Institution: EaStCHEM

Unit of Assessment: 8; Chemistry

Title of case study: Albachem: Commercialisation of the chemical synthesis of biologically active human proteins

1. Summary of the impact

Impact: EaStCHEM spin out Albachem (1994), subsequently incorporated into the Almac group, enabling the latter company to become a world leader in the provision of chemically synthesised proteins.

Significance: Chemical synthesis is competitive with recombinant methods for commercial production of the therapeutic polypeptides that represent ~50% of drugs in big pharma pipelines and have a market value in 2008 of over $13B. The value attributable to Ramage's methods for polypeptide syntheses over the REF period is estimated at approximately £6M.

Beneficiaries: Drug manufacturers, contract research organisations, patients, clinicians.

Research: Studies (1993-6) led by Ramage (at the University of Edinburgh) on new methods for high-yield total syntheses and purification of long polypeptides.

Reach: Almac's protein-manufacturing team remains in the UK with 24 staff members. The Almac Group, headquartered in N. Ireland, has 3300 employees globally (1300 outside UK) and sells to 600 companies worldwide.

2. Underpinning research

Proteins and polypeptides are attractive research tools and therapeutic agents. They combine biological activity, low toxicity and high selectivity. But they are expensive. While solid-phase peptide synthesis was already a routine procedure, the cumulative outcome of sub-100% yields for each of many steps in the process had limited the length of peptides that could be produced and purified in useful quantities to < 50-60 amino acid residues. Most biologically or clinically interesting proteins are bigger. Furthermore, many proteins of interest to biotechnology and pharmaceutical companies contain disulfide bonds, creating additional challenges for synthesis.

Professor Robert Ramage, FRS, of EaStCHEM recognised an unmet need for total protein synthesis using solid-phase chemistry as a versatile alternative to recombinant gene expression. From 1993-5 the Ramage group published, [1, 2] and patented [6] new solid-phase and protecting group methodologies for peptide synthesis. The work allowed the total synthesis of hitherto inaccessible long polypeptides. He demonstrated the usefulness of these techniques by manufacturing biologically active proteins that provided detailed insights into structure-function relationships.[3-5]

Ramage recognised that resolution of the product from acetylated truncated peptides was key. He researched methods for derivatising the peptide N terminus with a removable tag for affinity purification on a solid support. He introduced the base-labile N(α)-protecting group, tetrabenzo[a,c,g,i]fluorenyl-17-methoxycarbonyl (Tbfmoc) for affinity purification on porous graphitised carbon.[1,2] In a key paper, he described a convenient synthesis of Tbfmoc and its application to the production of proteins containing up to 85 amino acids. The hydrophobicity of Tbfmoc was further exploited to simplify peptide purification by reverse-phase HPLC. The Ramage group showed that Tbfmoc could be added directly to the N terminus rather than being incorporated as a Tbfmoc-Gly(cine)-OH. Thus, crucially, any peptide could be made with retention of its native N-terminal residue. This discovery was patented (US Patent No: 6,566,520, 2003) [6] and paved the way for the spin out of Albachem (1994). The University supported Albachem by contributing infrastructure, funding, collaborations and technical assistance to transfer crucial knowhow from academia into the commercial environment. Albachem remained within the

University until 2002.

The utility of Tbfmoc was dramatically demonstrated in the production of a biologically active cytokine (monocyte chemoattractant protein 1, MCP-1). This contained 76 residues and two disulfide bonds.[4] Critically, Tbfmoc was removable under mild conditions, preserving the nascent protein product in a reduced state (-SH). Subsequent oxidative folding yielded the correct disulfide pattern and fully active cytokine. Albachem thus demonstrated to the world its capability of producing large quantities of purified cell-signaling proteins. This established its credentials as a world-leader. Cumulatively, the 1993, 1995 and 1996 papers underpinned Albachem's establishment, subsequent growth and assimilation into CSS that later became the Almac group.

The Ramage group's work expanded protein therapeutics by facilitating access to multiple-gram quantities of material suitable for use in laboratories, animal models and humans. Future prospects are exciting. Routine recombinant methods for protein production, besides being expensive, provide little flexibility for new developments because the building blocks are constrained to genetically encoded amino acids. No such limitations apply to synthetic proteins of the kind accessible *via* strategies based on Tbfmoc and its successors that can incorporate a vast range of chemistries.

Key researchers:
R. R. Ramage: PI, lead inventor on patents, from 09/84 to retirement in 09/01.
A. R. Brown, S. I. Irving, G. Raphy, C. Jamieson: PhD students and PDRAs in Ramage group.
Y. A. Lam, C. M. Pickart: collaborators, School of Public Health, Johns Hopkins Univ., USA.
A. Alban, M. Landon, R. J. Mayer, R. Layfield: collaborators in University of Nottingham Medical School.

3. References to the research

Underpinning research has been published in international, high-quality, peer reviewed, academic journals and receives citations from across the research area:

Journal articles

[1] * Affinity purification of synthetic peptides and proteins on porous graphitized carbon
A. R. Brown, S. I. Irving, R. Ramage
Tet. Letters 1993, 34, 7129-7132. doi:10.1016/S0040-4039(00)61617-9. 26 cits, JIF 2.4.

[2] * (17-tetrabenzo[a,c,g,i]fluorenyl)methylchloroformate (tbfmocCl) a reagent for the rapid and efficient purification of synthetic peptides and proteins
A. R. Brown, S. I. Irving, R. Ramage, G. Raphy
Tetrahedron, 1995, 51, 11815-11830. doi:10.1016/0040-4020(95)00743-R. 17 cits, JIF 2.8.

[3] * A solid phase approach to quinolones using the DIVERSOMER® technology.
A. A. MacDonald, S. H. DeWitt, E. M. Hogan, R. Ramage
Tet. Letters, 1996, 37, 4815-4818. doi:10.1016/0040-4039(96)00944-6. 45 cits, JIF 2.4.

[4] The total chemical synthesis of monocyte chemotactic protein-1 (MCP-1).
A. R. Brown, M. Covington, R. C. Newton, R. Ramage, P. Welch
J. Pept. Sci. 1996, 2, 40-46. doi:10.1002/psc.46.o. 8 cits, JIF 2.1.

[5] Inhibition of the ubiquitin-proteasome system in Alzheimer's disease
Y. A. Lam, C. M. Pickart, A. Alban, M. Landon, C. Jamieson, R. Ramage, R. J. Mayer, R. Layfield.
Proc. Natl. Acad. Sci, USA. 2000, 97, 9902-9906. doi:10.1073/pnas.170173897. 198 cits, JIF 9.7.

Patent

[6] US Patent No: 6,566,520, 'Support for synthesis and purification of compounds' filed 1999, issued 2003). S. H. DeWitt, A. A. MacDonald, R. R. Ramage.

4. Details of the impact

Ramage's innovations in synthesis and purification of long polypeptides rapidly established Albachem as a world leader in the supply of chemically synthesised immunomodulatory proteins. The subsequent synthesis of biologically active peptides that afforded insights into the structure-function relationships of antimicrobial peptides [3], cytokines [4], and the ubiquitination process [5] demonstrated to the world the usefulness of Albachem's procedures. The Almac group (at that time CSS) noticed Albachem's success and recognising the future earnings potential, acquired Albachem – thus taking a "quantum leap into protein synthesis".[S1] "*The Albachem acquisition allowed Almac to add very significant synthetic chemistry expertise to its core competencies and, critically, to establish a position at the interface between chemistry and biology. Access to Ramage's innovations transformed [Almac's] market differentiation by allowing provision of specialist services to its clients worldwide with a focus on the pharmaceutical industry*".[S2]

1. Economic:

The skills and expertise resident in Albachem and derived from Ramage's original research, were key [S3] to the Almac group's subsequent success in worldwide protein and peptide sales (> £6M revenue in REF period attributed to the technology described in Professor Ramage's papers, not including revenue generated through Almac's GMP capability that was built on technical expertise flowing from Albachem[S4]). Elaboration of the methodologies described in Section 2 for the chemical synthesis of long polypetides allows Almac to produce proteins in high yield and purity. Almac has made over 7000 peptides. Almac can also make polypeptides with non-natural amino acids that offer new research avenues to their customers. They specialise in chemically synthesised cytokines. There are >60 of these in their catalogue with biotinylation and site-specific fluorescent labelling or PEGylation available. "*A legacy of a research focus developed at … Edinburgh and in Albachem is our ongoing development of protocols for synthesis of chemokines... As well as being long polypeptides, chemokines are challenging to manufacture because they contain … cysteines that form disulfide bridges following oxidation. Starting from methods that are founded on Ramage's original technologies, we have robust laboratory protocols that have been applied to several GMP campaigns on a range of chemokine products.*" [S4] In effect, Albachem became the specialist R&D arm of the Almac group that offers protein and peptide services. "*it is easy to trace a clear pathway from research carried out in Professor Ramage's lab in the early 1990s, through the spinning out of Albachem and its eventual acquisition by Almac, to significant past, present and future economic and health benefit impacts*".[F1]

Almac is nowadays a global-reach company with 3300 staff (2000 in UK) and a £300M turnover with £14.3M profit in 2012 and total profits 2008-12 of £55M based on sales to 600 companies including all the market leaders. Peptide-based therapeutics represents the fastest-growing class of new drugs, accounting for ~2% of drugs on the market, but comprising ~50% of drugs in the pipelines of major drug manufacturers. The market for peptide drugs is growing by 7.5% annually and will be more than $13 billion this year.

2. Human Capital:

A total of 14 Almac employees (eight PhD level) remain in Gladsmuir, East Lothian, Scotland, while large-scale GMP polypeptide manufacture occurs in a dedicated facility established (2006) by Almac in Craigavon, employing a further 14 people. In the REF period "*we have trained many chemists in peptide synthesis, in both full-time appointment and on placements generating significant human capital contribution to the economy*".[F1]

If it were not for Ramage's pioneering work with Tbfmoc, Almac – who employ people around the world - would have looked overseas when it chose to buy into the protein synthesis business. They may have not been able to make such rapid inroads into the market and certainly would not be employing any researchers in Scotland.

Impact Development Timeline:

1994 Albachem spun out from Ramage's research. Distinguishes itself by its unique capacity to produce cytokines.

2002 Current Operations Manager (PhD, University of Edinburgh 1995) joins Albachem.

2004 CSS/Almac acquires and expands Albachem.[S3]

2005 The Almac Group further expands peptide synthesis efforts, introduces GMP.

2006 Almac establishes a dedicated facility in Craigavon, employing a further 14 staff.

2007 Almac announces' 'First in Man' approach to peptides, embraces development of peptide synthesis, full analytical support, and GMP [batch production, and CMC (Chemistry, manufacturing and controls) documentation].[S1]

2008 The current Almac Operations becomes Senior Group Leader in protein synthesis at Almac.[S4]

2013 Almac is fully established as a global-reach company with 3300 staff (2000 in UK), £275M turnover and £15M profit.

5. Sources to corroborate the impact

[S1] A 'First in Man' approach to peptides, article containing 'quantum leap' quote. http://www.avakado.eu/dev/node/417.

[S2] 'Integrating Technologies for Complete Chemical Synthesis'. Article highlights the importance of Ramage/Albachem to protein synthesis in Almac http://www.almacgroup.com/wp-content/uploads/Integrating_Technologies.pdf.

[S3] 'Broadening the Custom Synthesis portfolio'. Article explains how crucial Albachem was seen to be by CSS (CSS became part of Almac) http://www.almacgroup.com/wp-content/uploads/SP2_S_Barr_profile_July_20051.pdf.

[S4] 'Modern Perspectives on Peptide Synthesis'. Article by current Almac Operations Manager in his capacity as leader of protein synthesis for Almac. http://www.almacgroup.com/wp-content/uploads/Modern-Perspectives-on-Peptide-Synthesis.pdf.

[F1] Comments in corroborating letter made by the Operations Manager for Almac.