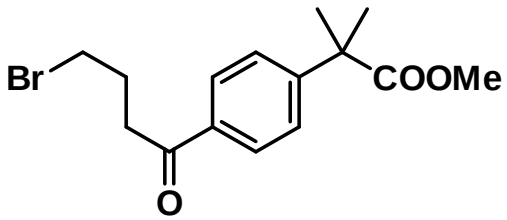
10.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.93 (d, 2H, *J* = 8.3 Hz), 7.44 (d, 2H, *J* = 8.3 Hz), 3.60 (s, 3H), 3.36 (t, 2H, *J* = 6.3 Hz), 3.22 (s, 3H), 3.03 (t, 2H, *J* = 7.2 Hz), 1.85 (quint, 2H, *J* = 6.7 Hz), 1.52 (s, 6H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 199.0 (C), 175.8 (C), 149.6 (C), 135.1 (C), 128.0 (CH), 125.9 (CH), 71.1 (CH_2), 57.7 (CH_3), 52.1 (CH_3), 46.4 (C), 34.6 (CH_2), 26.1 (CH_3), 23.8 (CH_2).

FT-IR (KBr): ṽ = 2978, 2931, 2877, 1731, 1683, 1606, 1464, 1407, 1367, 1256, 1148, 1097, 992, 845 cm^{-1}.

ESI-MS m/z 279.3 $[M+H]^+$ (calcd for $C_{16}H_{22}O_4$, 278.2).



11.4

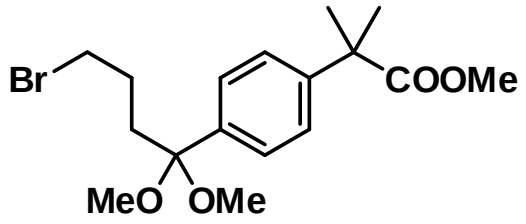
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.94 (d, 2H, *J* = 8.3 Hz), 7.46 (d, 2H, *J* = 8.3 Hz), 3.60 (m, 5H), 3.17 (t, 2H, *J* = 7.0 Hz), 2.17 (quint, 2H, *J* = 6.7 Hz), 1.53 (s, 6H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 198.1 (C), 175.7 (C), 149.7 (C), 134.9 (C), 128.0 (CH), 125.9 (CH), 52.1 (CH_3), 46.4 (C), 36.3 (CH_2), 34.3 (CH_2), 27.0 (CH_2), 26.0 (CH_3).

ESI-MS m/z 327.2/329.2 $[M+H]^+$ (calcd for $C_{15}H_{19}BrO_3$, 326.1/328.1).

4.5.5 Synthesis of methyl 2-(4-(4-bromo-1,1-dimethoxybutyl)phenyl)-2-methylpropanoate (12.4)

To a solution of methyl 2-(4-(4-bromobutanoyl)phenyl)-2-methylpropanoate (**11.4**, 0.376 mol, 123.0 g) in methanol (123 mL) were added sulfuric acid (0.038 mol, 2.0 mL) and trimethylorthoformate (0.451 mol, 49.4 mL). The resulting solution was heated to reflux temperature for 2 hours. At the end of the reaction, the solution was cooled down to 20°C and methanolic NaOMe ($30\%_{w/w}$, 7.0 mL) was added. The solvent was evaporated under reduced pressure and toluene (250 mL) and water (100 mL) were added. The phases were separated and the solvent was removed under reduced pressure to give methyl 2-(4-(4-bromo-1,1-dimethoxybutyl)phenyl)-2-methylpropanoate (**12.4**, 125.8 g, 90% yield) as a brown oil.



12.4

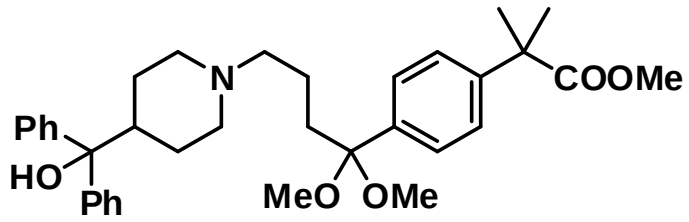
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.36 (d, 2H, *J* = 8.0 Hz), 7.32 (d, 2H, *J* = 8.6 Hz), 3.59 (s, 3H), 3.36 (t, 2H, *J* = 7.4 Hz), 3.05 (s, 6H), 1.97 (m, 2H), 1.51 (s, 6H), 1.41 (m, 2H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 176.1 (C), 143.9 (C), 138.6 (C), 126.6 (CH), 125.1 (CH), 102.5 (C), 51.9 (CH_3), 48.0 (CH_3), 45.9 (C), 35.1 (CH_2), 34.5 (CH_2), 26.9 (CH_2), 26.3 (CH_3).

ESI-MS m/z 373.2/375.2 $[M+H]^+$ (calcd for $C_{17}H_{25}BrO_4$, 372.1/374.1).

4.5.6 Synthesis of methyl 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)-phenyl)-2-methylpropanoate (13.4)

To a solution of $NaHCO_3$ (0.472 mol, 39.7 g) and KI (0.034 mol, 5.6 g) in water (250 mL) azacyclonol (0.337 mol, 90.1 g) was added. A solution of methyl 2-(4-(4-bromo-1,1-dimethoxybutyl)phenyl)-2-methylpropanoate (**12.4**, 0.337 mol, 125.8 g) in toluene (500 mL) was added and the resulting reaction mixture was heated to reflux for 5 hours. At the end of the reaction, the mixture was allowed to warm to room temperature and the phases were separated. The organic layer was washed with water (250 mL) and the organic solvent was removed under reduced pressure to give 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)phenyl)-2-methylpropanoate (**13.4**, 165.6 g, 88% yield) as a brownish oil.



13.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.49 (d, 4H, *J* = 7.4 Hz), 7.36 (d, 2H, *J* = 8.6 Hz), 7.30 (m, 6H), 7.11 (t, 2H, *J* = 7.3 Hz), 4.92 (s, 1H), 3.57 (s, 3H), 3.07 (s, 6H), 2.61 (bd, 2H, *J* = 11.0 Hz), 2.36 (tt, 1H, J_1 = 11.6 Hz, J_2 = 3.1 Hz), 2.05 (bt, 2H, *J* = 7.0 Hz), 1.87 (m, 2H), 1.75 (bt, 2H, *J* = 10.7 Hz), 1.51 (s, 6H), 1.43 (qd, 2H, J_1 = 12.2 Hz, J_2 = 2.7 Hz), 1.23 (m, 2H), 1.06 (quint, 2H, *J* = 7.5 Hz).

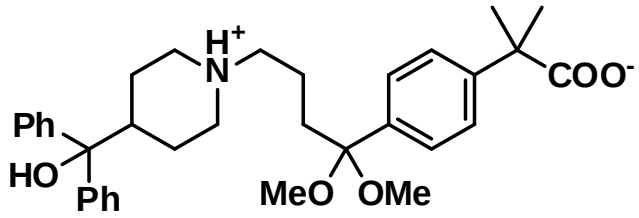
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 176.2 (C), 147.3 (C), 143.7 (C), 139.1 (C), 127.7 (CH), 126.7 (CH), 125.7 (CH), 125.0 (CH), 103.1 (C), 78.5 (C), 57.5 (CH_2), 53.6 (CH_2), 51.9 (CH_3), 48.1 (CH_3), 45.9 (C), 43.8 (CH), 34.2 (CH_2), 26.4 (CH_3), 26.0 (CH_2), 20.6 (CH_2).

FT-IR (KBr): ṽ = 3528, 2952, 2931, 2810, 2771, 1956, 1889, 1748, 1490, 1448, 1261, 1150, 752, 707 cm^{-1}.

ESI-MS m/z 560.5 $[M+H]^+$ (calcd for $C_{35}H_{45}NO_5$, 559.3).

4.5.7 Synthesis of 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)phenyl)-2-methylpropanoic acid (14.4)

To a solution of 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)phenyl)-2-methylpropanoate (**13.4**, 0.296 mol, 169.8 g) in methanol (450 mL) aqueous NaOH (30%$_{w/w}$, 0.503 mol, 50.4 mL) was added dropwise, maintaining the temperature below 25°C. At the end of the addition the reaction mixture was heated to reflux for 4 hours. At the end of the reaction, the resulting solution was allowed to warm to room temperature and acetic acid (0.503 mol, 28.8 mL) was added dropwise over a period of 30 minutes. Precipitation of the product was observed. The resulting suspension was stirred at 18°C for 1 hour, in order to maximize the precipitation of the product then was filtered, the solid washed with methanol/water 2:1 (2x40 mL) and dried in a vacuum oven. The crude was suspended in methanol (890 mL) and the resulting suspension was heated to reflux. Water (44 mL) was added and the mixture was stirred for 1 hour. The suspension was cooled down to 15°C and was stirred for 30 minutes, in order to maximize the precipitation of the product. The solid was filtered, washed with methanol/water 2:1 (2x40 mL) and dried in a vacuum oven to give 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)-phenyl)-2-methylpropanoic acid (**14.4**, 148.6 g, 92% yield) as a white solid.



14.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.48 (d, 4H, *J* = 7.3 Hz), 7.33 (s, 4H), 7.24 (t, 4H, *J* = 7.7 Hz), 7.11 (t, 2H, *J* = 7.3 Hz), 4.27 (bs, >2H, OH + NH + moisture), 3.04 (s, 6H), 2.65 (bd, 2H, *J* = 11.1 Hz), 2.37 (tt, 1H, J_1 = 11.6 Hz, J_2 = 3.0 Hz), 2.06 (bt, 2H, *J* = 7.1 Hz), 1.80 (m, 4H), 1.45 (s, 6H), 1.40 (m, 2H), 1.20 (bd, 2H, *J* = 12.5 Hz), 1.04 (quint, 2H, J = 7.8 Hz).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 177.9 (C), 147.2 (C), 145.1 (C), 138.4 (C), 127.8 (CH), 126.4 (CH), 125.8 (CH), 125.3 (CH), 103.1 (C), 78.5 (C), 57.1 (CH_2), 53.1 (CH_2), 48.1 (CH_3), 45.8 (C), 43.3 (CH), 34.2 (CH_2), 26.7 (CH_3), 25.5 (CH_2), 20.2 (CH_2).

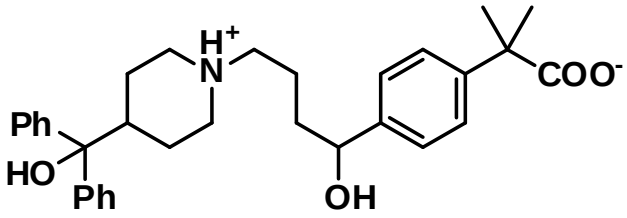
MP 191.1°C (DSC).

FT-IR (KBr): ṽ = 3300-2200 (br), 2956, 2828, 1577, 1471, 1447, 1391, 1347, 1074, 1041, 746, 706 cm^{-1}.

ESI-MS m/z 546.3 $[M+H]^+$ (calcd for $C_{34}H_{43}NO_5$, 545.3).

4.5.8 Synthesis of fexofenadine (15.4)

To a suspension of 2-(4-(4-(hydroxydiphenylmethyl)pyperidyn-1-yl)-1,1-dimethoxybutyl)phenyl)-2-methylpropanoic acid (**14.4**, 0.272 mol, 148.6 g) in methanol (445 mL) aqueous HCl ($37\%_{w/w}$, 0.299 mol, 24.8 mL) was added dropwise over a period of 30 minutes. The resulting solution was stirred for 1 hour. At the end of the reaction, aqueous NaOH ($30\%_{w/w}$, 0.598 mol, 60.0 mL) was added dropwise over a period of 30 minutes, maintaining the temperature below 30°C (slightly exothermic). At the end of the addition, the resulting reaction mixture was cooled down to 18°C and $NaBH_4$ (0.163 mol, 6.2 g) was added portionwise over about 30 minutes. At the end of the addition, the resulting reaction mixture was stirred at 20°C for 4 hours. At the end of the reaction charcoal (5 g) was added, the mixture was filtered on a Celite® pad and the filter washed with methanol/water 2:1 (2x20 mL). To the resulting solution acetic acid (0.707 mol, 40.5 mL) was added dropwise over a period of 30 minutes. Precipitation of the product was observed. The solid was filtered, washed with methanol/water 2:1 (2x40 mL) and dried in a vacuum oven to give fexofenadine (**15.4**, 132.1 g, 97% yield) as a white solid.



15.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.54 (d, 4H, *J* = 7.7 Hz), 7.29 (m, 8H), 7.14 (t, 2H, *J* = 7.2 Hz), 4.88 (bs, >3H, 2xOH + NH + moisture), 4.53 (t, 1H, *J* = 6.0 Hz), 2.88 (bs, 2H), 2.46 (bt, 1H, *J* = 10.7 Hz), 2.32 (bt, 2H, *J* = 6.6 Hz), 1.99 (bt, 2H, *J* = 10.9 Hz), 1.64 (m, 2H), 1.52 (m, 4H), 1.49 (s, 6H), 1.32 (bd, 2H, *J* = 12.2 Hz).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 178.3 (C), 147.2 (C), 144.4 (C), 144.1 (C), 127.9 (CH), 125.8 (CH), 125.6 (CH), 125.3 (CH), 78.5 (C), 72.0 (CH), 57.5 (CH_2), 53.1 (CH_2), 53.0 (CH_2), 45.9 (C), 43.2 (CH), 37.4 (CH_2), 26.9 (CH_3), 25.4 (CH_2), 22.5 (CH_2).

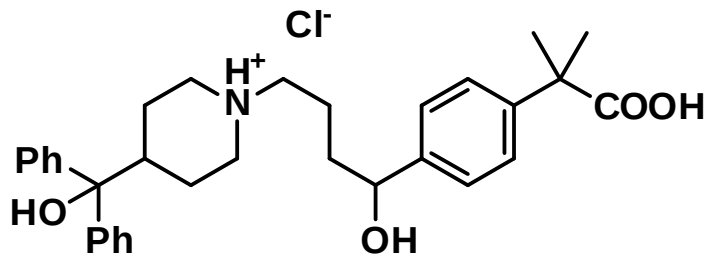
MP 226.4°C (DSC).

FT-IR (KBr): $\tilde{\nu}$ = 3640, 3324 (br), 3050, 3028, 2989, 2961, 2867, 3000-2200 (br), 1664, 1624, 1578, 1499, 1447, 1390, 1324, 1165, 1067, 859, 755, 708 cm^{-1}.

ESI-MS m/z 502.3 $[M+H]^+$ (calcd for $C_{32}H_{39}NO_4$, 501.3).

4.5.9 Synthesis of fexofenadine·HCl (1.4)

To a solution of fexofenadine base (**15.4**, 0.263 mol, 132.1 g) in ethyl acetate (1320 mL) aqueous HCl (37%$_{w/w}$, 0.289 mol, 23.9 mL) was added dropwise over a period of 30 minutes. At the end of the addition, a white solid starts to precipitate. The resulting suspension was stirred for 2 hours, in order to allow the complete precipitation of the product. The white solid was filtered and washed with ethyl acetate (3x50 mL) and dried in a vacuum oven at 45-50°C. The solid was suspended in acetone (780 mL) and heated to reflux for 5 hours. The reaction mixture was allowed to cool down to 40°C and was filtered. The solid was washed with acetone (3x50 mL) and dried in a vacuum oven to give fexofenadine·HCl (**1.4**, 130.0 g, 92% yield) as a white solid.



1.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 10.04 (bs, 1H), 7.52 (d, 4H, *J* = 7.7 Hz), 7.29 (m, 8H), 7.16 (t, 2H, *J* = 7.2 Hz), 5.40 (bs, 1H), 4.56 (t, 1H, *J* = 6.0 Hz), 3.36 (bs, 1H), 3.32-2.72 (m, 10H), 1.83 (m, 4H), 1.67 (m 2H), 1.49 (s, 6H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 177.6 (C), 146.7 (C), 144.1 (C), 143.5 (C), 128.0 (CH), 126.2 (CH), 125.8 (CH), 125.3 (CH), 78.3 (C), 71.3 (CH), 56.1 (CH_2), 51.6 (CH_2), 45.5 (C), 41.0 (CH), 36.1 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 20.0 (CH_2).

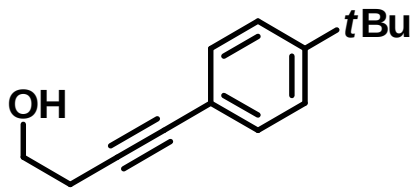
MP 168.9°C (DSC).

FT-IR (KBr): ṽ = 3528, 3311, 3055, 3024, 2943, 2885, 3000-2450, 1716, 1508, 1491, 1446, 1264, 750, 702 cm^{-1}.

ESI-MS m/z 502.3 $[(M\cdot HCl)-Cl]^+$ (calcd for $C_{32}H_{40}ClNO_4$, 537.3).

4.5.10 Synthesis of 4-(4-*t*-butylphenyl)but-3-yn-1-ol (17.4)

A solution of 1-bromo-4-*t*-butylbenzene (**16.4**, 0.024 mol, 5.0 g) in triethylamine (0.059 mol, 8.2 mL) was stirred under nitrogen for 15 minutes. Triphenylphosphine (0.14 mmol, 37 mg) and CuI (0.12 mmol, 22 mg) were added and the reaction mixture was stirred until complete dissolution was obtained. To the resulting solution dichlorobis(triphenylphosphine) palladium(II) (0.05 mmol, 33 mg) was added and the reaction was heated to 70°C. 3-Butyn-1-ol (0.026 mol, 2.0 mL) was added dropwise over a period of 4 hours. At the end of the addition the resulting reaction mixture was stirred at 70°C for 7 hours. The reaction mixture was allowed to warm to room temperature and toluene (15 mL) was added. Aqueous HCl (3.5M, 9 mL) was added dropwise, maintaining the temperature under 30°C (slightly exothermic). At the end of the addition the phases were separated, and the organic layer was washed with water (3x9 mL). Charcoal (0.25 g) was added and the mixture was stirred for 30 minutes then was filtered off on a Celite® pad. The filter was washed with toluene (2x5 mL) and the solvent was removed under reduced pressure to give 4-(4-*t*-butylphenyl)but-3-yn-1-ol (**17.4**, 4.5 g, 94% yield) as an orange oil.



17.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.34 (d, 2H, *J* = 8.9 Hz), 7.31 (d, 2H, *J* = 8.9 Hz), 4.90 (t, 1H, *J* = 5.7 Hz), 3.59 (q, 2H, *J* = 3.6 Hz), 2.55 (t, 2H, *J* = 6.9 Hz), 1.22 (s, 9H).

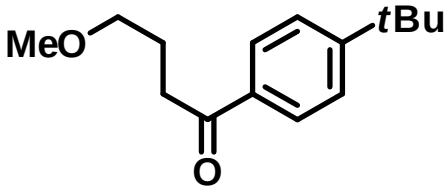
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 151.0 (C), 131.6 (CH), 125.8 (CH), 120.9 (C), 88.2 (C), 81.6 (C), 60.5 (CH_2), 34.9 (C), 31.5 (CH_3), 23.9 (CH_2).

FT-IR (KBr): ṽ = 3363, 2960, 2904, 2869, 1678, 1605, 1504, 1463, 1363, 1269, 1109, 1044, 834 cm^{-1}.

ESI-MS m/z 202.1 $[M+H]^+$ (calcd for $C_{14}H_{18}O$, 202.1).

4.5.11 Synthesis of 4-bromo-1-(4-*t*-butylphenyl)butan-1-one (19.4)

A solution of 4-(4-*t*-butylphenyl)but-3-yn-1-ol (**17.4**, 0.023 mol, 4.5 g) in methanol (10 mL) was cooled down to 10°C and aqueous HBr ($48\%_{w/w}$, 0.023 mol, 1.3 mL) was added dropwise, maintaining the temperature below 25°C. At the end of the addition, the reaction mixture was heated to reflux temperature for 4 hours. The solution was then cooled down to room temperature and the solvent was removed under reduced pressure to give a mixture of 4-methoxy-1-(4-*t*-butylphenyl)butan-1-one (**18.4**) and 4-bromo-1-(4-*t*-butylphenyl)butan-1-one (**19.4**) as a brown oil. A small aliquot of the mixture was subjected to column chromatography (eluant hexane-ethyl acetate 98:2), obtaining an analytical sample of **18.4**. The whole residue was cooled down to 5°C and acetic HBr ($33\%_{w/w}$, 4.5 mL) was added dropwise, maintaining the temperature below 20°C. At the end of the addition the resulting mixture was stirred at room temperature for 4 hours. At the end of the reaction toluene (15 mL) and water (10 mL) were added. The phases were separated, and the product was extracted with toluene (5 mL). The combined organic phases were washed with aqueous $NaHCO_3$ saturated solution (15 mL) and the solvent was removed under reduced pressure to give 4-bromo-1-(4-*t*-butylphenyl)butan-1-one (**19.4**, 5.9 g, 91% yield) as a brown oil.



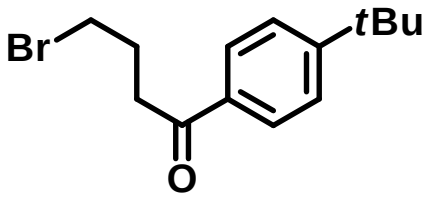
18.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.90 (d, 2H, *J* = 8.3 Hz), 7.47 (d, 2H, *J* = 8.0 Hz), 3.37 (t, 2H, *J* = 6.4 Hz), 3.22 (s, 3H), 3.10 (t, 2H, *J* = 7.2 Hz), 1.88 (quint, 2H, *J* = 6.7 Hz), 1.26 (s, 9H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 199.0 (C), 156.0 (C), 134.2 (C), 127.8 (CH), 125.4 (CH), 71.1 (CH_2), 57.8 (CH_3), 34.7 (C), 34.5 (CH_2), 30.8 (CH_3), 23.8 (CH_2).

FT-IR (KBr): $\tilde{\nu}$ = 2961, 2928, 2869, 1681, 1605, 1406, 1364, 1270, 1190, 1117, 995, 840 cm^{-1}.

ESI-MS m/z 234.3 $[M+H]^+$ (calcd for $C_{15}H_{22}O_2$, 234.2).



19.4

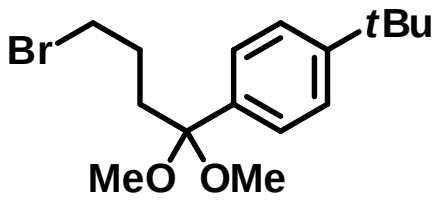
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.88 (d, 2H, *J* = 8.3 Hz), 7.48 (d, 2H, *J* = 8.6 Hz), 3.57 (t, 2H, *J* = 6.7 Hz), 3.13 (t, 2H, *J* = 6.9 Hz), 2.14 (quint, 2H, *J* = 6.9 Hz), 1.25 (s, 9H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 197.9 (C), 156.1 (C), 133.9 (C), 127.8 (CH), 125.4 (CH), 36.2 (CH_2), 34.7 (C), 34.2 (CH_2), 30.7 (CH_3), 27.1 (CH_2).

ESI-MS m/z 283.2/285.2 $[M+H]^+$ (calcd for $C_{14}H_{19}BrO$, 282.1/284.1).

4.5.12 Synthesis of 1-(4-bromo-1,1-dimethoxybutyl)-4-*t*-butylbenzene (20.4)

To a solution of 4-bromo-1-(4-*t*-butylphenyl)butan-1-one (**19.4**, 0.021 mol, 5.9 g) in methanol (10 mL) were added sulfuric acid (0.002 mol, 0.1 mL) and trimethylorthoformate (0.025 mol, 2.8 mL). The resulting solution was heated to reflux temperature for 2 hours. At the end of the reaction, the solution was cooled down to 20°C and methanolic NaOMe ($30\%_{w/w}$, 0.3 mL) was added. The solvent was evaporated under reduced pressure and toluene (20 mL) and water (10 mL) were added. The phases were separated, and the solvent was removed under reduced pressure to give 1-(4-bromo-1,1-dimethoxybutyl)-4-*t*-butylbenzene (**20.4**, 6.1 g, 88% yield) as a brown oil.



20.4

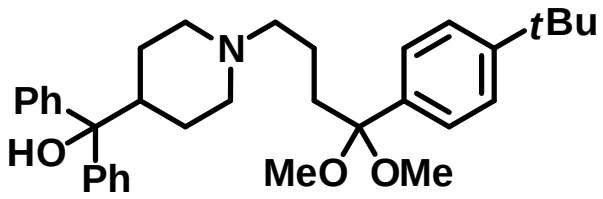
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.39 (d, 2H, *J* = 8.3 Hz), 7.32 (d, 2H, *J* = 8.3 Hz), 3.37 (t, 2H, *J* = 6.6 Hz), 3.07 (s, 6H), 1.98 (m, 2H), 1.45 (quint, 2H, *J* = 8.0 Hz), 1.27 (s, 9H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 149.8 (C), 137.3 (C), 126.2 (CH), 124.6 (CH), 102.5 (C), 48.0 (CH_3), 36.1 (CH_2), 34.6 (CH_2), 34.1 (C), 31.1 (CH_3), 27.0 (CH_2).

ESI-MS m/z 329.1/330.2 $[M+H]^+$ (calcd for $C_{16}H_{25}BrO_2$, 328.1/330.1).

4.5.13 Synthesis of (1-(4-(4-*t*-butylphenyl)-4,4-dimethoxybutyl)piperidin-4-yl)diphenylmethanol (21.4)

To a solution of $NaHCO_3$ (0.027 mol, 2.2 g) and KI (0.002 mol, 0.32 g) in water (35 mL) azacyclonol (0.019 mol, 5.1 g) was added. A solution of 1-(4-bromo-1,1-dimethoxybutyl)-4-*t*-butylbenzene (**20.4**, 0.019 mol, 6.1 g) in toluene (70 mL) was added and the resulting reaction mixture was heated to reflux for 5 hours. At the end of the reaction, the mixture was allowed to warm to room temperature and the phases were separated. The organic layer was washed with water (35 mL) and the organic solvent was removed under reduced pressure to give (1-(4-(4-*t*-butylphenyl)-4,4-dimethoxybutyl)piperidin-4-yl)diphenylmethanol (**21.4**, 8.8 g, 90% yield) as a brownish oil.



21.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.49 (d, 4H, *J* = 7.6 Hz), 7.34 (s, 4H), 7.24 (t, 4H, *J* = 7.5 Hz), 7.11 (t, 2H, *J* = 7.2 Hz), 4.92 (s, 1H), 3.07 (s, 6H), 2.62 (bd, 2H, *J* = 10.7 Hz), 2.35 (bt, 1H, *J* = 11.5 Hz), 2.05 (t, 2H, *J* = 6.7 Hz), 1.86 (bt, 2H, *J* = 8.0 Hz), 1.76 (bt, 2H, *J* = 11.2 Hz), 1.43 (qd, 2H, J_1 = 11.7 Hz, J_2 = 2.7 Hz), 1.28 (m, 11H), 1.08 (quint, 2H, *J* = 7.6 Hz).

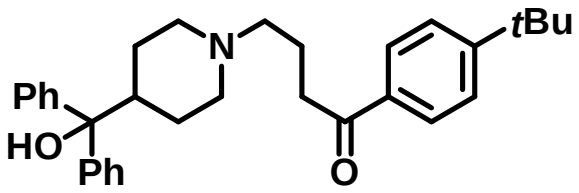
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 149.6 (C), 147.3 (C), 137.8 (C), 127.6 (CH), 126.4 (CH), 125.8 (CH), 124.5 (CH), 103.2 (C), 78.5 (C), 57.6 (CH_2), 53.7 (CH_2), 48.0 (CH_3), 44.0 (CH), 34.3 (CH_2), 34.1 (C), 31.1 (CH_3), 26.1 (CH_2), 20.6 (CH_2).

FT-IR (KBr): ṽ = 3517, 3443, 3354, 3090, 3047, 3024, 2954, 2897, 2827, 2808, 2769, 1652, 1447, 1121, 1065, 749, 703 cm^{-1}.

ESI-MS m/z 515.4 $[M+H]^+$ (calcd for $C_{34}H_{45}NO_3$, 515.3).

4.5.14 Synthesis of 1-(4-*t*-butylphenyl)-4-(4-(hydroxydiphenylmethyl)piperidin-1-yl)butan-1-one (22.4)

To a solution of (1-(4-(4-*t*-butylphenyl)-4,4-dimethoxybutyl)piperidin-4-yl)diphenylmethanol (**21.4**, 0.016 mol, 8.0 g) in methanol (30 mL) aqueous HCl ($37\%_{w/w}$, 0.017 mol, 1.4 mL) was added dropwise over a period of 30 minutes. The resulting solution was stirred for 1 hour. At the end of the reaction, aqueous $NaHCO_3$ (30 mL) was added dropwise over a period of 30 minutes, maintaining the temperature below 30°C (slightly exothermic). EtOAc (30 mL) was added and the phases were separated. The organic layer was washed with brine (15 mL) and the solvent was removed under reduced pressure to give 1-(4-*t*-butylphenyl)-4-(4-(hydroxydiphenylmethyl)-piperidin-1-yl)butan-1-one (**22.4**, 7.4 g, 97% yield) as a colourless oil.



22.4

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.92 (d, 2H, *J* = 8.6 Hz), 7.57 (d, 4H, *J* = 8.0 Hz), 7.53 (d, 2H, *J* = 8.2 Hz), 7.28 (t, 4H, *J* = 7.7 Hz), 7.15 (t, 2H, *J* = 7.2 Hz), 2.98 (t, 2H, *J* = 7.0 Hz), 2.89 (bd, 2H, *J* = 7.0 Hz), 2.46 (bt, 1H, *J* = 11.9 Hz), 2.36 (t, 2H, *J* = 6.9 Hz), 1.97 (bt, 2H, *J* = 10.9 Hz), 1.84 (quint, 2H, *J* = 7.1 Hz), 1.56 (qd, 2H, J_1 = 11.8 Hz, J_2 = 2.0 Hz), 1.34 (m, 11H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 199.0 (C), 155.6 (C), 147.0 (C), 134.3 (C), 127.5 (CH), 127.3 (CH), 125.6 (CH), 125.4 (CH), 125.0 (CH), 78.4 (C), 57.1 (CH_2), 53.5 (CH_2), 43.8 (CH), 35.5 (C), 34.4 (CH_2), 30.5 (CH_3), 25.9 (CH_2), 21.4 (CH_2).

FT-IR (KBr): $\tilde{\nu}$ = 3460, 1677, 1601, 822, 757 cm^{-1}.

ESI-MS m/z 470.3 $[M+H]^+$ (calcd for $C_{32}H_{39}NO_2$, 469.3)

4.6 References

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5. Ravidasvir

The work of the third year of the PhD was focused on the selected API ravidasvir **1.5** (*Figure 1.5*).

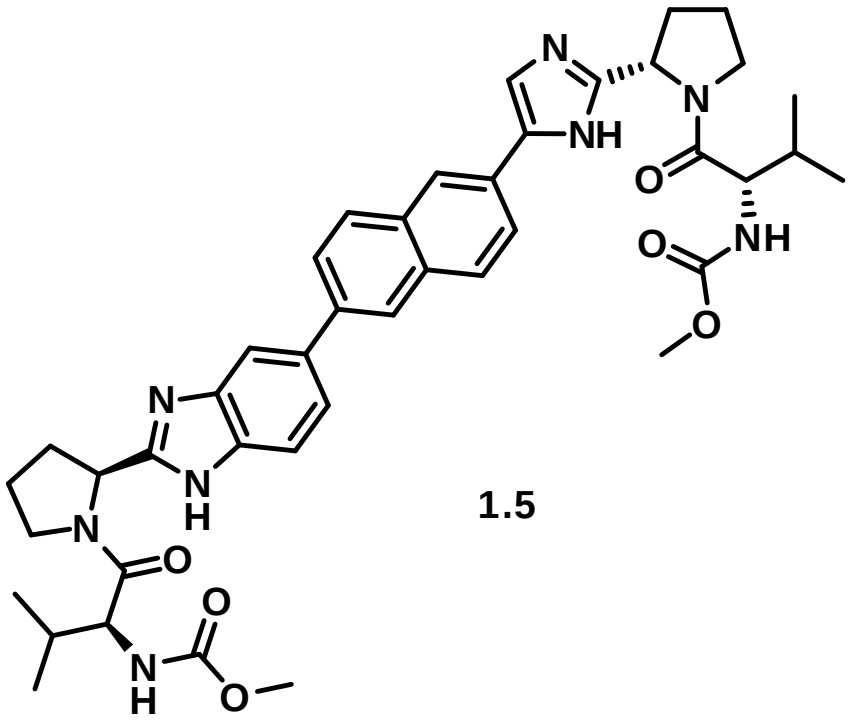


Figure 1.5 - Ravidasvir

This important API, commercialized as the hydrochloride and formerly known as PPI-668, is a novel Direct-Acting Antiviral (DAA) used for the treatment of chronic hepatitis C.

Actually the molecule of ravidasvir is in Phase 3 clinical trials.[1.5]

The aim of this work is to pursue an efficient and scalable preparation for this API, alternative to the existing synthetic protocols actually covered by patents.

5.1 Chronic hepatitis C

The hepatitis C virus (HCV) is a major human pathogen. It causes chronic hepatitis C infection, a very important public health problem, with about 71 million people worldwide living with one of the six major genotypes of HCV.[2.5,3.5] Each year, hepatitis C causes approximately 399.000 deaths worldwide, mostly from cirrhosis and hepatocellular carcinoma (HCC).[4.5]

According to WHO (World Health Organization), about 3-4 million new cases of infection are annually reported. HCV is considered a major health issue, since the infection frequently progress to cirrhosis and liver cancer (*Figure 2.5*). The main cofactors influencing the progression of the disease, terminating with cirrhosis and HCC, are the metabolic syndrome and the alcohol abuse. Each year, liver transplantation is performed on about a third of patients with complication associated with infection by hepatitis C virus.[3.5]

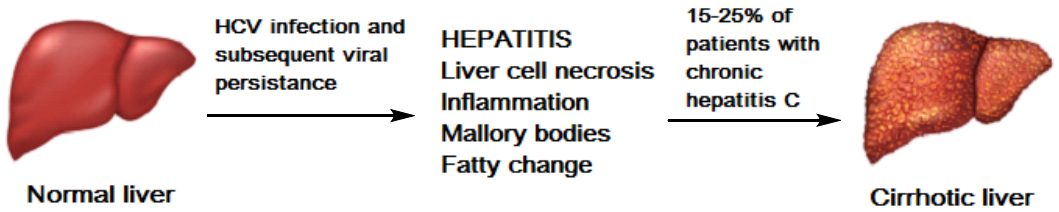


Figure 2.5 - Pathophysiology of HCV infection

In addition to hepatic disease, HCV infection has also been found to causes a several number of extra-hepatic diseases, affecting eyes, joints, thyroid, salivary glands, nervous and immune systems, skin and kidneys. These extra-hepatic diseases produce an important burden for health care systems and society, since are reported in up to threequarters of the patients affected by HCV.[2.5]

First therapy for HCV infection consisted in the use of pegylated interferon-alpha (peg-IFN-α), in monotherapy or in association with ribavirin (RBV). Due to the virus resistance and an high number of side effects of peg-IFN-α and RBV, like fatigue, mood and sleep disorders, skin reaction, dyspnea and hematological disorders, a new therapy was needed for the treatment

of chronic hepatitis C. A new treatment based on Direct-Acting Antivirals (DAAs) opened the way to the viral eradication without substantial side effects in almost all infected patients.[2.5]

5.2 Direct-Acting Antivirals for the treatment of HCV infection

In 2011, boceprevir and telaprevir (first generation DAAs) were approved for the treatment of HCV infection. The mechanism of action of these two drugs consist in an inhibition of the non-structural protease NS3/4A, combined with peg-IFN-α and RBV to prevent virus resistance. From early 2014, the second generation of DAAs was introduced and classified in four drug classes on the base of their target of action:

- NS3/4A protease inhibitors (*e.g.* simeprevir);
- NS5B polymerase inhibitors, nucleoside analogues (*e.g.* mericitabine, sofosbuvir);
- NS5B polymerase inhibitors, non-nucleoside analogues (*e.g.* setrobuvir, beclabuvir);
- NS5A inhibitors (*e.g.* elbasvir, ledipasvir, daclatasvir, ravidasvir, *Figure 3.5*).

Ravidasvir (PPI-668) is a very potent NS5A inhibitor for the treatment of chronic hepatitis C virus infection.

After Phase 1 and 2 trials, this drug showed high efficacy, even in hardly treatable cirrhotic patients and proved to have an excellent margin of safety and tolerability.[1.5,5.5]

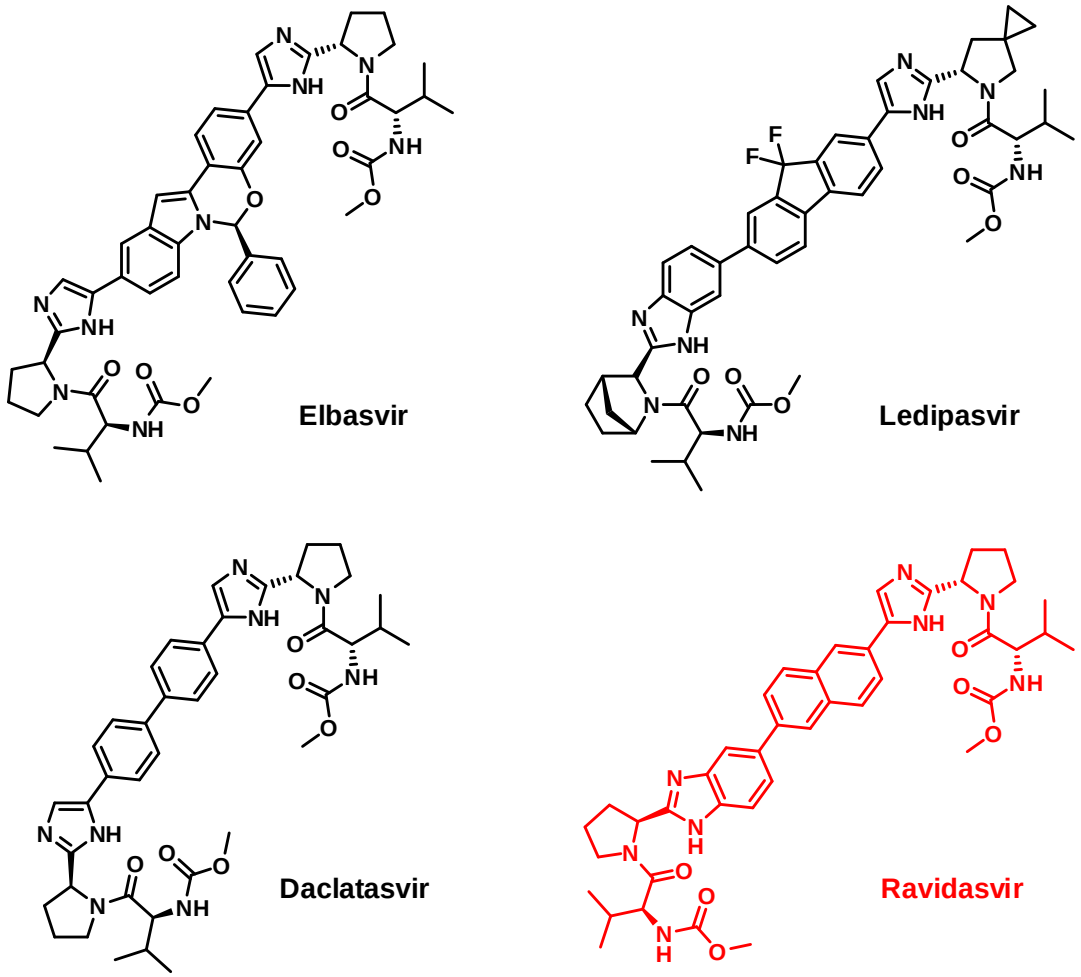


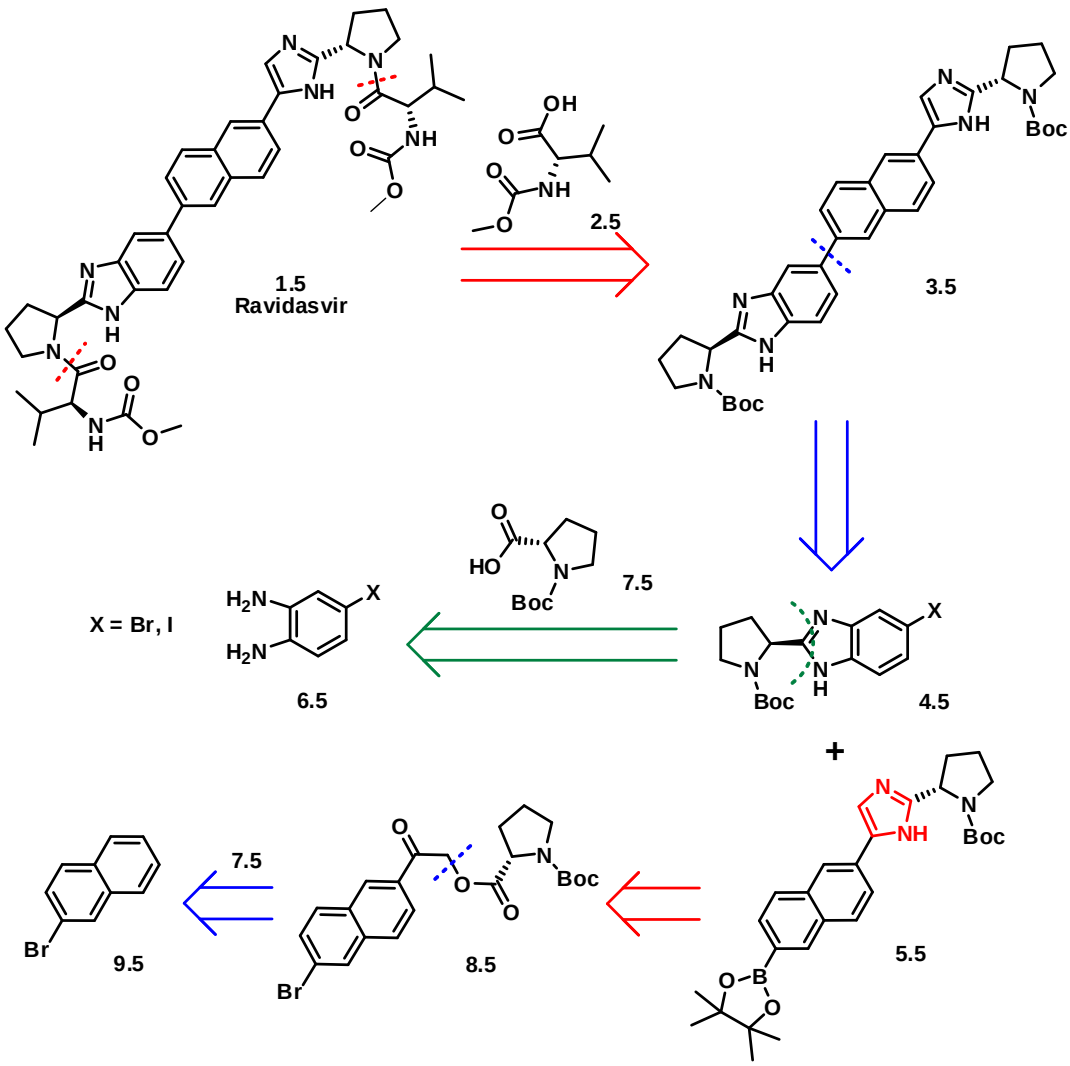
Figure 3.5 - NS5A inhibitors (-asvir)

5.3 Results and discussion

The strategies reported to date for the synthesis of ravidasvir hydrochloride **1.5** are extremely conservative. Since this API is still in Phase 3 of the clinical trials, all the reported preparation shares the same precursor/key intermediates, according to the originator's way of synthesis (*Scheme 1.5*). [6.5]

The two key retrosynthetic steps of these route of synthesis are:

1) the introduction of the Moc protected L-Val-L-Pro (represented by compounds **2.5** and **7.5**);
2) the synthesis of the two intermediates **4.5** and **5.5** and subsequent coupling to generate the entire back-bone of the final product.



Scheme 1.5 - Retrosynthetic strategy of the prior-art syntheses

Initially, the double disconnection of two Moc-protected L-valine residues generates the compound **3.5**, which contains two Boc-protected pyrrolidines sharing a stereocenter with the same chirality. These two

moieties are derived from the common previous ancestor, represented by Boc-protected L-proline **7.5**.

The intermediate **3.5** is then fragmented in two compounds: the benzimidazole **4.5** and the 5-naphthyl-imidazole **5.5**, both suitably functionalized with selected functional groups. The choice of the halogen atom (X = -Br[7.5,8.5], -I[9.5]) on the benzimidazole **4.5** and a boronic group on the naphthyl residue of the compound **5.5**, paves the way for their connection, performed through a Pd-catalyzed Suzuki coupling, generating the intermediate **3.5**.

The synthesis of the compound **4.5** is achieved by reacting 4-halo-1,2-diaminobenzene **6.5** (X = -Br[7.5,8.5], -I[9.5]) with the Boc-protected L-proline **7.5** (the latter representing the precursor of the chiral pyrrolidine moiety). By disconnection of the imidazole moiety of the compound **5.5**, the α-acyloxy ketone **8.5** was obtained. The latter was synthesized starting from the bromonaphthalene **9.5**, by performing three reactions: Friedel-Crafts acylation, bromination of the obtained ketone and nucleophilic substitution with **7.5**. The intermediate **8.5** can be transformed in the desired compound **5.5** by a simple heterocyclization step, generating the imidazole ring. Another patent describes the possibility to exchange the halogen group and the boronic group, for the Suzuki reactions, between the compounds **3.5** and **4.5**.[10.5]

All the starting materials used in these approaches are commercially available and cheap and for this reason, we decided to use some of them (*e.g.* the natural aminoacids L-valine and L-proline) to start with the search of a new and efficient synthetic way to ravidasvir hydrochloride **1.5**.

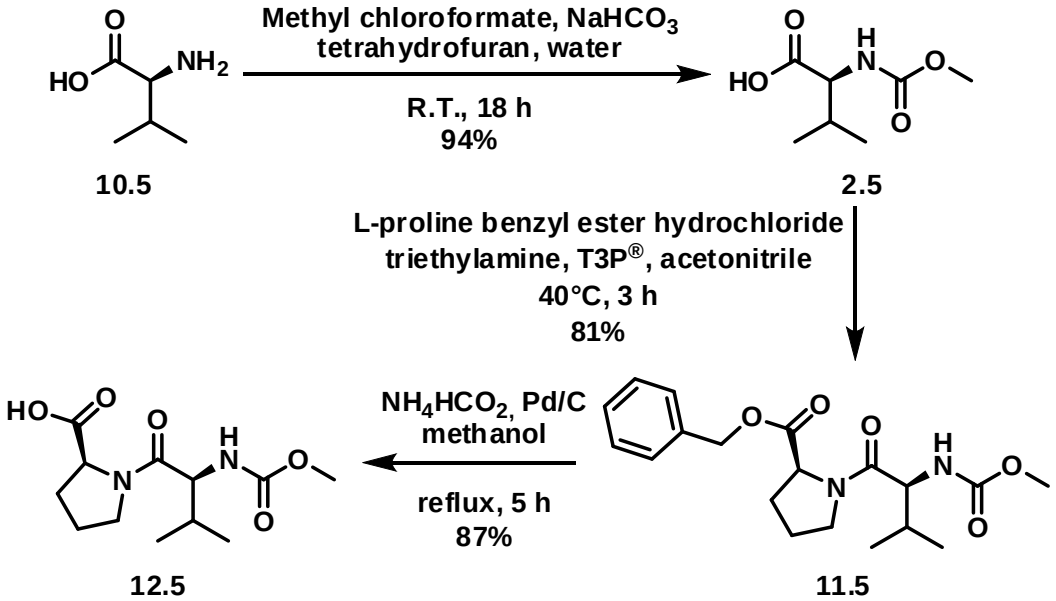
We explored the possibility to perform a different approach in the assembly of the molecular skeleton of ravidasvir. Previous approaches rely on a semiconvergent synthesis starting with the central core of ravidasvir, represented by the biarylmoiety (naphthalene-benzimidazole). The outer aminoacid-derived residues (pyrrolidine and Moc-L-valine) are then added in the final steps of the synthesis.

Our approach is based on the observation that the outer parts of the molecule of ravidasvir share a common residue consisting in a Moc-L-Val-pyrrolidine, the latter bearing two aminoacid-derived stereocenters (one

from L-Val and the stereocenter of the 2-substituted pyrrolidine ring arising from L-Pro). This common residue could be built as a key intermediate in a convergent synthesis, reducing the overall number of steps. Moreover, this alternative strategy has the additional advantage of avoiding claimed intermediates, generating instead new and patentable compounds.

The synthesis starts with a simple protection of the commercially available L-valine by using methyl chloroformate, $NaHCO_3$ as base in a water/tetrahydrofuran mixture (*Scheme 2.5*). After 18 hours of reaction at room temperature, the Moc-protected L-valine **2.5** was obtained in high yield (94%).

The following step is the formation of a peptide bond between **2.5** and the natural aminoacid L-proline. The carboxylic group of the proline must be protected (*e.g.* as ester), in order to avoid the generation of byproducts (arising for example from the homo-coupling); the commercially available L-proline benzyl ester hydrochloride was then used. After the treatment with triethylamine, necessary to generate the proline free base, the condensing agent T3P® (commercially available as acetonitrile solution) succeeded in the formation of the intermediate **11.5** (40°C, 3 hours, 81% yield).



*Scheme 2.5 - Synthesis of the novel key intermediate **12.5***

Hydrogenation of the benzyl ester of **11.5** was performed by using ammonium formate as the hydrogen donor, in the presence of palladium on carbon as the catalyst, in refluxing methanol for 5 hours. The Moc-protected dipeptide **12.5** was isolated in 87% yield (final step) and 66% overall yield.[11.5] This compound represent the first important key intermediate, clearly recognizable in both the distal portions of the molecule of ravidasvir. This result paves the way to the development of the second part of the synthesis, involving the preparation of the two aryl "partners" for the Suzuki coupling*en route* to the biaryl core.

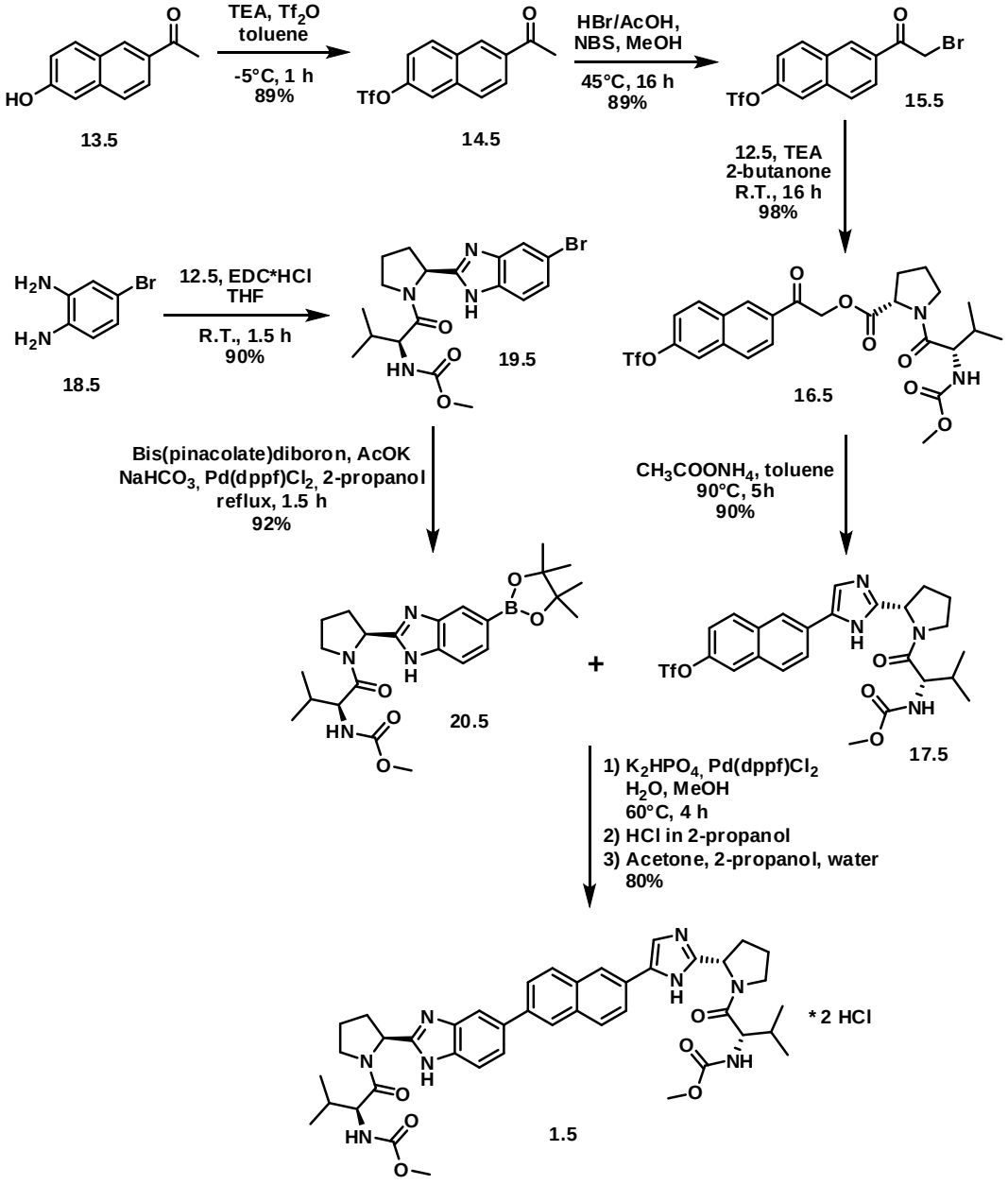
The synthesis of the first compound *(Scheme 3.5)*, for the Suzuki reaction, started with the trifluoromethanesulfonation of the cheap starting material **13.5**, achieved by using trifluoromethanesulfonic anhydride, triethylamine as base in toluene at -5°C for 1 hour. The corresponding trifluoromethanesulfonate **14.5** was obtained in 89% yield. The functional group aryl-triflate, introduced in this reaction, can be used in the last synthetic step instead of the halogen group, as reported in literature.[12.5,13.5]

Bromination of the keto group of the compound **14.5** was performed with *N*-bromosuccinimide in methanol, in the presence of catalytic HBr/AcOH (45°C, 16 h, 89% yield). The corresponding α-bromoketone **15.5** bearing the leaving group for the subsequent nucleophilic substitution of the dipeptide **12.5**. This reaction ran in 2-butanone at room temperature for 16 hours, with triethylamine as base. The product **16.5**, almost quantitatively obtained (98% yield), contains an α-acyloxyketone group which has the role of precursor of the imidazole moiety, present in the final API.

Cyclization of the α-acyloxyketone **16.5** was achieved by using ammonium acetate in toluene at 90°C. After 5 hours of reaction, the corresponding naphthylimidazole **17.5** was isolated in a good yield (90%), representing the first key intermediate for the final coupling.

The second key intermediate was prepared starting with the commercially available at low price 4-bromo-1,2-diaminobenzene **18.5**. The latter was reacted with the dipeptide 12.5, generating the benzoimidazole **19.5**. This synthetic step was performed with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride as condensing agent and tetrahydrofuran as solvent (room temperature, 1.5 hours, 92% yield).

The subsequent step consisted in the transformation of the bromide group of the compound **19.5** in a suitable boronic group. We found in literature that the boronic esters have good reactivity in the Suzuki coupling[14.5] and are also very simple to prepare.



*Scheme 3.5 - New synthetic route of ravidasvir hydrochloride **1.5***

We choose bis(pinacolate)diboron as the reagent to perform this transformation, since it is commercially available and relatively cheap. By using the conditions reported in *Scheme 3.5* (potassium acetate, sodium bicarbonate as base, [1,1′-bis(diphenylphosphino)ferrocene]dichloro-palladium(II) as catalyst, refluxing 2-propanol, 1.5 hours), the corresponding pinacol boronic ester **20.5** was obtained in 92% yield.

We finalized the last synthetic step of this original preparation of the selected API by mixing the two key intermediates **17.5** and **20.5**, in the presence of potassium hydrogen phosphate and catalytic [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II). After 4 hours of reaction (60°C in a water/methanol solvent mixture), ravidasvir free base was obtained and subsequently salified as hydrochloride (crude **1.5**) by treatment with a solution of HCl in 2-propanol (20%$_{w/w}$).

Final crystallization in a mixture 2-propanol/acetone led to analytically pure ravidasvir hydrochloride **1.5**, collected by simple filtration as white crystalline powder, in 80% yield (referred to final step and purification).[11.5]

5.4 Conclusion and future developments

In the final year of the PhD we developed an efficient and scalable synthetic approach for the preparation of the important NS5A inhibitor ravidasvir hydrochloride **1.5**, for the treatment of chronic hepatitis C virus infection. This novel synthesis is based on the preparation of a key dipeptide **12.5**, used for the subsequent generation of other two advanced intermediates **17.5** and **20.5**, finally connected to obtain the desired product. This synthetic strategy significantly reduces the number of the synthetic steps (8 $_{vs}$ 11) and circumvents existing patents by introducing the Moc-L-valine-L-proline moiety before the Pd-catalyzed Suzuki coupling. Moreover, the isolation of all intermediate and the final product requires only simple work-up procedures and avoids chromatographic separations, allowing the entire protocol to be transferred on a kilolab scale then on the industrial scale. The overall yield of the above described process is 46%,[11.5] referred to the longest linear step.

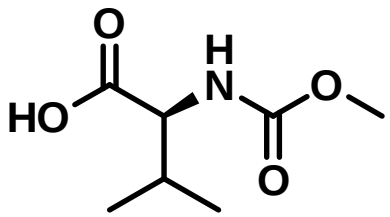
5.5 Experimental section

5.5.1 Material and methods

The chemicals used for the experiments were of the highest analytical grade. Nuclear magnetic resonance spectra (^{1}H, ^{13}C-NMR) were recorded on a JEOL ECP300 (7.04 T) spectrometer or a Bruker Avance Neo (9.4 T) spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (*J*) are quoted in Hertz (Hz). Mass spectra (MS) were recorded on a Thermo Finnigan TSQ700 triple-quadrupole instrument equipped with an electrospray ionization source.

5.5.2 Synthesis of (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (2.5)

To a suspension of *L*-valine (**10.5**, 0.85 mol, 100.0 g) in tetrahydrofuran (500 mL), water (500 mL) and $NaHCO_3$ (2.65 mol, 215.1 g) were added and the resulting mixture was cooled down to 15°C. Methyl chloroformate (1.02 mol, 79.3 mL) was added dropwise and the resulting suspension was stirred at room temperature for 18 hours. At the end of the reaction, aq. HCl (37%$_{w/w}$, 79.3 mL) was added dropwise. The phases were separated, and the organic layer was washed with saturated aq. NaCl (100 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (**2.5**, 141.0 g, 94% yield) as a white solid.



2.5

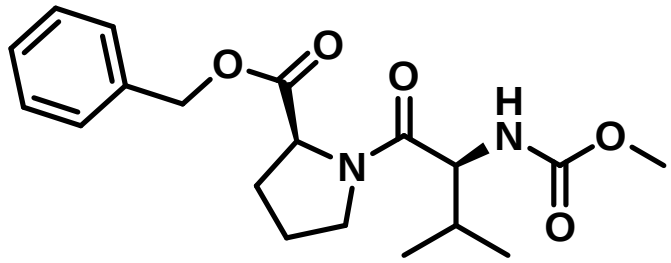
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 12.50 (bs, 1H), 7.23 (d, 1H, J = 8.6 Hz), 3.86 (bt, 1H, J = 7.2 Hz), 3.54 (s, 3H), 2.03 (m, 1H), 0.88 (d, 3H, J = 5.8 Hz), 0.86 (d, 3H, J = 6.1 Hz).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 173.5 (C), 157.2 (C), 59.7 (CH), 51.6 (CH_3), 29.8 (CH), 19.3 (CH_3), 18.1 (CH_3).

ESI-MS m/z 176.3 $[M+H]^+$ (calcd for $C_7H_{13}NO_4$, 175.2).

5.5.3 Synthesis of (*S*)-benzyl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (11.5)

To a suspension of (*S*)-benzyl pyrrolidine-2-carboxylate hydrochloride (0.37 mol, 89.7 g) and (*S*)-2-(methoxycarbonylamino)-3-methylbutanoic acid (**2.5**, 0.37 mol, 65.0 g) in acetonitrile (180 mL), triethylamine (1.48 mol, 207.0 mL) was added dropwise maintaining the temperature below 25°C. T3P® solution (50%$_{w/w}$ in acetonitrile, 0.44 mol, 381.8 mL) was added dropwise and the resulting suspension was heated to 40°C for 3 hours. At the end of the reaction the solvent was removed under reduced pressure and ethyl acetate (450 mL) and water (200 mL) were added. The phases were separated and the organic layer was washed with water (100 mL), aq. HCl (2N, 100 mL), saturated aq. $NaHCO_3$ (100 mL) and saturated aq. NaCl (100 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give (*S*)-benzyl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**11.5**, 108.1 g, 81% yield) as a white solid.



11.5

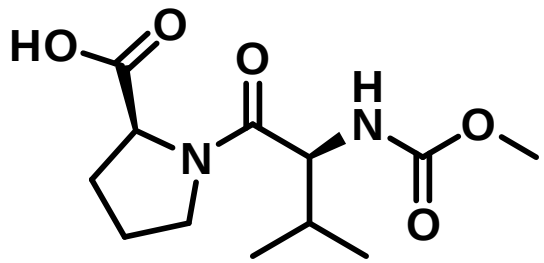
^{1}H-NMR (400 MHz, d_6-DMSO): δ (ppm) = 7.36 (m, 6H), 5.13 (s, 2H), 4.41 (q, 1H, *J* = 4.4 Hz), 4.05 (t, 1H, *J* = 8.5 Hz), 3.82 (q, 1H, *J* = 7.5 Hz), 3.60 (q, 1H, *J* = 7.5 Hz), 3.53 (s, 3H), 2.19 (m, 1H), 1.89 (m, 4 H), 0.89 (m, 6H).

^{13}C-NMR (100.6 MHz, d_6-DMSO): δ (ppm) = 171.7 (C), 170.6 (C), 156.8 (C), 136.0 (C), 128.4 (2xCH), 128.0 (CH), 127.8 (2xCH), 65.8 (CH_2), 58.6 (CH), 57.8 (CH), 51.4 (CH_3), 46.9 (CH_2), 29.9 (CH), 28.8 (CH_2), 24.6 (CH_2), 18.7 (CH_3), 18.5 (CH_3).

ESI-MS m/z 363.5 $[M+H]^+$ (calcd for $C_{19}H_{26}N_2O_5$, 362.4).

5.5.4 Synthesis of (*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (12.5)

To a solution of (*S*)-benzyl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**11.5**, 0.22 mol, 79.0 g) in methanol (550 mL), ammonium formate (0.38 mol, 23.8 g) and palladium on carbon (10%$_{w/w}$, 0.01 mol, 16.2 g) were added and the resulting suspension was heated to reflux temperature for 5 hours. At the end of the reaction, the mixture was cooled down to room temperature and filtered on a Celite® pad. The solvent was removed under reduced pressure then ethyl acetate (300 mL) and water (50 mL) were added. The phases were separated and the organic layer was washed with water (2x50 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give (*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (**12.5**, 52.0 g, 87% yield) as a white solid.



12.5

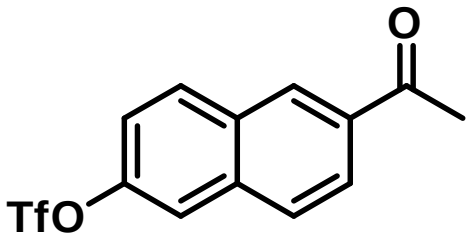
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 7.25 (d, 1H, *J* = 8.2 Hz), 4.25 (m, 1H), 4.03 (t, 1H, *J* = 8.1 Hz), 3.78 (m, 1H), 3.56 (m, 1H), 3.52 (s, 3H), 2.13 (m, 1H), 1.90 (m, 4H), 0.93 (d, 3H, *J* = 6.4 Hz), 0.88 (d, 3H, *J* = 6.4 Hz).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 173.4 (C), 170.5 (C), 156.9 (C), 58.7 (CH), 57.9 (CH), 51.5 (CH_3), 47.0 (CH_2), 30.1 (CH), 28.9 (CH_2), 24.7 (CH_2), 18.9 (CH_3), 18.6 (CH_3).

ESI-MS m/z 273.4 $[M+H]^+$ (calcd for $C_{12}H_{20}N_2O_5$, 272.3).

5.5.5 Synthesis of 6-acetylnaphthalen-2-yl trifluoromethanesulfonate (14.5)

A solution of 1-(6-hydroxynaphthalen-2-yl)ethanone (**13.5**, 1.61 mol, 300.0 g), triethylamine (2.09 mol, 291.7 mL) and toluene (3000 mL) was cooled down to -5°C and trifluoromethanesulfonic anhydride (1.77 mol, 297.6 mL) was added dropwise, maintaining the temperature below -2°C, and then was stirred for 1 hours. At the end of the reaction, water (750 mL) was added and the resulting mixture was allowed to warm to room temperature and the phases were separated. The organic layer was washed with aq. Na_2CO_3 (10% $_{w/w}$, 750 mL), aq. HCl (2N, 750 mL) and saturated aq. NaCl (750 mL). The organic phase was then filtered on a Celite®/charcoal pad and the solvent was removed under reduced pressure to give 6-acetylnaphthalen-2-yl trifluoromethanesulfonate (**14.5**, 456.7 g, 89% yield) as a brownish solid.



14.5

^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 8.67 (s, 1H), 8.25 (d, 1H, *J* = 9.0 Hz), 8.06 (m, 3H), 7.61 (bd, 1H, *J* = 9.0 Hz), 2.67 (s, 3H).

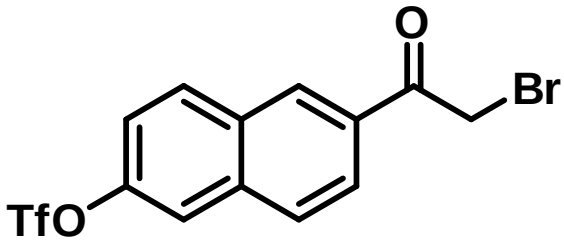
^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 189.5 (C), 148.3 (C), 135.2 (C), 135.1 (C), 132.8 (CH), 131.4 (C), 130.1 (CH), 128.5 (CH), 125.1 (CH), 120.4 (CH), 119.3 (CH), 118.3 (CF_3, *J* = 321 Hz), 26.6 (CH_3).

ESI-MS m/z 319.4 $[M+H]^+$ (calcd for $C_{13}H_9F_3O_4S$, 318.3).

5.5.6 Synthesis of 6-(2-bromoacetyl)naphthalen-2-yl trifluoromethanesulfonate (15.5)

A solution of 6-acetylnaphthalen-2-yl trifluoromethanesulfonate (**14.5**, 1.41 mol, 450.0 g) and acetic HBr (33%$_{w/w}$, 0.14 mol, 34.6 g) in methanol (4500 mL) was heated to 45°C. *N*-Bromosuccinimide (1.84 mol, 327.1 g) was added portionwise and the resulting mixture was stirred for 16 hours. At the end of the reaction the solution was cooled down to 15°C and water (5000 mL) was added. The resulting suspension was filtered and the solid was washed with water (3x1000 mL). The solid was dissolved in 2-butanone and aq. HBr (62%$_{w/w}$, 0.63 mol, 83 mL) was added. The resulting solution was stirred at room temperature for 16 hours then was washed with water (3x1500 mL) and saturated aq. NaCl (1500 mL). The organic phase was dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. 2-Propanol (1350 mL) was added and the suspension was heated to 55°C until complete dissolution then cooled down to 10°C.

Precipitation of the product starts and the suspension was stirred for 30 minutes. The solid was filtered and washed with cold 2-propanol (3x100 mL). The solid was dried in a vacuum oven to give 6-(2-bromoacetyl)naphthalen-2-yl trifluoromethanesulfonate (**15.5**, 495.0 g, 89% yield) as a white solid.



15.5

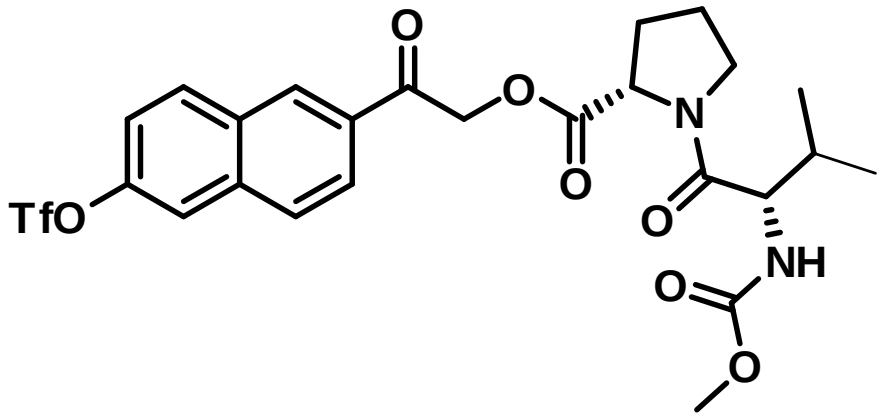
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 8.84 (s, 1H), 8.31 (d, 1H, *J* = 8.8 Hz), 8.20-8.08 (m, 3H), 7.68 (dd, 1H, J_1 = 9.2 Hz, J_2 = 1.8 Hz), 5.07 (s, 2H).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 191.4 (C), 148.5 (C), 135.4 (C), 132.8 (CH), 132.4 (C), 131.2 (C), 130.6 (CH), 128.8 (CH), 125.5 (CH), 120.7 (CH), 119.4 (CH), 118.3 (CF_3, *J* = 321 Hz), 33.9 (CH_2).

ESI-MS m/z 397.3/399.3 $[M+H]^+$ (calcd for $C_{13}H_8BrF_3O_4S$, 396.2/398.2).

5.5.7 Synthesis of (*S*)-2-oxo-2-(6-(trifluoromethylsulfonyloxy)naphthalen-2-yl)ethyl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (16.5)

To a solution of 6-(2-bromoacetyl)naphthalen-2-yl trifluoromethanesulfonate (**15.5**, 1.01 mol, 400.0 g) and (*S*)-1-((S)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (**12.5**, 1.21 mol, 331.2 g) in 2-butanone (4000 mL), triethylamine (1.01 mol, 139.6 mL) was added dropwise in about 5 hours, maintaining the temperature below 25°C. At the end of the addition, the resulting suspension was stirred for 16 hours. At the end of the reaction HCl 2N (1000 mL) was added. The phases were separated and the organic layer was washed with saturated aq. $NaHCO_3$ (3x1000 mL) and saturated aq. NaCl (1000 mL). The solvent was removed under reduced pressure to give (*S*)-2-oxo-2-(6-(trifluoromethylsulfonyloxy)naphthalen-2-yl)ethyl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**16.5**, 581.0 g, 98% yield) as an orange oil.



16.5

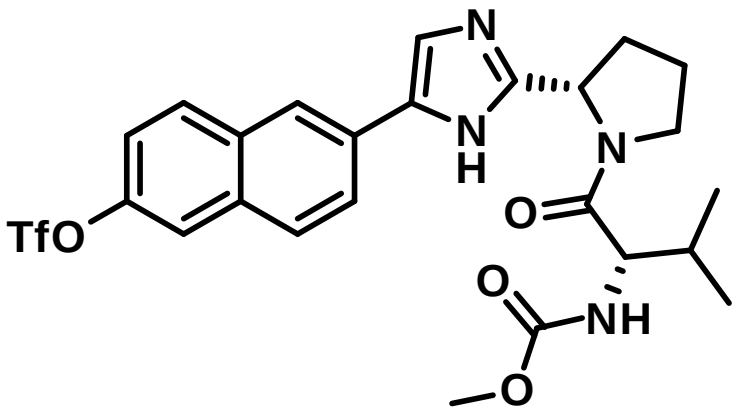
^{1}H-NMR (300 MHz, d_6-DMSO): δ (ppm) = 8.78 (s, 1H), 8.29 (d, 1H, *J* = 9.0 Hz), 8.16 (s, 1H), 8.03 (d, 1H, *J* = 9.0 Hz), 7.66 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.8 Hz), 7.37 (d, 1H, *J* = 8.2 Hz), 4.65 (q, 2H, *J* = 5.5 Hz), 4.56 (m, 1H), 4.03 (m, 2H), 3.80 (m, 1H), 3.54 (s, 3H), 2.28 (m, 1H), 1.98 (m, 4H), 0.91 (d, 3H, *J* = 7.3 Hz), 0.88 (d, 3H, *J* = 8.0 Hz).

^{13}C-NMR (75.6 MHz, d_6-DMSO): δ (ppm) = 192.7 (C), 171.7 (C), 171.0 (C), 157.1 (C), 148.7 (C), 135.8 (C), 133.1 (CH), 132.4 (C), 131.5 (C), 130.0 (CH), 129.1 (CH), 124.9 (CH), 121.0 (CH), 119.7 (CH), 118.5 (CF_3, *J* = 323 Hz), 66.8 (CH_2), 58.6 (CH), 58.0 (CH), 51.7 (CH_3), 47.2 (CH_2), 30.1 (CH), 29.0 (CH_2), 24.7 (CH_2), 18.8 (CH_3), 18.6 (CH_3).

ESI-MS m/z 589.7 $[M+H]^+$ (calcd for $C_{25}H_{27}F_3N_2O_9S$, 588.6).

5.5.8 Synthesis of 6-(2-((*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)naphthalen-2-yl trifluoromethanesulfonate (17.5)

To a solution of 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**16.5**, 0.780 mol, 460.0 g) in toluene (4600 mL), ammonium acetate (2.73 mol, 210.4 g) was added and the resulting suspension was heated at 90°C for 5 hours. At the end of the reaction, the solution was cooled down to room temperature and water (3000 mL) and $NaHCO_3$ (300 g) were added. The phases were separated and the organic layer was washed with water (2x1000 mL) and saturated aq. NaCl (1000 mL). The organic layer was filtered on a Celite®/charcoal pad and the filter was washed with toluene (3x500 mL). The solvent was removed under reduced pressure to give 6-(2-((*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)naphthalen-2-yl trifluoromethanesulfonate (**17.5**, 398.0 g, 90% yield) as a pale yellow solid.



17.5

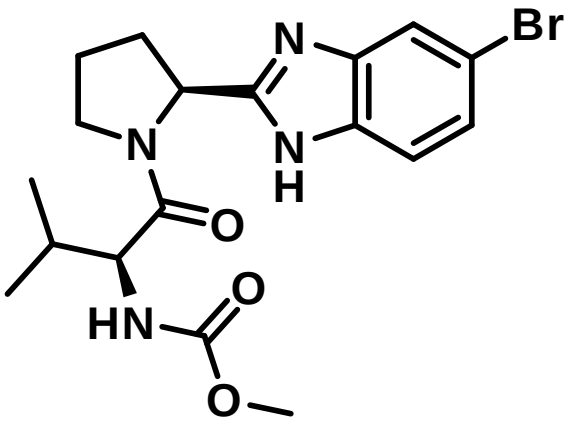
^{1}H-NMR (400 MHz, d_6-DMSO): δ (ppm) = 11.92 (bs, 1H), 8.33 (s, 1H), 8.04 (m, 4H), 7.68 (s, 1H), 7.53 (dd, 1H, J_1 = 8.9 Hz, J_2 = 1.9 Hz), 7.30 (d, 1H, J = 8.4 Hz), 5.15 (m, 1H), 4.12 (t, 1H, J = 8.2 Hz), 3.85 (bs, 2H), 3.57 (s, 3H), 2.18 (m, 2H), 2.08 (m, 3H), 0.94 (d, 3H, J = 6.7 Hz), 0.88 (d, 3H, J = 6.6 Hz).

^{13}C-NMR (100.6 MHz, d_6-DMSO): δ (ppm) = 170.5 (C), 156.9 (C), 146.2 (C), 132.7 (2xC), 131.6 (2xC), 131.5 (C), 130.6 (2xCH), 128.2 (CH), 125.3 (CH), 121.3 (CH), 119.8 (CH), 119.2 (CH), 118.8 (CF_3, J = 318 Hz), 58.1 (CH), 54.4 (CH), 51.4 (CH_3), 46.9 (CH_2), 30.9 (CH_2), 29.9 (CH), 24.4 (CH_2), 19.0 (CH_3), 18.5 (CH_3).

ESI-MS m/z 569.7 $[M+H]^+$ (calcd for $C_{25}H_{27}F_3N_4O_6S$, 568.6).

5.5.9 Synthesis of (*S*)-5-bromo-1H-benzo[*d*]imidazol-2-yl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (19.5)

A solution of (*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (**12.5**, 0.367 mol, 100.0 g) and 4-bromobenzene-1,2-diamine (**18.5**, 0.367 mol, 68.5 g) in tetrahydrofuran (500 mL) was cooled down to 0°C. 3-(Ethyliminomethyleneamino)-*N*,*N*-dimethylpropan-1-amine hydrochloride (0.380 mol, 73.6 g) was added and the resulting mixture was stirred at room temperature for 1.5 hours. At the end of the reaction the solvent was removed under reduced pressure and ethyl acetate (850 mL) and water (250 mL) were added. The biphasic mixture was filtered on a Celite® pad then the phases were separated. The organic layer was washed with water (150 mL) and saturated aq. NaCl (150 mL) and the solvent was removed under reduced pressure to a residue of about 300 mL. Acetic acid (0.75 mol, 42.9 mL) was added and the resulting solution was heated to reflux temperature for 2.5 hours. At the end of the reaction, the mixture was cooled down to room temperature and ethyl acetate (700 mL), water (250 mL) and $NaHCO_3$ (100.0 g) were added. The phases were separated, and the organic layer was washed with water (200 mL) and saturated aq. NaCl (200 mL) and the solvent was removed under reduced pressure. The obtained residue was dissolved in methyl *t*-butyl ether (300 mL) and the solution was added dropwise to heptane (1300 mL). The suspension was stirred for 1 hour to maximize the precipitation of the product. The solid was filtered and washed with heptane/methyl *t*-butyl ether mixture (4:1, 250 mL) to give methyl (*S*)-1-((*S*)-2-(5-bromo-1H-benzo[d]imidazol-2-yl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (**19.5**, 139.4 g, 90% yield) as a white solid.



19.5

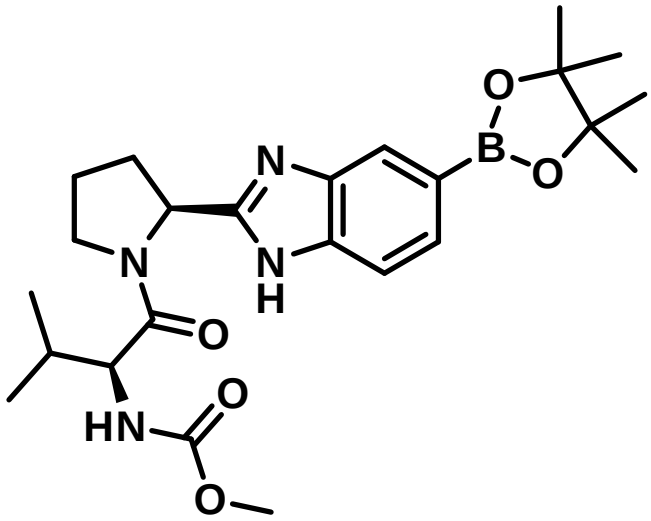
^{1}H-NMR (400 MHz, d_6-DMSO): δ (ppm) = 12.39 (bs, 1H), 7.68 (s, 1H), 7.45 (d, 1H, *J* = 8.5 Hz), 7.27 (m, 2H), 5.19 (m, 1H), 4.09 (t, 1H, *J* = 8.3 Hz), 3.85 (m, 2H), 3.55 (s, 3H), 2.21 (m, 2H), 2.00 (m, 3H), 0.85 (d, 3H, *J* = 7.3 Hz), 0.83 (d, 3H, *J* = 8.2 Hz).

^{13}C-NMR (100.6 MHz, d_6-DMSO): δ (ppm) = 170.7 (C), 157.2 (C), 156.9 (C), 124.2 (3xCH), 113.6 (C), 66.4 (C), 58.0 (CH), 54.7 (CH), 51.5 (CH_3), 47.0 (CH_2), 30.9 (CH_2), 29.7 (CH), 24.5 (CH_2), 19.1 (CH_3), 18.4 (CH_3).

ESI-MS m/z 423.4/425.4 $[M+H]^+$ (calcd for $C_{18}H_{23}BrN_4O_3$, 422.3/424.3).

5.5.10 Synthesis of (*S*)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[*d*]imidazol-2-yl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (20.5)

To a solution of (*S*)-5-bromo-1H-benzo[*d*]imidazol-2-yl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**19.5**, 0.472 mol, 200.0 g) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (0.567 mol, 144.0 g) in 2-propanol (1600 mL), potassium acetate (0.944 mol, 92.8 g), $NaHCO_3$ (0.472 mol, 39.7 g), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (complex with dichloromethane, 0.009 mol, 7.71 g) and water (0.472 mol, 8.5 mL) were added. The resulting mixture was heated to reflux temperature for 1.5 hours. At the end of the reaction, the mixture was cooled down to room temperature and water (1400 mL) and toluene (1000 mL) were added. The phases were separated and water (800 mL) and aq. HCl (37%w/w, 500 mL) were added. The biphasic mixture was filtered on a Celite®/charcoal pad and the filter was washed with aq. HCl (2N, 2x400 mL). The phases were separated and dichloromethane (1000 mL) and $NaHCO_3$ (1.424 mol, 80.0 g) were added to the aqueous layer. The phases were separated, the organic layer was washed with aq. NaCl (2N, 400 mL) and the solvent was removed under reduced pressure to give (*S*)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[*d*]imidazol-2-yl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**20.5**, 205.2 g, 92% yield) as a yellowish solid.



20.5

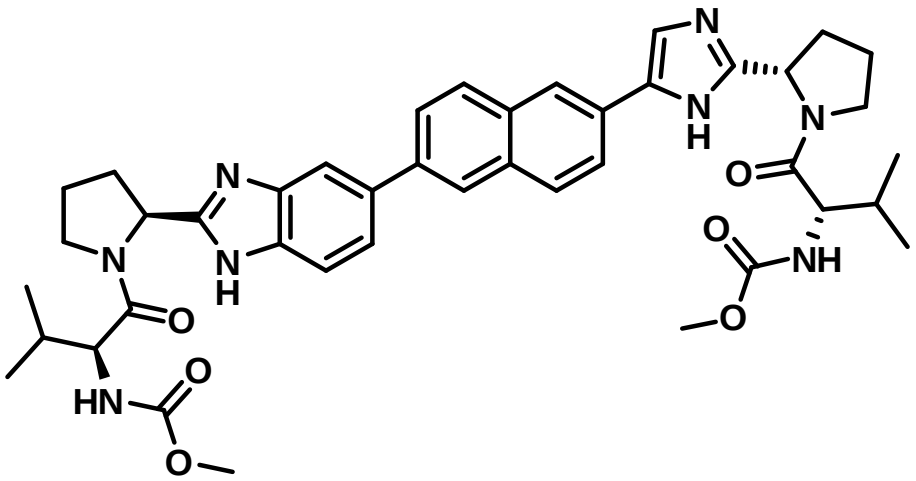
^{1}H-NMR (400 MHz, d_6-DMSO): δ (ppm) = 12.25 (d, 1H, *J* = 16.0 Hz), 7.81 (d, 1H, *J* = 22.7 Hz), 7.49 (m, 2H), 7.29 (d, 1H, *J* = 6.3 Hz), 5.21 (bs, 1H), 4.10 (t, 1H, *J* = 8.2 Hz), 3.87 (m, 2H), 3.56 (s, 3H), 2.24 (m, 2H), 1.98 (m, 3H), 1.29 (s, 12H), 0.84 (m, 6H).

^{13}C-NMR (100.6 MHz, d_6-DMSO): δ (ppm) = 170.6 (C), 156.9 (C), 145.6 (C), 142.8 (C), 136.6 (C), 133.9 (C), 127.9 (CH), 127.0 (CH), 125.3 (CH), 83.4 (C), 83.3 (C), 58.0 (CH), 54.8 (CH), 51.5 (CH_3), 47.0 (CH_2), 30.9 (CH_2), 29.8 (CH), 24.7 (4xCH_3), 24.5 (CH_2), 19.0 (CH_3), 18.4 (CH_3).

ESI-MS m/z 471.5 $[M+H]^+$ (calcd for $C_{24}H_{35}BN_4O_5$, 470.4).

5.5.11 Synthesis of ravidasvir hydrochloride (1.5)

To a solution of (*S*)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[*d*]imidazol-2-yl 1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidine-2-carboxylate (**20.5**, 0.893 mol, 420.0 g) in methanol (11000 mL) 6-(2-((*S*)-1-((*S*)-2-(methoxycarbonylamino)-3-methylbutanoyl)pyrrolidin-2-yl)-1H-imidazol-5-yl)naphthalen-2-yl trifluoromethanesulfonate (**17.5**, 0.616 mol, 350.0 g), K_2HPO_4 (1.540 mol, 268.3 g), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (complex with dichloromethane, 0.031 mol, 25.2 g) and water (1200 mL) were added and the resulting mixture was heated to 60°C for 4 hours. At the end of the reaction, the mixture was cooled down to 40°C and filtered on a Celite®/charcoal pad and the filter was washed with methanol (2x900 mL). The solvent was removed under reduced pressure, dichloromethane (5000 mL) was added and the phases were separated. The organic layer was washed with water (1500 mL). HCl in 2-propanol (20%$_{w/w}$, 200.0 g) was added and the solvent was removed under reduced pressure to give crude ravidasvir hydrochloride **1.5**. The crude product was suspended in 2-propanol (1500 mL) and the suspension was heated to reflux temperature until compete dissolution. The resulting solution was cooled down to 60°C and acetone/water (99:1, 2000 mL) was added dropwise during a period of 1 hours. The mixture was stirred for 2 hours, allowing the complete precipitation of the product. The solid was filtered and washed with acetone (3x600 mL) to give pure ravidasvir hydrochloride (**1.5**, 412.0 g, 80% yield) as a white solid.



1.5

^{1}H-NMR (400 MHz, d_6-DMSO): δ (ppm) = 15.65 (bs, 1H), 15.12 (bs, 1H), 8.65 (s, 1H), 8.28 (d, 2H, *J* = 3.3 Hz), 8.10 (m, 3H), 7.93 (m, 4H), 7.31 (d, 1H, *J* = 8.2 Hz), 7.27 (d, 1H, *J* = 8.3 Hz), 5.38 (t, 1H, *J* = 6.4 Hz), 5.31 (t, 1H, *J* = 6.4 Hz), 4.20 (m, 2H), 4.13 (m, 2H), 3.85 (bs, 2H), 3.55 (s, 6H), 2.42 (m, 4H), 2.24 (m, 6H), 2.05 (m, 2H), 0.86 (d, 6H, *J* = 6.2 Hz), 0.77 (d, 6H, *J* = 4.7 Hz).

^{13}C-NMR (100.6 MHz, d_6-DMSO): δ (ppm) = 171.2 (C), 171.0 (C), 157.0 (2xC), 155.6 (C), 149.6 (C), 137.7 (C), 137.6 (C), 133.0 (C), 132.1 (C), 131.6 (C), 130.5 (C), 129.3 (CH), 128.9 (CH), 126.4 (CH), 125.9 (CH), 125.2 (CH), 124.9 (C), 124.0 (CH), 123.7 (CH), 112.4 (CH), 114.5 (CH), 111.9 (CH), 57.9 (CH), 57.8 (CH), 53.5 (CH), 52.9 (CH), 51.6 (2xCH_3), 47.3 (2xCH_2), 31.1 (2xCH_2), 29.0 (2xCH), 25.1 (2xCH_2), 19.8 (CH_3), 19.7 (CH_3), 17.8 (CH_3), 17.7 (CH_3).

ESI-MS m/z 836.9 $[M+H]^+$ (calcd for $C_{43}H_{52}Cl_2N_8O_8$, 835.8).

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6. Discussion

Active Pharmaceutical Ingredients were the main subject of this PhD in Chemistry & Biology. The work was focused on the research and development of innovative, efficient and convenient synthetic processes for the preparation of APIs. IP issues had to be tackled, too, avoiding claimed compounds and problems related to the originator's way of synthesis.

After the selection of the API, we started with the design of novel synthetic strategies for the selected molecules and we tested them in the laboratory on a small scale. In this thesis, for the sake of conciseness, we usually report, among the many different synthetic routes explored for the selected APIs, the one giving the best results in terms of overall yield, purity of the final molecule and costs.

In a second time all the steps of the reported syntheses were scaled up to a 10-100 g scale, in order to gain additional information required for the following transfer of the manufacturing methods to the industrial scale.

6.1 Chapter 3 - Vildagliptin

During the first year, the work was centered on the important API vildagliptin, currently employed for the treatment of type 2 diabetes mellitus.

The critical step of the actual syntheses was the S_N2 reaction between halo acylproline derivatives and 3-amino-1-adamantanol. This nucleophilic displacement gave a hardly separable mixture of the desired product vildagliptin and of bis-alkylation byproducts, with complex work-up procedures and a drop in the overall yield of the process.

To avoid these issues and to prevent the formation of byproducts, we designed a synthesis based on a one-pot formylation and carboxymethylation of the nitrogen atom of the 3-amino-1-adamantanol. This represented the best alternative for the simultaneous introduction of the C_2-linker moiety on the nitrogen atom and a formyl- as a protecting group, leading to a key monoalkylated-monoformylated intermediate and completely avoiding the formation of the bis-alkylated compounds.

By using this approach, we developed a 4-step scalable synthesis for vildagliptin, with high overall yield (63%) and simple isolation of the final product. We ran the entire synthetic protocol on a 200 g scale.

6.2 Chapter 4 - Fexofenadine

Fexofenadine hydrochloride, an API largely employed for the treatment of allergic rhinitis, was the target of the second year of the PhD.

The syntheses reported to date share a common intermediate bearing an alkyne moiety as a precursor of the hydroxyl group contained in the final molecule. The key step of the transformation of the functional group of this intermediate is represented by the hydration of the C≡C triple bond. Reported protocols for this reaction usually require harsh conditions and toxic reagents (HgO/H_2SO_4 or $Cu(BF_4)_2$ in refluxing methanol), hardly compatible with large scale production.

With the aim to eliminate the use of toxic metal ions, we started to study an alternative protocol for this alkyne hydration and we found that treatment of an intermediate alkynol with aqueous HBr gave a mixture of two products, identified as a γ-methoxyketone and a γ-bromoketone. Subsequent reaction with HBr in acetic acid, achieved the conversion of this raw mixture toward a single product, represented by the abovementioned γ-bromoketone. The latter represented the key intermediate for the preparation of fexofenadine hydrochloride, obtained with a 8-step synthesis in a satisfying 59% overall yield.

Additional work involved: i) the scale up of this new route of synthesis to a 100 g scale, ii) the mechanistic analysis of the new hydration reaction highlighting, the important role of the OH group of the alkynol in assisting intramolecularly the addition to the alkyne C≡C triple bond. Moreover, to test the generality of this transformation, the protocol was successfully adapted to the preparation of a cognate API (terfenadine hydrochloride).

6.3 Chapter 5 - Ravidasvir

The third year of the PhD was focused on a member of a novel class of Direct-Acting Antivirals (NS5A inhibitors, *-asvir*), recently introduced for the

treatment of hepatitis C virus infection, represented by ravidasvir hydrochloride.

The strategies reported to date for the synthesis of this rather complex molecule were based on a semiconvergent synthesis, starting with the assembly of the central core of the molecule, represented by a biaryl moiety (benzimidazole-naphthalene). The two external residues, connected to the two extremities of the core, are composed by aminoacid-derived residues (pyrrolidine and Moc-L-Val) and are added in the final step of the synthesis.

We observed that the outer moieties of ravidasvir share the same substructure and stereochemistry and for this reason we explored the possibility to build this common residue as a key and early intermediate, to be used in a convergent synthesis, allowing to reduce the overall number of steps and relying on new and patentable intermediates. The key intermediate (a protected dipeptide), was successfully prepared starting from L-valine and L-proline benzyl ester hydrochloride and subsequently employed to build two distinct portions of the final molecule. These as partners in a Pd-catalyzed Suzuki coupling we obtain the desired API ravidasvir hydrochloride in high purity and overall yield (46%). This strategy reduced the number of steps compared to existing synthetic protocols.

6.4 PhD student's contribution

My contribution to the work of the PhD project may be summarized as follow: after an initial study of the hypothetical synthetic strategies for the preparation of selected APIs, I identified suitable starting materials and reagents in terms of costs and commercial availability. I tested the reaction of the proposed way of synthesis in a gram scale and isolated all the product and byproduct for their full characterization (*e.g.* NMR, IR, ESI-MS). After this fundamental work I tried to change the experimental conditions in order to increase the yield of the synthetic steps and the purity of the obtained product. When the syntheses were adequately optimized I proceeded with a first scale up (100-200 g scale) to test if the synthetic protocol was robust and efficient for a future transfer to the industrial scale. Finally I contribute to write internal reports, scientific articles and patents of the selected APIs.

6.5 List of publications

A concise and efficient synthesis of vildagliptin

Michele Castaldi,[a,b] Marco Baratella,[b] Ivan G. Menegotto,[c] Graziano Castaldi,[b] Giovanni B. Giovenzana[a,c,*]

[a]Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2/3, I-28100, Novara, Italy

[b]Chemelectiva srl, Strada due ponti 12, I-28100, Novara, Italy

[c]CAGE Chemicals srl, Via Bovio 6, I-28100, Novara, Italy

[*] Corresponding author

Tetrahedron Lett., **2017**, 58, 3426-3428. DOI: 10.1016/j.tetlet.2017.07.062.

An efficient and scalable synthesis of fexofenadine hydrochloride

Michele Castaldi,[a,b] Marco Baratella,[b] Mauro Gaboardi,[b] Graziano Castaldi,[b] Giovanni B. Giovenzana[a,*]

[a] Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2/3, I-28100, Novara, Italy

[b] Chemelectiva srl, Strada due ponti 12, I-28100, Novara, Italy

[*] Corresponding author

Chemistry Select, **2019**, 4(1), 428-431. DOI: 10.1002/slct.201802808

A robust and scalable synthesis of ravidasvir hydrochloride

Michele Castaldi,[a,b] Graziano Castaldi,[b] Mauro Gaboardi,[b] Giovanni B. Giovenzana[a,*]

[a] Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2/3, I-28100, Novara, Italy

[b] Chemelectiva srl, Strada due ponti 12, I-28100, Novara, Italy

[*] Corresponding author

Organic Process Research & Development, submission in progress.

6.6 Abstract

Active Pharmaceutical Ingredients (APIs) are a fundamental class of chemicals due to their important role in the human health. APIs are protected by intellectual property (IP), allowing the owners of the newly invented drug to have a temporary but complete exclusivity in terms of use, production and commercialization. The activity of the PhD is focused on the research of efficient and alternative synthesis of APIs, for which product/process patents are no longer valid or they are about to expire. The molecules selected for the research of alternative syntheses were vildagliptin (treatment of type 2 diabetes mellitus), fexofenadine hydrochloride (therapy for allergic rhinitis) and ravidasvir hydrochloride (a novel API, not yet approved, for the treatment of chronic hepatitis C). The aim of this work is to design alternative preparations for these selected APIs, optimising all the synthetic steps by adjusting reactions conditions and maximizing the yield of the processes and finally scaling up the whole syntheses to transfer the processes to the industrial scale.