Prof. dr. Nathaniel I. Martin

Antibiotics:
The Molecular Marvels of Modern Medicine

Universiteit
Leiden
The Netherlands

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The Molecular Marvels of Modern Medicine

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Prof. dr. Nathaniel I. Martin

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Mijnheer de rector magnificus, geacht faculteitsbestuur, zeer gewaardeerde toehoorders. Ik geef deze lezing in het Engels.

Today I am honored to formally accept the title of professor and to share with you some of my academic inspirations, achievements, and future ambitions. Specifically, I will focus on those aspects of my research that relate to the molecular marvels that are antibiotics. I will begin by sharing some of my favorite tales of medical breakthroughs enabled by natural products up to and including the antibiotic era. I will also address the societal challenge we now face due to antibiotic resistance and how my research efforts aim to address this threat.

Gifts from Nature

When considering the various themes I might touch on to begin this lecture, I found myself bouncing a question around with friends and colleagues: what is the most valuable, *naturally occurring*, material?

The history and advancement of human civilization is intimately linked with our capacity to discover and use natural materials to our benefit. The ancient bronze and iron ages marked humanity's ability to smelt raw metals and cast tools, instruments, and weapons that led to global expansion. Over the past millennia precious metals like gold and platinum, as well as gemstones like diamond and emerald, have risen to prominence and today are the most costly materials, by weight, to be stably found in nature.

It is the scarcity of these materials in Nature that makes them so valuable. In fact, it was the allure of transmuting cheaper, more abundant metals like lead and copper into the much rarer gold that drove the alchemists of the Middle Ages. While the practice of alchemy was ultimately abandoned during the Enlightenment, it did serve as the starting point for what we consider modern chemistry, including the field that I find most inspiring, organic chemistry.

Organic chemistry is, generally speaking, concerned with the study of molecules that are associated with life - molecules often possessing what we call biological activity. The biological activity of organic molecules covers an array of possibilities ranging from their color, scent, and taste, to their ability to act as poisons or, in special cases, as medicines.

The organic chemist is actually something of a modern day alchemist with the skills to transform inexpensive and readily available organic molecules into more complex compounds with new properties by means of what is called chemical synthesis. Over the past century synthetic organic chemistry has grown to be an incredibly powerful and useful branch of science. That said, the vast majority of (if not all) organic chemists agree, that the most impressive organic chemist of all time is, and continues to be, Mother Nature.

So, getting back to the question of the most valuable materials provided by Nature – are there naturally occurring, organic compounds that can rival precious metals and gems? Historically, the answer to this question is a resounding yes. Consider the economic forces that drove the Dutch "golden age" of the 17th century. It was largely the lucrative market for spices like pepper and nutmeg, that spawned the creation of the famous VOC, the Dutch East Indies Company. It is the biologically active organic compounds made by the producing plants that give these spices their desirable properties. Such spices were so sought after that in the early 1600s pepper was worth more by weight than gold, giving rise to the Dutch term, "peperduur", an adjective still commonly used today to describe something very expensive.

Many of you will also be aware that the Dutch were the first Europeans to settle the island of Manhattan, with the founding of a city aptly named New Amsterdam (which is of course today's New York). In 1667 the Dutch swapped Manhattan with the British for a tiny volcanic island, in what is now part of Indonesia. This small island was one of a cluster of ten (the

other nine already owned by the Dutch) that were the only locations in the World where nutmeg could be grown. Nutmeg was at the time worth more than both pepper and gold. This island swap gave the Dutch a worldwide monopoly on Nutmeg production that lasted for many decades bringing vast wealth to cities like Leiden [1].

These examples of natural organic materials are, or were, financially valued due to their scarcity. I would argue however, that when it comes to Nature's most important gifts to humanity, monetary worth is a rather poor gauge of real value. More to the point, none of the previously mentioned natural materials are medicines capable of preventing or curing disease. And so to focus the question, I would ask what naturally occurring substance has had the greatest impact on human health?

Considering the focus of much of my research is on antibiotics, I am naturally inclined to emphasize their importance. However, centuries prior to Fleming's discovery of penicillin another naturally occurring "wonder drug" saved untold millions of lives while transforming our world. The antimalarial drug quinine is an organic compound produced by the Cinchona tree and can be extracted from the tree's bark. In the 16th century, Jesuit missionaries in South America observed that the indigenous peoples of Peru and Bolivia drank a beverage prepared from the bark of the Cinchona tree to treat the symptoms of Malaria, most often severe fever. For this reason the Cinchona had become colloquially known as the "fever tree" by the locals [2].

By the early 19th century methods for the large scale isolation and purification of quinine from Cinchona bark were established in Europe. With ready access to the antimalarial drug in pure form the British Empire rapidly pushed its way into India and Africa. During this period British officers serving in these regions were provided a quinine ration that they were instructed to consume daily as an effective prophylaxis against malaria. There was one problem however: the incredibly bitter taste of quinine. In response, the officers found that by dissolving their daily dose of quinine in sugar-flavored soda water mixed with gin, a much more palatable option was obtained and so was born the gin and tonic. Years later Winston Churchill would be quoted as saying "*The gin and tonic* has saved more Englishmen›s lives than all the doctors in the Empire." It is also worth noting that the isolation of quinine from the Cinchona tree, and not chemical synthesis, remains the most effective means of producing the drug – Nature is an amazing organic chemist.

Perhaps not surprisingly, the Dutch also played an important role in quinine's rise to prominence. By the mid 1800's, the world's supply of Cinchona bark was tightly controlled by the Peruvian government with exports to Europe exceeding six million pounds per year. To safeguard their interests, the Peruvians banned the export of cinchona seeds, creating an incentive for smugglers. However, in 1865 Englishman Charles Ledger succeeded in transporting several kilos of Cinchona seeds out of South America. When Ledger failed to secure a buyer for the seeds in England, he offered them to Dutch merchants who eagerly paid the asking price. These seeds were used to sow the first Cinchona plantations on the Dutch-held Indonesian island of Java. The plants thrived in their new environment and by the early 1900s Dutch plantations were exporting 20 million pounds of Cinchona bark annually amounting to 97% of the world market [3]. The Dutch maintained this dominant position in the quinine world market until the Second World War. Under Nazi occupation at home and with the Japanese controlling Indonesia, the Dutch could not supply the allied forces with quinine. As a result tens of thousands of British and American soldiers are estimated to have died due to Malaria infections acquired in the African and Pacific theatres.

Quinine is generally credited as being the first natural product used to treat and prevent infectious disease. Quinine does not,

however, work against bacterial infections. For countries in the Northern hemisphere, Malaria is not a major concern given the equatorial habitat preferred by the Anopheles mosquito that transmits the disease. Rather, in Europe and North America bacterial infections were historically a much greater problem.

The Antibiotic Era

For people born in the Netherlands in 1900, the average life expectancy was 46 years for men and 48 years for women. While today heart disease and cancer present the leading causes of death, 100 years ago, infectious disease was the biggest killer. At that time bacterial diseases like cholera, diphtheria, pneumonia, typhoid fever, plague, tuberculosis, typhus, syphilis were rampant, claiming the lives of young and old alike. This of course dramatically changed with the widespread introduction of antibiotics in the mid 1940s. With the availability of antibiotics, average life expectancies rose sharply in the 20th century and it is estimated that since its introduction more than 200 million lives have been saved thanks to penicillin alone. I think it's safe to say that penicillin is one of Nature's greatest gifts to mankind.

Many of you will be familiar with the story – the most common story - of the discovery of penicillin. As it goes, Scottish biologist Alexander Fleming discovered penicillin in 1928 when he observed that a fungus, that had contaminated one of his experiments, appeared to excrete a compound that killed bacterial cells. As any good scientist does, Fleming wrote up his findings and submitted them for publication [4]. The potential impact of this discovery was not, however, immediately clear to the scientific community. Fleming's findings would flounder in relative obscurity for more than a decade before Howard Florey and Ernst Chain, working at Oxford University, became the first to demonstrate penicillin's capacity to cure bacterial infections in animals. With the outbreak of the Second World War, a massive collaborative research effort was then initiated with the goal of transforming penicillin from an academic curiosity to an anti-infective agent to support the allied forces. With financial backing from the governments of Great Britain and the United States as well as major drug companies including Merck, Pfizer, and Eli Lilly, the large-scale production of penicillin was realized. When the allies landed on the beaches at Normandy in 1944 they carried with them a million doses of penicillin, a significant advantage in the treatment of battlefield infections against the Germans who had not been successful in developing effective antibiotics.

The Development of Penicillin in the Netherlands

This is the most commonly known historical account of the discovery and development of penicillin. There is however, another chapter in the story that unfolded much closer to where we stand today. In the Netherlands from 1943-1945, a covert operation saw a team of Dutch researchers succeed in secretly producing penicillin while under Nazi occupation. These efforts took place in the laboratories of the *Nederlandsche Gist- en Spiritusfabriek*, in English "The Netherlands Yeast and Alcohol Company". Founded in 1869 by Jacques van Marken, a graduate from the TU Delft, this company was arguably one of the world's first biotechnology companies, using fermentation-based technologies to produce a range of products. By the early 1900's the company had become hugely successful and was one of Europe's largest suppliers of the yeasts used in baking and brewing as well as a range of other products including the famous Dutch gin Jenever.

Following the 1940 German occupation of the Netherlands, the Nazis saw the economic value in keeping this company operational. As such, employees were given "essential worker" status protecting them from forced labor in Germany. Also, as the story goes, they placed just a single guard to watch over the company's day-to-day operations. This guard apparently had little interest in microbiology or fermentation technology but did have a definite appreciation for Jenever. Company management saw to it that his thirst was satisfied on a daily basis leaving the researchers inside the laboratories to pursue their interests uninterrupted [5].

The head of the company's research division at the time was Francois Gerard Waller and in early 1943, Waller and his team caught wind of the British and American effort to produce penicillin. By some accounts the Dutch scientists' interest in penicillin had been sparked by information about the new wonder drug contained in the leaflets called *De Vliegende Hollander (the Flying Dutchman)* dropped by British pilots. Though completely cutoff from his colleagues in Britain and America, Waller decided that given his team's world leading position in fermentation technology, they too would join the hunt for penicillin.

While Allied researchers had the full support of the US and UK governments, the team in Delft had to work on a shoestring budget all the while keeping their efforts hidden from their occupiers. Starting in 1940 the Allies had imposed a strict embargo on the publication of any research relating to penicillin. As such Waller and his team had little to go on, relying primarily on Fleming's original 1928 publication. In his seminal paper Fleming had noted that it was a strain of *Penicillium* mould that produced the antibiotic agent he later named penicillin. Notably, one key advantage that the Delft team had was proximity to the world's most extensive fungal strain collection, the *Centraalbureau for Schimmelculture* (CBS) housed near Utrecht.

In late 1943, Waller's team contacted prof. Johanna Westerdijk, the curator of the CBS, and the first female professor in the Netherlands, with a request for any *Penicillium* strains in her collection. Professor Westerdijk responded by sending them twenty-one strains, six of which Waller's team confirmed produced a substance capable of killing *Staphylococcus aureus* bacteria. The highest levels of antibiotic production were observed with a strain named *Penicillium baculatum* leading Waller to designate his team's clandestine efforts as "project bacinol." Waller rightly knew that the Nazis were also keenly interested in obtaining penicillin so a code name was essential to keeping his team's work a secret.

In the months that followed, the Delft team worked diligently to develop an effective procedure for the production of penicillin, which they continued to call bacinol. Limited access to infrastructure required resourcefulness - in fact, the first large scale fermentations of *Penicillium baculatum* where carried out in hundreds of empty milk bottles, the only available vessels by the summer of 1944. Working in complete isolation, Waller's team devised their own optimal growth media and isolation protocols to achieve high production levels of their bacinol. By the end of 1944 they demonstrated that bacinol was also capable of clearing infections in mouse and rabbit models.

Along with the liberation of the Netherlands on May 5, 1945 came access to American made penicillin. A month later in June 1945 Waller and his colleagues excitedly confirmed that bacinol was in fact identical to penicillin. In the months that followed, the production of penicillin in Delft was scaled up. In November 1945, a mere 6 months following the liberation, 21-year old Maria Geene became the first person to be treated with Dutch made penicillin and was completely cured within two weeks. In early 1946 clinical trials where conducted here at the Leiden Academic Hospital and by the end of that year penicillin was being widely used in Dutch hospitals.

The fact that the Waller's team had developed their own means of producing penicillin also had important intellectual property implications. Given that the Americans used a different strain of *Penicillium* and not *Penicillium baculatum* to produce their penicillin, there was no patent clash. The Nederlandsche Gist- en Spiritusfabriek, later renamed *Gist-Brocades,* would go on to become one of the world's largest producers of penicillin.

The Golden Age and the Discovery Void

The discovery that a naturally-produced organic compound like penicillin could safely cure bacterial infections in humans ignited the hunt for other naturally occurring antibiotics. What followed was the "golden age" of antibiotic discovery, a

20-year period spanning the mid 1940s to mid 1960s, wherein the majority of the antibiotics that we still use today were found and brought to the clinic.

Interestingly, most of the antibiotics discovered and developed in this period are produced by strains of bacteria isolated from soil samples. As it turns out, many soil dwelling bacteria produce the small organic molecules that we call antibiotics to limit the growth of other microorganisms competing for the same environment. Amazingly, the antibiotics found during the golden age of discovery work extremely well in treating infections inside a human body. This is a point that is worth emphasizing and a phenomenon that still amazes me. The bacteria that produce our clinically most important antibiotics did not evolve to live in human hosts. The fact that so many of the antibiotics that soil dwelling bacteria produce are able to function at all as medicines in a human body represents a huge stroke of luck for humankind.

To put this good fortune into perspective it is worth considering just how challenging it is for researchers who work in the field of drug discovery to design new medicines that are not derived from natural products. The field of drug discovery is very much a numbers game where failure far exceeds success. It is estimated that the pharmaceutical industry produces only one new drug for every 10,000 novel organic compounds it generates [6]. The reasons for this high failure rate are many but are primarily attributable to efficacy and safety issues: some new drug candidates work well in artificial lab-based experiments that we refer to as "*in vitro* assays" but then fail to work in much more complex animal models, called "*in vivo*" assays. Even more frustrating, many experimental medicines can show great activity in both *in vitro* and *in vivo* animal studies but when it comes to human trials fail to show a beneficial effect or even worse, can be toxic. In this light it really is astonishing that so many naturally occurring antibiotics can be used directly as medicines – very much a case of nature delivering us a gift on a silver platter.

By the 1970s the general consensus was that all readily abundant, safe, and effective naturally-occurring antibiotics had been found. This so-called "low hanging fruit" was identified using systematic approaches for identifying antibacterial activity from diverse environments and samples. Furthermore, modern chemical and biochemical techniques became increasingly available with the power to elucidate the molecular structures and working mechanism of these important new drugs.

With bacterial infections largely deemed to be under control thanks to a broad arsenal of new antibiotics, the focus of drug developers began to shift to other disease areas that had comparatively fewer therapeutic options - areas like cancer, heart disease, and neurodegenerative disease. Also, not unimportant, are the financial considerations that drove the decision making at large drug companies: with the low hanging fruit of naturally occurring antibiotics having been plucked in the previous decades, antibiotic discovery programs became increasingly challenging, offering a poorer return on investment. Simply put, other disease areas began to present more attractive business cases. The impact of these decisions are clear to see today: the past 50 years have witnessed amazing advancements in the development of innovative new medicines to treat heart disease and many cancers. By comparison, only three new classes of antibiotics have been brought to the clinic in the same time period. It boggles my mind to think that the vast majority of the antibiotics we still use today were discovered before I was born. The so called "discovery void" in antibiotic discovery is particularly worrisome in light of an issue I'm sure many of you will be familiar with: the emerging threat of antibiotic resistance.

Antibiotic Resistance

In the golden age of antibiotic discovery, deaths due to bacterial infections plummeted. Today, however, life-threatening infections are on the rise as many bacteria are able to resist the action of antibiotics. Globally, each year 700,000

deaths are estimated to be due to infections with drug resistant bacteria – infections that might have been treatable in the 1960s and 1970s before these strains became resistant. Even more worrisome are recent projections that suggest without serious investment and innovation in the field, by the year 2050 antibiotic resistance may claim as many as 10 million lives per year, more than cancer [7].

Effective antibiotics are not only necessary to cure serious bacterial infections, they are also foundational to the modern medical establishment. Invasive medical procedures ranging from minor hip or knee surgeries to organ transplantations are performed safely and routinely thanks to the use of prophylactic antibiotics that limit the risk of post-operative infection. Antibiotics are also often used by people receiving treatment with chemotherapeutic or immunosuppressing drugs, both of which can leave an individual prone to bacterial infection. Given the role antibiotics play as a cornerstone of modern medicine, the threat of resistance cannot be ignored.

The ability for bacteria to become resistant to antibiotics is in fact a completely natural evolutionary response to our use of antibiotics in medicine and agriculture. It is important to realize that for as long as bacteria and other organisms have produced antibiotics, resistance to these compounds has also circulated in the environment. This is most clearly understood from the perspective of a bacterium that produces an antibiotic to give itself a competitive advantage in its environment. Clearly, the producing strain must be able to resist the antibiotic it produces itself, otherwise it wouldn't survive very long. While genes that encode for antibiotic resistance are generally guarded by the strains that use them, a process known as gene transfer can lead to otherwise sensitive strains acquiring new resistance mechanisms. Another driver of antibiotic resistance is due to the process of genetic mutation. Mutations in the genes of a bacteria species occur spontaneously at a rate of about one in ten million. A bacterial infection represents a population of billions and billions of single celled bacteria. Due to spontaneous mutation there will always be a few cells that differ slightly from the others. While such mutations are rare and often do not provide an advantage to the mutant cell, in some cases they can allow a bacterium to resist the effect of an antibiotic. For example, a mutation that changes the structure of a bacterial protein that is targeted by an antibiotic can significantly reduce the effect of the antibiotic. Such a mutation will initially only impart a small number of bacteria with this advantage while the vast majority of the others remain sensitive to the antibiotic. Bacteria, however, reproduce at an amazing rate, in some cases doubling their population within 20 minutes. This means that an initially small subpopulation of mutant bacteria, can over a period hours become the dominant player in an antibiotic resistant infection.

The process of acquired antibiotic resistance and the threat it posed was in fact observed and described by Fleming himself in his 1945 Nobel lecture. Fleming then stated:

"It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant."

It is a commonly held view that microorganisms use antibiotics and resistance genes as weapons and defenses against their bacterial competitors. More likely, however, is that in nature, antibiotics serve as signaling molecules, allowing for the establishment of harmonious microbial communities rich in bacterial diversity [8]. It is more likely that our "weaponizing" of antibiotics in the fight against infectious disease is responsible for the rapid and unpredictable rise in resistance.

The impact of our industrialized use of antibiotics has created the exact scenario Fleming warned against. Nature was using antibiotics for her own means well before modern humans appeared on earth. It is the scale of our global antibiotic production and application that is now driving the widespread and increasing wave of resistance. Each year antibiotics are globally produced and consumed on the multi-ton scale. This has led to an inevitable and dramatic increase in the quantities of antibiotics that we now find in the environment [9]. Regular exposure to sub-lethal concentrations of antibiotics in waste streams and other environmental pools provides bacteria with precisely the conditions for resistance development that Fleming warned against.

While our use of antibiotics is vital to maintaining effective health care, it has also led us into uncharted territory as a species. We are currently in the midst of a massive, real time biological experiment. Over the past decades we have presented the planet's pathogenic bacteria with an enormous evolutionary pressure to adapt to the antibiotics we use to keep them at bay. Since the golden age of the mid 20th century, we have injected more than a megaton of antibiotics into our environment [10]. How resistance rates will increase in the decades to come may not be simple to predict but the need for action is clear.

So how do we address the threat posed by antibiotics resistance? One approach is to reduce our use of antibiotics, so called stewardship, an approach that can slow the rate at which resistance appears. It is clear, however, that we cannot completely eliminate our use of antibiotics and that effective antibacterial therapies will continue to be a cornerstone of our medical system. Today there is growing interest in alternative technologies for the treatment of bacterial infections. You might have heard of phage therapy, an intriguing approach to clearing infections with small viruses that specifically target bacterial cells. There are also efforts to develop so-called "biologic drugs" based on large antibody molecules that can selectively target bacterial cells. While both phage therapy and biologic drugs are very promising, there remains much work to do to demonstrate their widespread applicability. In the meantime, we should not ignore the amazing historic success of antibiotic drugs based on naturally occurring small organic molecules.

During the golden age of antibiotic discovery, the "low hanging fruit" was plucked: those naturally occurring antibiotics that were found to be potent, effective, and safe for use in humans rapidly made their way to the clinic. Along the way however, many other classes of antibiotic compounds were also discovered that, while effective at killing bacteria in a petri dish, were not safe or effective in the human body. In the golden age such compounds were logically cast aside. Today, however, some researchers are returning to these molecular castaways as possible leads for the development of new antibiotic classes. This approach is part of the strategy that my research group uses in the hunt for new antibiotics by using organic chemistry-based strategies to address antibiotic resistance.

Addressing Antibiotic Resistance

One of the main themes in my group's research is the use of organic chemistry to structurally modify natural product antibiotics to enhance their activity and safety. This technique is commonly referred to as "semi-synthesis". In this approach we rely on Nature's ability to generate structurally complex, biologically active molecules and then use organic chemistry to enhance their properties. But what, exactly, do I mean when I say we can enhance the properties of a naturally occurring antibiotic?

Well, some of the antibiotic molecules that nature produces are very potent and effective in their native environments but in our body are rapidly digested and/or excreted. The human body is very well equipped to remove foreign compounds. An early study conducted by the first PhD student in my group

Timo Koopmans, working in collaboration with colleague Eefjan Breukink, demonstrated that the naturally occurring antibiotic nisin could be modified to generate analogues that are much more stable to degradation and excretion [11]. These findings garnered much interest from the community and inspired us to pursue similar strategies with other classes of antibiotics.

In some cases, enhancing an antibiotic can mean reducing its toxicity. In a more recent and not yet published study, Jaco Slingerland has shown that structural modifications can significantly reduce the toxicity of the polymyxin class of antibiotics. The polymyxins are very important antibiotics because they are among the few options that doctors still have when treating certain multi-drug resistant infections. The drawback to the polymyxins is their high kidney toxicity. Our new semisynthetic polymyxins may circumvent this problem.

Yet a third approach to enhancing a naturally occurring antibiotic is by modifying its structure in a way that enables it to overcome resistance. To this end we recently undertook studies aimed at modifying the structure of vancomycin, one of the most important antibiotics used in hospitals around the world. Unfortunately, vancomycin resistance is rapidly increasing and for some bacterial species vancomcyin resistance is now more common than not. To address this issue recent work by Emma van Groesen, has revealed that certain structural modifications can dramatically increase the activity of vancomycin towards resistant strains – in some cases by many thousands fold. Ongoing studies are aimed at establishing how well these new vancomycin variants work in treating infections *in vivo*.

Aside from modifying complex natural molecules by semi-synthesis, my group has also undertaken the total synthesis of various antibiotics. In some cases it is easier to completely synthesize a molecule than try to isolate it from natural sources. Total synthesis also offers a much broader range of possibilities for the generation of unnatural structural analogues. Most notable in this line of research is the work we've carried out on the family of calcium-dependent antibiotics. These antibiotics present promising leads for clinical development but many questions remain about how they actually kill bacteria. Early efforts in our group by Peter 't Hart demonstrated the feasibility of preparing calcium-dependent antibiotics by total synthesis [12]. This work was subsequently elaborated upon by Laurens Kleijn who, in collaboration with the group of Bert Janssen, succeeded in obtaining a crystal structure of the calcium-dependent antibiotic Laspartomycin C in complex with its bacterial target [13, 14]. This seminal work represents the first crystal structure of such an antibiotic bound to its bacterial target and provides key mechanistic insight. Ongoing efforts by Tom Wood and Karol Al Ayed are currently aimed at further elucidating the structure-activity relationships for Laspartomycin C and other members of this unique class of antibiotics.

The examples of our research that I've described so far are concerned with understanding and improving the properties of antibiotics by structural modification. There is, however, another approach one can take in trying to overcome drug-resistant bacteria: by interfering with the resistance mechanisms themselves. Some bacterial strains called Gram-negatives have an extra outer membrane that can prevent antibiotics from entering the cell. In this way Gram-negative bacteria are inherently resistant to many antibiotics. Current work in our group carried out by Charlotte Wesseling, in collaboration with the group of Suzan Rooijakkers, is aimed at developing compounds that can disrupt the Gram-negative outer membrane as a means of sensitizing them to antibiotics.

In another approach we are also working to target acquired resistance mechanisms where bacteria use specific enzymes to actively destroy antibiotics. Investigations initiated by Kamal Tehrani and Matthijs van Haren, and recently followed up by Nicola Wade and Ioli Kotsogianni, have led to the development

of a new class of small molecule inhibitors that blocks the activity of an especially dangerous family of resistance enzymes. Our *in vitro* studies reveal that when combined with one of our new inhibitors, conventional antibiotics are once again able to kill resistant bacteria. These findings are the basis of a patent application that we recently filed with the Leiden University Technology Transfer Office. In the coming months we will carry out the first *in vivo* studies with these new inhibitors. Exciting times!

As I hope I've illustrated with these examples from my own group's work, I am optimistic that a dedicated, global research effort can effectively address the problem of antibiotic resistance. Yes, the low hanging fruit was plucked long ago but today we have new insights and technologies to help in the fight. Furthermore, it is also to our advantage that bacterial cells are so different from the cells in our body. When trying to treat diseases like cancer it can be very difficult to distinguish diseased cells from healthy cells. Bacterial cells on the other hand offer many unique targets and metabolic processes that the cells in our body do not employ. In addition, the resistance mechanisms that bacteria develop in response to antibiotic pressure can also present an opportunity for the development of new therapeutic strategies. As the famous Dutch philosopher Hendrik Johannes Cruijff once said, "*Elk nadeel heb zijn voordeel*". If we closely monitor the emergence of new resistance mechanisms, and understand them at the molecular level, we may also be able to exploit them.

The simple fact of the matter is that our use of antibiotics will continue to drive resistance. This is unavoidable and places us in an arms race of sorts where we find ourselves pitted against a highly adaptable bacterial foe.

The Future of Antibiotic Development

While I am confident about the scientific community's capacity to innovate in response to the threat of antibiotic resistance, current economic realities provide less reason for optimism. Simply put, with the "low hanging fruit" already plucked, antibiotics currently present a very poor business case. Today, it costs about the same number of dollars to develop a new antibiotic as it does to develop a new medicine for any other disease. The problem is that antibiotics tend to earn much less money compared to drugs for other disease areas [15].

As for any new drug, a company that brings a new antibiotic to market hopes to recoup the costs invested in developing the new medicine as well as the costs sunk on the inevitable failures along the way, remember the "one in ten thousand" number. While drugs for cancer and autoimmune diseases, which are taken for extended periods, if not for life, are very expensive, antibiotics are cheap and typically only require a week to ten days to cure a patient's infection. Furthermore, the likelihood of resistance development to any new antibiotic provides additional disincentive for drug makers.

As a class of drugs antibiotics present a perplexing paradox. When they work, antibiotics are the best deal in health care for patients: they are low cost, safe medicines that generally offer a complete cure in just days. On the other hand, antibiotics are no longer profitable enough for companies to justify working on. In 1980 there were twenty-five large pharmaceutical companies with active antibiotic discovery programs worldwide, today there are three.

So what can be done? While it's tempting to wag one's finger at drug companies for not solving the problem, this will not lead to change. Drug companies, like any other businesses, exist to produce products that will keep them in business. Antibiotics simply do not represent such a product in the current marketplace. This is a clear case of market failure and requires a different approach.

Today there is a growing appreciation for the foundational role that antibiotics play in our health care system. This view

of "antibiotics as infrastructure" is spurring discussions about new economic models that might give companies a reason to once again consider antibiotic development as a viable business model [16]. In July 2019 the UK's National Health Service announced a novel compensation scheme wherein companies will be paid a "license fee" for providing access to a new antibiotic. This subscription style model will see drug companies paid upfront for their new antibiotics, regardless of the number of prescriptions written by doctors. The American government is also reviewing similar proposals designed to provide incentives for drug developers to once again turn their gaze towards antibiotic development.

I would also like to take a moment to address the potential role that academic researchers might play in delivering the antibiotic of the future. Increasingly we as researchers are being asked to communicate the societal relevance of our work. Anyone who has submitted an NWO grant in recent years is no doubt familiar with the "Utilization Potential" section. Most of us will agree that the Academy is, and should remain, the home of fundamental, curiosity-driven research. That said, it can also be a place where focused, goal-oriented work is conducted. While not every academic research line will lead to patents and new medicines, those that might should be provided the chance to make an impact beyond the walls of the university.

The Leiden BioScience Park is one of the top science parks in Europe and presents a unique ecosystem for university researchers looking to create innovative spin out companies based on their academic findings. If encouraged and properly supported, such activities can deliver great benefit to researchers, the university, and society. I very much look forward to working with members of the science faculty and executive board in creating opportunities to realize the translational potential of the research conducted at this fine University where I am honored to accept the title of Professor today.

ßAcknowledgements and Thanks
In the closing minutes of this lecture I would like to acknowledge those who have contributed to my being appointed Professor. I express my sincere thanks to the boards of the University and of the Science Faculty, specifically Carel Stolker and Geert de Snoo. I am also deeply grateful to the IBL, and in particular, to former and current scientific directors, Herman Spaink and Gilles van Wezel. Thank you for welcoming my team and I to the IBL, and for providing us with the brilliant new labs we now call home. I would also like to thank my new colleagues in the LIC, LACDR, and LUMC for their warm welcome. New collaborations have already been struck with many of you and I look forward to our joint success.

Scientifically speaking I cannot express enough the gratitude I feel to the PhDs and postdocs who have made my research group what it is. I have already named many of you here today but there are also a number of people whose research did not feature in this lecture. It is therefore my pleasure to also thank by name: Gosia Sleszynska, Alen Sevšek, Yongzhi Gao, Ray Zhang, Paolo Innocenti, and Ned Buijs. It is truly my pleasure to oversee such a wonderful group of young scientists who are dedicated not only to their own work but also the collective success of the team. I can safely say that I have succeeded in following Mark Twain's advice by hiring smarter and more skilled people than myself. Thank you all for making my job a constant pleasure and for making our group a driven yet supportive research environment.

I am also grateful to the many colleagues I enjoyed working with during my more than ten years at Utrecht University. I would specifically like to thank Rob Liskamp for agreeing to host me when I arrived from Berkeley with a VENI grant back in 2007. Special thanks also go to Roland Pieters whose advice and mentorship was much appreciated as I adapted to life in the Dutch academic system. I am also grateful for the many fruitful collaborations I have enjoyed, and continue to

enjoy, with colleagues in Utrecht and elsewhere. Though too many to thank by name, your openness and cooperation has undoubtedly strengthened and broadened my research group's profile.

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Prior to beginning my independent scientific career in the Netherlands I had the pleasure of pursuing my PhD under the guidance of John Vederas at the University of Alberta. Upon completing my PhD in 2004 I moved to UC Berkeley where I joined the group of Michael Marletta for postdoctoral studies. In both labs I enjoyed great scientific freedom and saw what true mentorship looks like. I am also grateful to the many teachers who challenged and inspired me as a student.

I wish also to thank my mother and father, Evelyn and Brian Martin, who I am pleased could be here for this special day. Growing up with an artist and a physicist for parents I learned at a young age both how to draw a three dimensional object on a two dimensional page as well as how to build a rudimentary electric motor using copper wire and a magnet – perhaps little surprise I became an organic chemist. Thanks mom and dad for providing a home full of love and big ideas.

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Prof. dr. Nathaniel I. Martin

Experience and Education

06/2018 - present	**Full Professor** of Biological Chemistry Institute of Biology Leiden, Faculty of Science, Leiden University, Leiden, The Netherlands
01/2016 - 06/2018	**Associate Professor** Department of Medicinal Chemistry and Chemical Biology, Utrecht University, Utrecht, The Netherlands
09/2010 - 12/2015	**Assistant Professor** Department of Medicinal Chemistry and Chemical Biology, Utrecht University, Utrecht, The Netherlands
09/2007 - 09/2010	**Junior Group Leader** Department of Medicinal Chemistry and Chemical Biology, Utrecht University, Utrecht, The Netherlands
06/2004 - 06/2007	**Postdoctoral Fellow** Department of Chemistry, University of California Berkeley, Berkeley California, USA
09/1999 - 06/2004	**Ph.D. Researcher** Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada
09/1995 - 04/1999	**Bachelor of Science** Department of Chemistry, The King's University College, Edmonton, Alberta, Canada

Biography

Nathaniel Martin obtained his PhD in 2004 from the Department of Chemistry at the University of Alberta (Canada) on research into naturally occurring antimicrobial peptides. Upon completion of his PhD he moved to the University of California Berkeley (USA) as a postdoctoral fellow where he developed a number of inhibitors and mechanistic probes for the enzyme nitric oxide synthase. In 2007 Martin began his independent research career at Utrecht University where he built a dynamic research group working in the fields of medicinal chemistry and chemical biology.

Fundamental to the work carried out in the Martin group is the application of synthetic organic chemistry to address biologically interesting and medically relevant questions. Specifically, Martin's research focuses on using (bio)chemical approaches to combat infectious disease as well as developing new molecular tools with which to study epigenetic processes.

Martin has received a number of grants and awards in support of his research including the NWO VENI (2007) and VIDI (2010) grants as well as the ERC consolidator grant (2016). He was also recently named as one of the top three young medicinal chemists in Europe (in voting for the 2016 EFMC Prize Young Medicinal Chemists in Academia).

In 2018 Martin moved his research group to Leiden University where he was appointed full professor and is currently head of the Biological Chemistry group and leader of the research theme "Bioactive Molecules" within the Institute of Biology Leiden (IBL).

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The Netherlands