# Pharmaceuticals Through The Looking Glass.

**By**
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This lecture will firstly give an overview of the pharmaceutical industry in Ireland, then outline how drugs are discovered and, in particular, how this process has changed in recent years, and finally focus on the significance of asymmetry in bioactive compounds used as pharmaceutical agents, illustrating this with some of our recent research in this area.

The reference to the Lewis Carroll's famous work *Though the Looking Glass* may seem obscure at the outset – is it simply a ploy to make a Chemistry lecture appear more attractive by raising curiosity in the audience? Or does it refer to the nature of scientific research where everything appears 'curiouser and curiouser..'. By the end of the lecture the relevance should become clear.

***Pharmaceutical Industry in Ireland***

Globally the pharmaceutical sector is one of the most successful industrial sectors.[1] It is distinguished from other industrial sectors by the very high investment of its sales on R&D, typically 15-20% of sales are reinvested in discovering and developing new drugs, much higher than the typical single digit investment in most sectors. The reason for this is very simple – discovering and developing new drugs is an extremely resource intensive activity, requiring large numbers of highly skilled staff, state-of-the-art equipment and facilities, and involves high risk to a company who invest enormous amounts in a drug which may fail at the final hurdle. High standards required by regulatory authorities to ensure the safety of pharmaceuticals require costly and lengthy studies into efficacy, toxicity *etc.* The future economic growth of the sector is assured – currently 80% of pharmaceuticals are consumed by 20% of the world's population, a sad reflection of economic inequity. However, as third world economies improve, providing their population with access to more expensive drug therapies, the market for pharmaceuticals will grow rapidly.

Within Ireland the sector is dominated by multinationals; the first investment here in 1968 was by Pfizer in Ringaskiddy, and since this time the sector has grown steadily, underpinned by a competitive operating environment and the ready availability of a skilled work force.[1] Nine of the top ten pharmaceutical companies in the world, including Pfizer, Eli Lilly, GlaxoSmithKline., Bristol-Myers Squibb, Merck *etc.*, now have facilities in Ireland, and a significant proportion of the world supply of pharmaceuticals is manufactured here. The presence of a critical mass of companies within Ireland ensures that the expertise required for pharmaceutical production is highly developed within Ireland. Approximately 40% of the companies here are involved in the synthesis of the active ingredient of the drugs – so called Active Pharmaceutical Ingredient (API) manufacturers - while approximately 60% are involved in drug product manufacture – formulation, tabletting *etc*.

The pharmaceutical sector in Ireland is characterised by stability and growth[1] – despite the downturn in the economy worldwide in recent years, the pharmaceutical

[1] Embedding the PharmaChem Industry in Ireland, ICSTI statement, 2003.

sector in Ireland has displayed continued growth in contrast to most other sectors. Employment in the sector is now >20,000 (cf. ~13,000 in 2000, ~11,000 in 1997) and has grown very rapidly in recent years reflecting the enormous expansion underway during this time. Significantly up to 50% of the employees in the sector are graduates or hold higher level degrees, reflecting the fact that the sector is highly technology based. It is also one of the most highly regulated sectors, with involvement of the Food and Drug Administration (FDA) and the Irish Medicines Board (IMB) focusing of drug safety, the Environmental Protection Authority (EPA) focused on the environmental impact and the Health and Safety Authority focussing on the impact on employees and the community at large.

The economic importance of the sector is reflected in the growth of exports illustrated below :

| | |
|---|---|
| 1973 | €100.3 million |
| 1990 | €2 billion |
| 1995 | €6.4 billion |
| 2000 | €27.22 billion (33% of total exports) |
| 2001 | €32 billion (35% of total exports). |

Furthermore, capital investment in the sector over the 25 year period 1973-97 totalled €4,051 M, while in the next 3 year period 1998-200 €2,382 M was invested, reflecting an enormous capital expansion in the sector in recent years, providing an expansion in career opportunities as outlined earlier. Indeed most Irish plants are international benchmarks in terms of standards. The sector is the largest corporation tax contributor to the Exchequer (€700M in 2001).

One of the fundamental reasons for the remarkable stability of the pharmaceutical sector compared to other sectors is the enormous capital investment in a pharmaceutical production plant – no company can afford to walk away from these extremely valuable facilities. Furthermore the lifetime of a pharmaceutical plant is relatively long compared to other sectors *e.g.* the ICT sector where technology changes very rapidly, rendering equipment obsolete on a short time frame.

Over the past few years a key strategic development is underway in the pharmaceutical sector in Ireland[1] – in the early 1990's the sector was almost entirely focussed exclusively on manufacturing, with little research activity. In the past 5 years or so there is significant evidence of increased activity in Process R & D – the development and scale-up of the processes used for pharmaceutical production. This positions the Irish plants higher up the value chain in the overall company structure globally, and provides more interesting career opportunities for chemists and engineers working in the sector. This development is essential as Ireland is shifting towards a knowledge based economy – we can no longer afford to focus exclusively on manufacture for our future economic success in a competitive global economy. Expertise in Process Development will make Ireland the first choice for initial process scale-up; once processes are developed and optimised here, the interesting and challenging part of the work, they can be transferred to lower tax environments for longer scale production.

Employment opportunities in the Pharmaceutical Industry include research in medicinal chemistry *i.e.* drug discovery, process development, laboratory work for

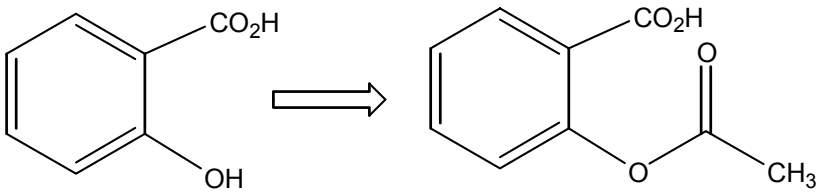
pilot scale development, production, quality assurance and quality control, management and administration, and sales and marketing. Ironically, the one area which most people identify, when they think of the sector, is the area of drug discovery, although there is relatively little activity in this area in Ireland, as most multinationals conduct this work near their headquarters in a high tax regime.

**Drug Discovery – the Traditional Approach**

Humankind has used Nature's bounty of bioactive compounds in plant sources since prehistoric times, evidently not understanding the mode of action or the nature of the active component but relying on herbal lore.[2] According to the WHO 80% of the world's inhabitants still rely on traditional medicines based on plants for their primary health care. Indeed both random and targeted screening of natural sources of plant, marine and microbial origin has produced a rich diversity of pharmaceutical agents, as illustrated by the following examples:

- In 1929 Fleming's serendipitous discovery of penicillin from a fungal source, *Peniciliium notatum*, led to the development of antibiotics which are now accepted as a routine aspect of healthcare
- Taxol, an important anti-cancer agent, was isolated from the bark of *Taxus brevifolia* as part of a random screen. Identification of a precursor to this key compound from the leaves of the tree which can be subsequently converted to taxol semi-synthetically provided a renewable source for clinical material.
- The anticancer alkaloids vincristine and vinblastine isolated from the Madagascar periwinkle *Catharanthus roseus* were discovered during an investigation into the use of these plants as a potential source of hypoglycaemic agents on the basis of their use by various cultures for treating diabetes.
- Immunosuppressive drugs such as cyclosporins, cholesterol lowering agents such as lovastatin, and antiparasitic agents such as ivermectins were all isolated from microbial sources.
- Epibatidine, isolated from the skin secretions of poisonous frogs used on arrow-tips by Ecuadorian tribes, was found to be a potent painkiller.

[2] Nature's Bounty, G. Cragg and D. Newman, *Chemistry in Britain*, 2001, January, 22.




**Salicylic acid** **Aspirin - acetylsalicylic acid**

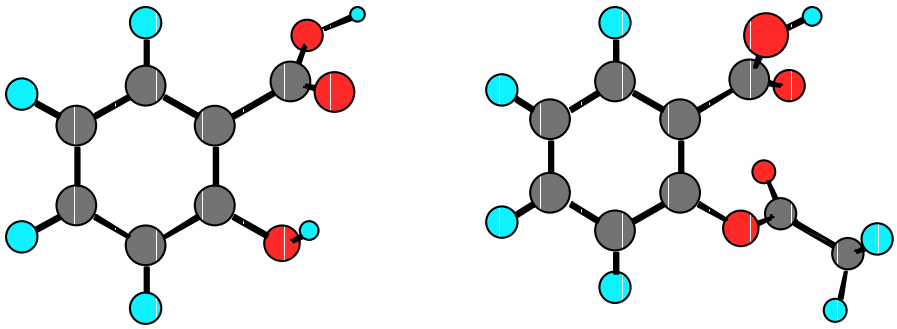
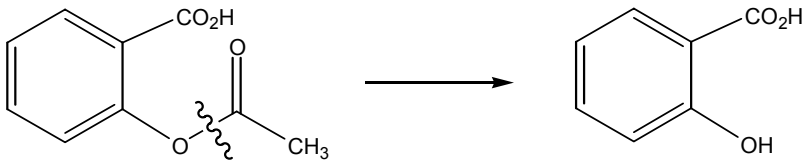


**Figure 1**

Perhaps one of the longest used and best known drugs is Aspirin; over 100,000 million tablets of this very cheap drug are taken annually worldwide.[3] It can be said that clinical trials on this began over 3,000 years ago as extracts from the bark of willow tree were used in traditional medicine to relieve pain and reduce fever and inflammation. The ancient Egyptians knew of the pain-relieving properties of salicylate-containing plants, while in 400BC Hippocrates was describing the use of willow leaves to relieve labour pains. In the early 19th century the active ingredient, salicylic acid was isolated from the willow tree extract. Salicylic acid was used successfully for many years as an anti-inflammatory, anti-pyretic (temperature reducing) and analgesic (painkilling) agent, but when used long term, for example in the treatment of rheumatoid arthritis, leads to severe gastric bleeding. It also has a very bitter taste. In 1894, a pharmacist, Hoffmann, at Bayer synthesised acetylsalicylic acid which was found to have fewer side-effects; Bayer patented this as Aspirin in 1899, see Figure 1.




**Scheme 1**

The mode of action of aspirin was proposed in 1971 by Vane – salicylic acid irreversibly inhibits cyclooxygenase (COX) a key enzyme involved in the inflammatory response. Aspirin is metabolised in the body releasing salicylic acid (Scheme 1), thereby resulting in COX inhibition. In addition to its best known use as

[3] Aspirin: New Life for an Old Drug, *Chemistry in Britain*, 1996, June, 8.

a pain killer, aspirin prevents blood clotting and thereby has a preventative and therapeutic role in cardiovascular disease. It is also used to reduce the risk of rejection of foreign materials such as artificial valves, promote acceptance of vascular grafts, an in the prevention of pre-eclampsia – the main cause of maternal death in developing countries. Exploratory studies into the use of Aspirin in AIDS treatment, delaying the onset of Alzheimer's disease and as a chemopreventative agent in cancer therapy have been undertaken.

Clearly Aspirin merits the title 'Wonder Drug' and illustrates how a lead from nature can be developed into a successful drug – the traditional approach to drug discovery.

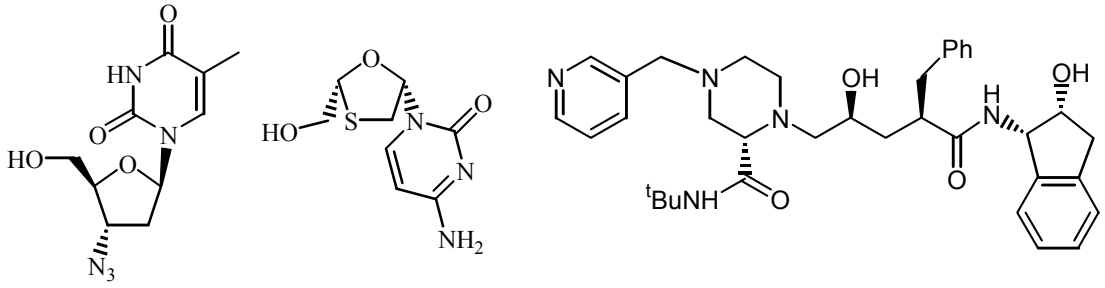
**Drug Discovery Revolution**

Over the past decade or so there has been a major revolution in the way drugs are discovered – mainly because of a significantly increased understanding of complex biological systems at a molecular level, which enables chemists and biologists to collaborate fruitfully on drug discovery. Indeed the Chemistry-Biology interface is one of the most exciting areas of research worldwide. Within UCC this area is underpinned by the establishment of the Analytical and Biological Chemistry Research Facility and the School of Pharmacy – major centres for interdisciplinary research.

Biological systems are based on very complex and elegant organic chemistry. With modern techniques it is possible to study and determine the nature of these systems at a molecular level. The future of healthcare and drug discovery lies in

- Understanding and control of biochemical processes at a molecular level
- Development of techniques and material to examine and manipulate molecular interactions in biological systems
- Design of pharmaceuticals based on this knowledge.

Advances in genomics has led to an understanding of protein structure and thereby **rational design** of molecules to interact with receptors, enzymes *etc.* This represents an enormous step where instead of random screening, drugs can now be designed in a logical fashion based on scientific understanding of the disease or condition.

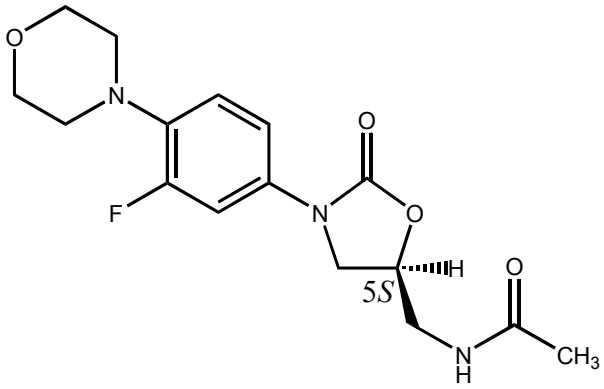



**AZT** **3TC** **Crixivan**

**Figure 2**

To illustrate this approach – HIV combination therapy involves the use of a cocktail of 2 drugs *e.g.* AZT, 3TC designed to inhibit a key enzyme *Reverse Transcriptase* and a protease inhibitor *e.g.* Crixivan thereby targeting the virus through two mechanisms, Figure 2. Furthermore Pharmacia, now part of Pfizer, recently introduced a new class of antibiotics based on oxazolidinones *e.g.* Linezolid, Figure 3 – the first new series of

antibiotics in over 30 years, which is of particular importance due to issues with antibiotic resistance. Linezolid has a novel mechanism of action in that it blocks protein synthesis.




Linezolid

**Figure 3**

The life cycle of a drug is illustrated in Figure 4 : it typically takes 10-14 years from discovery to market and is currently estimated to cost €800M for each new drug developed. Attrition is very serious, as for every new drug placed on the marketed typically 10,000 novel compounds are synthesised and tested, approximately 10 of these will be progressed to development stage, but only 1 will make it to the market. Others may fail due to side effects, for example.

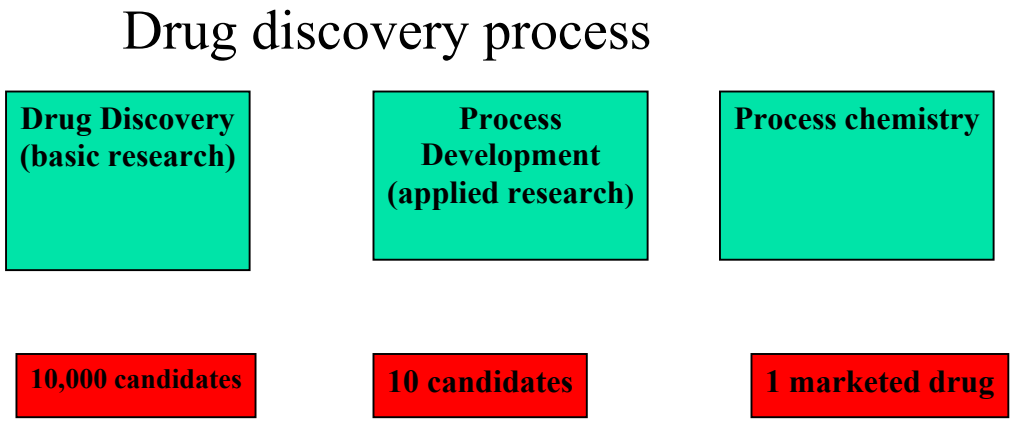
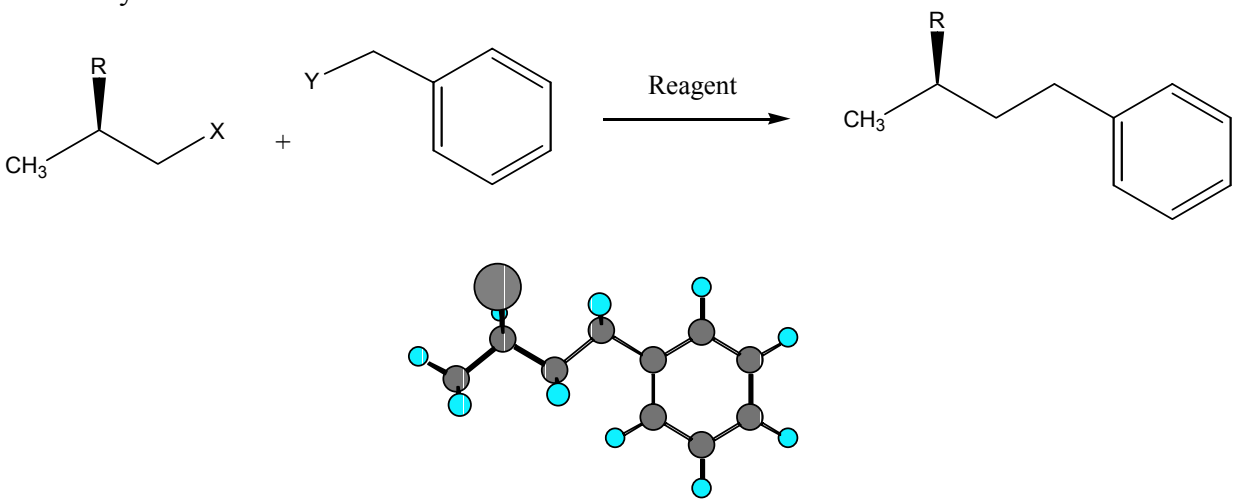



**Figure 4**

Development of new techniques such as Combinatorial Chemistry, High Throughput Screening, Molecular Biology, Biotechnology, Biocatalysis, Proteomics, Genomics, Molecular Modelling, Peptidomimetics have had an enormous impact on the way pharmaceuticals are discovered all contributing to Rational Drug Design. However, all of these are underpinned by Synthesis – there is no point in designing the most wonderful drug unless an efficient, robust, safe route for its production can be developed. What is meant by Synthesis in this context? Synthesis involves the rational construction of organic molecules through key bond forming steps as illustrated in

Scheme 2. Note the two dimensional representations relate to three dimensional structures in reality – these symbols provide a universal language for Organic Chemistry.




**Scheme 2**

**Asymmetry**

Asymmetry or chirality occurs widely in everyday objects[4] – chiral objects are non-superimposable with their mirror images *e.g.* our hands, car doors *etc.* In a symmetrical environment this has no impact – for example both of our hands can pick up a symmetrical object such as a book in exactly the same way. However, when placed in an asymmetric environment, the situation changes completely – imagine forcing your left hand into a right glove. Therefore, in an asymmetric environment the two mirror image forms of chiral objects behave very differently. In chemistry the two mirror image forms of molecules are described as enantiomers.

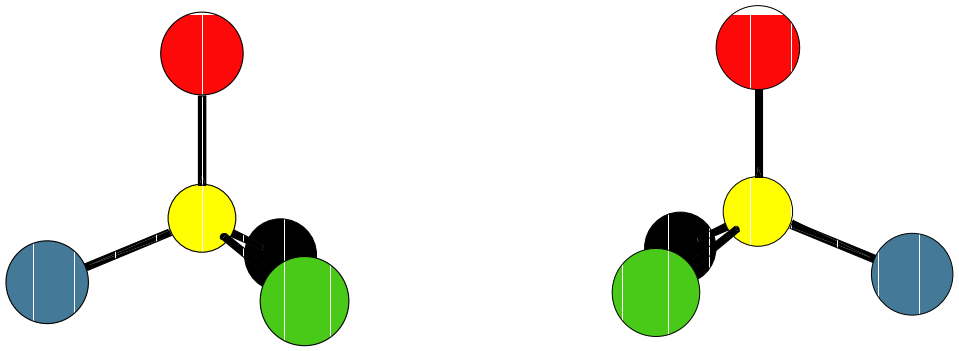


**Figure 5**

Due to the nature of the carbon atom one of its principal modes of bonding in molecules is tetrahedral as illustrated in Figure 5. When all four substituents on the carbon are different, this results in chirality as the two molecules are non-

[4] Asymmetric Synthesis, G. Proctor, Oxford University Press, NY, 1996

superimposable mirror images or enantiomers. As a result many organic compounds exist in two mirror image forms. Many biomolecules such as amino acids and proteins, enzymes, receptors, carbohydrates, nucleic acids are chiral molecules. Critically they occur in nature in one of the two enantiomeric forms only. When a molecule is designed to interact with an enzyme or receptor, for example, the 'left hand' enantiomer will fit into the site in a very different way to the 'right hand ' enantiomer – the hand in glove analogy - and therefore the biological activity of the two enantiomers of a compound used as a drug can be very different. In some cases one enantiomer will be inactive and simply have no effect, but in the worst case scenario the wrong enantiomer will lead to undesirable side effects.

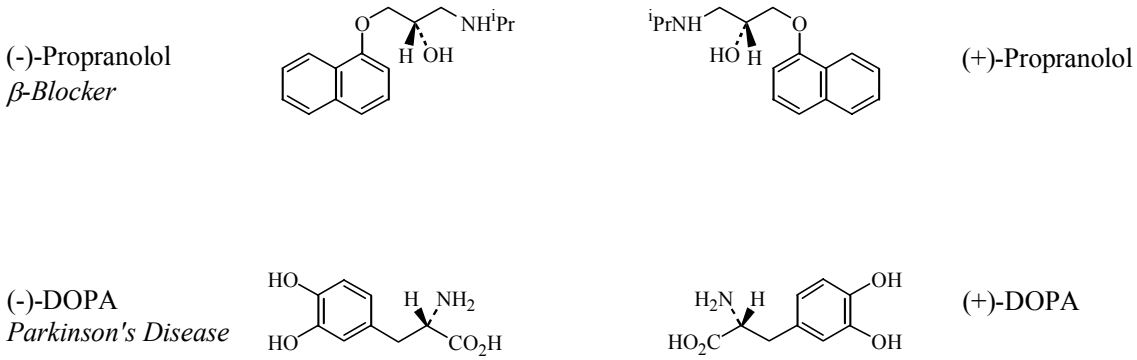



**Figure 6**

Some examples of compounds which are used in enantiopure form are illustrated in Figure 6. For example, one enantiomer of propranolol is used as a β–blocker in the treatment of cardiac disease; the enantiomer of propranolol has different biological properties. L-DOPA, the classical drug for treatment of Parkinson's Disease, is another interesting example. In this case DOPA is a pro-drug which is converted into the active species, dopamine, by enzymatic decarboxylation in the brain. Dopamine, an achiral compound, cannot be administered directly due to transport issues – accordingly DOPA is used instead. However, only one enantiomer of DOPA can be decarboxylated enzymatically and therefore if the drug is administered as a mixture of enantiomers, the unactivated version builds up to dangerous levels in the brain. This is a particularly interesting case where the active component is not chiral itself, but administration of an enantiopure prodrug is necessary.

Accordingly, regulatory agencies now require information on the bioactivity profile of both enantiomers of any new compound developed for use as a pharmaceutical agent, and a very high proportion of new drugs introduced to the market are in single enantiomer forms as a result, see table 1. For example, in the context of the new antibiotic class introduced by Pharmacia which was mentioned earlier, Linezolid is marketed as an enantiopure drug with the 5-*S*-configuration essential for antibacterial activity as illustrated in Figure 3.

**Table 1 New Drugs which are Enantiopure**

| | |
|---|---|
| 1983 | 3% |
| 1991 | 21% |
| 1996 | 62% |
| 1998 | 69% |

Recognising the need to produce compounds in single enantiomer forms is only the starting point, however. This presents the challenge of synthesising the compounds as single enantiomers, a task which is more complex than it might appear at first glance. Asymmetric synthesis of compounds is now one of the most active areas of synthetic organic chemistry,[4] driven by the greater understanding of biochemical mechanisms, *e.g.* three dimensional drug - receptor interactions, and FDA requirements. The increasing economic importance of asymmetric synthesis is highlighted by the fact that in 1998, enantiopure drugs represented 30% of total sales ($99 billion) while in 1999 the figures were 32% of sales ($115 billion).

Among the strategies employed for synthesis of enantiopure drugs are the use of :

- The Chiral Pool – use of the naturally occurring enantiopure compounds Nature has provided, *e.g.* amino acids, carbohydrates *etc.* as starting materials for the compounds. This is very attractive if a suitable precursor can be identified and obtained. However, it suffers the major disadvantage that usually only one enantiomer is readily available by this route – the unnatural series can be difficult to obtain and very expensive.
- Chiral Auxiliaries – attachment of a chiral directing group to a molecule to control the stereochemistry of a key transformation and then removal of the auxiliary afterwards, has proven very successful. It has the disadvantage of requiring 2 additional steps – attachment and removal of the auxiliary - but has the advantage that, in principle at least, recycling of the valuable chiral auxiliary is possible.
- Chiral Reagents – this avoids the need for attachment and removal steps but requires one mole of chiral reagent for one mole of chiral product
- Asymmetric Catalysis – this is by far the most attractive strategy as a small amount of a chiral catalyst is employed to produce a large amount of chiral material. Two distinct approaches can be identified – use of chemical catalysts especially those based on transition metal catalysts such as copper, palladium or rhodium, and use of biocatalysts – Nature's enzymes. Indeed, we conduct research on both approaches.

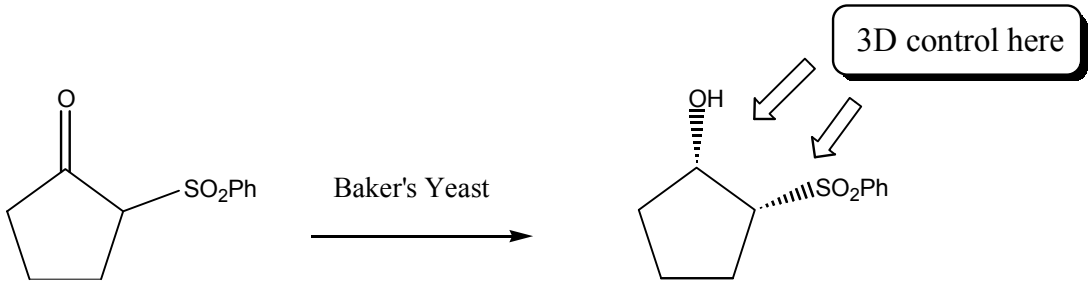
**Biocatalysis in Organic Synthesis**

Biocatalysis refers to the use of isolated enzymes or whole cell systems as catalysts for synthetic transformations.[5] The advantages include the fact that typically reactions can be conducted under very mild conditions of temperature, pH and pressure compared to conventional chemical techniques. However, the most important factor is the fact that enzymes are superb for asymmetric synthesis due to their intrinsic chirality and excellent selectivity for one enantiomer of a compound over the other.

[5] Biotransformations in preparative Organic Chemistry, H.G. Davies, R.H. Green, D.R. Kelly, and S. M. Roberts, Academic Press, London, 1989.

Either water or organic solvents can be employed for biocatalysis – typically when solvents such as hexane or ether are employed, small amounts of water are also added to maintain the enzyme activity. In some cases enzymatic transformations are achieved which are difficult by traditional chemical techniques. Finally there is an environmental advantage in using enzymes to effect transformations in synthetic chemistry – 'green appeal'.

Given the exquisite selectivity of enzymes, Nature's catalysts, the question of how enzymes can be used in synthetic chemistry outside of their 'natural' application is a valid one. The key is to use substrates which are closely related to the natural substrate so the molecule will still fit in the active site of the enzyme and undergo a transformation which is similar to the natural transformation, but not identical.



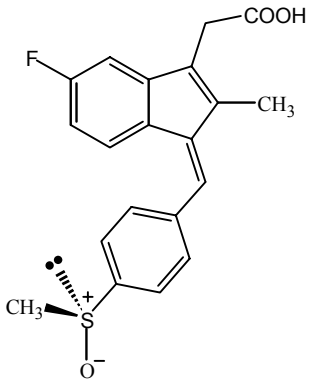

Ketone Alcohol

**Scheme 3**

One of the projects underway in our lab involves investigation of baker's yeast *Saccharomyces cerevisiae* as a biocatalyst – this is very easy to use, readily available, safe and inexpensive.[6] Furthermore, it has a broad substrate scope; the yeast alcohol dehydrogenases present in the cells reduce a wide range of carbonyl groups to alcohols with control of the three dimensional structure. One reaction which we have recently developed is illustrated in Scheme 3.[7] 2-Sulfonyl cyclopentantones are very efficiently reduced to the analogous cyclopentanols with excellent enantioselectivity, due to the manner in which the ketone is bound in the active site of the enzyme. This work has been applied to the asymmetric synthesis of a series of insect pheromones.

[6] Baker's Yeast - good for more than baking bread, A.R. Maguire, Irish Scientist Millennium Year Book, 2000, 187.

[7] Dynamic Kinetic Resolution in the Baker's Yeast Mediated Reduction of 2-Benzenesulfonylcycloalkanones
A.R. Maguire and N. O'Riordan, *Tetrahedron Lett.*, 1999, **40**, 9285-8.




**Sulindac**

**Figure 7**

Another project underway in the lab resulted in the first asymmetric synthesis of Sulindac, Figure 7.[8] Sulindac is a classical non-sterodial anti-inflammatory drug (NSAID) used as a painkiller for over 30 years. However, in recent years it has attracted renewed attention in cancer chemoprevention, especially for colon cancer. Sulindac is a chiral compound due to the presence of the sulfoxide group. However, it is used as a mixture of the two enantiomers because it is transformed *in vivo* into an achiral compound, which is the active component in terms of its anti-inflammatory effect. However, the precise mode of action in terms of cancer chemoprevention is unknown, and therefore it was important to conduct an asymmetric synthesis of sulindac and evaluate both enantiomers separately in the anti-cancer context. We reported the first asymmetric synthesis of Sulindac in 2001, based on a transition metal catalysed oxidation.[8]

These two examples serve to highlight the importance of asymmetric synthesis especially for bioactive compounds.

**Through the Looking Glass**

Returning to Lewis Carroll's *Through the Looking Glass* – Lewis Carroll, a contemporary of a famous German chemist Emil Fischer who worked on the structure of sugars, was fascinated by mirror image asymmetry. In 1872 he published *Through the Looking Glass* containing the following passage. He was absolutely correct: looking glass milk would contain the mirror image forms of proteins and carbohydrates – the beneficial parts of the milk - which would be of no nutritional benefit – but the less healthy fats, which are achiral, would be absorbed from the looking glass milk.

"Well then, the books are something like our books, only the words go the wrong way; I know that, because I've held up one of our books to the glass, and then they hold up one in the other room.

[8] Enantioselective Synthesis of Sulindac
A.R. Maguire, S. Papot, A. Ford, S. Touhey, R. O'Connor, and M. Clynes, *Synlett*, 2001, 41-4.

How would you like to live in Looking-glass House, Kitty? I wonder if they'd give you milk in there? *Perhaps Looking-glass milk isn't good to drink--* But oh, Kitty! now we come to the passage. You can just see a little peep of the passage in Looking-glass House, if you leave the door of our drawing-room wide open: and it's very like our passage as far as you can see, only you know it may be quite different on beyond. Oh, Kitty! how nice it would be if we could only get through into Looking-glass House! I'm sure it's got, oh! such beautiful things in it!"

*(This lecture was delivered at the UCC Science Faculty Public Lecture Series 2002-2003, on February 4, 2003.)*