

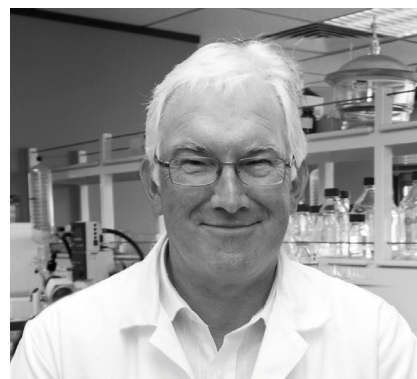
From Pesticides to Paint *via* Pharmaceuticals - the Evolution of New Zealand's Applied Carbohydrate Chemistry R&D

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About the Senior Author

Richard H. Furneaux BSc(Hons), PhD, FNZIC, FRSNZ, founded, and leads a world-renowned team of chemists at Industrial Research Ltd. (IRL) whose focus is on the discovery and commercialization of *Glycotherapeutics*—drugs and dietary supplements based upon knowledge of the role of complex carbohydrates in biological processes. They partner nationally and internationally for biology and biochemistry. He has been involved with (and for a year managed) the GlycoSyn business unit at IRL, which undertakes process development and cGMP manufacturing by organic chemical synthesis of small molecule drugs for use in proof-of-concept human clinical trials. Together these units total 55 technical and support staff.



Richard was born in Wellington and educated at Scots College. He gained an Honours degree in physical chemistry before turning to the art of organic synthesis and a PhD under Prof. Robin Ferrier's supervision. He had post-doctoral positions with Prof. Fred Shafizadeh at the University of Montana and then back at VUW, before joining DSIR Chemistry in 1980; it became IRL in 1992. Richard, a Fellow of the Royal Society of New Zealand, was awarded its Hector Medal in 2006. He has authored 186 original papers and 25 reviews or book chapters. He is the NZ representative on the International Carbohydrate Organization. His research team has grown from 3 to 32 members since its inception in 1985, and since 1995 has produced 281 publications, 27 reviews, 29 published patent series and 57 other reports.

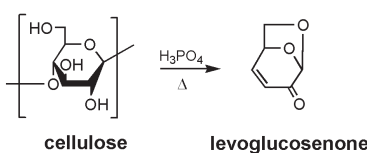
The team is focused on the rational design and synthesis of drug candidates based on knowledge of the role of carbohydrates in biology, in partnership with World Class Biologists. The flagship project is the collaboration with the biochemistry/biology team of Prof. Vern Schramm at Albert Einstein College of Medicine in New York, which is focused on inhibitors of carbohydrate and nucleoside processing enzymes for use as pharmaceuticals.

Carbohydrates are ubiquitous in nature and highly varied in their structures. From crystalline cellulose to the sweetest of sugars and even DNA itself, they pervade life on Earth. New Zealand is rich in carbohydrate resources with its diverse forest botany, virtual mountains of lactose as it comprises 2 – 8% of milk by weight, and a vast coastline draped with seaweeds.

The Carbohydrate Chemistry Group, based at Industrial Research Limited (IRL) in Lower Hutt, has seen these resources as an opportunity upon which to build arguably the largest and most productive carbohydrate chemistry team in the world. This is the story of how the now Group Manager, Dr. Richard Furneaux led the team to become the 32-strong international group that it is today.

Richard began work as a Research Chemist in the Applied Chemistry Group of the Chemistry Division of the Department of Science and Industrial Research (DSIR) under Dr Ian Miller.¹ Chemistry Division was the perfect place for the applied research that he had a passion to do, with a supportive management and freedom to pursue ideas to enhance NZ's economic well-being. Early adventures were in the carbohydrate chemistry of seaweed

polysaccharides and the transformation of cellulose to a class of cyclic ether herbicides *via* levoglucosenone using Michael or conjugate addition chemistry (Scheme 1).²

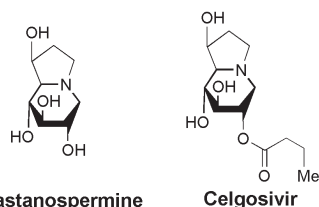


Scheme 1. Acid catalysed pyrolytic conversion of cellulose to levoglucosenone

The lead compound, named PT-9 from Dr. Peter Tyler, was sent to Jealot's Hill Research Station, part of ICI Agrochemicals, for testing and was found to be active at concentrations as low as 1–10 g/ha. In a significant collaboration, a plethora of analogues were synthesized and tested, but PT-9 was hard to beat. However, it was active but not very selective, and was a little too volatile and water soluble. Although PT-9 was only four synthetic steps from waste paper, up against Roundup® (non-selective) and Treflan™ (selective, less potent, but very cheap) it could not compete on cost-of-goods. Subsequently, at a review meeting at ICI in 1988, it was agreed to cease work on the project.

New collaborations with the National Institutes of Health (NIH) in the US led to their testing of compounds for biological activity that ranged from NZ seaweed-derived polysaccharides to iminosugars. Later, other plant-based extracts were included and so began a fifteen year relationship with the NIH/NCI (National Cancer Institute) to screen natural products and IRL's own drug candidates. As part of this relationship, the Carbohydrate Chemistry Group also re-synthesized lead compounds for the NCI under two consecutive five-year contracts. Of particular interest was a range of iminosugar enzyme inhibitors – sugar-shaped alkaloids where the ring oxygen is replaced by a nitrogen atom. These molecules, primarily made by plants, are used to fool the enzymes of potential predators that metabolise sugars, and interfere with their normal function.

The head of the ICI Corporate Carbohydrate Chemistry Group expressed an interest in the biological activities of castanospermine derivatives, especially the anti-HIV lead 6-*O*-butanoylcastanospermine (Celgosivir).³ A collaborative research programme was established utilising pharmaceutical screens at ICI to test the castanospermine-based targets synthesized by the Carbohydrate Chemistry group. This work led to an interest in iminosugars⁴ in general, together with their associated biological activity. Nearly 200 castanospermine analogues⁵ were made and tested in both agrochemical (herbicides and fungicides) and pharmaceutical (cancer and glycosidase) screens. Other compounds that were part of the ICI collaboration included allosamidin—a potent chitinase inhibitor, tagetotoxin—a potential herbicide, and the insecticide dysghomoerythrine.⁶



Group numbers had grown to thirteen by the time the team became part of the newly established CRI, Industrial Research Limited, in 1992. A conscious decision was made then to increase the group's commercial revenue in the event that government funding priorities changed. So began the creative tension between commercial work and discovery science that continues to this day.

In 1993, Richard Furneaux met the Head of MAF's Wallaceville Animal Research Centre, Dr. Paul Atkinson who had returned to NZ in 1991 after twenty three years at the Albert Einstein College of Medicine (AECOM). Atkinson mentioned that a former colleague, Professor Vern Schramm,⁷ was having difficulty finding a chemistry group to synthesize a target molecule he had designed. Under a US/NZ Co-operative Science Program Grant, Drs. Furneaux and Tyler met Schramm at a New York Yacht Club to discuss the blueprint design that he had for an inhibitor of nucleoside hydrolases. Nucleoside hydrolases and phosphorylases are salvage enzymes and in humans are implicated in a variety of immune disorders.⁸

Schramm's hypothesis was that the *virtual* molecule in question was potentially the perfect inhibitor of such enzymes. Tyler recognized that the enzyme purine nucleoside phosphorylase (PNP) would be the best and most commercially relevant target, and inhibitors could be used to treat a variety of human ailments from rheumatoid arthritis to T-cell cancers. Rate enhancements of 10^{15} are common in enzymology, and such inhibitors could, therefore, provide unprecedented utility in drug design. Armed with a bar napkin bearing the structure of the proposed enzyme inhibitor (coincidentally containing an iminosugar at its core) and an agreement with AECOM to share all royalties from their collaboration 50:50 (with the proviso that AECOM would take the lead in commercialising the attendant IP), then Furneaux and Tyler returned home and set to work.

Realising Schramm's blueprint design took considerably longer than was first envisaged but, in 1997, immucillin-H (ImmH; immucillin: penicillin for the immune system)⁹ eventually was synthesized and sent to be assayed in his biochemistry group's enzyme screens, particularly against the now preferred target, PNP. Screening revealed that against bovine PNP, ImmH afforded inhibition constants at concentrations far lower than previously reported for other compounds and bore out the predictions of transition state theory.^{10,11} With a lead compound identified, work began on turning the synthetic process from a *twenty one* step linear synthesis into a practical *twelve* step convergent synthesis.¹²

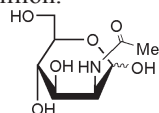
A thirty month-long process then began to find an investor to license the patented technology. One company, BioCryst Pharmaceuticals, already had a drug in Phase III clinical trials targeting PNP. In 1997, this compound (BCX-34) failed a Phase III clinical trial against psoriasis but management still believed that PNP was a good biological target for the treatment of T-cell mediated diseases. Consequently, BioCryst showed considerable interest in licensing the IRL collaborative IP in the area of PNP inhibitors, and more specifically Immucillin-H, in order to plug the gap left by the failure of BCX-34. So, in June 2000, BioCryst signed an agreement with IRL and AECOM to license all PNP inhibitors generated as a result of the collaboration. Since that time compounds from an additional three generations of inhibitors have fallen under this license.

Inextricably linked with the license agreement was the supply of kilogram quantities of ImmH to BioCryst in order for animal toxicology and human clinical trials to progress. Furneaux had recognized that although the intellectual property package had value for IRL, real value for NZ could only be gained if scalable processes to synthesize the immucillins could be developed by IRL, and the technology then transferred to a local chemical manufacturer. To realise this step-change in manufacturing, IRL first required the facilities to safely take chemical processes from a gram to a kilogram scale. In August 2000, Furneaux presented a proposal to the IRL Board to build a fit-for-purpose facility, which would house equipment to synthesize kilogram quantities of new chemical entities (NCEs) as well as assure the quality of the prod-

uct and the safety of processes.

A small process development team under Dr. Paul Benjes was seceded from the Carbohydrate Chemistry group in 2005. Its purpose was to specialize in laboratory scale development work, intermediate scale synthesis of good laboratory practice (GLP) material, and kilogram scale current good manufacturing process (cGMP) synthesis. The IRL GlycoSyn group is based now in the facilities Furneaux proposed in 2000 and officially opened by the Prime Minister, Helen Clark, in 2003.

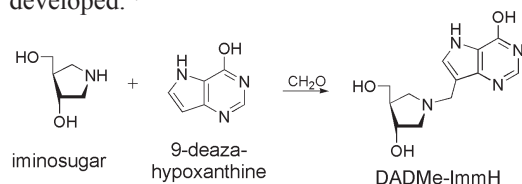
In an innovative marketing approach, a chemical sales website (now GlycoFineChem) was begun in the late 1980s offering for sale small quantities of 10–20 imino-sugars. Acting as an internet *shop window* for the Carbohydrate Chemistry group, the business has enabled the initial sale of compounds such as *N*-acetylmannosamine, worth only a hundred dollars, to grow into multi-million dollar sales. Following some further work on the production process from glucosamine, both at IRL and New Zealand Pharmaceuticals (NZP), the technology package was licensed to NZP and is now worth more than \$NZ10 million.



***N*-acetylmannosamine**

ImmH moved through animal toxicity trials and into BioCryst's first human patient in July 2002. Science progress was significant also, with the biological activity of a selection of immucillins featured on the cover of the ACS journal *Biochemistry*.¹³ In 2004, ImmH was granted orphan drug status by the US FDA for the treatment of T-cell cancers. This enabled BioCryst to receive pre-filing regulatory guidance as well as reduced filing fees, and meant potential market exclusivity in the US for a period of seven years, should ImmH receive approval by the FDA.

A second generation family of inhibitors was based on work by Schramm's group to clone and express the human form of PNP and generate a blueprint for an inhibitor of this enzyme. Dr. Gary Evans completed the twenty-two step linear synthesis of DADMe-ImmH in early 2001 and screening against the human enzyme showed it was four times as potent as ImmH.¹⁴ Soon, a convergent process was patented¹⁵ using the Mannich Reaction¹⁶ and a scalable chemoenzymatic synthesis of the iminosugar was developed.¹⁷



Scheme 2. Scalable synthesis of DADMe-ImmH.

Other compounds were made to target methylthioadenosine phosphorylase (MTAP), an enzyme impli-

cated in the control of polyamine synthesis which is up-regulated in proliferating cells such as cancer cells.¹⁸ The compounds were exquisite inhibitors of the human MTAP enzyme and, when assayed against tumour cell lines, showed strong activity against head and neck tumours that are notoriously difficult to treat.¹⁹ Ultimately, it was shown that the mode of action was probably the inhibition of whole body MTAP, the consequent alteration of methylthioadenosine levels in the body and the subsequent effect this has on DNA methylation.²⁰

Schramm had also identified a biological target that would potentially disrupt quorum sensing in bacteria – the way bacteria communicate that ultimately results in an infection. Methylthioadenosine nucleosidase (MTAN) was the target and modified immucillins were designed and synthesized in order to inhibit this enzyme. These compounds were more potent than any inhibitor that had been synthesized to-date and had some of the lowest inhibition constants ever reported for any enzyme, in the femtomolar (10^{-15} molar) range. The work was so significant that the prestigious international journal, *Journal of Biological Chemistry* featured an excerpt of this work on its cover.²¹

Another class of compounds that were a focus for the group were the phosphatidylinositol manno-oligosaccharides (PIMs) from the cell walls of bovine tuberculosis.²² When isolated, these natural PIMs were shown to be potent immunomodulators and Carbohydrate Chemistry group members set about the challenging task of synthesizing and characterising them. The PIMs have proven to be valuable tools in probing immune responses and ultimately may have commercial worth as adjuvants.²³

In late 2005, Roche and BioCryst announced an exclusive sub-licence to develop and commercialize DADMe-ImmH for the prevention of acute rejection in transplantation and for the treatment of autoimmune diseases. The deal, the fifth largest Biotech/Pharma one of 2005, meant that Roche would obtain worldwide rights to DADMe-ImmH in exchange for a \$US25 million up-front payment, a \$US5 million payment for the supply of material during the first two years of the collaboration, and future event payments potentially reaching \$US530 million in addition to royalties on product sales. Following this, early in 2006, a sub-licence agreement was announced between BioCryst and Mundipharma to develop ImmH for markets outside North America. The agreement involved an initial \$US10 million payment and future event payments of as much as \$US155 million. But the big reward and big risk nature of the *pharmaceutical game* was clearly bought home in May 2008 when Roche returned the rights to DADMe-ImmH to BioCryst following an inconclusive Phase II trial. Subsequently, in 2010, BioCryst announced positive results for a Phase II trial of DADMe-ImmH in gout.

Other applications for the immucillins have included the treatment of malaria through the inhibition of malarial PNP. Initial work looked at inhibiting the isolated enzyme and generated some beautiful crystal structures of the inhibitor in the active site of the enzyme, which were

featured on the cover of the *Journal of Biological Chemistry*.²⁴ This work was supported by the NZ and US governments and the Medicines for Malaria Venture, financed through the Bill and Melinda Gates Foundation.

In 2009, when Resene approached Furneaux to help prepare their entry for the IRL-run competition *What's Your Problem New Zealand?* he jumped at the chance to help. Their vision was to develop new waterborne paint, based on resins made from up to 80% sustainable ingredients, and so break a long-term reliance on gas and oil for the product. The Resene bid won the \$NZ1 million prize on offer and this work has now attracted additional FRST funding to support the research over the next four years.

Richard Furneaux is forever chasing opportunities that only he sees, and at times, only his team can realise. He is justly proud of what his group has accomplished, just as the group members themselves are proud of his achievements. He is also the first to acknowledge the assistance of support and business development staff and senior managers, without whom none of this would have been possible.

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References

- Parker, S. *The Littlest Clue*, Industrial Research Ltd., Lower Hutt, **2002**, 62-66.
- Henzell, R. F.; Furneaux, R. H.; Tyler, P. C. *Pesticide Sci.* **1990**, *30*, 59-66.
- Durantel, D. *Curr. Opin. Investig. Drugs* **2009**, *10*, 860-870.
- Furneaux, R. H.; Tyler, P. C.; Whitehouse, L. A. *Tetrahedron Lett.* **1993**, *34*, 3609-3612; Furneaux, R. H.; Tyler, P. C. *Tetrahedron Lett.* **1993**, *34*, 3613-3616; Furneaux, R. H.; Lynch, G. P.; Way, G.; Winchester, B. G. *Tetrahedron Lett.* **1993**, *34*, 3477-3480.
- Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C. *Tetrahedron* **1994**, *50*, 2131-2160; Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C. *Tetrahedron Lett.* **1994**, *35*, 3143-3146; Furneaux, R. H.; Mason, J. M.; Tyler, P. C. *Tetrahedron Lett.* **1995**, *36*, 3055-3058; Furneaux, R. H.; Gainsford, G. J.; Hartlet, O.; Mason, J. M., *et al. Tetrahedron* **1995**, *51*, 12611-12630; Furneaux, R. H.; Gainsford, G. J.; Hartlet, O.; Mason, J. M., *et al. Tetrahedron* **1997**, *53*, 245-268; Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C. *Carbohydr. Res.* **2004**, *339*, 1747-1751.
- Hart, J. B.; Mason, J. M.; Gerard, P. J. *Tetrahedron* **2001**, *57*, 10033-10038.
- Schramm, V. L. *J. Biol. Chem.* **2009**, *284*, 32201-32208.
- Giblett, E. R.; Anderson, J. E.; Cohen, F.; Pollara, B.; Meuwissen, H. J. *Lancet* **1972**, *2*, 1067-1069.
- Mazella, L. J.; Parkin, D. W.; Tyler, P. C.; Furneaux, R. H.; Schramm, V. L. *J. Am. Chem. Soc.* **1996**, *118*, 2111-2112; Furneaux, R. H.; Limberg, G.; Tyler, P. C.; Schramm, V. L. *Tetrahedron* **1997**, *53*, 2915-2930; Furneaux, R. H.; Schramm, V. L.; Tyler, P. C. *Bio-org. Med. Chem.* **1999**, *7*, 2599-2606; Evans, G. B. *Aust. J. Chem.* **2004**, *5*, 837-854.
- Miles, R. W.; Tyler, P. C.; Furneaux, R. H.; Bagdassarian, C. K.; Schramm, V. L. *Biochemistry* **1998**, *37*, 8615-8621.
- Fedorov, A.; Shi, W.; Kicska, G.; Fedorov, E., *et al. Biochemistry* **2001**, *40*, 853-860.
- Evans, G. B.; Furneaux, R. H.; Hutchison, T. L.; Kezar, H. S., *et al. J. Org. Chem.* **2001**, *66*, 5723-5730.
- Kicska, G. A.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H., *et al. Biochemistry* **2002**, *49*, 14489-14498.
- Lewandowicz, A.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H.; Schramm, V. L. *J. Biol. Chem.* **2003**, *278*, 31465-31468.
- Evans, G. B.; Tyler, P. C. *Process for Preparing Inhibitors of Nucleoside Phosphorylases and Nucleosidases*, PCT Int. Appl. 2004, WO 2004069856.
- Evans, G. B.; Furneaux, R. H.; Tyler, P. C.; Schramm, V. L.; *Org. Letters* **2003**, *5*, 3639-3640.
- Clinch, K.; Evans, G. B.; Furneaux, R. H.; Lenz, D. H., *et al. Org. Biomol. Chem.* **2007**, *5*, 2800-2802.
- Singh, V.; Shi, W.; Evans, G. B.; Tyler, P. C., *et al. Biochemistry* **2004**, *43*, 9-18.
- Baso, I.; Cordovano, G.; Das, I.; Belbin, T. J., *et al. J. Biol. Chem.* **2007**, *282*, 21477-21486.
- Basu, I.; Locker, J.; Cassera, M. B.; Belbin, T. J., *et al. J. Biol. Chem.* **2011**, *286*, 4902-4911.
- Lee, J. E.; Singh, V.; Evans, G. B.; Tyler, P. C., *et al. J. Biol. Chem.* **2005**, *280*, 18274-18282.
- Severn, W. B.; Furneaux, R. H.; Falshaw, R.; Atkinson, P. H. *Carbohydr. Res.* **1998**, *308*, 397-408.
- Denis, M.; Ainge, G. D.; Larsen, D. S.; Severn, W. B.; Painter, G. F. *Immunopharmacol. Immunotoxicol.* **2009**, *31*, 577-582.
- Shi, W.; Ting, L.-M.; Lewandowicz, A.; Tyler, P. C., *et al. J. Biol. Chem.* **2004**, *279*, 18103-18106.



The IRL Carbohydrate Chemistry Group