https://dx.doi.org/10.21577/0103-5053.20220044

J. Braz. Chem. Soc., Vol. 33, No. 7, 664-680, 2022

Review

Sustainable Synthetic Strategies for the Preparation of Low Molecular Weight Drugs by Biotech Routes

Patricia G. Ferreira,[a] *Alcione S. de Carvalho,*[b] *Wilson C. Santos,*[a] *Luana S. M. Forezi,*[b,c] *Fernando C. da Silva*[*,b,c] *and Vitor F. Ferreira*[*,a,b,d]

[a]*Programa de Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, 24241-000 Niterói-RJ, Brazil*

[b]*Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, 24020-141 Niterói-RJ, Brazil*

[c]*Departamento de Química Orgânica, Instituto de Química, Universidade Federal Fluminense, 24020-141 Niterói-RJ, Brazil*

[d]*Departamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal Fluminense, 24241-000 Niterói-RJ, Brazil*

For billions of years, the phenomena of life and biocatalysis have been intertwined. If in the beginning biocatalysis was fundamental for the origin of life, currently it is very important for the cleaner production of pharmaceuticals and fine chemical intermediates. There is no doubt that drugs have brought great benefits to humanity, but currently, the expectations of modern society are focused on drugs with greater safety, less environmental impact, more sustainable practices, and less energy use. This review intends to show how the challenges for the production of some low molecular weight drugs produced by synthetic routes that involve at least one biotechnological step using microorganisms or enzymes were faced. These biotechnological drug production routes are more sustainable than conventional synthetic routes, as they produce a much smaller amount of waste, use moderate reaction conditions, have lower energy consumption, and have lower metal consumption, in addition to being more selective. Additionally, many natural products have structures too complex to be produced exclusively by chemical routes. The large-scale and economical production of these drugs is of great importance for fighting cancer as well as inflammatory, infectious, autoimmune, metabolic, hormonal, cardiovascular, and neurological diseases, among others.

Keywords: medicines, organic synthesis, sustainability, green chemistry

1. Introduction

Modern organic chemistry is based on solid mechanistic concepts and the diversity of reactions that allow it to carry out the most challenging and difficult tasks, namely, the total synthesis of complex natural products.[1] The challenge of organic synthesis of a molecule must be faced with tenacity and resilience, and despite being one of the most successful areas of chemistry, it is also where the greatest failures related to the production of undesirable chemical waste occur. However, it is very important to highlight that this is the area of science that most impacts the longevity and quality of life of humanity through the synthesis of drugs and biopharmaceuticals. The number of molecules of varying sizes and complexities that have been made over more than a hundred years is remarkable. With the advancement of physicochemical analysis techniques, it was possible to elucidate complex natural products with no resemblance to the synthetic targets of the past.

The current emphasis on chemical reactions has shifted toward a more ecological bias since the principles of green chemistry were established by its creator, Professor Paul Anastas. Since then, there have been a significant number of reports in the literature detailing new discoveries of reactions and innovations in production processes that were already consolidated. The creativity within green chemistry, associated with the criteria of environmental

*e-mail: fcsilva@id.uff.br; vitorferreira@id.uff.br
Editor handled this article: Teodoro S. Kaufman

sustainability, demonstrated that it is possible to make more environmentally recommendable chemistry. Today, it is necessary to be concerned with many other issues when drawing up a synthesis, such as waste, efficiency, sustainability, renewable resources, system perspective, energy, product lifecycle, and waste and supply chain management.[2,3]

2. Challenges for Synthetic Organic Chemicals

Many syntheses of complex molecules have been carried out and represent the art and intellectual capacity of synthetic organic chemicals, but today, they are no longer used. Many complex substances could be cited that were synthesized by well-designed routes, but we highlight two milestones from different eras that are shown in Figure 1.

The first milestone selected is the vitamin B12 synthesis performed by Woodward[4] and Eschenmoser[5,6] in the 1970s, which was considered the pinnacle of achievement in organic synthesis. The synthetic work took 12 years to complete and involved more than 90 separate reactions performed by over 100 researchers. During the development of the synthetic route, many stereochemical challenges were faced and led to the establishment of the Woodward-Hoffman rules, which were the basis for understanding how molecules are reorganized as a function of the symmetry of the orbitals. The other outstanding example was the synthesis of the potent marine neurotoxin ciguatoxin CTX3C produced by the dinoflagellate *Gambierdiscus toxicus* (Figure 1). This substance is responsible for the poisoning of more than 20,000 people by fruit contaminated by this neurotoxin. The synthetic challenge in constructing the ciguatoxin CTX3C ring system was very great, as its ladder-shaped structures contain only carbon, hydrogen and oxygen, thirty stereogenic centers, twelve *trans* fused rings with between five to nine members, and a spiroketal ring.[7,8]

The greatest challenge for synthetic organic chemists is to produce enantiomerically pure molecules by selective reactions,[9] as most natural products are chiral and their biological properties depend on their recognition by chiral receptors. Scientist Phil Baran, in his account[10] of his syntheses of complex molecules, makes a statement that should guide those who venture into this area: "The successful total synthesis of such molecules demands a high degree of innovation, which in turn enables the discovery of new reactivity and principles for controlling chemoselectivity".

There are several motivations for developing the synthesis of a simple or complex molecular target, such as the intellectual challenge that stimulates the development of a synthetic route to a complex, beautiful and intriguing structure; the need for practical, short, and more efficient routes of an important synthetic target; and the opportunity to prove some intrinsic property of a substance or to prove its stereochemistry. In this context, when planning the approach to a complex target, covalent bond disconnections are traced to form fragments to maximize skeletal simplification, especially when the proposed chemistry has little preceding knowledge or is completely unknown. Although total synthesis aims for the

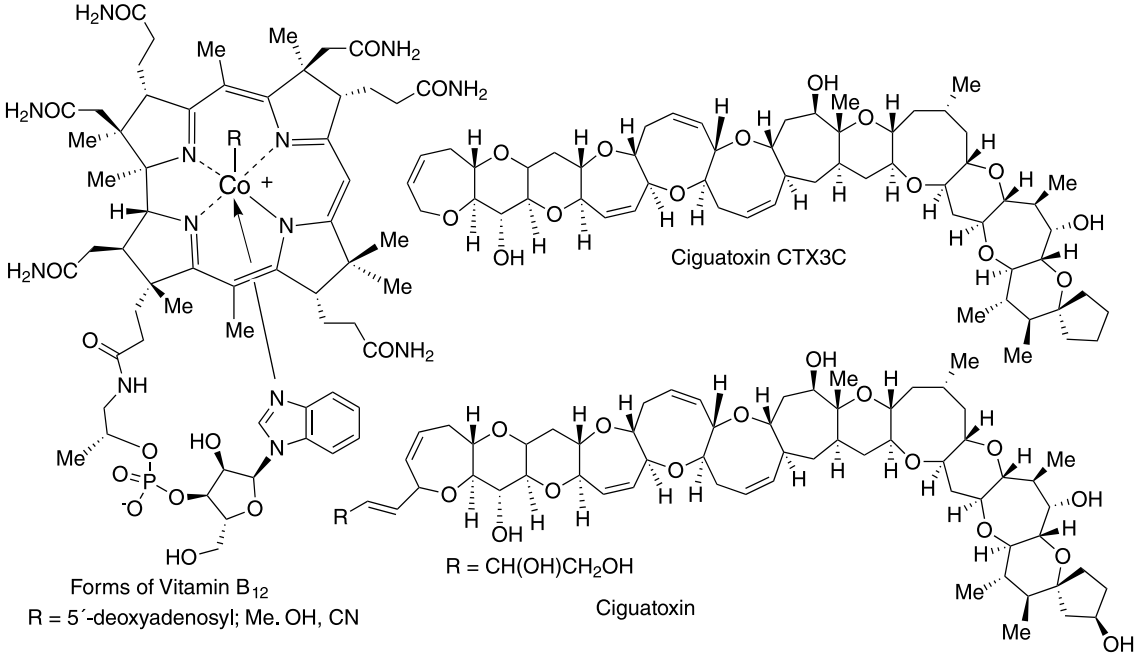


Figure 1. Examples of complex natural products.

fewest possible steps and uses low-cost starting materials available in large renewable quantities, the opportunity to develop new reactions in the chosen route with less waste is especially important. In addition to the synthesis of the desired product, new green reactions are discovered; that is, innovation and invention also become the goal of total synthesis.

The well-being of modern society depends heavily, among others, on natural and synthetic products from the pharmaceutical industry. The pharmaceutical manufacturing task has enormous demands and responsibilities in balancing the knowledge and robustness of chemical and/or biological processes. However, on the one hand, the syntheses need to be chemo-, regio- and enantioselective, and on the other hand, there has been a growing awareness that the syntheses need to be increasingly sustainable; therefore, the biocatalytic processes are more ecological, green and sustainable when compared to their chemical alternatives, which are often not easily conducted by classical organic chemistry or by substituting various chemical steps. This alternative is in fact very advantageous from the point of view of green synthesis for the modern organic chemistry and extremely important for industrial biotechnology. It can be carried out in water at room temperature and neutral pH, without the need for high pressure and extreme conditions, saving energy for processing.

The atom efficiency or atom economy concept is an extremely useful tool for rapid evaluation of the amounts of waste that will be generated by alternative processes. It is calculated by dividing the molecular weight of the product by the total sum of the molecular weights of all substances formed in the stoichiometric equation for the reaction involved. In the late 1980s, Roger Sheldon[11] generated the environmental impact factor (E factor) (kg waste *per* kg product) to evaluate the environmental impact of manufacturing processes, with a higher E factor indicating a greater waste burden and a more negative environmental impact on the earth. In 1992, he initially estimated E factors for various chemical industries, which revealed that the pharmaceutical industry had the highest E factor among those chemical industries (Table 1).

Table 1. Environmental impact of chemical industries

Industry	Annual product / (tons year^{-1})	E factor / (kg waste *per* kg product)
Petrochemical industry	10^6-10^8	< 0.1
Bulk chemical industry	10^4-10^6	< 1-5
Fine chemical industry	10^2-10^4	5-> 50
Pharmaceutical industry	10-10^3	25-> 100

The E factor concept has played a major role in focusing the attention of the chemical industry worldwide, particularly the pharmaceutical industry, on the problem of waste generation in chemical manufacturing. It provided, and continues to provide, the impetus for developing cleaner, more sustainable processes.[12]

In 2021, Sheldon and co-workers[13] collaborated across the International Consortium for Innovation & Quality in Pharmaceutical Development, and the American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable[14] introduced the innovation green aspiration level (iGAL). They developed a statistical model for the metric named the innovation green aspiration level, iGAL 2.0, in which, with the yields of the reaction and some key sustainability indicators, included a new formula for convergence with potential applicability in computer-assisted synthesis planning algorithms. The improved statistical model of iGAL 2.0 represents an extension to the common active pharmaceutical ingredient (API) process waste metrics, process mass intensity (PMI) and complete E factor, and thus, it can be used in sustainable development efforts to measure the degree of relative process greenness in API manufacturing processes.

The production of vinegar dates back approximately 2,000 years, and perhaps it is the oldest microbial biotransformation, among many other reactions and processes that have been developed throughout its history. In this context, there is a great advantage in choosing syntheses that involve microorganisms and enzymes for the transformation of synthetic chemical products under more favorable conditions of temperature and atmospheric pressure. Microorganisms have numerous intracellular enzymes that operate within a highly structured and protected environment, while extracellular enzymes are secreted by the cell, thus acting in the environment surrounding the microorganism. Certainly, clean chemoenzymatic processes with improved optimization protocols positively impact alternative drug routes, leading to new, more sustainable opportunities.

In this review, the general aspects of some biopharmaceuticals and products that have some type of biological effect and that have at least one biotechnological step will be discussed, such as fermentation, biotransformation by microbial, animal or vegetable whole-cells, transformations catalyzed by organelles or enzymes, cloning, and enzyme expression and targeted enzyme evolution to improve selectivity (Figure 2). Sustainability is the motivation for using biocatalysis over conventional routes and chemical catalysis, including shorter synthetic routes and milder reaction conditions.

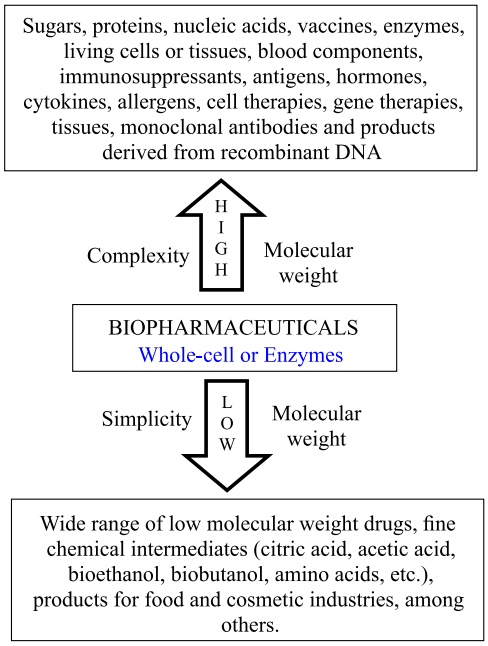


Figure 2. Alternatives for preparing highly and less complex biopharmaceuticals.

Pharmaceutical industrial biotechnology is the area of science that covers all technologies used for the production of biopharmaceuticals or biological medicines by biotechnological routes, including the extraction of living systems; that is, the active principle of the medicine is obtained through the industrial use of microgenetically modified organisms or cells. This technology has a beneficial effect on greenhouse gas emissions and simultaneously supports the agricultural sector by producing important raw materials. Rapid advances in biotechnology have had a significant impact on drug discovery.[15] Achievements in human genomics, proteomics, and bioinformatics have led to enormous possibilities to unveil new biological targets.

More recently, modern pharmaceutical industrial biotechnology has included protein engineering, metabolic engineering, synthetic biology, systems biology and downstream processing, gene cloning, and recombinant deoxyribonucleic acid (DNA) technology in its lines of research.[16] Gene cloning and recombinant DNA technology are powerful tools in discovering new biopharmaceuticals. Gene cloning is the process in which a gene of interest is located and cloned from DNA extracted from one organism and implanted in another organism. Recombinant DNA technology, or gene splicing, involves altering genetic material outside an organism, for instance, inserting a segment of a different DNA into a DNA molecule. This recombinant DNA technology allows for the modification of microorganisms, animals, and plants so that they produce pharmaceutically useful substances. These highly complex biopharmaceuticals are obtained from biological sources such as humans, animals, or microorganisms and are quite diverse, such as sugars, proteins, nucleic acids, vaccines, enzymes, living cells or tissues, therapeutic agents such as blood components, immunosuppressants, antigens, hormones, cytokines, allergens, inputs for cell and genetic therapies, tissues, stem cells, monoclonal antibodies and products derived from recombinant DNA.[17]

Some biopharmaceuticals that were initially extracted from animals and plants are currently produced by biotechnology routes specially designed for their production. Therapeutic insulin, for instance, has long been extracted from islets in the pancreas of pigs and is now being produced by a biotechnology route since 1982, using recombinant DNA with *Saccharomyces cerevisiae* or *Escherichia coli*. This was the first licensed drug produced with recombinant DNA technology that still occupies a large market estimated to reach 29.9 billion USD by 2025.[18,19]

The complex biopharmaceutical market is highly segmented by the type of drug and driven by the growth of the geriatric population, the increase in chronic diseases, and investments in frontier research. It was valued at approximately $325.17 billion in 2020, with the prospect of achieving revenue of $496.71 billion in 2026 with a compound annual growth rate (CAGR) of 7.32% between 2021 and 2026.[20]

With pharmaceutical biotechnology, it is also possible to prepare structurally simpler drugs of low molecular weights, fine chemical intermediates (citric acid, acetic acid, bioethanol, biobutanol, amino acids, etc.), and special products for the food and cosmetic industries. Importantly, most drugs exhibit a molecular weight of approximately 500 Daltons, usually less than 1,000 Daltons. Due to this small size, any chemical modification in a small molecule drug can dramatically alter its pharmacological activity and usually lead to new drugs for new uses or new indications. In this type of biotechnological production, there is a special motivation for natural products, especially when related to food, health products, and agricultural areas. It is guided by the unambivalent purpose of the United Nations Sustainable Development Goal 12, which aims at substantially reducing waste production by 2030, and driven by a vision to catalyze greener API manufacturing around the globe.[21,22]

In the last few years, the biocatalytic production of fine chemicals has been expanding rapidly. Industrial processes based on biocatalytic methods have many advantages over classical chemical synthesis and extraction from natural sources. Most enzymes used in biotechnological processes are hydrolytic enzymes, transferases, oxidoreductases, and lyases. However, it is important to emphasize that in food

products, microbial enzymes also play an important role in the formation of chemical compounds that give them flavor. Importantly, enzyme production and plant cell culture can help alleviate the pressure on the supply of natural flavors in a sustainable way.

The global enzyme market was $9.9 billion in 2019 and is expected to grow at a CAGR of 7.1% between 2020 and 2027. Demand for industrial enzymes will increase in several areas, such as sustainable drug preparation with waste reduction, the pharmaceutical industry, food additives, biofuels, etc. Genetic engineering for the production of new types of enzymes is one of the new ways of applying enzymes, with consequent innovation, development of new products, and catalysis of reactions that do not occur naturally.[23] On the other hand, the microorganism market is very segmented and is growing at a moderate pace with substantial growth rates in recent years, but it is estimated that it will grow significantly between 2020 and 2027.[24]

Pharmaceutical industrial biotechnology has a long tradition in the production of biopharmaceuticals by fermentation methods in cell culture microorganisms as well as in enzymatic bioprocessing.[25] Low molecular mass drugs have been the target of biotechnology since the 19th century, and the greatest expression of this scientific endeavor came with the discovery of benzylpenicillin or penicillin G by Alexander Fleming[26] in 1928 and the large-scale production of this antibiotic. Penicillin G was obtained from the fermented broth of the fungus *Penicillium notatum*, which secretes this substance to eliminate Gram-positive and Gram-negative pathogenic bacteria. This biopharmaceutical is considered to be the first antibiotic that was successfully used in clinical medicine and represents a family of β-lactam heterocycle compounds fused to a thiazolidine ring (5 members). In fact, since its discovery, there has been a long history of drugs reaching the medical clinic, but it soon became a major area of research and development of antibiotics obtained by conventional synthetic and biotechnological routes.[27] Cephalosporin C is another antibiotic biopharmaceutical isolated in 1961 from a fungus of *Cephalosporium acremonium*. This substance served as an inspiration for the preparation of much more active synthetic unnatural antibiotics (Figure 3).[28]

3. Chemoenzymatic Synthesis of some Drugs

Many complex molecules were synthesized by long chemical routes and, therefore, are of little industrial viability. Subsequently, their preparations were studied by selective enzymatic routes and were more sustainable where part of the synthesis was carried out by some type of biotechnological process. Whenever there are difficulties in carrying out a selective chemical transformation, biotechnological processes are used.

(*S*)-α-Methyldopa (L-α-methyl-3,4-dihydro-oxy-phenyl-alanine) is a decarboxylase inhibitor prodrug metabolized in the central nervous system as a potent presynaptic α-2-adrenoceptor agonist, resulting in decreased sympathetic flow and decreased blood pressure, especially in gestational hypertension and preeclampsia.[29] Recently, pharmacological studies[30,31] have confirmed the important role of (*S*)-α-methyldopa in inducing postpartum depression through hormonal changes, reducing cerebral blood flow and decreasing neuronal function. This drug is sold under three trademarks, but it is also available as a generic product. There are many chiral and racemic syntheses for the drug (*S*)-α-methyldopa. One involves the use of the industrially produced Gram-positive bacterium *Rhodococcus rhodochrous* to catalyze the conversion of acrylonitrile to acrylamide. The key step of this synthesis was the enzymatic reduction transformation of the functional groups as desymmetrization (98.2% enantiomeric excess (ee)).[30,31] After hydrolysis of the other functional groups, it was possible to obtain the drug (*S*)-α-methyldopa (Scheme 1).

Another interesting example is γ-aminobutyric acid (GABA), an important inhibitory neurotransmitter that plays a pivotal role in the central nervous system. Imbalance of this neurotransmitter can cause many diseases, such as epilepsy, anxiety disorders, neuropathic pain, and social phobias. Many drugs based on the GABA structure were prepared and entered the pharmaceutical market, such as gabapentin, pregabalin, and baclofen (Figure 4). There are several methods for the synthesis of these drugs that include chiral pools, kinetic resolution processes, and enantioselective reactions. Some methods have disadvantages, such as expensive chiral starting materials and unwanted side products.

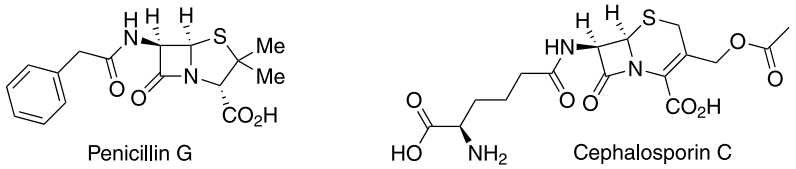
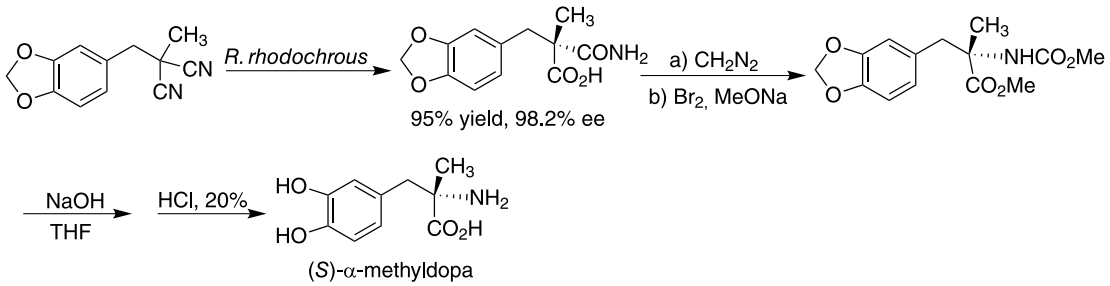


Figure 3. Chemical structures of penicillin G and cephalosporin C biopharmaceuticals.



Scheme 1. Synthetic route to the drug (*S*)-α-methyldopa.

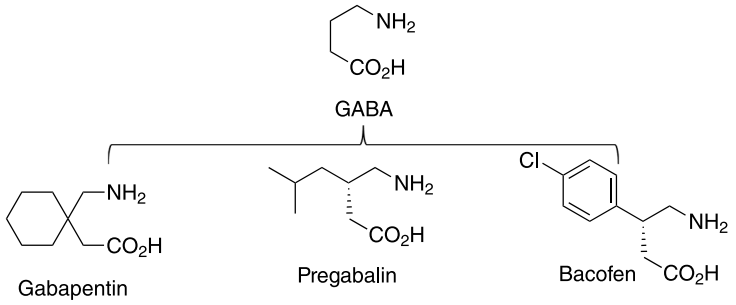
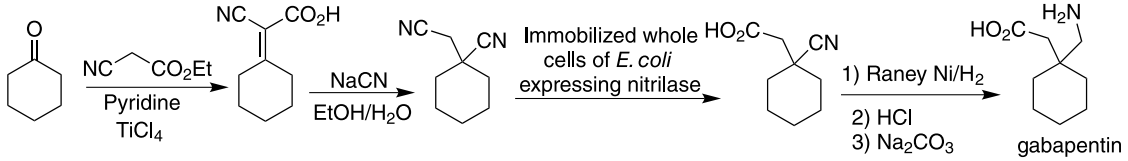


Figure 4. Some drugs based on the GABA structure.

Gabapentin (2-[1-(aminomethyl)cyclohexyl]acetic acid) is a controlled drug that is indicated for the treatment of epilepsy and neuropathic pain in adults, with an estimated annual production of 1000 tons. This drug is a structural analog of the neurotransmitter GABA, which does not cross the blood-brain barrier, whereas gabapentin penetrates the central nervous system (CNS), and its activity may be related to GABA.[32] There are many conventional syntheses based on the Hofmann rearrangement for gabapentin, whose structure is not highly complex.[33,34] Zheng and co-workers[35] developed a gabapentin preparation route based on a simple chemical step process and an efficient chemo-whole-cell acid transformation step from more ecological (1-cyanocyclohexyl)acetonitrile. Whole cells of recombinant *Escherichia coli* expressing the nitrilase enzyme were immobilized to obtain high conversion and facilitate (1-cyanocyclohexyl)acetic acid synthesis, which could be efficiently converted to gabapentin. The proposed approach will allow for the most economical and environmentally attractive production of gabapentin (Scheme 2).

Pregabalin is a drug launched in 2005 for the treatment of diseases related to GABA levels and neuropathic pain, postherpetic neuralgia and epilepsy. Its structure maintains the γ-aminobutyric framework present in GABA. The patent expired in 2018, and its market share with generic manufacturing companies has increased. The market size for pregabalin is expected to reach $890 million by 2025, growing at a CAGR of 3.67% during the 2020-2025 forecast period.[36] The initial manufacturing process used a racemic synthesis of pregabalin followed by a classical resolution with (*S*)-mandelic acid, but other routes were studied,[37] such as using L-leucine as starting material, via the Stobbe approach and enzymatic resolution of gamma-isobutylglutaric acid. The biocatalytic route to pregabalin uses the same racemic diester starting material as the classical resolution route, namely, Knoevenagel condensation of isovaleraldehyde and diethyl malonate, followed by cyanation. The biocatalytic step by the kinetic resolution of the diester to the (*S*)-monoester was performed using the commercially available lipase obtained from *Thermomyces lanuginosus* (Scheme 3a). Although this process involves a kinetic resolution of enantiomers, it has several advantages over other synthetic routes, as it eliminates the use of organic solvents and enables the recycling of the unwanted



Scheme 2. Chemoenzymatic synthesis of gabapentin using immobilized whole cells of *E. coli* expressing nitrilase.

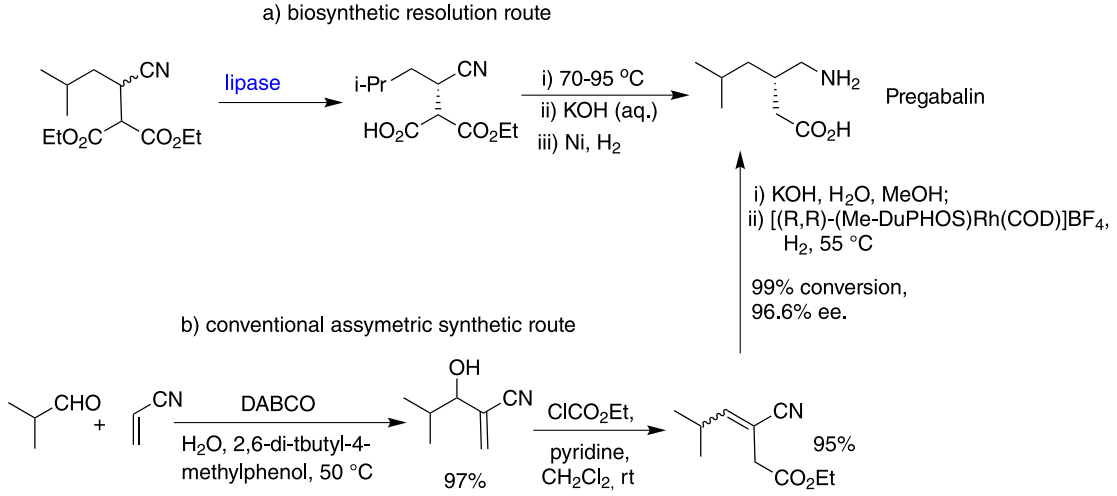
isomer.[38] The sequence is completed in three steps that include hydrolysis, reduction and decarboxylation, all performed in a single vessel with a single isolation step (Scheme 3a). Other synthetic routes via enantioselective reactions with chiral catalysts were developed and are also presented as good alternatives for the preparation of this drug (Scheme 3b).[39,40]

In nonenantioselective asymmetric syntheses, there is an undesirable loss of half of the material, as racemization is thermodynamically favorable due to the increase in entropy. Usually, enantiomers have different toxicities and biological activities. Racemate resolution is an alternative, but more often, the alternative is kinetic resolution using a microorganism in which virtually all racemic material is converted to a single stereoisomer. This is a good process on an industrial scale to search for intermediates for the syntheses carried out by the pharmaceutical industries.[41]

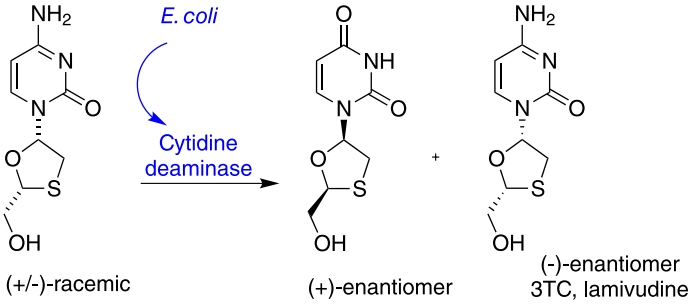
Nucleosides are chiral substances that are associated with DNA nucleic acids. Many chiral structural analogs have been prepared and have become drugs with diversified biological activities, such as anti-HIV (human immunodeficiency virus), antitumor, and antiviral activities, and therefore are of great pharmaceutical value. In this context, there are several strategies for the synthesis of nucleosides, such as the preparation of 3TC (lamivudine) from racemic 2'-deoxy-3'-thiacytidine (Scheme 4). This is a drug that is in the therapeutic arsenal as an antiretroviral used in combination with other antiretroviral drugs, such as zidovudine and abacavir, to treat HIV. The two enantiomers of the racemate are equipotent in terms of anti-HIV activity, but the (+)-enantiomer is more cytotoxic than its optical antipode, (–)-enantiomer (3TC). The separation of these enantiomers was done through an enzyme-catalyzed resolution, where the amino group was enantioselectively deaminated from (+)-enantiomer. Cytidine deaminase is able to perform this enantioselective deamination in 76% yield, making the resolution process simpler. This enzyme was produced by cloning *Escherichia coli* and overexpression of the gene, and its immobilization allowed its reuse several times.[42]

Other antivirals, such as abacavir and (+)-carbocyclic 2'-deoxy-5-[(*E*)-2-bromovinyl]uridine (c-BVdU), selective inhibitors and potent antivirals for the treatment of HIV infections, have also been prepared with the same type of chemoenzymatic strategy to obtain active enantiomers.[43]

To overcome multidrug resistance to 1st generation HIV protease inhibitors (PIs) (zidovudine, lamivudine, and abacavir), compounds of 2nd generation were developed and



Scheme 3. Two synthetic routes for preparing pregabalin.



Scheme 4. Enzymatic production of optically pure (2'*R-cis*)-2'-deoxy-3'-thiacytidine (3TC, lamivudine).

FDA (Food and Drug Administration) approved (darunavir), which all contain the (3*R*,3a*S*,6a*R*)-hexahydrofuro [2,3-*b*]furan-3-ol moiety (Figure 5). A cost analysis of various synthetic pathways to darunavir showed that the bis-tetrahydrofuran (THF) alcohol moiety contributed to roughly half the cost of synthesizing the active ingredient. This explains why numerous routes for its synthesis have been described.[44-46]

In a study by Sheldon and co-workers,[47] the greenness and sustainability of the three most recent and innovative routes and new route were assessed (Scheme 5). Bis-THF was initially obtained in racemic form, and enzymatic kinetic resolution using lipase was the key step in obtaining the desired enantiomer. It was found to be the most efficient, scalable, inexpensive, environmentally friendly, and least health hazardous pathway for assembling bis-THF alcohol. The reaction of furan with Cbz-protected aldehyde under photocatalytic conditions gave rise to bicyclic racemates, which were hydrogenated in the presence of palladium to give racemates. This compound immediately undergoes rearrangement to give the desired bis-THF alcohol in racemic form. The final resolution step was performed using porcine pancreatic lipase (PPL) in the presence of propionic anhydride in methyl *tert*-butyl ether (MTBE) to give propionate and the desired bis-THF alcohol (–)-enantiomer in 99% ee (Scheme 5). This procedure adhered to several principles of green chemistry through the use of renewable, CO_2 neutral, and environmentally acceptable solvents and photocatalytic and biocatalytic reactions under ambient operating conditions.[48]

The vast majority of drug syntheses use a mixed process in which part of the synthesis is carried out by conventional synthesis and one or more steps are carried out by biotechnological processes. This strategy has grown greatly in the synthesis of pharmaceutical products due to the commercial availability of many enzymes, bacteria and microorganisms. The synthesis of artemisinin is a classic case, where the production of the carbon skeleton is biosynthesized in the first step.

Artemisinin is a natural drug obtained from the Chinese plant *Artemisia annua* L., known as "qinghaosu" in Chinese medicine.[49] This species is the only economically viable

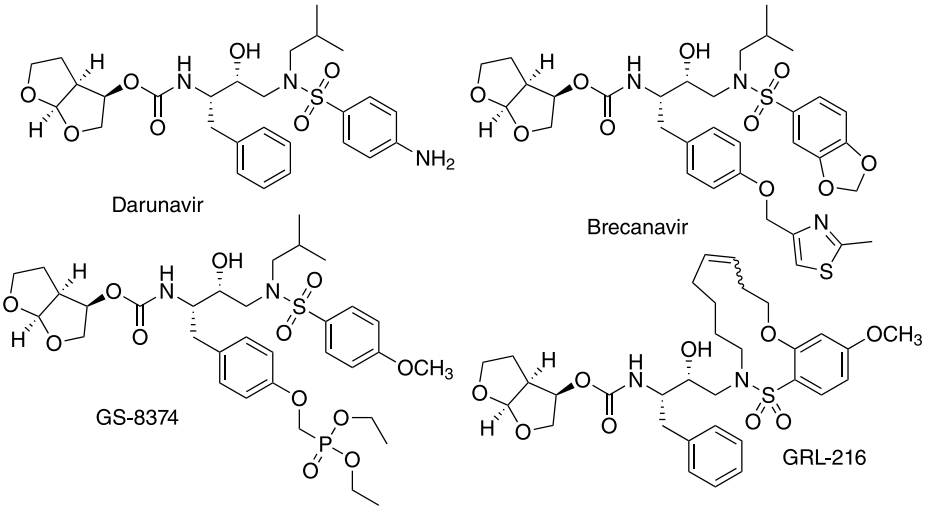
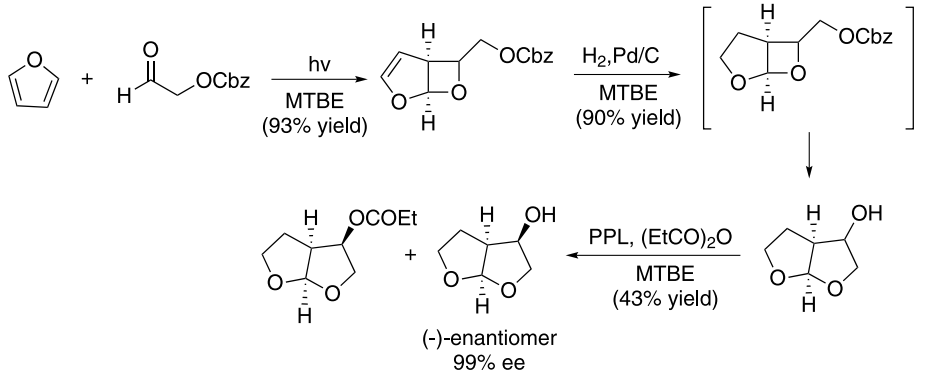


Figure 5. Structures of HIV protease inhibitors containing the bis-THF alcohol moiety.



Scheme 5. Route that employs an enzymatic kinetic resolution.

source for the extraction of this substance, which, despite being able to be acclimated in other countries, e.g., Brazil, does not always meet the world demand. This substance has become a widely used drug in the treatment of malaria and is also the main component of the combined therapies recommended by the World Health Organization.[50] From artemisinin, several other antimalarial drugs (artemether, arteether, and artesunate) were prepared (Figure 6).[51-53]

The production of artemisinin through conventional chemical synthesis was not economically viable due to the high number of steps (13 steps), the complexity of the reactions even starting from the natural monoterpene (–)-isopulegol, and the low total yield of the synthetic route.[54] However, from this route, the photooxygenation of the olefin in the six-membered ring with hydrogen peroxide is stereoselective.

An alternative that proved to be viable for the manufacture of artemisinin in commercial quantities was the development of the semisynthetic route from a natural precursor, amorpha-4,11-diene, obtained by microbiological transformation.[55] The production of this bioproduct was carried out by fermentation using several modified strains of *Saccharomyces cerevisiae* or *Escherichia coli*.[48] Amorpha-4,11-diene is a chiral sesquiterpene with 4 stereogenic centers that undergoes a simple conventional 8 step chemical conversion. This semisynthesis in a few steps was economically viable and became the best alternative for the production of this drug. The latest technology has combined the use of liquid CO_2 and a dual-function fixed photocatalyst in a continuous flow reactor in which the only inputs are dihydroartemisinic acid, oxygen, and light, and the output is pure crystalline artemisinin (Scheme 6).[56]

A straightforward chemoenzymatic synthesis of enantiomerically pure rivastigmine, described for the treatment of mild to moderate dementia of the Alzheimer's type, has been efficiently carried out under mild reaction conditions, with *Candida antarctica* lipase B responsible for the acetylated (*R*)-enantiomer yielding the acetamide in enantiomerically pure form (> 99% ee) and the remaining (*S*)-amine (> 97.5% ee) in very high optical purity (Scheme 7). An exhaustive enzymatic study[57] has

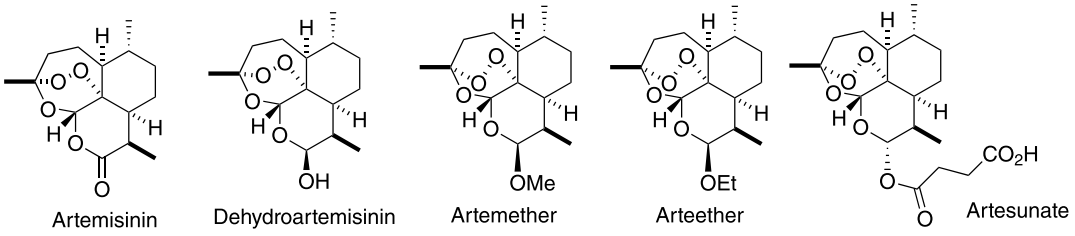
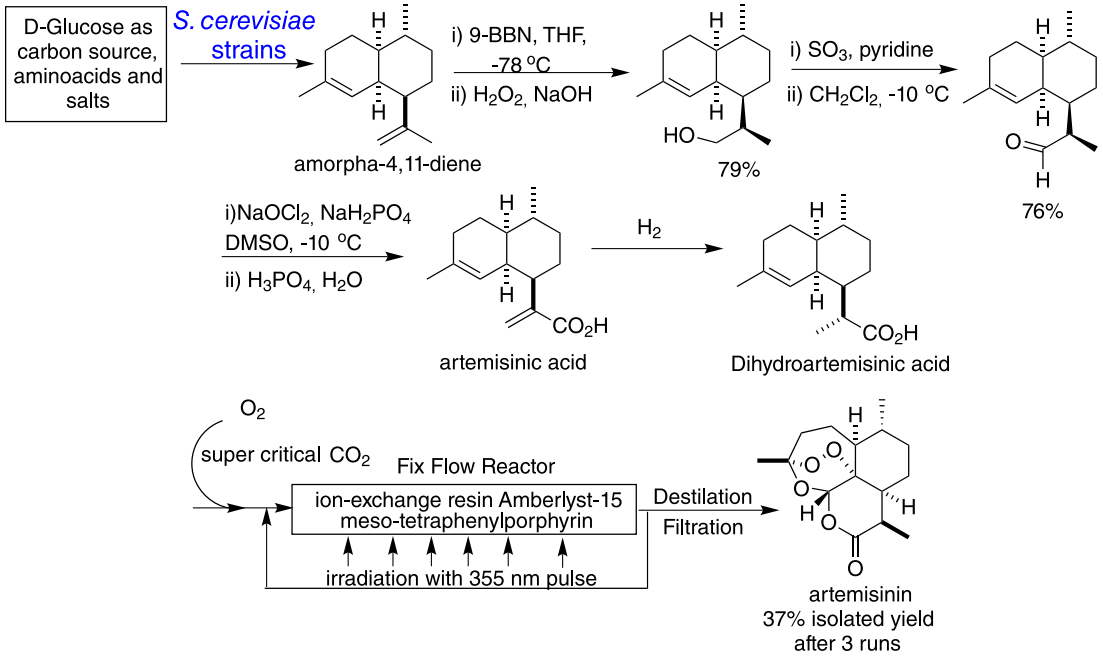
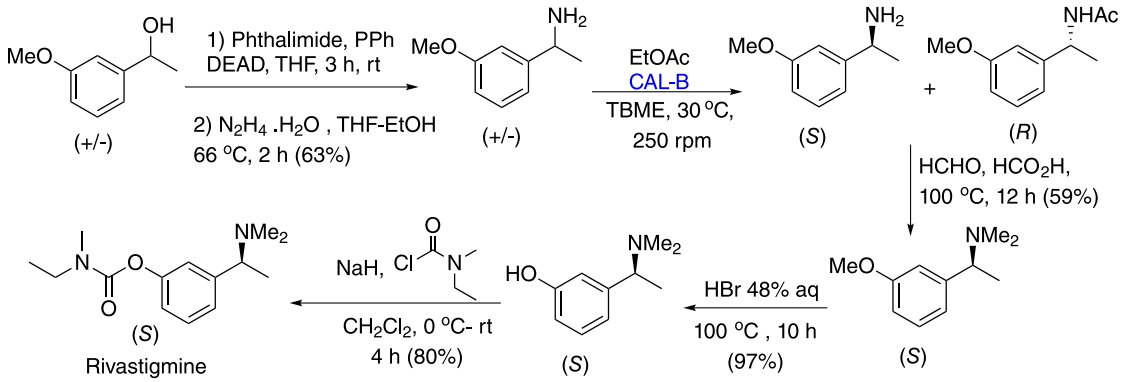


Figure 6. Artemisinin and analogues drugs.



Scheme 6. Partial total synthesis of artemisinin.



Scheme 7. Chemical synthesis of (*S*)-rivastigmine from enzymatic kinetic resolution.

been developed exploring the possibilities of carrying out enzyme recycling, scaling up the enzymatic process and developing a dynamic kinetic resolution procedure for the production of adequate enantiomerically pure precursors of rivastigmine.

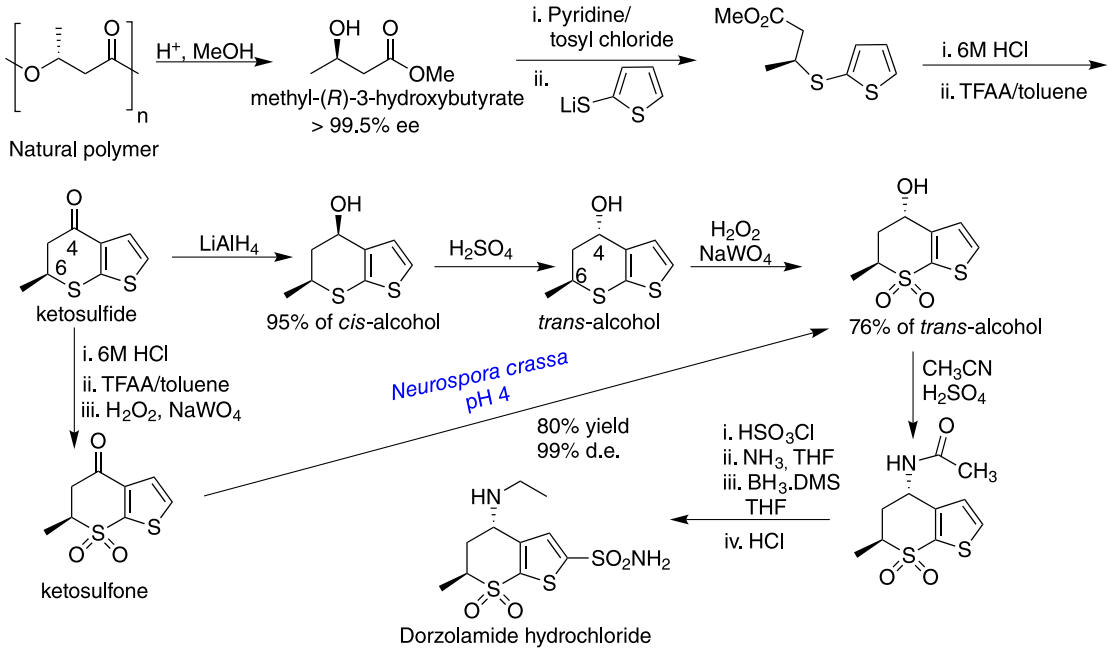
Another example of this type of approach that is worth mentioning is the synthesis of the drug dorzolamide hydrochloride (MK-0507), a carbonic anhydrase inhibitor. This drug is used as an ophthalmic solution indicated to reduce high intraocular pressure and treat glaucoma. Glaucoma is an eye disease that elevates intraocular pressure and, if left untreated, can lead to blindness.

The structure of dorzolamide is not very complex, but its challenges include two chiral centers in the dihydrothiopyran ring and the C-4 stereochemistry in the sulfone ring.[58] The conventional synthesis and route with a biotechnological step start from the same chiral substance (methyl-(*R*)-3-hydroxybutyrate) whose configuration of the methyl group of C-6 is already defined (Scheme 8). This starting product is produced by depolymerization with methanol in an acidic medium of the natural biodegradable homopolymer produced by some microorganisms on an industrial scale of several tons. This process produces methyl-(*R*)-3-hydroxybutyrate with a chemical purity > 98% and > 99.5% ee.[59-61] It is important to note that optically active methyl-(*R*)-3-hydroxybutyrate can also be prepared by asymmetric hydrogenation of ethyl acetoacetate by catalyzed reduction with rhodium or ruthenium catalysts with optically active phosphine ligands. The most important step in the synthesis involves the chiral center (C-4).[62] The chiral environment of the intermediate ketosulfide was then used to induce chirality in the second center during reduction. However, this reversal is incomplete and leads to the undesirable product *cis*-alcohol. It is possible to invert the stereochemistry to the desired *trans*-alcohol. Through the biological reduction of the ketone functionality, the problem of epimerization at C-6 was solved. To carry out this step, ketosulfone was used, which has a greater solubility in water than the intermediate ketosulfide. Many microorganisms are able to carry out this reduction,[63] but the fungus *Neurospora crassa* was chosen due to its ability to grow at low pH values, which avoids the problem of racemization. Under these conditions, *trans*-hydroxysulfone was obtained with very high optical purity.[58,64] Despite this synthesis starting from a raw material from a renewable source and having a more efficient biocatalytic step, many other steps still continue without utilizing green reactions.

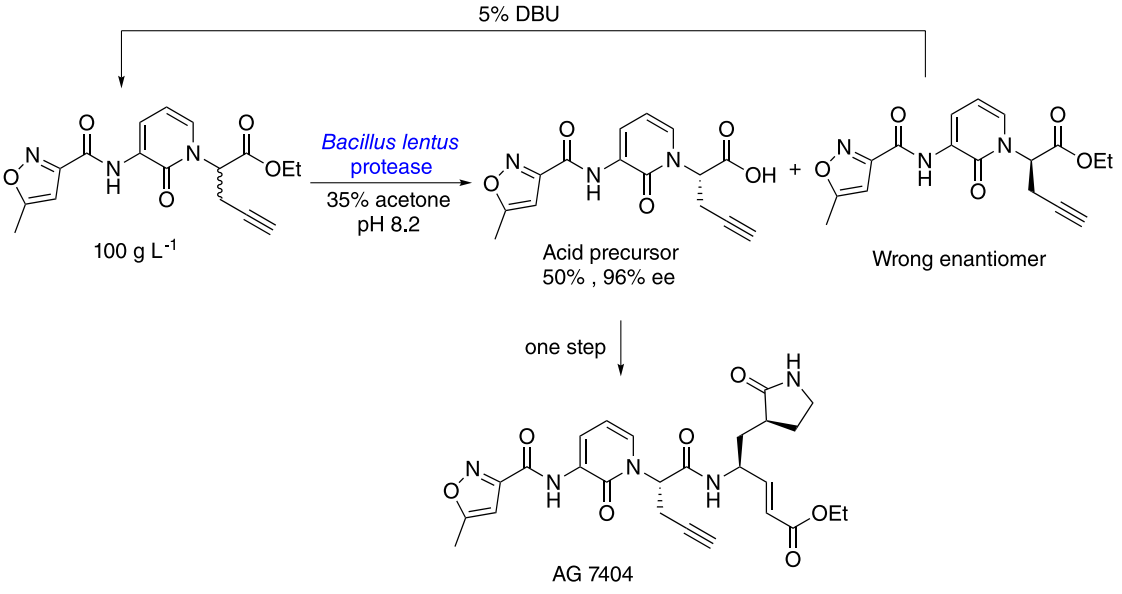
The chemoenzymatic process is much more cost-effective and high-yielding than the classic resolution route. For example, in the synthesis of AG7404, a rhinovirus protease inhibitor for the treatment of the common cold, the key intermediate is an acid precursor. The existing chemical resolution is inefficient, suffering low yields. Through a 96-well plate-based screening of a comprehensive library of hydrolases, *Bacillus lentus* protease (BLP) was identified as the best hit. In the presence of 35% acetone and 100 g L^{-1} racemic ester, excellent enantioselectivity (96% ee) was obtained with a conversion of 50% at a pH of 8.2 after 24 h (Scheme 9). Moreover, the incorrect enantiomer can be readily recycled using a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[65]

The fermentation of plant cell cultures or microorganisms isolated from various environmental sources or modified is a powerful tool for producing molecules of low and high degrees of complexity. It is seen as an alternative for the production of drugs and their fine chemical intermediates.[66] Many natural products produced by conventional farming have problems that can be circumvented by cell culture. These issues include environmental factors, pests, diseases, crop adulteration, energy cost and storage. Drugs obtained by cell culture can be produced under a controlled process throughout the year in the consumer country, with production controlled by demand and without solid biomass residues.

There are many drugs produced by cell culture that can be highlighted. Paclitaxel commercial drug (Figure 7)



Scheme 8. Strategies for the synthesis of dorzolamide hydrochloride.



Scheme 9. Chemoenzymatic synthesis of the rhinovirus protease inhibitor AG7404.

is the first anticancer drug worth billions of dollars ($986 *per* dose). Its structure is quite complex, with several chiral centers, and it has a long history of clinical development since the extraction of diterpene from the bark of the Pacific yew *Taxus brevifolia* at 0.0004%.[67] There are two published[67] total syntheses of approximately 40 steps with total yields of approximately 2%, and they are therefore unfeasible for large-scale production of paclitaxel commercial drug. Alternatively, the leaves and branches of the yew *Taxus baccata* have approximately 0.1% of the substance 10-deacetylbaccatin (10-DAB), which is the central tetracyclic core of paclitaxel commercial drug but without the side chain. To arrive at the commercial drug, conventional side chain synthesis was performed and coupled to the 10-DAB central nucleus; thus, the drug synthesis was shortened. This semisynthetic process was developed on a larger scale and produced the drug with viable commercial value. However, the switch from the extraction of Pacific yew bark to European yew leaves and branches followed by synthetic transformation into paclitaxel commercial drug continued to have environmental problems.

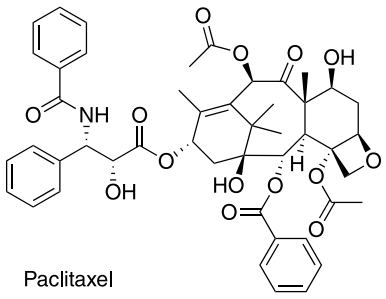


Figure 7. Chemical structure of paclitaxel.

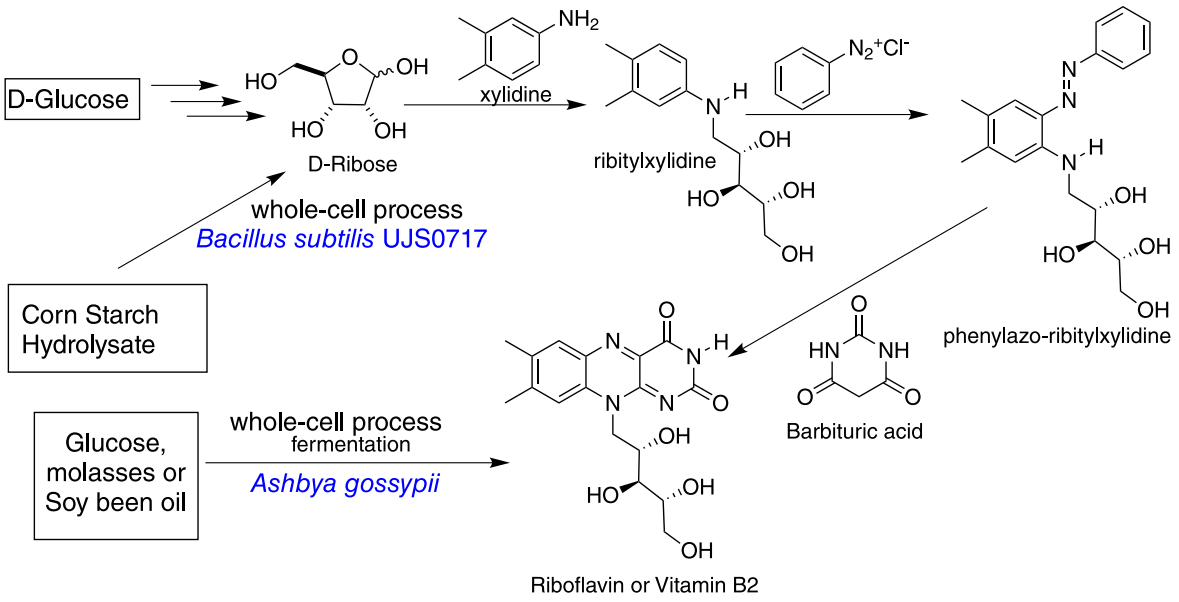
The ultimate solution for an environmentally sustainable solution for the production of paclitaxel commercial drug was the fermentation of plant cells from the Chinese yew *Taxus chinensis* in an aqueous medium. Calluses are propagated in a completely aqueous medium in large fermenters under controlled conditions of temperature, ambient pressure, pH, rotation speed, carbon sources, nitrogen sources, precursors, inducers and inhibitors.[68] The raw material for cell growth consists of renewable nutrients, sugars, amino acids, vitamins and trace elements. The crude drug was recovered from the fermentation broth by liquid/liquid extraction with a mixture of isobutyl acetate and isopropanol. Chromatographic purification of the raw broth followed by crystallization yields paclitaxel commercial drug at a much lower cost and with many advantages in terms of sustainability and environmental stresses, as there are many quantities of materials eliminated by the switch to cell culture technology. The production of paclitaxel commercial drug by the cell culture of microorganisms is not a closed research topic. Recently, the discovery of new microbial strains, which are this drug producers, that present increased production was resumed.[69,70] The new endophytic fungus *Epicoccum nigrum* TXB502 strain produces a paclitaxel commercial drug precursor with an initial yield of 61.35 μg L^{-1} and was isolated from *Taxus baccata* and identified by morphological and molecular tools.[71]

Another example to be highlighted involving whole-cell transformation is related to vitamin B2 or riboflavin. Vitamins are organic molecules that act as essential micronutrients for the metabolism of humans and animals, performing several functions. However, they are not always biosynthesized in sufficient quantities for proper functioning of biochemical functions; therefore, ingestion through diet and dietary supplements is necessary. The body only needs small amounts daily to balance the immune system in perfect working order. This need is the key driver for the growth of the global vitamin market, which will generate $17.54 billion in revenue in 2021 with a growth trend until 2025.[72]

Vitamin B2 is a water-soluble additive produced by all plants and most microorganisms. This vitamin is essential for the growth and reproduction of humans and animals, human nutrition, dietary supplements, pharmaceutical products, cosmetics and animal feed as a growth promoter. It also acts as an antioxidant and a water-soluble intensive yellow colorant (E101) for coloring fatty foods and cosmetics. Pharmaceutical and animal feed segments account for the majority of the use of vitamin B2, as it is an essential micronutrient for protection against cardiovascular diseases and cancers, reduces the risk of cataracts, reduces osteoporosis, and reduces migraines.[73] Its deficiency during pregnancy increases the risk of preeclampsia. These applications explain the large global market for riboflavin, which is predicted to grow between 2020 and 2025 at a CAGR of 4.5%.[74]

The conventional synthetic route, for many years and with some changes, was the only way to synthesize riboflavin (Scheme 10). The reaction is carried out from D-glucose, which is sequentially transformed into potassium gluconate, D-arabinose and D-ribose. The reaction of D-ribose under reductive addition conditions with xylidine forms the product ribitylxylidine. The addition of the diazonium salt of aniline to ribitylxylidine forms phenylazo-ribitylxylidine. The final step in the chemical synthesis reaction is the cyclocondensation of phenylazo-ribitylxylidine with barbituric acid, which produces riboflavin as a product.[75] This synthetic route achieved riboflavin yields of almost 30% based on D-glucose. The complexity, low cost-benefit and large amount of waste meant that this synthetic route was replaced by fermentation using recombinant microorganisms. In 1990, the annual production of riboflavin by fermentation was only 5% of the total production. However, biotech production completely supplanted chemical production, which increased to 75% in 2002. Currently, riboflavin production is fermentative and has completely replaced chemical synthesis, as it is more economical and sustainable. This is an excellent example where the starting product (D-ribose)[76] and the riboflavin[77] of a conventional synthesis were replaced by whole-cell processes (Scheme 10).

Many bacteria, fungi and yeasts (e.g., *Ashbya gossypii*, *Candida famata* var. *flareri* and *Bacillus subtilis*) are able to produce riboflavin by fermentation[78,79] using various inexpensive renewable raw materials or industrial waste as a growth medium, such as sucrose, xylose, glucose, fructose, beet-molasses, gelatin, vegetable oils and corn molasses.[80,81] However, it was up to one company to show that the genetically modified *Ashbya gossypii* fungus produces 40,000 times more vitamin B2 than it needs for its own growth. This process is the most economically

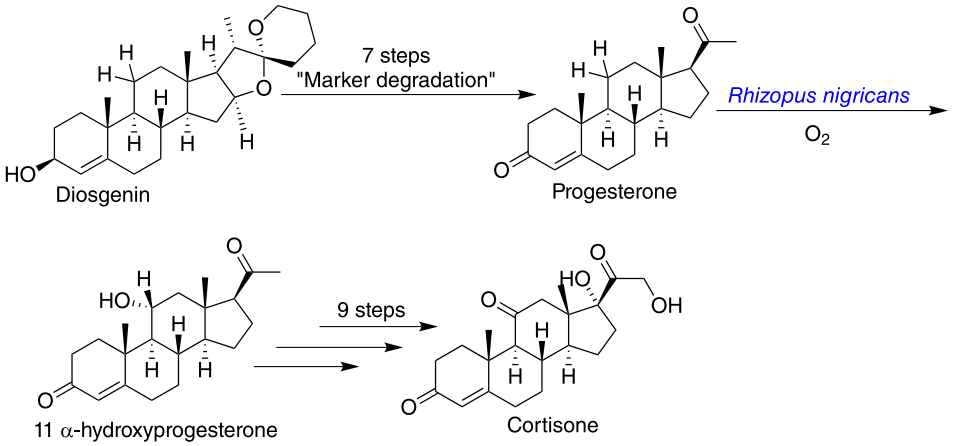


Scheme 10. Conventional and biotechnological synthetic routes for the preparation of vitamin B2.

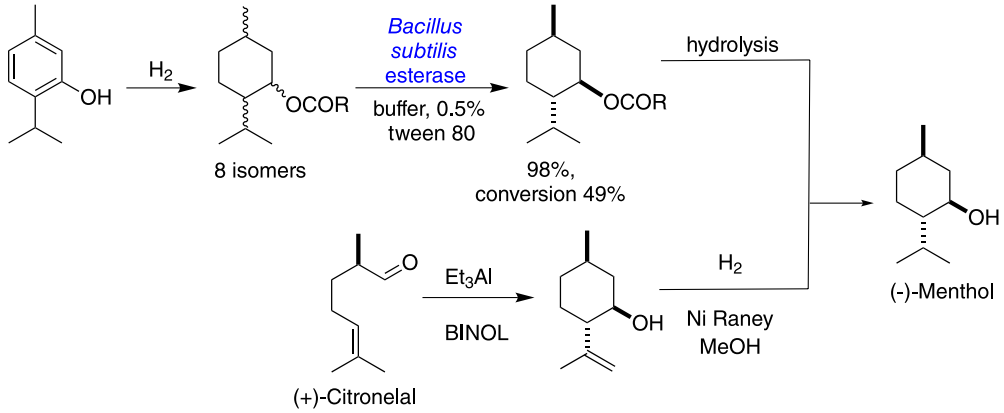
viable and aligned with the principles of green chemistry for riboflavin production.[82] The world market for riboflavin production for human and animal use has more than doubled in 13 years, from 4000 ton *per* year in 2002 to 9000 ton *per* year in 2015.[77]

Corticosteroids are terpenoid lipids that have been widely used in the pharmaceutical industry and in therapeutic approaches, mainly as immunosuppressive, anti-inflammatory, antirheumatic, diuretic, sedative, anabolic, and contraceptive agents.[83] They are substances that, despite not having complex structures, have different peculiarities, such as oxidation at position 11. One company introduced a microbial hydroxylation step with the fungus *Rhizopus nigricans* in a regio- and enantiospecific manner of progesterone at position 11 to produce α-hydroxyprogesterone (Scheme 11), which has become the most important intermediate in the commercially viable synthesis of cortisone, replacing a conventional 31-step synthesis. This microbiological hydroxylation step paved the way for the commercial success of steroid hormones and is still the subject of research on increasing the oxidative capacity of genetically modified *Rhizopus nigricans*.[84] The progesterone used in this synthesis is prepared from diosgenin[85] extracted from the Mexican plant *Dioscorea barbasco*[86] and many other plants. The process is called marker degradation,[87] which is a sequence of reactions that removes most of the side chain atoms and transforms diosgenin into progesterone. The size of the global progesterone market has increased to $622.68 million and will reach $1240.28 million in 2027, growing at a CAGR of 10.31% between 2021 and 2027.[88]

(–)-Menthol is an important flavoring agent with a characteristic peppermint odor used in the food, pharmaceutical and cosmetic industries. Menthol has



Scheme 11. Preparation of cortisone from progesterone.



Scheme 12. Strategies that can be used to prepare (–)-menthol.

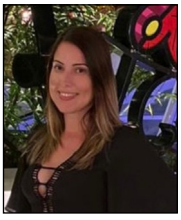
anesthetic and anti-inflammatory properties and is widely used in pharmaceuticals to fight inflammation in the throat. Scheme 12 shows the various strategies that can be considered in preparing this important terpene. Three sources of starting materials can be used for the production of menthol: two renewable sources and one nonrenewable source, but one step involves an enzymatic process. Many lipases isolated from microorganisms hydrolyze menthyl esters and prefer (–)-menthyl esters, whereas (+)-menthyl esters are not hydrolyzed at all. It is also possible to use whole cells (immobilized or not) for this hydrolysis of d,l-menthyl esters, such as the hydrolysis of d,l-menthyl succinate by *Rhodotorula minuta* var. *texensis*[89] or the hydrolysis of d,l-menthyl benzoate by the esterase of *Bacillus subtilis*.[90]

4. Final Remarks

Throughout human history, microorganisms have been fundamental in the preparation of beverages and foods and, therefore, have always been important elements of the economy and food for society. After many centuries of their use in these areas, it has been discovered that they can carry out biotransformation of chemical substances by well-defined reactions. In this brief review, we describe some sustainable synthetic strategies using biotechnological tools, exemplified in the preparation of some low molecular weight drugs. We believe that in the future, there will be an increasing number of processes in the chemical and pharmaceutical industries driven by biotechnology, as its success is recommendable for industrial processes with advantages for the circular economy, given that fossil feedstocks are increasingly smaller, prices of raw materials are growing and global warming is already affecting the planet. The reality is that synthetic routes for the production of drugs need to be rethought and updated with replacement by whole-cell processes, reduction of steps using products obtained from renewable sources or obtained in biotechnological manners. These factors greatly influence the growth of industrial biotechnology associated with genetic information to manipulate metabolic pathways. We sincerely hope that this essay will be useful in the academic field and helpful to obtain a strategic view of industrial biotransformations.

Author Contributions

Patricia G. Ferreira, Alcione S. de Carvalho, Wilson C. Santos and Luana S. M. Forezi were responsible for the manuscript writing; Fernando C. da Silva and Vitor F. Ferreira were responsible for the coordination and manuscript writing.

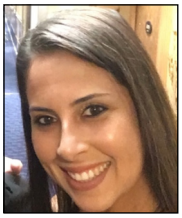


Patricia G. Ferreira *graduated in Pharmacy from Federal University of Rio de Janeiro in 2012 and Master (2015) and PhD (2019) in Applied Sciences in Health, both from Federal Fluminense University. She is currently conducting a post-doctoral research stage at the Federal Fluminense University acting in the development of solid lipid nanoparticles of potentially bioactive molecules.*

Alcione S. de Carvalho *received her bachelor's degree in Chemistry in 1993 from Humanity of Pedro II Faculty and she has obtained MSc (1996) and PhD (2000) in Science from Federal University of Rio de Janeiro. In 2000, she became researcher at FIOCRUZ planning and developing projects in the field of Pharmaceutical Research and Development, where she stayed for 19 years. In 2021, she obtained her MBA in Project Management from the Center for Research in*

Planning and Management (NPPG/UFRJ). Currently, since 2019, she is post-doctoral researcher at the Federal Fluminense University researching the synthesis of small bioactive molecules with emphasis on neglected diseases. She is member of two Technical Chambers of the CRQ, the Technical Chamber of Cosmetics, Pharmaceuticals and Sanitizing Products and the Technical Chamber of Technology, Innovation and Competitiveness.

Wilson C. Santos *received his bachelor's degree in Pharmacy in 1986 from UFRJ, and he has obtained MSc (1997) and PhD (2001) in Pharmacology from UNIFESP, with a stage at Universidad Autónoma de Madrid (UAM, Spain). He carried out some post-doctoral studies at Departamento de Farmacología de la Facultad de Medicina de la UAM (2008, 2011, 2012, 2013), where he is also an Associate Researcher at Instituto Teófilo Hernando de I+D del Medicamento. Dr Wilson is currently a Full Professor at the Pharmacy Administration Department of Federal Fluminense University, researching the Pharmacology of natural products and the smooth muscle contraction. He was also the Dean of Pharmacy Faculty (2007-2015).*



Luana S. M. Forezi *received her bachelor degree in Chemistry in 2008 from Federal University of Juiz de Fora and obtained her MSc (2011) and PhD (2014), with stage at the University of Aveiro (Portugal) at the Laboratory of Synthesis of Porphyrin Compounds, both from the Fluminense Federal University. She carried out two post-doctoral studies, one at the Federal University of Rio de Janeiro (2015), and another at the Fluminense Federal University (2015-2020). Currently, since 2020, she is an adjunct professor at Department of Organic Chemistry of the Fluminense Federal University. Her research interests focus is synthesis of coumarins, nucleosides, quinolones, 1,2,3-triazoles, quinones and porphyrins.*

Fernando C. da Silva *received his bachelor degree in Industrial Chemistry in 2002 and his PhD in 2007, both from the Fluminense Federal University. He then completed a postdoctoral stage at the University of Aveiro (Portugal) at the Laboratory of Synthesis of Porphyrin Compounds. Currently, he is an associate professor at Department of Organic Chemistry of the Fluminense Federal University. He was an affiliate member of the Brazilian Academy of Science (2016-2020) and the research interests focus are synthesis of quinones, 1,2,3-triazoles, coumarins, carbohydrates, diazocompounds, β-enaminones and porphyrins.*

Vitor F. Ferreira *got his bachelor degree in Chemistry in 1976 and a masters in Natural Products Chemistry in 1980, both from the Federal University of Rio de Janeiro. In 1984 he finished his PhD in Organic Chemistry at the University of California, San Diego. In 1986 he became professor of Organic Chemistry at Organic Chemistry Department of Universidade Federal Fluminense (UFF), in Niterói, RJ, and in 1995 became full professor. In 1998 he spent one-year in a post-doctoral research stage at the University of Oklahoma. Professor Ferreira is currently a full professor at the Pharmaceutical Technology Department of UFF, researching the synthesis of small molecules with focus on the development of new methods in organic synthesis in the search of bioactive compounds and their pharmaceutical formulation. He is a full member of the Brazilian Academy of Science and was president of the Brazilian Chemical Society from 2012 to 2014. He is currently the advisor of the presidency of the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).*

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Submitted: January 19, 2022
Publioshed online: March 10, 2022