

Clarke, Modet & C^o

COLOMBIA

P-1922

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. **06-030.455**

Solicitud de patente a nombre de **NOVARIS AG.**

Yo, DILIA MARIA RODRIGUEZ D'ALEMAN identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, obrando como apoderada sustituta de acuerdo con la sustitución presentada el 21 de septiembre de 2010, de la sociedad **NOVARIS AG.**, domiciliada en Basilea, Suiza, solicitante de la patente de la referencia, en respuesta al oficio No. 13179 notificado por fijación en lista el 26 de agosto de 2011, relacionado con el concepto técnico de fondo No. 2494, nos permitimos manifestar lo siguiente:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio donde se realizó la siguiente modificación.

Se especificó dentro de la nueva reivindicación 3, que los polinucleótidos que codifican el anticuerpo humanizado de las reivindicaciones 1 ó 2 son aquellos que comprenden un polinucleótido de SEQ ID No. 34 ó un polinucleótido de SEQ ID No. 35 y un polinucleótido de SEQ ID No. 13 ó un polinucleótido de SEQ ID No. 14 ó 33.

El sustento para esta modificación se encuentra desde la página 26, línea 22 hasta la página 33, línea 4 de la memoria descriptiva.

Sobre este particular nos permitimos manifestar que las anteriores modificaciones no involucran ampliación alguna de la materia originalmente presentada y se dan como respuesta a las objeciones expuestas por el señor examinador en su concepto técnico No. 2494.

Teniendo en cuenta el concepto técnico emitido por el Señor Superintendente Delegado para la propiedad intelectual GianCarlo Marcenaro Jiménez a la doctora Ana Maria Castro Wey, que da alcance a las comunicaciones No. 09-001524 y 09-012566, no se presenta una tasa adicional, toda vez que la solicitud fue presentada con anterioridad al 1 de enero de 2009.

2. Claridad

En relación con la antigua reivindicación 3, el señor examinador podrá corroborar que los polinucleótidos mencionados en tal reivindicación se encuentran ahora caracterizados a partir de su secuencia nucleótida de acuerdo con lo establecido en la memoria descriptiva y definen el producto de la invención de acuerdo con la amable sugerencia del señor examinador.

De otra parte, en relación con la objeción de claridad de las reivindicaciones 4 a 6 nos permitimos manifestar que las mismas son claras, ya que por ejemplo el vector definido en la reivindicación 4, se encuentra definido por las características técnicas que ahora se establecen en la reivindicación 3 de la cual dependen. Lo mismo sucede con las reivindicaciones 5 y 6. Así las cosas, respetuosamente solicitamos sean retiradas tales objeciones toda vez que las reivindicaciones 4 a 6 se encuentran definidas por sus características técnica esenciales.

3. Nivel Inventivo

Con respecto al argumento del señor examinador relacionado con la carencia de altura inventiva de la invención en estudio en vista de las enseñanzas de WO02/072832 (D1) y el artículo de Dunn-Walters y colaboradores (D2). Respetuosamente nos permitimos manifestar lo siguiente:

Consideramos que la Oficina de Patentes acierta en pensar que la única diferencia entre los anticuerpos de la invención reclamada y aquellos expuestos en D1 es la mutación N73A en la secuencias VH, esto es, donde un residuo de asparagina ha sido deliberadamente mutado hacia un residuo de alanina. No obstante, consideramos también que la Oficina de Patentes no es acertada en formular la objeción de nivel inventivo basada en tal hecho.

En efecto, ese despacho considera que el problema técnico que se pretenden resolver en la presente invención es el de producir un anticuerpo anti CD45RO/RB que incluye un sitio de N-glicosilación. Respetuosamente nos permitimos manifestar que esto no es correcto. De hecho, el anticuerpo de la presente invención ahora carece del residuo de asparagina en la

posición 73. La razón por la cual el solicitante deliberadamente ha removido dicho residuo y lo ha reemplazado con un residuo de alanina es como se explica a continuación.

Podría ser argumentado por la mayoría de los fabricantes, si ellos son de anticuerpos o sustancias similares, que existe una gran necesidad de eliminar a un alto grado posible de éxito, la variabilidad existente en la producción entre distintos lotes. Los anticuerpos terapéuticos no son la excepción al respecto. La invención, como se refleja a través de la reivindicación 1, hace referencia al hallazgo de un *sitio de glicosilación parcialmente críptico o enigmático* en la región variable de los anticuerpos reportados en D1. Este sitio introduce en el proceso de fabricación, una fuente potencial de variabilidad en el producto final que es tanto innecesario como indeseable. Entonces, resulta claramente ventajoso poder remover o retirar este *sitio de glicosilación parcialmente críptico o enigmático* una vez encontrada su existencia y determinarse si será innecesario para su uso final. Sin embargo, de ninguna manera es cierto que tal sitio está identificado simplemente por la “lectura” de la secuencia de la región variable. Si el sitio se encuentra glicosilado o no, es un asunto de observación experimental.

Lo anterior se puede ver reflejado en el Artículo adjunto (Anexo 1) Bedford Colin y colaboradores “Glycomics: From glycobiology to Diagnostics and Therapeutics” Drug News Perspect 19(3), Abril de 2006, documento que no es técnica anterior, pero el cual proporciona evidencia de lo que el campo científico debe enfrentar en estos aspectos. En este sentido, amablemente dirigimos la atención del señor examinador a la página 170, al párrafo del medio en la columna de la mitad::

“The challenges posed by biopharmaceutical glycosylation are significant. In some cases even small changes in cell culture conditions during production can cause aberrant glycosylation, which may or may not be recognized during final QC.”

El resto del párrafo se encuentra luego explicando algunos de los problemas actuales que deben ser enfrentados en el campo científico debido a la glicosilación aberrante, principalmente las fallas “falsas” y “verdaderas” en los lotes, la afectación de la seguridad y la eficacia no estandarizada. En consecuencia, el campo científico reconoce que, incluso pequeños cambios pueden producir diferencias significativas y estas diferencias pueden o no ser reconocidas. Así las cosas, respetuosamente nos permitimos manifestar que el señor examinador no debe enfocarse sobre la diferencia estructural entre el anticuerpo reclamado y los anticuerpos divulgados en D1, sino considerar la significancia o importancia práctica de tal diferencia como se refleja en el campo científico en sí.

En ese orden de ideas, por supuesto que no existe sugerencia en D1 relacionada con la eliminación del sitio de glicosilación y la ventaja está dada entonces en hacerlo de tal manera que elimine una variable en la fabricación del producto final. Como se explica en el Artículo de Bedford y colaboradores, el campo técnico reconoce por sí mismo que no siempre se detectan estos pequeños, pero finalmente grandes problemas significativos, incluso así sea en la parte final del control de calidad (QC). Por consiguiente, la presente invención es inventiva sobre las enseñanzas de la técnica anterior.

En consecuencia, de manera atenta solicitamos a ese despacho conceder el privilegio de patente de invención para la solicitud en estudio o si es el caso, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional. De igual forma, si el examinador considera que subsisten razones para objetar la presente invención ruego expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que mi mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *"habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión que será motivada al menos en forma sumaria si afecta a particulares"* y *"(.) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso"*, respectivamente (El subrayado es nuestro).

Señor Superintendente,


DILIA MARIA RODRIGUEZ D'ALEMAN
CC. 41.654.882
TP 20824 CSJ

ANEXO 1

Artículo de Bedford Colin y colaboradores titulado “Glycomics: From glycobiology to Diagnostics and Therapeutics” Drug News Perspect 19(3),
Abril de 2006

MEETING REPORTS

*Highlights from the Royal Society of Chemistry,
Biotechnology Group Symposium, held December 12–13, 2005,
in London, U.K.*

Glycomics: From Glycobiology to Diagnostics and Therapeutics

by Colin Bedford,
Tony Cass,
Irene François
and Stephen Harding

Carbohydrates are ubiquitous, and their structural diversity allows them to perform crucial functions in a plethora of biological processes including cell–cell recognition, cell–cell communication, protein regulation, tissue development and immune response. Complexity, low bioavailability, difficult purification and analysis are just some of the reasons why the field of glycoscience is in the early stages of development. However, biological and chemical breakthroughs are being made that will overcome these drawbacks and open up this very important area of research. The Consortium for Functional Genomics (CFG; <http://www.functionalglycomics.org>), a large research initiative with 230 participating investigators worldwide, was set up to understand the role of carbohydrate–protein interactions at the cell surface in cell–cell communication.

Summary

The Royal Society of Chemistry Biotechnology Group and Chemical Biology Forum held a two-day symposium on December 12–13, 2005, in London. The meeting was designed to give an overview of the exciting new technologies being applied to study complex carbohydrates from their sequence analysis, characterization and function through to the development of novel pharmacological approaches to diagnose and alleviate polysaccharide-mediated diseases. The meeting, which also included a poster session, highlighted the multidisciplinary nature of the research and development and the exciting advances being made in this field. © 2006 Prous Science. All rights reserved.

One of the goals of the CFG is to generate resources for the use of investigators in studying the complex biology that governs the interactions of glycan-binding proteins (GBPs) and their ligands in mediating cell communication. Core resources, to date, include: carbohydrate compound library, GBP, antibodies, glyco-gene chip analysis, mouse lines/transgenics, analytics and glycan microarray screening.

The Royal Society of Chemistry Biotechnology Group and Chemical Biology Forum, held December 12–13, 2005, in London, opened with an excellent overview by Prof. James Paulson (The Scripps Research Institute, La Jolla, California, U.S.A.)

on the challenges being faced in decoding the cellular glycome. With the upcoming completion of the genomic sequences of humans and several other commonly studied model organisms, even more spectacular gains in the understanding of biological systems are anticipated. However, there is often a tendency to assume the following extension of the central paradigm: DNA → RNA → Protein → Cell → Organism. In actual fact, creating a cell requires two other major classes of molecules: lipids and carbohydrates.

All cells and many macromolecules in nature carry a dense and complex array of covalently attached sugar chains (called oligosaccharides

TABLE I. MAMMALIAN GLYCAN-BINDING PROTEINS (GBP)

GBP FAMILY	LIGANDS	FUNCTIONS
C-type lectins	Various	Cell adhesion Glycoprotein clearance Innate immunity
Siglecs (1-type lectins)	Sialosides	Cell signaling Cell adhesion
Galectins	Galactosides	Extracellular matrix cross linking
CD1/TCR	Glycolipids/Glycopeptides	Innate immunity Acquired immunity
M-type lectins	High mannose N-linked	Quality control in protein folding in endoplasmic reticulum
L-type lectins	Various	Protein sorting in endoplasmic reticulum
P-type lectins	Man-6-phosphate	Lysosomal enzyme sorting (post-golgi)
R-type lectins	Various	Enzyme targeting Hormone turnover

or glycans). Glycans are positioned to mediate the flow of information and recognition among cells. They are increasingly recognized for their roles in protein folding, pathogen recognition of host cells, antibody/antigen interactions, inflammation, signal transduction, apoptosis, fertilization, tissue formation, cell adhesion and intracellular trafficking. GBPs play a significant role in decoding the information content of glycans by recognizing and specifically binding to glycosylated protein and lipid ligands. Three major families of mammalian GBPs are involved in cell surface biology through recognition of glycan ligands: C-type lectins, siglecs and galectins (Table I).

Prof. Paulson (Director, CFG) highlighted the siglecs, of which there are 11 members. Siglecs are a subgroup of the Ig super family that has in common an NH₂-terminal Ig domain that binds sialic acid-containing carbohydrates of glycoproteins as a ligand. CD22 (Siglec-2), a co-receptor and regulator of B-cell receptor (BCR) signaling, is a member of the siglec family that has a high specificity for sialoside ligands containing the sequence Sia α 2-6Gal that often terminates N-linked carbohydrate groups of glycoproteins. The sequence recognized by CD22 is abundant on B and T lymphocytes. The precise ligand interactions that modulate CD22 function are not well understood, but both

cis (B-cell) and *trans* (adjacent cell, e.g., T-cell) glycoprotein ligands appear to modulate CD22 function as a regulator of BCR signaling.

These interactions are extremely complex and compounded by the absence of well-defined glycan libraries, and analytical screening methods have limited analysis of their specificities and elucidation of their biological function. The CFG has developed a glycan array¹ format that utilizes standard robotic microarray printing technology to couple amine functionalized glycans to commercially available amine-reactive *N*-hydroxysuccinimide-activated glass slides. This allows rapid covalent coupling of amine functionalized glycans or glycoconjugates. The array comprises more than 200 synthetic and natural structurally defined terminal sequences of glycoprotein and glycolipid glycans developed for an ELISA-based array. The utility of this array has been demonstrated for analysis of GBP specificity for most of the major classes of GBP and has potential in the development of glycotherapeutics. For example, it raises the possibility for identifying glycan-specific antibodies for the diagnosis of microbial infections, cancer and autoimmune diseases.

N-linked glycans play an important role in protein folding, a topic covered by Prof. Ari Helenius (Institute of Biochemistry, Swiss Federal

Institute of Technology, Zurich, Switzerland). The endoplasmic reticulum (ER) is dedicated to the import, folding and assembly of all proteins that travel along or reside in the secretory pathway of eukaryotic cells. Around half of human genes encode glycoproteins and ~90% of these are N-linked. Newly synthesized proteins are N-glycosylated and by default form disulfide bonds in the ER, but not elsewhere in the cell. In his presentation, Prof. Helenius discussed which features distinguish the ER as an efficient folding factory, how the ER monitors its output and how it disposes of folding failures.

ER-associated protein degradation (ERAD) eliminates misfolded or unassembled proteins from the ER. ERAD targets are selected by a quality control (QC) system within the ER lumen and are ultimately destroyed by the cytoplasmic ubiquitin-proteasome system. The spatial separation between substrate selection and degradation in ERAD requires substrate transport from the ER to the cytoplasm by a process termed dislocation. Advances in various aspects of ERAD and discussions on new findings on how substrate dislocation is achieved were summarized. The unfolded protein response leads to ER proliferation and is implicated in many human diseases.

Glycoprotein glucosyltransferase is a key component of the glycopro-

tein-specific folding and QC system in the ER.² By exclusively re-glucosylating incompletely folded and assembled glycoproteins, it serves as a folding sensor that prolongs the association of newly synthesized glycoproteins with the chaperone-like lectins calnexin (Cnx) and calreticulin (Crt).

Nascent and newly synthesized glycoproteins enter the Cnx/Crt cycle when two out of three glucoses in the core N-linked glycans have been trimmed sequentially by ER glucosidases I (GI) and GII.³ GI removes the outermost glucose immediately after glycan addition. However, although GII associates with singly glycosylated nascent chains, trimming of the second glucose only occurs efficiently when a second glycan is present in the chain. Consistent with a requirement for multiple glycans to activate GII, pancreatic RNase in live cells needs more than one glycan to enter the Cnx/Crt cycle. Thus, whereas GI trimming occurs as an automatic extension of glycosylation, trimming by GII is a regulated process. By adjusting the number and location of glycans, glycoproteins can instruct the cell to engage them in an individually determined folding and QC pathway.

Prof. Ten Feizi (The Glycosciences Laboratory, Imperial College, London) next described an elegant approach that utilizes oligosaccharide microarrays to decipher the glyco code and to challenge and develop the knowledge base of carbohydrate-binding proteins in the proteome. The diverse oligosaccharides that "decorate" glycoproteins, glycolipids, proteoglycans and polysaccharides are potentially a vast source of information, and could harbor a "glyco code" that is waiting to be deciphered in various contexts of biological and medical importance.

Since it is impossible to clone oligosaccharides, Prof. Feizi and her team have developed a carbohydrate microarray platform using lipid-linked oligosaccharide probes that can be uniquely generated from naturally

occurring sequences of glycoproteins, glycolipids, proteoglycans, polysaccharides, as well as chemically synthesized oligosaccharides and glycolipids. This approach has its foundations in the neoglycolipid (NGL) technology that was introduced in 1985 and further refined.^{4,5} It was devised to address the need for a microscale technology to present oligosaccharides in a clustered form for studies of carbohydrate-protein interactions, in particular assignments of the ligands of carbohydrate-binding receptors and the epitopes recognized by antibodies. The NGL approach, originally developed using sequence-defined O-glycans for antibody binding studies, has also been shown to be a powerful means of generating other types of oligosaccharide probes: from N-glycans, fragments of glycosaminoglycans and polysaccharides, and from diverse chemically synthesized oligosaccharides.

Glycolipids, both naturally occurring and synthetic, are included in the repertoire of probes. A key development has been to combine carbohydrate-protein interaction studies with mass spectrometry *in situ* to gain information on monosaccharide composition, sequence and branching pattern, as well as sulfate and phosphate substitution of the immobilized oligosaccharide probes at low picomole levels. NGL probes generated as mixtures from whole cells and tissues and probed with carbohydrate-recognizing proteins can be deconvoluted by TLC combined with mass spectrometry. "Designer" microarrays that are unique to this platform are aiding assignments of ligands for receptors that regulate leukocyte activation in the innate immune system.⁶

Prof. Anne Dell (Imperial College, London) described ultra-high sensitivity mass spectrometric strategies used to define the primary structures of glycoproteins in complex mixtures. These techniques, which include MALDI-MS and nano-electrospray (ES)-MS/MS, underpinned by knowledge of biosynthetic pathways, are

revolutionizing structural glycobiology in the field of glycomics and glycoproteomics. Determining the glycan repertoire in cells, tissues and organs is a first step to defining function. Prof. Dell and her team have devised MS strategies that employ MALDI mapping and ES-MS/MS sequencing of permethylated N- and O-glycans that enable the glycome of cells (use $\sim 10^6$ cells), tissues and organs to be examined and the glycoforms of individual glycoproteins to be identified.

The strategies were illustrated by data from collaborative research aimed at defining the glycosylation repertoire of model organisms and establishing the roles of glycans in cell-cell communication. Current research projects include glycomics and glycoproteomic studies of normal and knockout mice, parasitic nematodes, bacterial pathogens, mucins associated with cancer and cystic fibrosis, and glycoproteins implicated in mouse and human fertilization and reproduction. For example, in glycoproteomic studies, nanospray and on-line LC-ES-MS/MS technology has been used to yield important new information on the O-glycosylation of zona pellucida glycoproteins⁷ from normal and transgenic mice (the carbohydrate groups on the zona pellucida glycoproteins function as sperm receptors and binding of sperm to the zona pellucida is a receptor-ligand interaction with a high degree of species specificity) and the N-glycosylation of a variety of novel bacterial glycoproteins.

The development of a high-throughput glycosylation-monitoring technique for use in biopharmaceutical production was presented by Dr. Andrew Sutcliffe (Procognia Limited, Maidenhead, U.K.). Procognia uses protein arrays to understand the function of proteins and their post-translational modifications, particularly glycosylation, which impacts most biological properties. Four main factors influence glycosylation: host cell; clone; process parameters including culture medium; and fermentor

design. Glycosylation is also difficult to analyze, monitor and control, and biopharmaceutical batches may differ in glycosylation pattern.

Procognia's innovative technology analyzes the glycan structures of intact glycoproteins. The analysis is performed directly on crude samples in growth media, thus obviating the need for time-consuming clean-up processes and degradation steps. The U-c fingerprint technology allows the analysis of a glycoprotein to be performed in ~4 hours, and ~20 samples (sample size nM concentrations) can be run in parallel. The technology is applicable to all stages of clone selection and optimization, process development, manufacturing and QC. The method is based on lectin arrays (28 lectins) grouped with overlapping recognition specificities. The native glycoprotein, either purified or in growth media, is incubated with the lectin array, and binding is detected by applying one or more labeled probes. The probes can be lectins or entities that recognize the protein moiety such as antibodies. The resulting fingerprint is characteristic of the glycan profile of the glycoform mixture and is highly sensitive to changes in this profile. Procognia has constructed a database of lectin-glycan recognition rules using a large dataset of carefully chosen, well-characterized glycoproteins. Procognia uses this database and its proprietary algorithms to give quantitative analysis of glycan structures within 10% accuracy.

Next, Prof. Robert Nash (MNL Pharma, Aberystwyth, Wales) discussed the therapeutic potential for imino sugars.⁸ Imino sugars are monocyclic and bicyclic polyhydroxylated derivatives of the following ring systems: pyrrolidine; piperidine; pyrrolizidine; indolizidine; and nor-tropane. MNL Pharma is a drug discovery and development company whose main interest is in the field of immunotherapy. The company is building a franchise in imino sugar technology and has accrued a large library of both naturally occurring

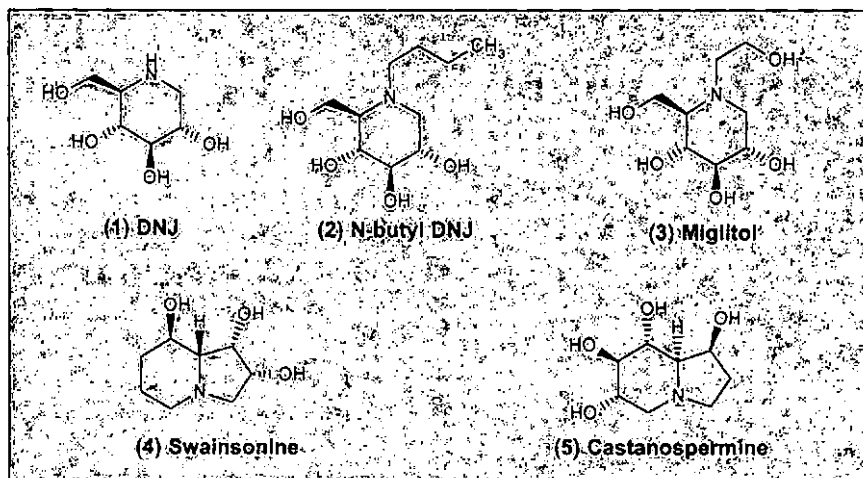


Fig. 1. Imino sugars.

imino sugars and synthetic analogues. Imino sugars are highly water soluble compounds and as such have been missed by drug discovery groups who tend to use fairly nonpolar solvents when extracting natural products from plants and soil samples. One of the first examples isolated is 1-deoxynojirimycin (DNJ; Fig. 1), a potent inhibitor of α -glucosidases, found in mulberry and other plant sources. Its *N*-butyl derivative is the oral drug *Zavesca* (Fig. 1), which is used for treating Gaucher's disease. The related derivative *Miglitol* (*N*-hydroxyethyl DNJ; Fig. 1) has been in the clinic for many years for the treatment of type II diabetes.

The discovery of the indolizidine alkaloids *swainsonine* and *castanospermine* (Fig. 1) increased the interest in imino sugars as therapeutic agents because of their potent inhibition of glycosidases, mannosidases and α -glucosidases, respectively, and their *in vitro* antiviral and *in vivo* anti-cancer activity. The use of these agents in the clinic has been limited due to their lack of selectivity and in the case of DNJ and castanospermine unacceptable GI disturbances through glucosidase inhibition at the high doses required for treating viral infections.

The key to the therapeutic application of imino sugars as glycosidase inhibitors is their ability to selectively

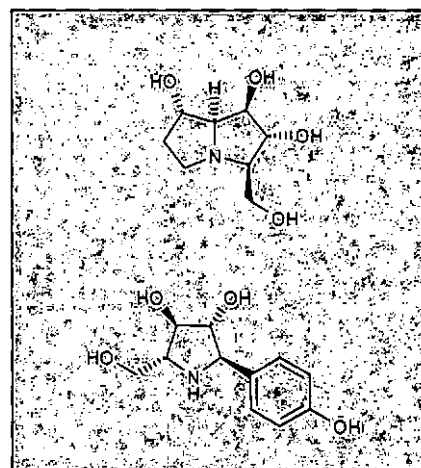


Fig. 2. Examples of new imino sugars with improved specificities.

alter *N*-linked glycans of viruses and cancer cells. Previous glycosidase inhibitors lacked specificity and were associated with adverse side effects, but MNL's research and development has uncovered compounds with good selectivity and favorable pharmacokinetic and pharmacodynamic profiles (Fig. 2). Clinical applications include: priming of the immune system with wide-ranging protective and healing effects; direct antiviral activity; indirect antiviral, anticancer and antibacterial activity via glycan changes/control; chaperoning of glycosidases aiding diseases caused by deficiencies (e.g., Gaucher's disease, Tay Sachs); reducing efficiency of nutrient uptake (decrease in weight gain); and potentiating insulin release, thus having antidiabetic potential.

MNL has synthesized imino sugars as immunomodulators acting via sugar receptors on immune cells including dendritic cells, macrophages and splenocytes. These small molecular weight sugar-like molecules, which illicit a potent immune response via the release of the cytokines IL-12 and IL-2, are being developed primarily for cancer, infectious diseases and as vaccine adjuvants. MNL Pharma is seeking partners for **MNLP462a**, a broad-spectrum immunomodulator and selective glucosidase inhibitor for cancer. This product is expected to enter the clinic in Q3 2006 and the company plans to progress studies to the end of phase IIa.

On day two, Prof. Kurt Drickamer (Division of Molecular Biosciences, Imperial College, London) opened proceedings with his presentation on combining structural, genomic and glycomic approaches to understanding carbohydrate specific receptors. Biological processes mediated by receptors that recognize glycans include cell-cell adhesion, serum glycoprotein turnover and immunity to viral, bacterial and fungal pathogens. Approximately 10 distinct groups of mammalian glycan-binding receptors have been described and structures of receptors in these groups can be used as a basis for identifying other potential receptors in genomic sequences. The results of such studies suggest that in humans there are roughly 100 different sugar-binding receptors that fall into these groups.

For example, serum mannose-binding protein (MBP)⁹ neutralizes invading microorganisms by binding to cell surface carbohydrates and activating MBP-associated serine proteases (MASPs) 1, 2 and 3. MASP-2 subsequently cleaves complement components C2 and C4 to activate the complement cascade. MASP-1 cleaves C2 almost as efficiently as MASP-2 does, but it does not cleave C4. Thus MASP-1 probably enhances complement activation triggered by MBP/MASP-2 complexes, but it cannot initiate activation itself.

TABLE II. BIOLOGICAL PROPERTIES OF PLANT POLYSACCHARIDES

IMMUNE MODULATORS	COMPLEMENT SYSTEM
Complement modifications	Thymus-dependent Ab res
Mitogenic activation	Regulation of specific cyclic Ab production
Macrophage activation	Regulation of IgM-IgG switch
Induction of chemotaxis	Modification of T- and B-cell proliferation

The human cell surface receptors dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN) and the closely related endothelial cell receptor DC-SIGN-related (DC-SIGNR) bind to oligosaccharide ligands found on human tissues as well as on pathogens including viruses, bacteria and parasites.¹⁰ The extracellular portion of each receptor contains a membrane-distal carbohydrate-recognition domain (CRD) and forms tetramers stabilized by an extended neck region consisting of 23 amino acid repeats. Both DC-SIGN and DC-SIGNR bind HIV and enhance infection. Screening an extensive glycan array demonstrated that DC-SIGN and DC-SIGNR have distinct ligand-binding properties. Furthermore, biochemical and structural comparisons reveal that they have very different physiological functions. DC-SIGN has dual ligand-binding properties and functions both in adhesion and in endocytosis of pathogens, whereas DC-SIGNR binds a restricted set of ligands and has only the properties of an adhesion receptor.

Studies of receptors with C-type CRD reveals that many of these receptors recognize broad classes of glycans, while only a few show highly selective binding to specific subsets of oligosaccharides. From these results, and similar studies of other receptor families, it is difficult to explain the biological significance of the enormous diversity of glycan structures, which significantly exceeds the number of receptors, identified using current genomic approaches.

Prof. Berit Smestad Paulsen (School of Pharmacy, University of Oslo, Norway) discussed the use of plant polysaccharides as medicines. In developing countries such as Mali¹¹

and also in most European countries there is a living tradition for the use of medicinal plants to treat different types of illnesses and ailments including wound healing. Traditionally, water extracts are used from which several plant pectin polysaccharides have been identified that have an effect on the immune system, often exemplified by the effect on the complement cascade (Table II).

Pectins consist of blocks of homogalacturonan, rhamnogalacturonan 1 (RG-I) and RG-II, but the distribution of each type varies. The overall structure of the polysaccharides has been determined as well as the structure of those parts of the polysaccharide that appear to be the most bioactive part of the plant polymers. The powdered roots from *Baccharoides adoensis* var. *kotschyana* in Malian folk medicine has been used for the treatment of gastritis, gastroduodenal ulcers, as an aid to ameliorate digestion and as a wound-healing remedy. The active components were identified as acidic polysaccharide fractions, containing pectic arabinogalactan type II structures, which showed both complement fixing ability and T-cell-independent induction of B-cell proliferation *in vitro*. Degradation studies show that the presence of galactose seems to be important for activity. The plant *Cochlospermum tinctorium* produces two peptic arabinogalactans (Ct50A1 and Ct50A2) and is also used for the treatment of gastric ulcers with good results. The extract shows dose-dependent activity with an IC₅₀ ~200 µg/ml. The roots were the part of the plant reported to be the most frequently used for medicinal purposes and the main indications are jaundice, gastrointestinal diseases, malaria, schistosomiasis and dysuria. The main components of the extract were identi-

fied as polysaccharides (59.3%) and polyphenols (9.3%).

A water extract of the aerial parts of *Biophytum petersianum* is a frequently used preparation for wound healing. One of the obtained polysaccharide fractions, BP100 III, isolated from a 100 °C water extract that has a monosaccharide composition typical for pectic substances, was shown to exhibit potent dose-dependent complement fixing activity. Degradation by endo- α -D-(1-4)-polygalacturonase produced three fractions obtained by gel filtration. The highest molecular weight fraction, BP100 III.1, had a more potent activity in the complement test system than the native polymer. It consists of galacturonic acid and rhamnose, with branches being present on both the rhamnose and galacturonic acid residues. Arabino-galactan type II is also present in the polymer, indicating that BP100 III.1 has a structure typical of the hairy region of pectins.

Glinus oppositifolius is a Malian medicinal plant used against various types of illnesses related to the immune response, like joint pain, inflammation, fever, malaria and wounds. Two pectin-type polysaccharides, GOA1 and GOA2, isolated from a 50 °C water extract from the aerial parts of *G. oppositifolius* were investigated for their bioactivity toward the complement system and different leukocyte subsets. The polysaccharide polymer in GOA1 was shown to contain considerable amounts of the neutral sugars arabinose (26.4 mol %) and galactose (42.9 mol %), and methylation analysis indicated the presence of arabinogalactans type I (AG-I) and type II (AG-II). GOA2 was rich in galacturonic acid (68.3 mol %), along with rhamnose, arabinose and galactose. Both GOA1 and GOA2 were shown to exhibit potent dose-dependent complement fixing activities, and induced chemotaxis of macrophages, T cells and NK cells.

The leaves from *Plantago major*, better known as the greater plantain,

have been used for wound healing. Studies testing the interaction of PMII, pectin that contains homogalacturonan and RG-1 with human complement systems show that it is a complement activator, both in the classical and in the alternative pathway.

Dr. Joseph Barchi (National Cancer Institute, Frederick, U.S.A.) updated results in an important new phase in carbohydrate nanotechnology.¹² Nanoglycotechnology is a fusion of materials science, carbohydrate chemistry and biotechnology to produce nanoscale devices that may be employed for important biological applications. As might be imagined from their ubiquitous and complex nature, the biological roles of glycans are quite varied. The diverse biological functions ascribed to glycans can be more simply grouped into two general classes: 1) structural and modulatory functions involving the glycans themselves or their modulation of molecules to which they are attached; and 2) specific recognition of glycans by lectins. The following is a well-known theme: monovalent carbohydrate-lectin binding tends to be of relatively low affinity, and such systems typically achieve their specificity and function by creating multivalent arrays of carbohydrate to enhance avidity. Mucins are high molecular weight glycosylated proteins that form a major part of a protective biofilm (mucus) covering the luminal surfaces of epithelial cells, where they can provide a barrier to particulate matter and bind microorganisms. Mucins contain numerous carbohydrate chains attached to the polypeptide core protein, mainly through O-glycosidic linkages.

It is well established that cell surface carbohydrates play unique roles in a host of biologically relevant events such as cell differentiation, adhesion and motility. Truncation of O-linked glycans on tumor mucins exposes underlying peptide epitopes presenting new "non-self" structures that can be recognized by the immune system and also contribute to tumor

aggressiveness and metastasis. Aberrant glycosylation results from genomic instability in tumor cells. Different expression of glycoprocessing enzymes causes additional branching of cell surface sugar chains. These alterations are considered to be relevant to the abnormal behavior of cancer cells, such as altered cell adhesion or metastasis and to the avoidance of immunological defense.

As described above, one problem with carbohydrate drugs is their weak monomeric binding constants (mMol). To enhance this affinity, one approach is to prepare multivalent carbohydrate constructs. In the pharmaceutical industry, nanoparticles such as dendrimers, liposomes, magnetic particles, nanoshells, nanotubes and quantum dots have been subjects of intense research and development during the last decade. The advantages of multivalent drugs are: increased selectivity, increased potency, improved efficacy and increased duration of action.

Dr. Barchi's team is developing synthetic methodologies that will provide access to new classes of compounds intended to mimic the recognition function of carbohydrates. Two separate nanometer-sized particles were described that can display multiple copies of carbohydrates on their surface and be used for the study of carbohydrate-mediated cellular events implicated in inflammation, viral, bacterial and toxin infection. Three-dimensional self-assembled monolayers of gold atoms bearing various carbohydrates have been prepared and assessed for their antitumor properties. Gold glyconanoparticles (GNPs) have also been prepared by the Penades group in Spain as new multivalent tools that mimic glycosphingolipids on the cell surface. GNPs are highly soluble under physiological conditions, stable against enzymatic degradation and nontoxic. Thereby, GNPs open up a novel promising multivalent platform for biological applications.

TABLE III. GOLD NANOPARTICLES AS GLYCOTHERAPEUTICS

Nontoxic
High multivalency
Strict control of ligand numbers, composition and NP size
Increased storage stability
Increased biological stability against enzyme degradation
T-Ag coated gold nanoparticles can inhibit metastasis <i>in vivo</i> , thus these particles may be useful as therapeutics

TABLE IV. BIOPHARMACEUTICAL PROPERTIES OF THE NEW GLYCO-QUANTUM DOTS

Prepared by a robust, simple and highly flexible technology for conjugation of carbohydrates to the surface of light-emitting quantum dots
Biofunctionality of the attached carbohydrates is preserved after conjugation
Short oligoethylene glycols not only promote luminescence but also eliminate nonspecific binding of the quantum dots <i>in vivo</i>
Sugar-quantum dot bioprobes may find broad applications in immunocytochemistry, pathological diagnosis, live cell imaging and multiple target analysis in a range of biomedical applications

A novel, multivalent presentation platform was developed by linking the tumor-associated, cell-surface disaccharide and the Thomsen-Friedenrich T antigen (Gal- β -1-3-GalNAc- α -O-Ser/Thr) to the surface of gold nanoparticles. Tumor-associated antigens are found in more than 90% of carcinomas and are used clinically as prognostic markers. A potential application for this multivalent system may be as an antiadhesive tool to inhibit metastasis. Administration of these nanoparticles into mice bearing breast tumors was shown to inhibit lung metastases in this model. This technology establishes the "proof of principle" for possible biological applications of GNPs (Table III).

In addition, a new synthesis of carbohydrate-bearing quantum dots that possess size-tuneable luminescent features that make them attractive reagents for biological imaging has been developed. Quantum dots are monodispersed semiconductor nanocrystals (CdS, CdSe, CdTe, ZnS) covered with a stabilizing monolayer. Particle size, which dictates color, is in the range 2–10 nm. Characteristics of quantum dots include broad absorption, narrow emissions and resistance to photobleaching (Table IV). This GNP principle described has the

potential to integrate all the current knowledge and applications on processes that involve carbohydrate molecules.

Prof. Sabine Flitsch's (Department of Chemistry, University of Manchester, U.K.) presentation focused on some fundamental studies on enzyme-catalyzed reactions on a variety of porous and nonporous solid support. Her team has studied the thermodynamics, kinetics and stereoselectivity of enzyme-catalyzed reactions on solid support and have found that all three reaction characteristics can change dramatically when substrate is immobilized.

There is a current trend toward miniaturization and automation in chemistry, biology and medicine that has led to increasing interest in (bio) chemical reactions on substrates linked to a solid support, such as polymer beads, glass slides or microtiter plates. Such formats have been tremendously successful for nucleic acids and are being investigated for other biomolecules such as peptides and carbohydrates. The U.K. Glycochips Consortium (www.glycochips.org.uk) aims to generate a diversity of carbohydrate arrays as tools for monitoring protein-carbohydrate interac-

tions. The aim is to develop carbohydrate arrays (glycochips) as innovative tools to map out the carbohydrate and protein partners in these highly specific interactions of the glycome.¹³

Several interesting new methods have been developed to monitor enzyme action on substrates attached to a solid phase such as polymer beads glass or gold surfaces. These include fluorescence measurements, MALDI-TOF mass spectrometry, and the use of quartz crystal microbalances to measure weight changes of immobilized molecules directly on the surface. Approaches that allow spatial resolution in single beads were also reported. The ability of enzymes to reach the inside of beads is becoming better characterized, and new supports have been developed that allow improved accessibility.

Tentagel resin, which consists of polyethyleneglycol (PEG) attached to cross-linked polystyrene through an ether link, combines the benefits of soluble PEG support with the insolubility and handling characteristics of polystyrene beads. Trypsin a 23.5 kDa enzyme can penetrate to the core of 90 micron Tentagel beads, and the bead matrix permits molecular motion at a similar rate to that in solution. The beads act as a separate gel phase rather than a porous solid. PEGA resin, a beaded polyethyleneglycol dimethylacrylamide copolymer, has also been used as an affinity support for the purification of carbohydrate-binding macromolecules. Thermolysin, a 35 kDa enzyme, gets into PEGA beads (90% yield). The rate-limiting step is the diffusion of the enzyme into the bead, which is dictated by size, charge and polymer swelling.

Fluoresce microscopy is used to study reactions in porous beads in spatial resolution. The one photon process uses a continuous wave laser that emits at the absorbing wavelength range of the fluorescent stain to irradiate the sample. Multiphoton events occur where two or more photons are

simultaneously absorbed in a medium with the effect of combining their energy. Two-photon fluorescence microscopy was introduced as a tool to assess enzyme accessibility and to quantify enzyme reaction rates on solid supports.

Hydroxymethylphenoxy linkers that are commonly used in solid-phase peptide synthesis are surprisingly susceptible to efficient cleavage by the protease chymotrypsin with a broad range of amino acid residues being tolerated at the scissile bond; this enzyme-cleavable linker system has been applied to peptide and glycopeptide synthesis (Fig. 3).

How does solid phase influence thermodynamics of the reactions? It is a balance between synthesis and hydrolysis. Hydrolysis yields are strongly dependent on overall reaction volume. At a low volume the balance is shifted towards synthesis rather than hydrolysis. Protonation of the amine drives the reaction, but it is more likely due to the uptake of product amide into the bead. Functional groups that make surfaces resistant to adsorption include hydrophilic groups and H-acceptors, but not H-donors. The equilibrium position of reactions on the solid surface can be substantially shifted compared with reactions in solution, and this can be usefully exploited using hydrolases in reverse. Research is also starting to tackle the way in which kinetics are modified when the substrates are surface immobilized.

Glycosylation can significantly influence the safety and efficacy of therapeutic glycoproteins. Dr. Daryl Fernandes (Ludger Ltd, Oxford, U.K.) described ways to measure and control glycosylation to reduce the risk of aberrant glycan profiles during biopharmaceutical production. The importance of this issue was illustrated by the case of recombinant erythropoietin (EPO), the most commercially successful biopharmaceutical to date with worldwide sales now exceeding USD 10 billion. Throughout its development, EPO has been associated

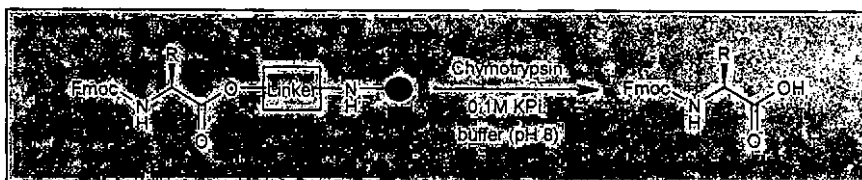


Fig. 3. Peptide and glycopeptide synthesis using an enzyme-cleavable linker system.

with serious manufacturing problems including QC failure of product batches and concerns about drug safety, and many of these problems relate to the characterization and control of EPO glycosylation. These difficulties apply to other therapeutic glycoproteins including monoclonal antibodies and arise because glycans can introduce great complexity and heterogeneity into the product, significantly influence the safety and efficacy profiles of biopharmaceuticals and vary considerably between product batches.

The challenges posed by biopharmaceutical glycosylation are significant. In some cases even small changes in cell culture conditions during production can cause aberrant glycosylation, which may or may not be recognized during final QC. This leads to a number of potential risks including true batch failure (the glycosylation pattern is out of specification), false batch failure (the glyco-profiling QC method falsely indicates out of specification glycosylation), safety compromise (e.g., the presence of nonhuman glycans) and non-standard efficacy *in vivo* (e.g., due to a change in the presence of key glycoforms). To deal with this, regulators such as the U.S. FDA and EMEA are increasing the requirements for biomanufacturers to demonstrate consistent glycosylation during biopharmaceutical production. Furthermore, these regulations are being harmonized internationally under the ICH Q6B and ICH Q5E guidelines.¹⁴

The key steps to implementing an effective ICH-compliant measurement and control program for biopharmaceutical glycosylation were overviewed and examples given for the case of biomanufacturing of monoclonal antibodies. Topics covered

included understanding glycosylation in the natural molecule, determining the importance of glycosylation *in vivo*, setting molecular specifications, setting measurement specifications, choosing the glycosylation parameters to measure, selecting appropriate glycoanalysis methods and implementing these in an integrated glycoprofiling system that satisfies the requirements of the ICH guidelines.¹⁵ Finally, the advantages of such a system to both biomanufacturers and regulators were explained.

Dr. Chris Urch (Glycoform Ltd, Abingdon, Oxford, U.K.) presented the research carried out at Glycoform. The Company's core expertise is in glycochemistry and cell-free glycosylation with direct applications in the fields of glycosylation of therapeutic proteins, including antibodies and glyco-targeted drug delivery. Glycoform's technology is based on the ability to synthesize complex carbohydrates and to add these in a controlled manner to therapeutic and other proteins.

About 70% of all human proteins, including antibodies, are glycosylated. Glycosylation increases stability by reducing degradation and solubility. However, glycosylation is not homogeneous and many glycoproteins occur naturally as a mixture of glycoforms, which have the same peptide sequence with different sugar patterns. As protein glycosylation is not under direct genetic control, the expression of therapeutic proteins in mammalian systems, for instance Chinese hamster ovary cells, have this issue of heterogeneity. Also, all commercial products are manufactured as mixtures (e.g., EPO and interleukins). Given that sugars are crucial to structure-activity relationships (SARs),

glycosylation at different sites affects biological properties: therapeutic response, side effects and efficacy. Thus, the ability to synthesize homogenous glycoproteins is a prerequisite for the optimization of the manufacture and biological and physicochemical properties of therapeutic glycoproteins.

Two parallel strategies can be employed:¹⁶ 1) electrophilic: whereby a cysteine-containing protein is converted into its corresponding phenylselenenylsulfide (PhSe-S-Protein), which then undergoes nucleophilic substitution by a 1-thio mono- or oligosaccharide; 2) nucleophilic: the 1-thio mono- or oligosaccharide is converted to their corresponding selenenylsulfide (glyco-SeS) derivative, which is subsequently coupled to a cysteine residue on the protein (protein-CH₂SH). The S-Se chemistry is exquisitely selective and obviates the need for the usual protection/deprotection steps. The resultant disulfide linkage is stable toward enzymatic carbohydrate extension and can also be processed by glucosyl transferases (Table V).

Glycoform's approach to the production of single glycoforms is to synthesize the oligosaccharide chemically, thus ensuring a homogeneous product. Each glycoform can then be studied and SARs developed so that the optimum glycoform can be chosen and then made selectively using proprietary techniques.

Dr. Pauline Rudd (Glycobiology Institute, Oxford, U.K.) closed the meeting with an upbeat overview of present status and future developments in glyco-chemistry/biology/biochemistry.¹⁷ Glycosylation is species, cell, protein and site specific, and processing pathways involve multiple interactions of the developing glycan chains with enzymes and sugar nucleotide donors. Glycan processing is restricted by the availability of these moieties as well as the local three-dimensional structure of the protein at the glycosylation site controls processing within the host cell.

TABLE V. PRODUCTION OF SINGLE GLYCOFORMS

Site-selective glycosylation creating pure isoforms
Site-specific chemical conjugation of carbohydrates to proteins in a highly chemo-selective reaction to give a single glycoform
Selective carbohydrate extension-glycoextend oligosaccharide enzymatically either before or after conjugation to the protein
The reaction can be pushed to completion

Is glycosylation an Achilles' heel? Primary amino acid sequence provides the signal (AsnXaaSer/Thr) for attachment of N-linked glycans. These cell- and protein-specific processes also depend on transport systems and cell signaling pathways, therefore glycosylation can be a sensitive marker of alterations in the internal and external environment of the cell. Glycosylation changes can both indicate the presence of disease and contribute to pathogenesis and are increasingly being assessed as potential diagnostic/prognostic markers and/or therapeutic targets. New analytical techniques discussed previously are proving useful for identifying and testing potential diagnostic markers as well as enabling the monitoring of disease progression and therapy.

Congenital disorders of glycosylation (CDGs), also known as carbohydrate-deficient glycoprotein syndromes, result in severe disease. In humans, most CDGs are N-glycosylation defects and only a few are in O-glycosylation. Glycan analysis of total serum glycoproteins can be used to identify the specific glycan processing steps that are compromised. In this collection of disorders, many glycoproteins are deficient or have reduced carbohydrate side chains. Subtypes of congenital disorders of glycosylation have been described based on the isoelectric focusing patterns of transferrin and on clinical features. Enzyme deficiencies have been reported in at least four of the subtypes: type Ia, phosphomannomutase; type Ib, phosphomannose isomerase; type Ic, glucosyltransferase; type II, glucosaminetransferase; and type IV, mannosyltransferase. For example, deficiency in N-acetylglucosaminyltransferase II CDG-IIa is characterized by

the following clinical disorders: dysmorphic features, hypoglycemia, epileptic episodes, and mental and severe psychomotor retardation. Rheumatoid arthritis is an autoimmune disease in which agalactosylated glycoforms of aggregated IgG may induce association with the mannose-binding lectin and contribute to pathology. Thus, the reduced level of IgG galactosylation is a distinctive disease marker following the disease into inactivity or remission. Terminal galactose levels (GO) on IgG Fc provide a diagnostic marker and insight into pathogenesis. Increased levels of IgGO levels are a prognostic marker for rheumatoid arthritis.

As more therapeutic options for cancer treatment become available, there is an increasing need for new biomarkers that will provide more sensitive and specific early detection of cancer. Potential serum biomarkers can be identified, segregated and quantified following detailed HPLC and MS analysis of >50 N- and O-linked glycans that comprise the serum glycome. Glycan analysis can also provide biomarkers and insights into pathogenesis, particularly when combined with proteomics or where oligosaccharides comprise recognition epitopes on a glycoprotein. For example, an increase in core fucosylation of proteins in HBV serum correlates with stages of liver cancer. The hepatocellular carcinoma marker, gp73, has been identified as a biomarker in more than 1,000 patients in clinical trials.

Gelatinase B is a matrix metalloproteinase (MMP-9) involved in tissue remodeling, development, inflammation and cancer. Altered glycosylation of MMP-9 is associated with

tumor metastasis in breast cancer. It is generally assumed that the primary mechanism by which MMPs promote cancer spread is by degradation of the extracellular matrix (ECM). Gelatinase B cleaves ECM, allowing cell(s) from the tumor mass—intravasation—to enter blood stream/lymphatic system.

Prostate-specific antigen (PSA) is a glycoprotein secreted by prostate epithelial cells. PSA is currently used as a marker of prostate carcinoma because high levels of PSA are indicative of a tumor situation. However, PSA tests are not specific enough to distinguish between benign prostate hyperplasia and prostate cancer. Core α (1–6) fucosylation of glycoprotein glycans is an important process in a number of normal and aberrant biological processes including cell adhesion, neurogenesis, the development of cancers, the natural suppression of cancer metastases and liver diseases. Recent studies have demonstrated that serum PSA from prostate cancer patients shows an increase in fucose residues. These findings may offer the basis for a PSA diagnostic test with improved specificity for prostate cancer.

Effective monoclonal antibodies (MAbs) to human immunodeficiency virus type 1 (HIV-1) are scant due to the virus having evolved various defensive mechanisms to enable it to resist neutralization. Human MAb IgG 2G12 can overcome these protective mechanisms. For neutralization the antibody requires a unique epitope on the surface of gp120 surface glycoprotein of HIV-1 that is not directly associated with the receptor-binding sites on this protein. The 2G12 epitope is destroyed when gp120 is treated with mannosidases. Further results confirm that this epitope is mannose dependent and consists of a cluster of mannose residues with little or no input from the gp120 polypeptide surface.

Interfering with glycosylation processing pathways has provided a suc-

cessful therapy for Gaucher's disease (Zavesca) and potential antiviral therapies for hepatitis B virus, hepatitis C virus and HIV. The discovery of a cluster of oligomannose glycans on HIV that is recognized by a unique domain swapped antibody suggests a new approach toward developing an HIV vaccine.

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REIVINDICACIONES

- 5 1. Un anticuerpo humanizado que tiene unión específica por CD45RO y CD45RB caracterizado porque comprende una región variable de cadena pesada de SEQ ID No. 31 ó SEQ ID No. 32 y una región variable de cadena ligera de SEQ ID No. 7 ó SEQ ID No. 8.
- 10 2. El anticuerpo humanizado de acuerdo con la reivindicación 1 que tiene unión específica por CD45RO y CD45RB caracterizado porque comprende:
 - 15 • Una región variable de cadena pesada de SEQ ID No. 31 y una región variable de cadena ligera de SEQ ID No. 7;
 - Una región variable de cadena pesada de SEQ ID No. 31 y una región variable de cadena ligera de SEQ ID No. 8,
 - Una región variable de cadena pesada de SEQ ID No. 32 y una región variable de cadena ligera de SEQ ID No. 7, ó
 - Una región variable de cadena pesada de SEQ ID No. 32 y una región variable de cadena ligera de SEQ ID No. 8.
- 20 3. Los Polinucleótidos que codifican el anticuerpo humanizado de las reivindicaciones 1 ó 2 que comprenden un polinucleótido de SEQ ID No. 34 ó un polinucleótido de SEQ ID No. 35 y un polinucleótido de SEQ ID No. 13 ó un polinucleótido de SEQ ID No. 14 ó SEQ ID No. 33.
- 25 4. Un vector de expresión que comprende la secuencia de polinucleótidos de la reivindicación 3 el cual es capaz de producir el anticuerpo humanizado cuando dicho vector está presente en una célula huésped compatible.

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Rvs modificadas
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5. Una célula huésped caracterizada porque comprende el vector de expresión de la reivindicación 4.
6. Una composición farmacéutica caracterizada porque comprende el anticuerpo de las reivindicaciones 1 ó 2 en asociación con al menos un vehículo o diluyente farmacéuticamente aceptable.