Germs and geothermals: A uniquely New Zealand collaboration

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Dr. Rob Keyzers from the School of Chemical and Physical Sciences is leading a long-running, uniquely New Zealand research project to help find new sources of antibiotics.

The collaboration is looking at a group of organisms called extremophiles—organisms that live in extremely hot or extremely cold environments unsuited to human habitation. For the past several years, the research—involving scientists from Victoria University of Wellington, GNS Science, the University of Auckland and the University of Canterbury—has focused on one organism that lives around geothermal vents in New Zealand.

And although the project has encountered many hurdles and setbacks, Dr. Keyzers says they can successfully point to research spanning organism discovery through to synthesis.

"This project was all about the right people coming together in the right environment with the right resources, all of which were found in New Zealand," Dr. Keyzers says.

"It started in 2011 when I was looking for new sources of antibiotics in the natural world," Dr. Keyzers says. "Nature has been a wonderful source of antibiotics so far, but we always need new drugs that kill pathogens in new ways. Extremophiles were an ecological niche that hadn't been explored much, so I thought it might be a good place to start looking."

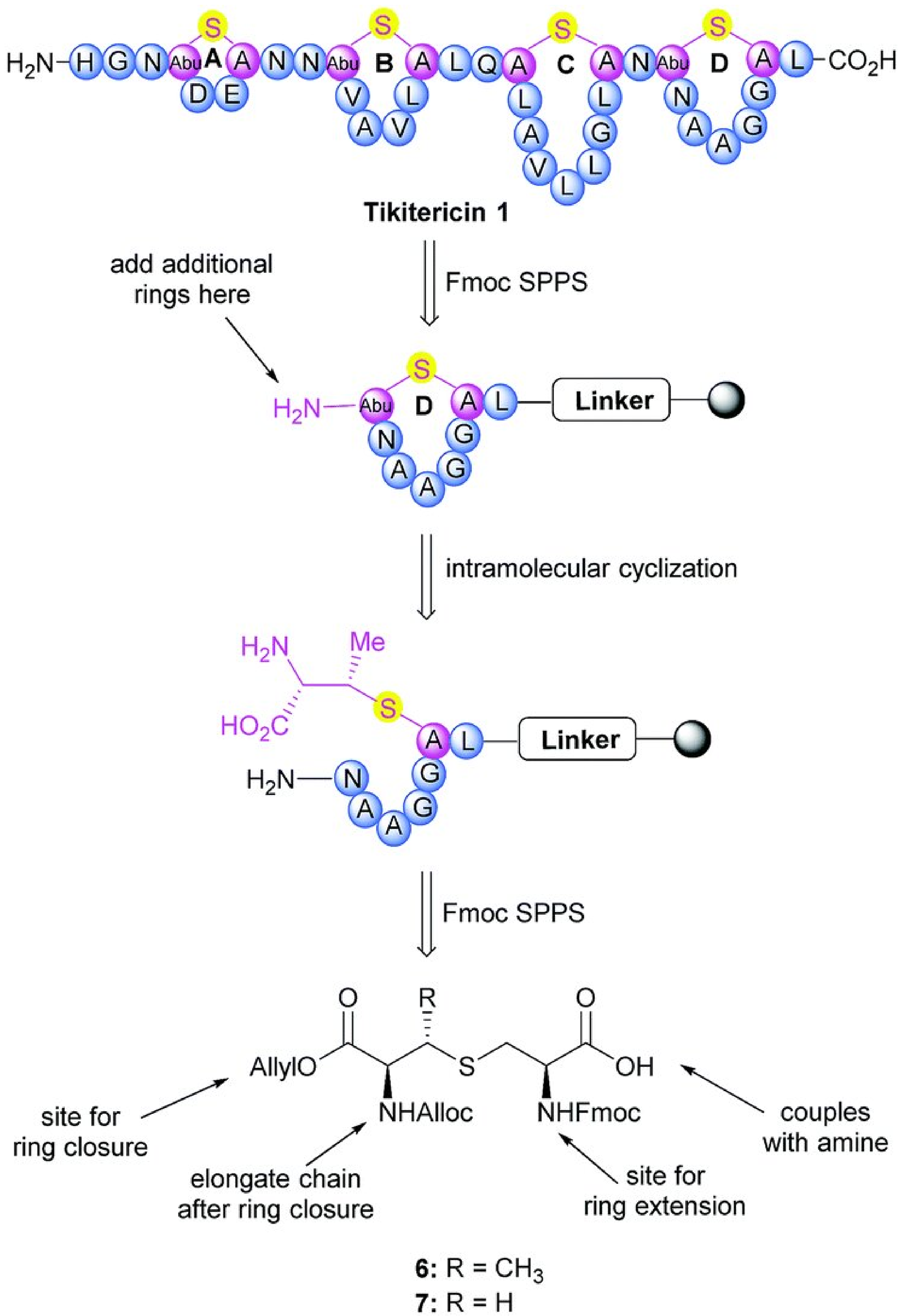
Dr. Keyzers contacted Matthew Stott, formerly at GNS Science and now the University of Canterbury, who is an expert in growing bacterial extremophiles from geothermal environments.

They started looking at one particular extremophile for natural products that might lead to a new antibiotic. Dr. Stott had recently sequenced the genome of that organism, which meant they could examine the DNA for sequences already known to be helpful in creating antibiotics.

"Matt's organism had the potential to make four useful molecules written into its genetic code," Dr. Keyzers says. "We had my Master's student, Emma Aitken, test the organism to see if any of these molecules were actually being produced, so we could test it for potential applications. She found one—a peptide that is part of a known class of antibiotics, which was very exciting."

The next step was gathering enough of this molecule to test it for potential applications. This turned out to be more of a challenge than Dr. Keyzers and his team were expecting—the organism would only grow under very specific conditions, and only produced a very small amount of the compound.

"Emma went through 1200 petri dishes to grow this organism," Dr. Keyzers says. "It would only make the molecule we wanted if we grew it in a petri dish on a certain type of agar. Even after that, we could only gather around 400 micrograms (0.4 g) of the molecule."


H2N-H G N Abu A A N N Abu B A L Q A C A N Abu D A L-CO2H
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Tikitericin 1
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H2N-Abu D A L Linker
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H2N-N A A G G
Fmoc SPPS
O R O
AllylO S OH
NHAlloc NHFmoc
site for ring closure
elongate chain after ring closure
site for ring extension
couples with amine
6: R = CH3
7: R = H

Retrosynthetic analysis of tikitericin

Dr. Keyzers began looking at options to synthesize the molecule and contacted Distinguished Professor Dame Margaret Brimble from the University of Auckland, a world-leading expert in synthetic chemistry. Coincidentally, Dame Margaret had also recently developed an interest in extremophiles.

"Dame Margaret and her team were able to synthesize the molecule using some very interesting chemistry techniques they had developed," Dr. Keyzers says. "They were only able to make a small amount, but it was enough to start testing the molecule."

Unfortunately, the tests were not promising—the molecule didn't seem to have any effect on the bacteria they tested it on. However, Dr. Keyzers and his colleagues were only able to test the molecule against a small group of bacteria.

"The molecule could be a successful treatment for other bacteria we couldn't test against, or it could be an anti-fungal," Dr. Keyzers says. "We would need to do further tests to find this out."

Although the tests themselves were unsuccessful, Dr. Keyzers says this research project has been hugely beneficial in other ways.

"We were able to get our work published in a well-regarded journal, *Chemical Science*, and put New Zealand on the map as a leader in this area of chemistry," Dr. Keyzers says. "There have been very few other cases where a research collaboration has been able to run the whole gambit from discovery to synthesis.

The research group also followed a vigorous identification method, which Dr. Keyzers hopes will set a high standard in this field, and Dame Margaret and her team at the University of Auckland were able to develop several improvements to the synthesis process as well, Dr. Keyzers says. They were also able to exploit an ecological niche —geothermal extremophiles—that is very New Zealand-centric and is an area where New Zealand can offer unique research possibilities in both geothermal and extremophile niches.

"It needed someone who knew about extremophiles and someone with knowledge of my area of chemistry, both of which are reasonably rare, as well as a microbiologist to provide material for me to test that I could then pass on to an expert in synthesis to create. We looked at a fairly unusual area with extremophiles and were able to achieve all these great things here in New Zealand."

"Collaborations like this are one of the benefits of living in New Zealand," Dr. Keyzers says. "We have a small community of researchers here, brought together through the Maurice Wilkins Centre of Research Excellence, who all know each other and can easily work together, using New Zealand's natural resources to push forward projects like ours."

Dr. Keyzers plans to continue his work on this project and hopes to bring in the expertise of School of Biological Sciences colleague, Dr. Jeremy Owen. Dr. Owen is a specialist in taking genetic codes that produce certain molecules from one organism and transplanting them into another organism to help them grow faster.

"Jeremy's expertise can help us produce molecules faster, as well as take DNA from anywhere and grow it," Dr. Keyzers says. "Along with

Margaret's expertise in synthesis, we can now discover and grow potentially helpful molecules much faster, which is a very exciting prospect."

More information: Buzhe Xu et al. Genome mining, isolation, chemical synthesis and biological evaluation of a novel lanthipeptide, tikitericin, from the extremophilic microorganism Thermogemmatispora strain T81, *Chemical Science* (2018). DOI: 10.1039/C8SC02170H

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