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Editorial

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Hans Vliegenthart was born on 7 April 1936 in Zuilen close to Utrecht, The Netherlands. While studying at the Rijks Hogere Burger School (a former type of Dutch high school) in Utrecht, he was already fascinated by questions from the life science area. But it was in fact his high school teacher in Biology who told him that for a good understanding of biological problems, the ultimate goal to strive for in the life sciences should be an understanding of biology at the molecular level. In fact, already in the early fifties, this teacher, without knowing it, confronted Hans with the concept of structural biology. These discussions between Hans and his teacher were the reasons that Hans decided not to study Biology, but Chemistry.

Hans studied Chemistry from 1953 to 1960 at Utrecht University, and his Master period comprised a major in organic chemistry (with Professor Dr. F. Kögl) and a minor in cell biology (with Professor Dr. M.T. Jansen). This was an unusual combination in those days; cell biology did not even belong to the chemistry course program. In 1960, he started his PhD work at Utrecht University under the supervision of Professor Dr. J.F. Arens, and in 1967, he got his PhD degree in Natural Sciences on a thesis entitled "Neurohypophysial hormones, a comparative study". He was involved in the isolation of the vasopressor and oxytocic principles of the finback whale, compounds identified by him as arginine vasopressin and oxytocin, as well as in the isolation and chemical characterisation of porcine neurophysin. Here, he was already intrigued by the phenomenon of interaction between biomolecules. Furthermore, he paid attention to the evolution of the vertebrate neurohypophysial hormones in relation to the genetic code, leading to a model that could predict primary structures of peptide hormones. For the analysis of amino acids and peptides that occur in the neurohypophysis, associated with the so-called Van Dyke's protein, a novel method was invented for the separation of amino acids and peptides based on the formation of complexes with Cu(II).

In 1968, Hans made one of the major decisions in his scientific life. After his appointment as an Assistant Professor of Bio-Organic Chemistry at Utrecht University, he started a research program on carbohydrates and glycoconjugates. Why did he not continue in the field of neurohypophysial hormones? Why a new topic, without any knowledge of that topic? Why in glycoscience, a field not very popular among protein biochemists? The reason was that by accident, he became involved in the isolation of a glycopeptide from the posterior lobe of pig pituitaries. Within a couple of months, he elucidated the structure of the 17-amino acid peptide backbone which contained an Asn–Ala–Thr sequence. But to elucidate the "glyco" component, that was a real problem. Here, Hans noticed a highly underdeveloped area. And the leading thread for his future became more and more visible to him. As he once stated: "I wanted to understand how carbohydrates function at the molecular level". And this statement still holds true today. I must confess that for some reason, the glycopeptide has never been found again in new extracts of pig pituitaries. Its structure has therefore never been established. Why this finding once and never again? Was it a message from higher spheres, showing Hans the way to go? We will never know. What we do know is that carbohydrate chemistry had taken roots in Utrecht.

So, with his organic chemistry/biochemistry background, Hans became interested in the biological significance of protein-bound carbohydrate chains and their interaction with complementary biomolecules. However, he realized at the

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same time that to gain insight into bioactivity, it is a prerequisite to know primary and three-dimensional (3D) structures. Therefore, in 1969, he initiated a systematic program on the structural analysis of oligosaccharides making use of mass spectrometry and NMR spectroscopy, with emphasis on the glycoprotein glycans. I was in the privileged position to join him as his first PhD student. However, it was not always easy to reach the planned aims. In 1975, we wrote in a grant application to the Dutch Research Council: “In the next years the attention of the group will be mainly focused on the primary analysis of glycoprotein glycans. After this period research will be focused also on the study of the interaction between carbohydrate chains and proteins which recognise the glycans”. To be honest, it turned out that we had to wait more than 20 years before real interaction studies could be carried out at the molecular level. We learned that glycoscience is not an area for quick wins.

Back to 1969. By means of electron-impact mass spectrometry, the identification of, at first, relatively simple oligosaccharide derivatives was carried out resulting in fragmentation rules that indicate the type of monosaccharide constituents (including ring form), the position of the glycosidic linkages and the sequence. Here, in particular, I must mention our long-term collaboration with Professor Dr. Roland Schauer on sialic acid analysis using GLC/EI-MS. We also explored the power of NMR spectroscopy for defining primary structures by means of ^{1}H- and ^{13}C-NMR spectroscopy of oligosaccharides and oligosaccharide derivatives. The combination of MS and NMR analyses was demonstrated to be of the utmost value for the characterisation of carbohydrates. PhD students involved in these early studies were, besides myself, Professor Dr. Johan Haverkamp and Dr. Dick Streefkerk. Twice a year, during weekends, on a small island in the north of The Netherlands, we taught each other carbohydrate chemistry. Although our wives always joined us, we kept our scientific hours in a strict way. They had their own social program. But of course, we also had our joint social hours, playing games, making puzzles, drinking, and walking along the beach. Fantastic memories.

On the introduction of 360 MHz ^{1}H-NMR spectroscopy to The Netherlands in 1975, Hans, already fascinated by the great possibilities of NMR spectroscopy as a non-destructive analysis method, immediately realized the importance of this new development for the analysis of carbohydrate chains of glycoproteins. In collaboration with several international groups, the Utrecht group (starting with Dr. Bert Dorland as PhD student) developed an NMR structural reporter-group concept which allowed the translation of a one-dimensional (1D) ^{1}H-NMR spectrum into a primary structure; this method was applied to the analysis of N-linked carbohydrate chains of glycopeptides and of released oligosaccharides/oligosaccharide alditols from *N*-glycoproteins. Later, a second structural-reporter-group concept based on similar principles was developed for oligosaccharide alditols generated from *O*-glycoproteins (started with Dr. Herman van Halbeek as PhD student). Hundreds of structures could be unravelled unambiguously. These concepts could be applied not only to the structural analysis of isolated carbohydrate chains, but also in studies focused on the biosynthesis (e.g. branch specificity of glycosyltransferases) and catabolism (e.g. sialidosis) of glycoprotein glycans. All these studies could not have been carried out without many international collaborations. At the start of the NMR program in 1975, the collaboration with the Lille group of Professor Dr. Jean Montreuil (Dr. Geneviéve Spik, Dr. Bernard Fournet, Dr. Gérard Strecker) was of the utmost importance. Major results in the NMR characterisation of glycoprotein glycans were also reached in collaboration with the groups of Professor Dr. Karl Schmid (Boston), Professor Dr. Philippe Roussel (Lille), Professor Dr. Dirk van den Eijnden (Amsterdam), and Profesor Dr. Leo März (Vienna). The intensive collaborations with Professor Dr. Kazuyuki Sugahara (Kobe) in the proteoglycan field also have to be mentioned, as well as the sabbatical period Professor Dr. Harry Schachter spent in our laboratory, teaching us to play with glycosyltransferases of lower animals. In recent years, the technology has grown even more powerful by the addition of various two-dimensional NMR techniques. The elucidation of the type and precise position of non-carbohydrate substituents in glycoprotein-derived glycans was made feasible by means of newly developed NMR techniques, as demonstrated for alkyl, acyl, phosphate and sulfate groups. To determine the position of phosphate groups, heteronuclear 1D ^{1}H{^{31}P} Relayed Spin-Echo Difference Spectroscopy was developed (Dr. Pieter de Waard as PhD student). Overall, the knowledge of primary structures of glycoprotein glycans has contributed highly to the basis for international research programs focused on glycobiological aspects, and stimulated organic carbohydrate chemistry activities. The generation of the knowledge of the regular biosynthetic pathways forms the basis of the modern MS approaches for structural analysis. Several detailed NMR studies on recombinant glycoproteins, glycoprotein-related inborn errors of metabolism, and glycoproteins of lower animals, bacteria and plants were carried out by many PhD students of the Utrecht group.

Besides the primary structure analysis of glycoprotein glycans, primary structure analysis of bacterial and plant polysaccharide analysis also had the attention of Hans. Bacterial polysaccharides comprised mainly those excreted by lactic acid bacteria. Because lactic acid bacteria are food grade, these viscous exopolysaccharides are of interest for the food industry. Systematic NMR structural studies of partial oligosaccharide elements of arabinoxylans generated by the action of endoxylanases (collaborations with Unilever Vlaardingen, The Netherlands, and the group of Professor Dr. Fons Voragen, Wageningen University, The Netherlands) lead to the defining of structural-reporter groups for the characterisation of arabinoxylans of various biological origins and for the elucidation of the substrate specificity of different endoxylanases. Even cellulose and starch research programs were carried out under the supervision of Hans Vliegenthart.

Part of the glycoscience program of Hans has always been focused on organic synthesis of oligosaccharide fragments of glycoprotein glycans and polysaccharides. Started in 1973, because reference compounds were needed for the structural studies, the program has evolved over the years more and more into the direction of investigations directed to carbohydrate-based vaccines and diagnostics as well as (mimic) substrates for interaction studies. In this context, the long-term fruitful collaboration with the group of Professor Dr. András Liptak (Debrecen, Hungary) has to be mentioned. Typical examples comprise the preparation of oligosaccharide (pure)–protein conjugates, whereby the pure oligosaccharides, being fragments of capsular polysaccharides of *Streptococcus pneumoniae* serotypes, are synthesised along chemical/enzymatic routes from monosaccharides. These conjugates were tested for their immunogenicity in animal models, and have already proven their potential as a new generation of vaccines.

From the beginning of the NMR studies, the 3D structure of carbohydrate chains was part of the program. During the last 10 years, the Utrecht group paid much attention to the 3D structure of human chorionic gonadotropin (collaboration with the protein NMR group of Professor Dr. Rolf Boelens at Utrecht University) taking into account its glycosylation pattern. The α-subunit has been studied in high detail (started in 1990 by Dr. T. de Beer as a PhD student). It turned out that part of the polypeptide backbone (the Asn52-containing part) is highly disordered, a phenomenon that did not exist in the α–β subunits complex as reported by others using X-ray studies. Recently, attention has also been paid to the development of general calculation methods for constructing conformational models in water of heteropolysaccharides built up from repeating units (started by Dr. Albert van Kuik). In addition to NMR as a method to study interactions between oligosaccharides and complementary biomolecules, the potential of surface plasmon resonance (started by Dr. Simon Haseley) has also been evaluated recently. Fascinating model studies have been carried out by these methods focused on understanding the cellular aggregation of sponges, for which self-recognition of defined carbohydrate epitopes is essential. The latter study is a typical example of the Utrecht approach, whereby structural analysis of the epitopes as part of the relevant sponge proteoglycans, organic synthesis of the established epitopes, and interaction studies with the synthetic epitopes come together.

At this point, I will stop discussing examples that illustrate the interest of Hans in carbohydrate research and glycobiology. It is a broad interest, covering many aspects of the glycoscience field. For those interested in more details, I refer to the list of PhD theses (below). But the interests of Hans go even further. For him certainly holds the statement: "A man should not live with carbohydrates alone. Each day a cup of unsaturated fatty acids mixed with a spoon of lipoxygenase keeps the brain fresh". A contact with Professor Dr. J. Boldingh in 1966 brought him into contact with the lipoxygenases. These enzymes, widely distributed in nature, occurring in plants as well as in animals, catalyse stereospecifically the deoxygenation of fatty acids containing 1*Z*,4*Z*-pentadiene moieties like linoleic acid, linolenic acid and arachidonic acid, to *E*,*Z*-conjugated monohydroperoxides. Over the years, together with Professor Dr. Gerrit Veldink, Hans was involved in the unraveling of many aspects of these enzymes.

Finally, some details about the scientific career of Hans in terms of awards, distinctions, and (inter)national functions. After having been appointed as full professor of Bio-Organic Chemistry at the Faculty of Chemistry of Utrecht University in 1980, he was chairman of the Department of Bio-Organic Chemistry during the period 1988–2001. He was the stimulating person behind the foundation of the Bijvoet Center, a joint institute of the Netherlands Foundation for Chemical Research and Utrecht University, and acted as Research Director during the period 1988–2000. The Bijvoet Center comprises the departments of Bio-Organic Chemistry, NMR Spectroscopy, Crystal and Structure Chemistry, Biomolecular Mass Spectrometry and Biochemistry of Membranes. Hans was Dean of the Faculty from 1985 to 1989 and is Dean of the Faculty from 2000 to 2003. He is a member of the Royal Netherlands Academy of Arts and Sciences, a foreign member of the Royal Swedish Academy of Sciences, Doctor Honoris Causa of the L. Kossuth University (Debrecen, Hungary), Doctor Honoris Causa of the Université des Sciences et Techniques de Lille (Villeneuve d'Ascq, France), Doctor Honoris Causa of Stockholm University (Stockholm, Sweden), Knight of the Order of the Lion of the Netherlands. In 1994, he received the Claude S. Hudson Award in Carbohydrate Chemistry from the American Chemical Society. He is the Netherlands representative in the International Carbohydrate Organization, in the International Glycoconjugate Organization, and in the European Carbohydrate Organization. He was chairman of the Organizing Committees of the XIIth International Carbohydrate Symposium in Utrecht (1984), the 9th European Carbohydrate Symposium in Utrecht (1997), and the XVIth International Symposium on Glycoconjugates in The Hague (2001). Among many national and international functions, he was member of several Nomenclature Committees (IUBMB and IUPAC), and a member of the Scientific Advisory Board of the Science Frontier Program at the Riken Institute (Wako, Japan). He currently serves on the editorial boards of Carbohydrate Research, Glycoconjugate Journal, Carbohydrate Letters, Trends in Glycoscience and Glycotechnology, Advances in Carbohydrate Chemistry and Biochemistry, Biochemical Journal, Journal of Biomolecular NMR, and European Journal of Organic Chemistry. He is (co)author of over 650 scientific publications and supervised over 75 PhD theses.

On 6 April 2001, Hans reached the age of 65 years, the age for official retirement in The Netherlands. It is quite clear: for him, this does not mean the end of his scientific life. As a Professor emeritus, he will certainly remain active in the glycoscience field. Believe me! For me and for many of his former co-workers, he is and will be always the inspiring scientist, dynamic and flexible in his approaches to solve a problem.

List of Glycoscience theses

J.P. Kamerling (1972)—Structure determination of oligosaccharides; an investigation of pertrimethylsilyl derivatives by mass spectrometry and PMR spectroscopy.

D.G. Streefkerk (1973)—Conformational studies on pertrimethylsilyl derivatives of mono- and oligosaccharides by high resolution PMR spectroscopy.

J. Haverkamp (1974)—A study of permethylated carbohydrates by ^{1}H and ^{13}C NMR spectroscopy.

W.A.R. van Heeswijk (1977)—The synthesis of $(1 \rightarrow 5)$-linked disaccharides.

L. Dorland (1979)—Structure determination of the complex carbohydrate chains of glycoproteins by 360 MHz ^{1}H-NMR spectroscopy.

A.M.J. Fichtinger-Schepman (1980)—Structural studies on the polysaccharide associated with the coccoliths of the alga *Emiliania huxleyi* (Lohmann) Kamptner.

B.L. Schut (1981)—Determination of the primary structure of the carbohydrate chains of horse pancreatic ribonuclease.

D.J.M. van der Vleugel (1981)—The synthesis of $(2 \rightarrow 6)$-linked sialodisaccharides.

H. van Halbeek (1982)—500-MHz ^{1}H-NMR spectroscopy as a tool in the structural analysis of the carbohydrate chains of glycoproteins.

C.M. Deijl (1985)—Studies on sialidase and aldolase from *Clostridium perfringens.*

J.H.G.M. Mutsaers (1986)—Structural analysis of the carbohydrate chains of glycoproteins by 500 MHz ^{1}H-NMR spectroscopy.

J.A. van Kuik (1987)—Structural determination of the carbohydrate chains from arthropod and mollusc hemocyanin by means of 500-MHz ^{1}H-NMR spectroscopy.

S.A.M. Korrel (1988)—Structural studies on the N- and O-linked carbohydrate chains of human platelet glycoprotein 1b.

J.E.G. van Dam (1988)—Contribution to the development of a semi-synthetic vaccine to *Streptococcus pneumoniae*. Preparation of neoglycolipids with repeating oligosaccharide determinants of capsular polysaccharides.

J. van Pelt (1988)—Isolation and structural analysis of sialyloligosaccharides from sialidosis and galactosialidosis fibroblasts, placenta and urine.

J.N. Breg (1989)—Application of 1D- and 2D-NMR techniques for the structural studies of glycoprotein-derived carbohydrates.

J.B.L. Damm (1989)—A general strategy for the structural analysis of glycoprotein-derived carbohydrate chains.

J.B. Bouwstra (1989)—Conformational studies on *N*-glycoprotein related carbohydrate chains.

T.M. Slaghek (1989)—Synthesis of oligosaccharide fragments of the capsular polysaccharides of *Streptococcus pneumoniae* types 6A and 6B.

P. de Waard (1990)—Structural studies on carbohydrates by means of 1D, 2D, and 3D NMR spectroscopy.

A.M.P. van Steijn (1990)—Synthesis of oligosaccharide fragments of capsular polysaccharides from various *Streptococcus pneumoniae* strains.

R.A. Hoffmann (1991)—Structural characterisation of arabinoxylans from white wheat flour.

G.J. Gerwig (1991)—Characterization of O-linked carbohydrate chains of glycoproteins in bacterial cellulase complexes.

K. Hård (1991)—Structural analysis by ^{1}H-NMR spectroscopy of carbohydrates from naturally occurring and recombinant-DNA human glycoproteins.

B.R. Leeflang (1991)—Conformational analysis of oligosaccharides.

E.A. Kragten (1991)—Structural analysis of carboxymethyl- and sulfoethylcelluloses.

M. Gruter (1992)—Structural characterization of polysaccharides from lactic acid bacteria.

F.A.W. Koeman (1992)—Synthesis of oligosaccharide fragments of the capsular polysaccharide of *Streptococcus pneumoniae* types 8 and 14.

J.G.M. van der Ven (1993)—Synthesis of xylose-containing oligosaccharides occurring in *N*-glycoproteins.

H. Voshol (1993)—The role of cell surface glycoconjugates in the recognition of target cells by human natural killer cells and lymphokine-activated killer cells.

C.H. Hokke (1993)—Structure determination of glycoprotein glycans. Sialylation patterns and *N*-acetyllactosamine repeats in recombinant human erythropoietin, equine chorionic gonadotropin and porcine zona pellucida glycoproteins.

J.P.M. Lommerse (1994)—Structural and conformational analysis of xylose-containing glycoprotein *N*-glycans.

A.A. Bergwerff (1994)—Characterization of the carbohydrate chains of biotechnologically produced glycoproteins of therapeutical interest.

H. Mulder (1995)—Studies on the biosynthesis of *N*-glycoprotein glycans in the connective tissue of the freshwater snail *Lymnaea stagnalis.*

J.A.L.M. van Dorst (1995)—Exploring the substrate specificity of sialyltransferases. Synthesis of Hex1-4GlcNAcβ1-2Manα1-O$(CH_2)_7CH_3$ sequences and their application as acceptor probes.

J.J.G. van Soest (1996)—Starch plastics: structure–property relationships.

A.L.J. de Beer (1996)—Structural studies of human ribonuclease U_S and human chorionic gonadotropin α-subunit. Application of NMR spectroscopy to intact glycoproteins.

G.W. Robijn (1996)—Structural studies on exopolysaccharides produced by lactic acid bacteria.

P.B. van Seeventer (1996)—Synthesis of epitope-related fragments of the *N*-glycans of human Tamm-Horsfall glycoprotein and human lutropin.

M.J.L. Thijssen (1996)—Synthesis of oligosaccharide fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 6B.

C.W.E.M. van Zuylen (1997)—Structural features of free and covalently bound glycoprotein glycans of bovine peripheral myelin glycoprotein P0 and human chorionic gonadotropin α subunit.

J.W. Timmermans (1997)—Conformational analysis and constitution of inulin and methylated derivatives.

K.M. Halkes (1997)—Synthesis of oligosaccharide fragments of hyaluronic acid and *Schistosoma mansoni* antigens for biological assays.

P.H. Kruiskamp (1998)—Conformational analysis of model compounds to predict physical properties of cellulose and cellulose acetates.

J.J.M. van Rooijen (1998)—Studies on the glycans of human Tamm-Horsfall glycoprotein and human amniotic fluid transferrin.

C.T.M. Fransen (1999)—Structural analysis of soy bean polysaccharides and transgalactosylation products from lactose.

C.J.M. Stroop (1999)—Structural analysis of N-linked carbohydrate chains in secreted EGF receptor and α_2-macroglobulin.

Y.E.M. van der Burgt (1999)—Structural studies on methylated starch granules.

H.J. Vermeer (2000)—Synthesis of oligosaccharides and their use in biomolecular interactions—immunological studies related to the parasite *Schistosoma mansoni* and self-recognition phenomena of the sponge *Microciona prolifera.*

E.J. Faber (2000)—Investigation of the structure of exopolysaccharides produced by lactic acid bacteria.

P.J.A. Erbel (2000)—Structural studies on effects of glycosylation on the conformation and stability of human chorionic gonadotropin; development of a structure calculation method for intact glycoproteins.

R. Gutiérrez Gallego (2001)—Enzymatic synthesis and biomolecular interactions of glycoconjugates.

D.J. Lefeber (2001)—Defined neoglycoproteins as candidate vaccines against *Streptococcus pneumoniae* type 3.

A.L.M. Smits (2001)—The molecular organisation in starch based products; the influence of polyols used as plasticisers.

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