

BACKGROUND

BACK TO NATURE

John Whitfield

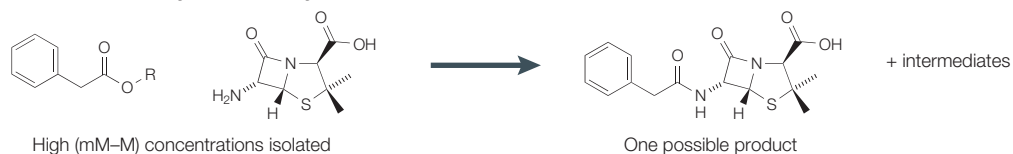
Chemists and organisms share the same philosophy — to create chemicals that provide survival advantage. However, scientists and cells carry out chemistry in very different ways, and learning how nature creates perfect compounds in the hurly-burly of the cell is teaching chemists new tricks.

Natural products lie at the roots of medicine and pharmacy. For millennia, before there were physicians and pharmacists, people have exploited medicinal plants, and plants continue to be a rich resource for bioprospectors. For example, the anticancer drugs paclitaxel (Taxol) and vinblastine (Velban, Velstar) were discovered in the Pacific yew and the Madagascar periwinkle, respectively; the periwinkle has also

yielded chemicals effective against diabetes and high blood pressure.

In the past half-century we have cast our drug-seeking net across an ever-expanding taxonomic range. From bacteria, we have taken antibiotics, and more — the cholesterol-lowering statins, currently the world's bestselling drugs, were discovered in moulds. Marine organisms are also a focus of much current research —

How chemists carry out chemistry



How nature carries out chemistry

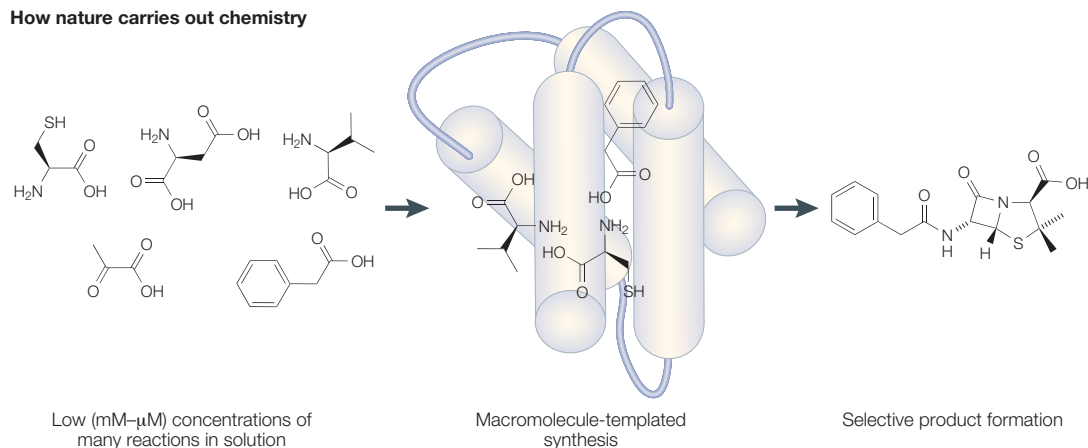


Figure 1 | **How chemists and nature create compounds.** Chemists and organisms both share a common goal: to create chemicals that provide survival advantage. But the similarity ends there as scientists and cells carry out chemistry in very different ways. Chemists typically create compounds by combining a few reagents at relatively high concentrations, and subsequently have to isolate the wanted product from byproducts and the original reagents. Cells, on the other hand, contain a soup of different chemicals, each present at a very low level. Chains of enzymes are used to control the reactions and to make sure that the correct product is made every time.

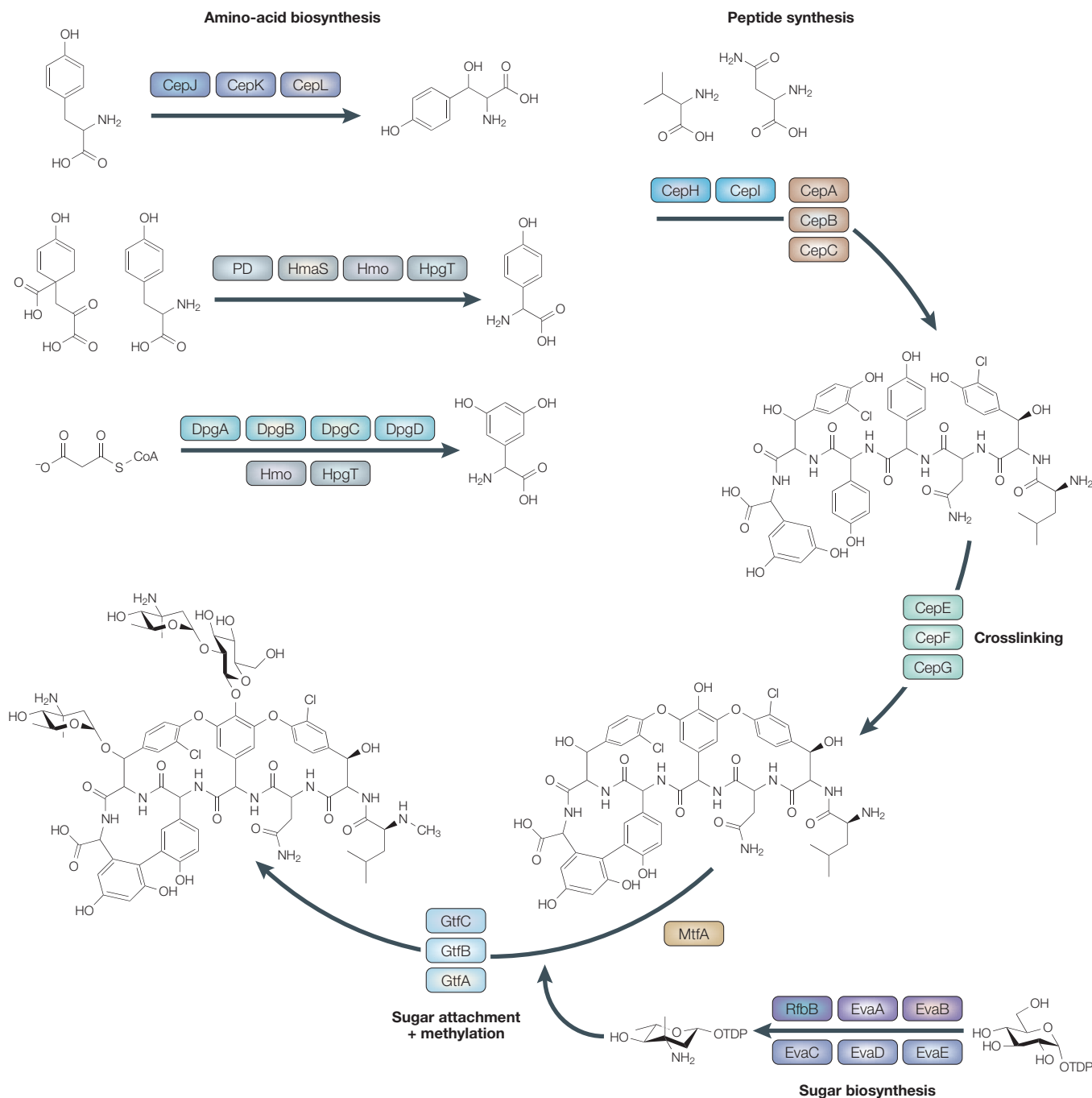


Figure 2 | **The challenge of creating vancomycin.** The antibiotic vancomycin has been used since 1959, but was not chemically synthesized until 1999. The figure shows the biosynthetic pathway of the closely related antibiotic chloroeremomycin, whose pathway has been elucidated. Although this is a small molecule containing only seven amino acids, it highlights the complexity that nature adopts to create molecules. A host of enzymes are recruited to create chloroeremomycin in several steps: amino-acid biosynthesis, peptide synthesis, crosslinking, sugar biosynthesis, and sugar attachment and methylation.

anticancer compounds have been isolated from sea squirts, and a toxin used by a cone shell to paralyze its prey might yield a non-addictive painkiller thousands of times more effective than morphine.

While chemists share the same goals as the organisms that inspire them — both want to make chemicals that provide survival advantage — scientists and cells carry out chemistry in very different ways (FIG. 1).

Chemists typically make compounds by combining a few reagents at relatively high concentrations, whereas the reactions in living systems involve a myriad of different chemicals, each present at a very low level. The cell uses molecular machines — enzymes — to keep this broth under control, using up a huge amount of energy to ensure that these reactions have the best chance of hitting the bullseye almost every time.

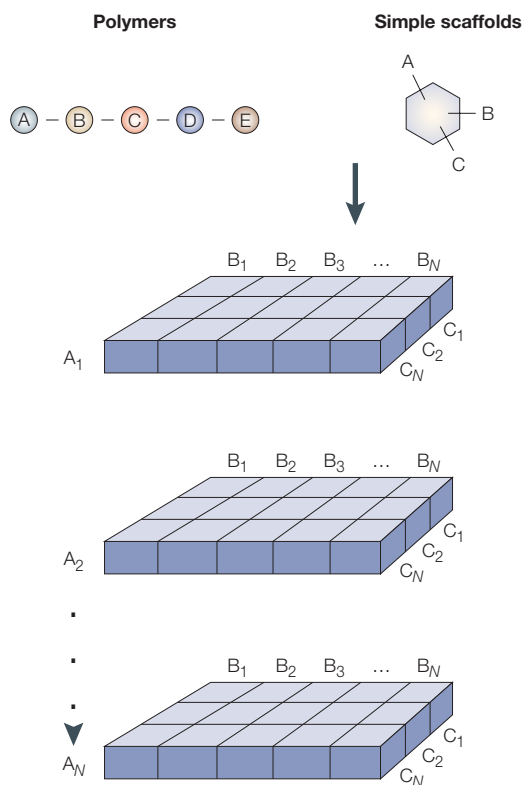


Figure 3 | **Combinatorial chemistry.** Combinatorial chemistry has expanded to encompass many variations on a theme. In one of its simplest forms, parallel synthesis, libraries of polymers and core scaffolds with points of randomization are created by varying the chemical nature of the building blocks A–E. In deep-well plates, randomization of a scaffold would be carried out by coupling A_1 to all of the compounds in plate A_1 , and this is repeated to the N th plate. In each plate, variants B_1 to B_N are added along the columns, and variants C_1 to C_N are added along the rows.

The antibiotic vancomycin, for example, was first used in the clinic in 1959. Yet the compound was not fully synthesized until 1999, after 20 years' work. Vancomycin is a dauntingly complex molecule to recreate (FIG. 2). The antibiotic consists of only seven amino acids, but they are folded back on themselves and interlinked, making a tightly packed final structure. The challenge in building it is like the difference between solving a three-dimensional jigsaw puzzle, in which every piece has only one correct position, and assembling a mobile, in which the pieces can move without the whole structure losing its integrity. In the end, two teams, one team led by K.C. Nicolaou and the other by David Evans, managed to produce the antibiotic by two different methods, showing that there are often many alternative synthetic routes to the same natural product.

Another reason to synthesize a natural product is that living organisms are not necessarily the best way to produce medicines on an industrial scale. For example, to get 60 g of discodermolide, an anticancer compound produced by the rare Caribbean sponge *Discodermia dissoluta*, would require 3,000 kg of dried sponge —

more sponge than exists throughout the world. So, chemists have tried to synthesize discodermolide. This was first achieved in 1993 in small quantities, but it wasn't until March this year that a team at Novartis announced that, after nearly two years' work, they had produced 60 g of synthetic discodermolide in a process containing 39 steps, and it is now in clinical trials against pancreatic cancer.

Being able to synthesize an antibiotic helps us understand how it works, and the drive towards 'total synthesis', as the technique is called, has led to the invention of many new methods in chemistry. Total synthesis can also lead to new drugs, as once we can manipulate a molecule we can make variations on its original structure. This is particularly important for antibiotics, as bacteria are constantly evolving resistance to treatments. Such variants can be created rationally — by analysing the molecule and deducing what tweaks might make it more effective — or by combinatorial chemistry, by creating as many variations as possible around a core chemical scaffold and then testing which variants have antibiotic properties (FIG. 3). Taking a natural molecular scaffold and modifying it has already given us several generations of next-generation synthetic antibiotics. We have reached the third generation of compounds descended from erythromycin, for example, and the fourth generation descended from penicillin.

As well as combating drug resistance, modifying antibiotic scaffolds can be a route to compounds with properties that go beyond killing bacteria. Most current drugs based on natural products are either antibacterials or anticancer drugs — indeed, nearly four-fifths of the former and three-quarters of the latter were developed from or inspired by natural chemicals. Fighting off infection and controlling cell division are universal biological problems, but there are many diseases unique to humans, and it might seem illogical to think that plants, insects or microbes have evolved chemical solutions to problems they never encounter, such as diabetes or schizophrenia.

On the other hand, there are good reasons to think that there might simply be something inherently 'drug-gable' about natural products in general and antibiotics in particular. The side effects of antibiotics are an obvious clue that their biological powers go beyond killing bacteria. The function of these molecules, after all, is to interact with other biomolecules. They have been adapted for penetrating cells and binding to proteins, properties that may allow them to be used on targets quite different from those they originally evolved to deal with. For example, in 2002, erythromycin was controversially trialled in India as a contraceptive. The neat antibiotic was not successful, but some researchers still hope that modified versions of the molecule may work as a contraceptive by blocking the action of gonadotropin-releasing hormone, one of the chemical messengers involved in fertility.

So, chemists are trying to borrow nature's tools, the enzymes, to help them make new and potentially useful compounds. To recreate in a test tube an entire biochemical pathway of the type that cells use to make

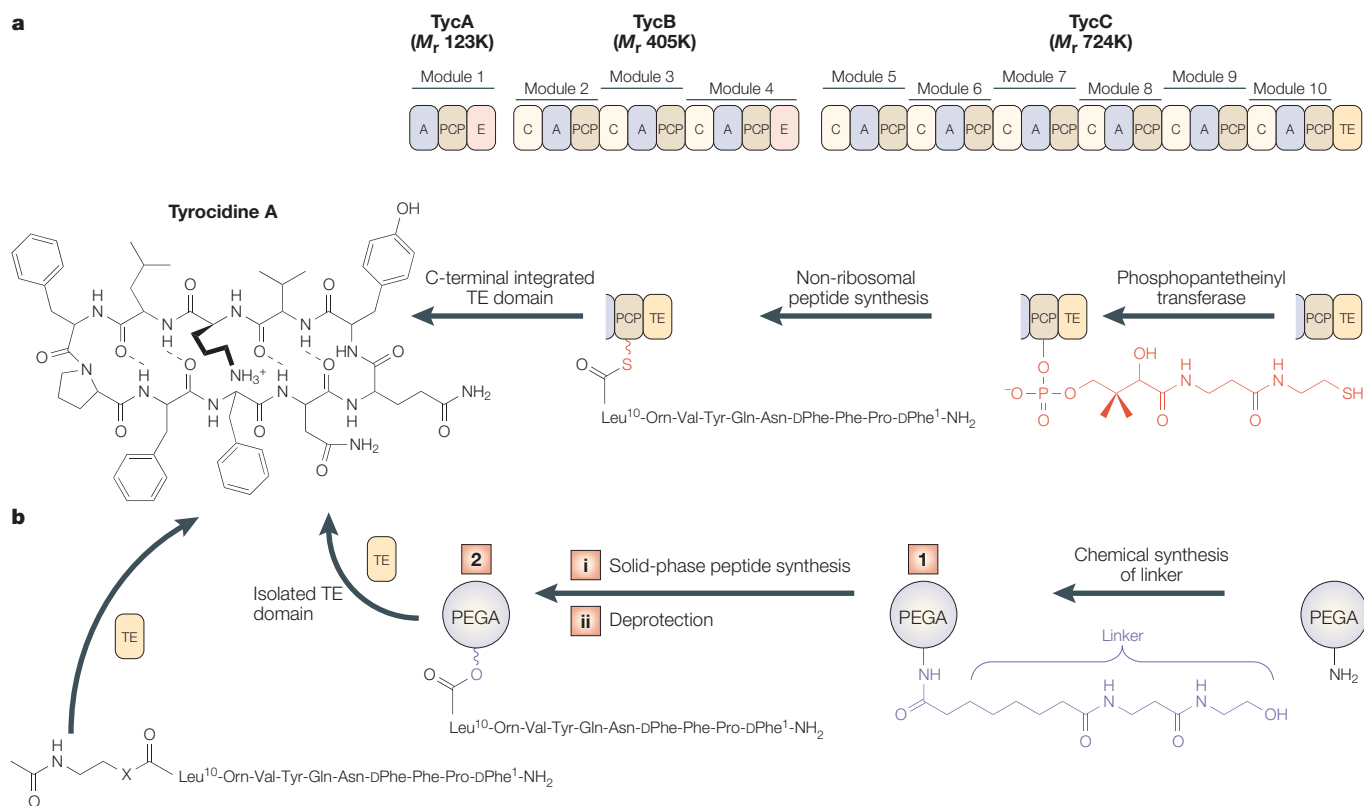


Figure 4 | Natural versus synthetic ways of circularizing antibiotics. a | In nature, an enzymatic assembly line is involved in biosynthesis of the cyclic cationic antimicrobial peptide, tyrocidine A. This consists of three large synthetase proteins, comprising ten modules in total. A carrier protein (PCP) in each module is loaded with a phosphopantetheine prosthetic group (red). The individual modules contain domains (A, adenylation; C, condensation; E, epimerization) that load amino-acid building blocks onto the thioester tether and condense via successive peptide-bond-forming reactions, giving the terminal PCP domain loaded with a linear decapeptide. The terminal thioesterase domain (TE) catalyses head-to-tail cyclization. **b** | In the biomimetic synthetic strategy, a linker molecule (purple) that mimics phosphopantetheine is chemically synthesized onto a solid-phase resin (1). Solid-phase peptide synthesis is used to construct (2), a tethered linear peptide, which can then serve as a substrate for cyclization by the TE domain excised from the synthetase proteins. The excised TE domain can catalyse cyclization of soluble peptidyl thioester and ester substrates. Modified from Kohli, R. M., Walsh, C. T. & Burkart, M. D. Biomimetic synthesis and optimization of cyclic peptide antibiotics. *Nature* **418**, 658–661 (2002) © Macmillan Magazines Ltd.

antibiotics would be daunting. But chemists can use enzymes selectively, to enhance their toolkits when such biological molecules can do things that their synthetic tools cannot.

One reaction that is a breeze for enzymes but a struggle for chemists is closing linear molecules up into circles. Nature often circularizes compounds to make them bioactive — a process called macrocyclization. Christopher Walsh and his team at Harvard University, USA, took a leaf from nature's book by using the enzyme thioesterase to circularize compounds created by standard synthetic techniques (FIG. 4). Thioesterase's original job is to make the natural cyclic peptide antibiotic, tyrocidine, but unlike many enzymes, it is not particularly fussy about the molecules it works on. By feeding it different linear peptide chains, Walsh's team have been able to make several new candidate antibiotics, some of which showed effectiveness against drug-resistant bacteria. This merging of natural chemistry with traditional solid-phase chemistry has many potential therapeutic applications, but Walsh believes that the enzymes will

probably be more useful in research, for generating libraries of chemicals for testing, than they will be in industrial drug production.

Many other chemists are exploring the use of enzymes. A team led by Chi-Huey Wong at the Scripps Institute, San Diego, USA, has used enzymes to join sugar groups, and to join sugars and proteins together, to form antibiotics. These sugar-based carbohydrates are usually found conjugated with proteins and lipids, and are essential for many processes; for example, constructing the cell walls of bacteria. Enzymes such as the glycosyl transferases, which are used to synthesize carbohydrates, are particularly good at controlling the geometry, or stereochemistry, of the molecules they make, which is often critical for their biological function. Wong's group is one of several hijacking these processes, by altering the specificity of these enzymes to produce a range of carbohydrate and carbohydrate-protein compounds, and is attempting to evolve antibacterial chemicals by multiple rounds of mutation and selection rather than designing them from scratch.

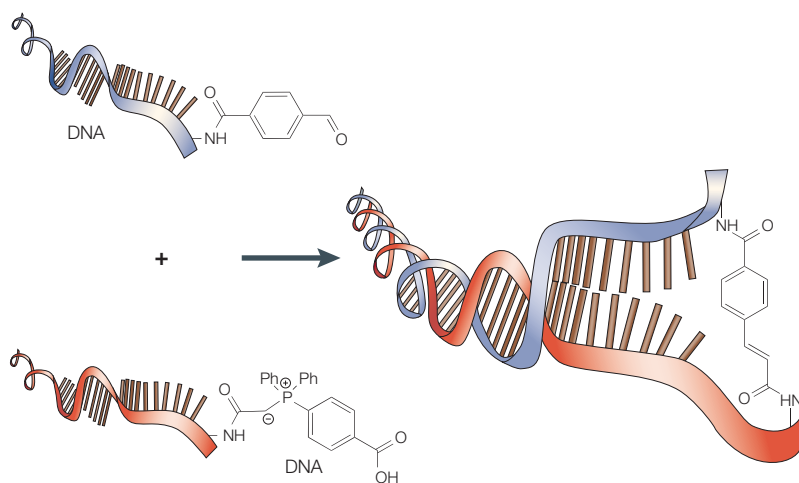


Figure 5 | **DNA-templated organic synthesis.** DNA-templated organic synthesis can direct a wide range of chemical reactions, even if the structures of the reactants or products do not resemble the DNA phosphodiester backbone. By tethering the chemicals they want to react to matching DNA strands — so that the chemicals react with each other when the complementary DNA strands anneal — chemistry can occur in a controlled manner at very low concentrations, as occurs in cells.

Another option is to repurpose biological molecules, making them do jobs different to those that they do in nature. A group led by David Liu at Harvard University uses DNA as an enzyme to direct a range of chemical reactions. By tethering the chemicals they want to react with each other to matching DNA strands — so that a chemical reaction occurs when their DNA carriers meet up — they have carried out precisely controlled chemistry at very low concentrations; just like cells do (FIG. 5). Like Walsh's method with thioesterase, this method, called DNA-templated organic synthesis, isn't fussy, and

can set up these reactions even if the added chemical structures don't resemble that of the normal DNA backbone. Recently, Liu's group has generated a library of DNA-linked macrocyclic fumaramides and selected for binding to a target protein, representing the first selection of a library of DNA sequences that encode small-molecule compounds rather than proteins.

The drive to use nature's tools to shape chemistry might be needed sooner than thought. A decade ago, pharmaceutical companies tried to go beyond natural products to create new drugs, using combinatorial chemistry to generate and screen vast libraries of synthesized compounds. But this approach has not yielded the hoped-for explosion in treatments. Combinatorial chemistry might have generated countless new compounds, but whether these are the right types of compound is a matter of great debate at present. Many say that these methods need to synthesize more 'drug-like compounds' — that is, efficacious compounds that are absorbed, distributed to the correct area, and metabolized and excreted effectively. Returning to nature could address that need, and it is therefore once more asserting itself as an invaluable source of inspiration for drug discovery.

FURTHER READING

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