Polymer-immobilized Carbohydrate Ligands: Versatile Chemical Tools for Biochemistry and Medical Sciences

N. V. Bovin
Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, Russian Federation
H.-J. Gabius
Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Veterinärstr. 13, D-80539 Munich, Germany

1 Introduction

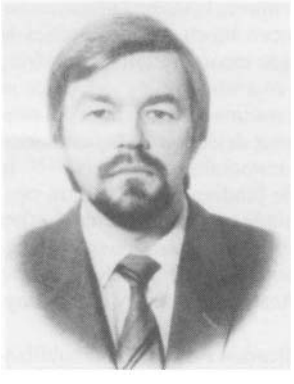
The exquisitely selective interplay of receptor molecules with suitable ligands is the prerequisite for efficient information exchange and transfer. Commonly, protein–protein, protein–nucleic acid and protein–hormone (small ligand) interactions are considered to guide physiologically relevant recognitive processes. Viewing the sequence of biopolymers as information-bearing code system, it is obvious that oligomers of the naturally occurring sets of amino acids and nucleotides fulfil essential requirements to display coding capacity like letters of an alphabet. However, the ability to store information is not limited to peptides and nucleic acids.

Remarkably, the extent of this property is surpassed by oligosaccharides formed from the sugar monomers galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine, mannose, fucose and sialic acid. These units primarily constitute the carbohydrate part of cellular glycoconjugates such as glycoproteins and glycolipids.[1] The other two heteropolymer classes in general have linear structures, and several factors explain the enhanced coding versatility of sugar compounds. The potential for formation of anomers, for variation in the positions of the glycosidic linkage, for branching and for further modifications like site-specific sulfation, phosphorylation, *O*-acetylation or lactonisation conspicuously increases the number of variants that can be generated from a small group of building blocks. Explicitly, theoretical calculations on the number of different hexamers with amino acids have yielded 46 656 structures, whereas $>1.05 \times 10^{12}$ hexasaccharide configurations could be devised from D-hexoses.[2] This result highlights the enormous coding potential of oligosaccharides. Our current knowledge of common sugar-chain biosynthesis, however, gives evidence that only certain linkage and branching types are actually formed from the theoretically possible panel. In this context it has yet to be noted that crucial biological functions are often mediated by rather unusual sugar determinants which thus have a restricted pattern of presentation.[3] This recent observation emphasizes the validity of the current notion to ascribe coding capacity to carbohydrate sequences and therefore to devote efforts to their characterization, preparation and functional analysis.

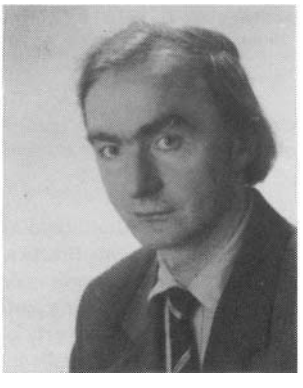
2 Glycostructures as Ligands in Biorecognition

As a component of glycoproteins the carbohydrates are able to serve a role in the folding of the nascent peptide chain, stabilization of protein conformations or protection against proteolytic degradation.[3] Beyond these rather passive functions, distinct glycoelements can act as ligands for sugar receptors, establishing recognitive

Nicolai V. Bovin was born in Moscow, Russian Federation. He obtained his MSc in 1976 for his work on 3,3-disubstituted cyclopropenes under the direction of I. G. Bolesov and Yu. S. Shabarov at the Moscow State University and his PhD in 1982 with a thesis on the synthesis of blood group-related oligosaccharides and their immobilization on polymeric matrices, performed under the direction of A. Ya. Khorlin at the Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow. After heading the group for synthetic antigens at the All Union Institute of Biotechnology, Ministry of Medical and Microbiological Industry, Moscow, from 1985—1988, he accepted the position of the head of the Laboratory of Carbohydrate Chemistry of the Shemyakin Institute of Bioorganic Chemistry. His research interests combine chemical and medical fields, currently focussing on the synthesis and structural analysis of oligosaccharides and their application in lectin and antibody research, e.g. in analysis of carbohydrate-mediated immunomodulation or cell adhesion and in tumour diagnosis and therapy.



Hans-Joachim Gabius was born in Bad Bevensen, Germany. He obtained his MSc in 1980 and his PhD in 1982 for chemical and biochemical studies on the proofreading mechanisms of aminoacyl-tRNA synthetases, under the direction of F. Cramer, Max-Planck-Institute for Experimental Medicine in Göttingen. 1981 was mostly spent in the laboratory of J. Abelson (Department of Chemistry, University of California, San Diego), investigating tRNA splicing. Work in tumour lectinology started in 1983 at the Max-Planck-Institute in Göttingen, and continued with post-doctoral research in the group of S. H. Barondes at UC San Diego (1984—1985) and appointments as assistant professor for biochemistry at the Max-Planck-Institute for Experimental Medicine in Göttingen (1987), as associate professor for pharmaceutical chemistry at the University of Marburg (1991) and as head of the Institute for Physiological Chemistry, Faculty of Veterinary Medicine , University of Munich (1993). Research awards include the Otto-Hahn-Medal (1983), the Award of the Dr. C. Duisberg-Foundation (1988) and the Award of the Paul-Martini-Foundation (1990). His research interests involve the chemical, biophysical and biochemical analysis of protein – carbohydrate interactions with relevance to biological and medical fields, such as the development of glycoscientific strategies for tumour diagnosis and therapy and the elucidation of functions of mammalian lectins.



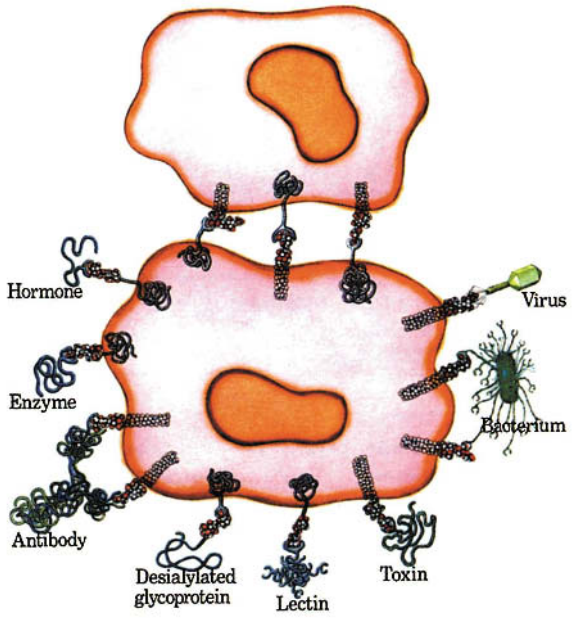


Figure 1 Schematic illustration of the recognitive interaction of various types of protein with carbohydrate ligands at the cell surface including cell adhesion events (from BioCarb, with permission).

protein–carbohydrate interactions, as illustrated for the cell surface in Figure 1. The deliberate depiction of cell–cell interactions, *e.g.* in formation of metastatic lesions in tumour progression or in interaction of leukocytes with activated endothelium in inflammation, and of the attachment of a virus or a bacterium to the cell surface *via* their lectins as first step to establish an infection exemplifies the assumed relevance of this type of biorecognition to help to understand the pathogenesis of diseases and to design rational therapeutical strategies.[4] Evidently, the analysis of expression of glycoconjugates and of glycoligand-specific receptors such as endogenous lectins (carbohydrate-binding proteins that are neither antibodies nor enzymes) can have a significant bearing on various biomedical fields (Figure 2). The present status of the molecular analysis of protein–carbohydrate interactions already clearly demonstrates the noticeable selectivity of this kind of recognition process, encouraging the design of concepts for biochemical and medical applications.[5]

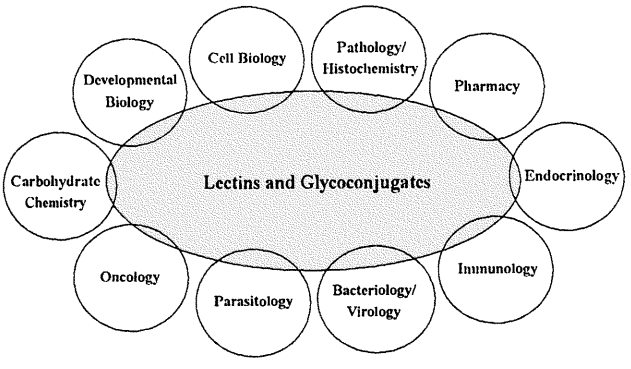


Figure 2 Representation of the interdisciplinary character of the research work on lectins and glycoconjugates, ranging from carbohydrate chemistry to clinical topics.

Development of reliable methods to detect carbohydrate-binding sites is essential to translate these considerations into practical approaches. Since the supposition of the existence of a sugar code system automatically implies the presence of suitable binding partners with the capacity for decoding, this fundamental property of the receptor(s) which at this stage are otherwise undefined, calls for an experimental strategy, employing the saccharide structure in search of respective binding sites such as lectins (Figure 3).

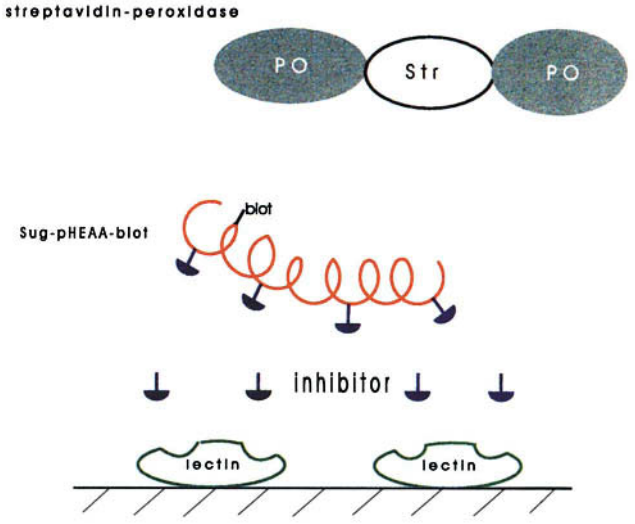


Figure 3 Schematic illustration of the application of the carbohydrate ligand-bearing conjugate as lectin-seeking probe, whose access to the sugar-binding sites can be blocked by presence of a free ligand (inhibitor).

Advances in chemical and chemoenzymatic oligosaccharide synthesis facilitate access to a rather unrestricted variety of testable structures.[6] However, their affinity as free units in solution for sugar receptors is often only in the millimolar range and they carry no label for localization or quantitation of binding. Further processing is thus essential for the construction of efficient probes for lectin detection. Conjugation of the ligand part to a macromolecular carrier addresses the given problems, namely to increase the avidity of a ligand for receptor sites by clustering and spatially to associate the ligand with a label. When a protein carrier is used as substrate, the product of the chemical attachment of a carbohydrate part to functional groups of amino acid side chains is termed a neoglycoprotein.[7] The prefix 'neo' denotes its origin by custom-made chemical synthesis.

3 Neoglycoproteins in Glycosciences

In contrast to natural glycoproteins that exhibit microheterogeneity in their carbohydrate composition the chemist can prepare a homogeneously glycosylated substance for any analytical purpose. A wide selection of derivatives that are in principle suitable such as *p*-aminophenyl glycosides and corresponding isothiocyanates or 2-imino-2-methoxyethyl 1-thioglycosides, and conjugation protocols involving amidation, thioureidation, diazo coupling, amidination, reductive amination and attachment of 1-amino or *N*-glycyl sugars or carboxy group-containing spacers to the protein matrix have been expertly described.[8] Their application yields well-defined products with exemplarily documented linkage regions between the ligand and its carrier (Figure 4). When employing glycosylated derivatives of aminotris(hydroxymethyl)methane, which is generally known as key buffer ingredient, clusters of carbohydrates can even be established which mimic branched oligosaccharide structures.[9] They exhibit enhanced biochemical affinities to various animal lectins. Following sugar incorporation the resulting product may require further addition of a label *e.g.* by iodination or biotinylation. Having performed this routine modification, the neoglycoprotein is ready for use in receptor detection and quantitation on tissue sections, cells or matrix-immobilized extracts.[4,8,10] In addition to initial analysis of specific binding with a certain neoglycoprotein that can contain a simple monosaccharide further structural refinements enable thorough characterization of specificity and geometry of the binding site(s) as well as selection of a ligand structure for subsequent purification by affinity chromatography.

It is necessary that chemical modification for ligand immobilization should not impair its recognitive properties. Therefore, a newly synthesized carbohydrate derivative should be tested in an assay

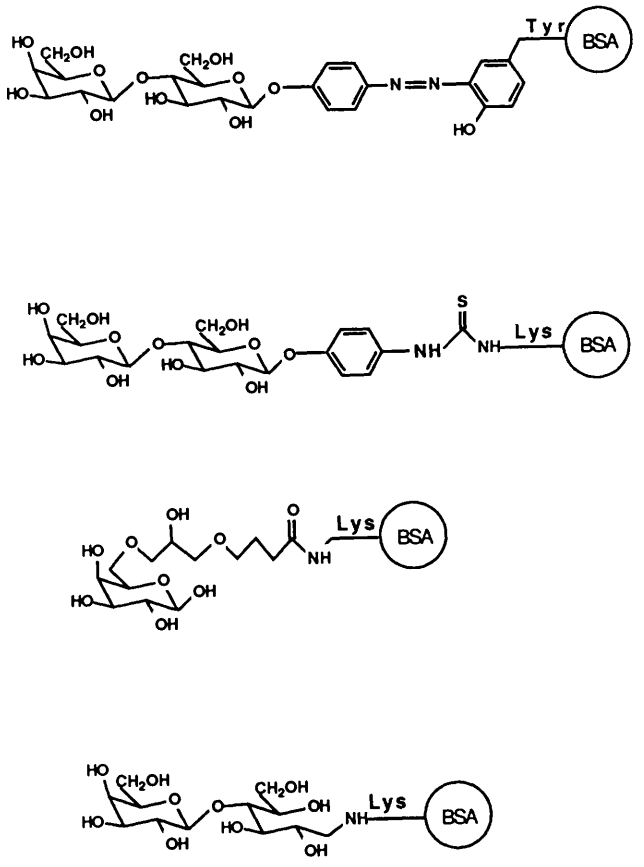


Figure 4 Structural representation of the linkage region between various carbohydrate derivatives and the respective amino acid side chain of the carrier protein, namely bovine serum albumin, in neoglycoproteins with specificity for β-galactoside-binding proteins.

system with a known lectin to reveal its principal potency to interact with a binding site. Since the aglycon and the ligand density can affect the binding properties, it is reasonable to prepare and to test several types of neoglycoprotein unambiguously to exclude false negative results. For example, experiments with β-galactoside-exposing probes on tissue sections have revealed quantitative differences in the extent of receptor localization which in several cases is primarily due to the presence of a certain lectin (galectin-1).[11] Its concomitant immunohistochemical detection corroborates the assumption of a ligand-dependent binding of the neoglycoprotein, constituting a control reaction.[11] False positive results can be obtained when the marker interacts with target molecules in the assay object by other parts of the molecule besides its supposedly crucial ligands. The carrier itself should thus be as inert as possible with no intrinsic binding properties. The alteration of net charge by the modification procedures that will cause enhanced ionic interactions should also be kept to a minimum. Addition of the unmodified carrier into the assay system to saturate any carbohydrate-independent specific binding sites, control experiments with carbohydrate-free, but labelled carrier and inhibition studies to verify sugar-specific recognition as well as to exclude marker binding by ionic interactions reliably prove the carbohydrate dependence of the binding process. When for example lysine residues of a carrier are involved in ligand coupling with ensuing increase of the net negative charge, application of a competitive sugar inhibitor or of formaldehyde-treated carrier backbone with similar isoelectronic point (pI) alteration can determine the relative contribution of sugar-dependent and sugar-independent binding, *e.g.* involvement of the scavenger receptor, as measured in isolated perfused rat livers.[12] To illustrate the impact of the presence of the competitive inhibitor on staining intensity with a labelled neoglycoprotein, two sections of a breast tumour metastasis are shown in Figure 5.

In general, serum albumin is used as a matrix for neoglycoprotein preparation. It combines several advantageous properties, namely lack of intrinsic glycosylation, good solubility, presence of adequate numbers of functional groups for sugar incorporation, low abundance of binding proteins primarily located in endothelial cells, and commercial availability. On the level of the organism such neoglycoproteins are instrumental to detect sugar-dependent clearance systems for serum glycoproteins, to purify the respective lectins, as for example shown for a Kupffer cell (phagocytes of the liver) lectin,[13] and to direct therapeutic drugs to certain cell types by lectin-mediated delivery.[10,14] The cytological and histological monitoring of presence of binding sites extends considerably our knowledge of sugar receptor expression. By custom-made marker synthesis any accessible sugar-binding sites that are not harmed by the preceding sample processing can be visualized using common procedures, allowing a convenient monitoring.[11,15] This analysis can be performed on any routinely available tissue material. With respect to tumour pathology, the examination of this diagnostic method has led reliably to distinguish various entities within a distinct class of tumour, *e.g.* small cell and non-small bronchial carcinomas, mesotheliomas (malignant tumours originating from the pleura) and metastatic adenocarcinomas in the pleura or five subtypes of meningiomas (a category of brain tumours of the same histogenesis with different extents of malignancy), and different stages of differentiation of a defined tumour.[16] Neoglycoproteins in this field are equal to diagnostically used monoclonal antibodies.

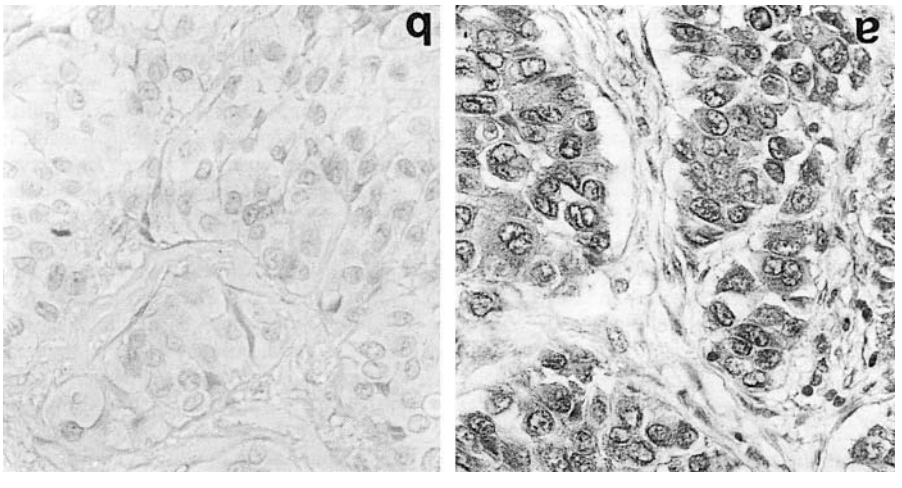


Figure 5 Light micrographs of sections of a lymph node metastasis of an invasive ductal mammary carcinoma after incubation with a T-antigen-bearing biotinylated neoglycoprotein in the absence (*a*) or in the presence of competitive inhibitor (*b*). Signal development after incubation with ABC kit reagents was followed by counterstaining with hematoxylin (×380).

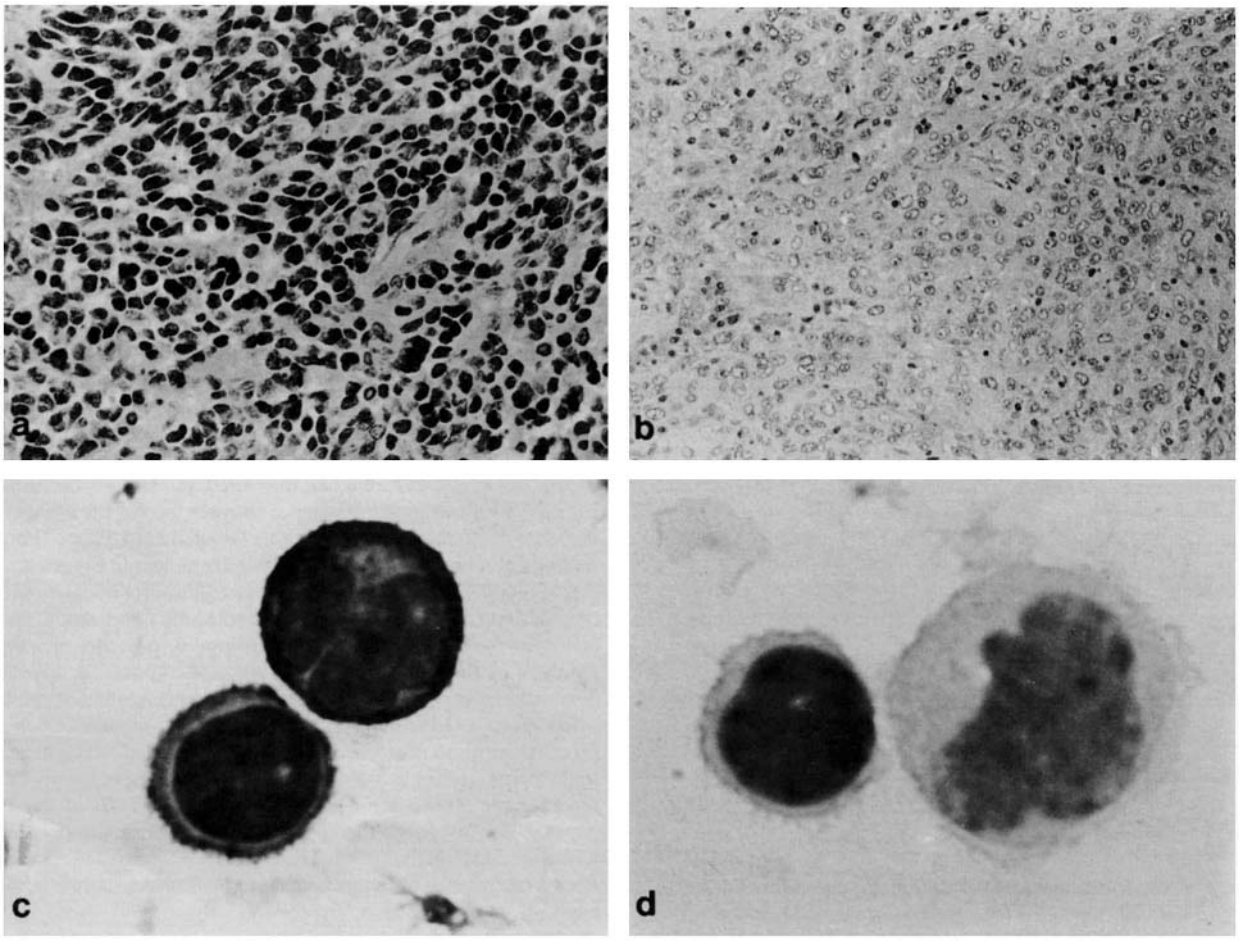


Figure 6 Visualization of binding sites for the carrier-immobilized sugar part from ganglioside GM_1 in a section of a small cell anaplastic lung carcinoma (*a*, ×200) and in a cytospin preparation of human peripheral blood lymphocytes and monocytes (*c*, ×800). Counterstaining was performed with hematoxylin or hemalaun. Comparatively weak staining was obtained with a ganglioside mixture containing GD_{1a}, GM_1, GT_{1b}, and GD_{1b} as donor of the sugar ligand for preparation of the biotinylated neoglycoprotein in the case of a section from a large cell anaplastic lung carcinoma (*b*, ×400). Presence of an excess of competitive inhibitor nearly completely abolished dye production for peripheral blood cells that had been counterstained (*d*, ×800).

An area of research with evident benefit is the correlation of glycohistochemical features to propensity of the primary tumour for metastasis formation, measurable in cell culture models and in comparison between primary tumours and their metastatic lesions.[11,16] Due to the complex nature of intercellular interactions in the metastatic cascade and the documented modulation of epitope expression by often ill-defined factors of the new organ microenvironment after homing of the tumor cell(s) to the secondary site, rapid progress should not be expected. Patient testing of a panel of probes can provide meaningful advances. Tailoring of the carbohydrate portion of the neoglycoprotein is inevitable. As a consequence of the inherent specificity of the tissue receptor(s) it leads to alteration in the staining intensity and its cellular distribution.[17] In contrast to monoclonal antibodies the binding properties of these glycohistochemical tools can thus be systematically varied to obtain optimal results.

In the course of the studies outlined the extension of the carbohydrate sequence is a consequent step. Besides products of synthetic origin, naturally occurring oligosaccharide chains from glycoproteins or glycolipids can be linked chemically with bifunctional crosslinkers or enzymatically with ceramide glycanase, endo-β-*N*-acetylglucosaminidase or transglutaminase in the form of a sugar–alkylamine substrate.[8] The application of such probes with increased complexity of the ligand part and their localization follows identical procedures, a result being demonstrated in Figure 6. The documented versatility to employ neoglycoproteins for receptor detection, analysis and isolation has encouraged considerations for *in vivo* applicability within drug targeting and receptor-selective blocking. Non-steroidal treatment of acute inflammation and prevention of docking of infectious pathogens are current topics of such a therapeutical approach. Due to envisaged *in vivo* testing it is desirable to construct well-defined and non-biodegradable carriers without immunogenicity. A backbone with the required properties is polyacrylate obtained chemically.

4 Polyacrylate-type Neoglycoconjugates

4.1 Synthesis and Characterization

The matrix should offer the possibility for quantitative and reproducible derivative incorporation to guarantee constant quality of the probe. Since the copolymerization of an olefinic group (allyl- or acryloyl)-containing carbohydrate with acrylamide can evince the inherent drawbacks of rather low reproducibility of yield, ligand density and structural characteristics of the copolymer as well as technical difficulties with batches of valuable substance of only 0.1—0.5 mg, alternative approaches have focused on the modification of the established polymer.[18] Initial attempts to activate polyacrylic acid or its copolymers with *N*-vinylpyrrolidone by *N*-hydroxysuccinimide, 4-nitrophenol or pentafluorophenol in the presence of carbodiimides have given unsatisfactory degrees of activation of $<10\%$ for subsequent ligand attachment. This result has prompted attempts to perform the activation at the monomer stage. An activated ester, namely 4-nitrophenyl acrylate has thus been synthesized and polymerized (1 mol l^{-1} in dry benzene) in the presence of 3% (*m*/*m*) azoisobutyronitrile (AIBN) as initiator at 70 °C for 50 h.[18] The final product of this reaction with a yield of 80% after reprecipitation is reproducibly obtained without

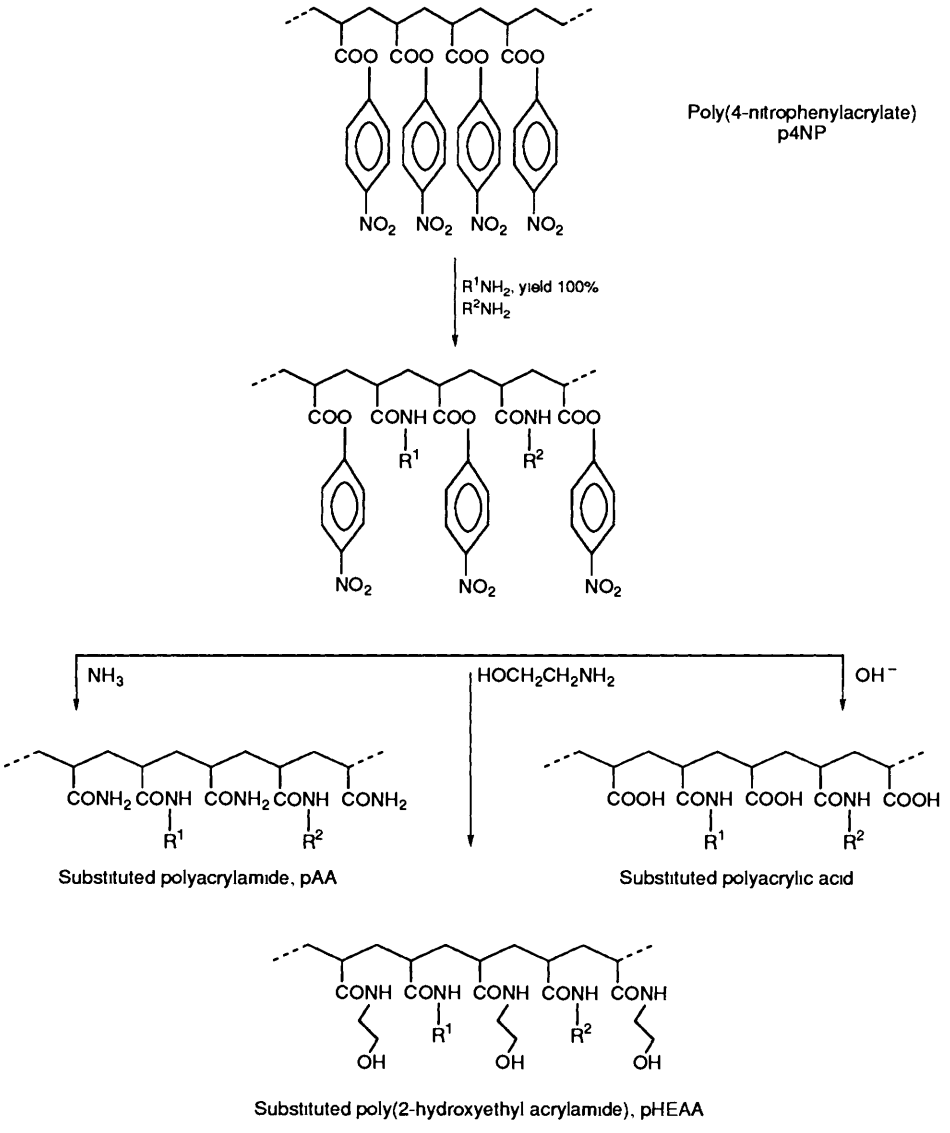


Figure 7 Synthesis of polyacrylate-based neoligandoconjugates in two stages, illustrating the attachment of amino group-containing substituents such as the ligand and a convenient label like a biotin derivative (R^1NH_2, R^2NH_2) to the activated polymer [poly(4-nitrophenylacrylate) p4NP] and treatment of the carrier with ammonia, ethanolamine or alkali to yield three conjugate variants, namely the polyacrylamide type (pAA), the ethanolamine type [poly(2-hydroxyethylacrylamide) pHEAA] and the polyacrylic acid type

alteration of the properties from batches of 100 mg to 10 g of monomer It exhibits good solubility in dimethylformamide and dimethyl sulfoxide which are used in further processing, shown in Figure 7 To be able to avoid any side reactions and obtain a quantitative outcome of conjugation, the ligand is added as a nucleophilic compound with a primary amino group, *e g* as the 3-aminopropyl derivative The detection of unreacted NH_2 groups on TLC plates with ninhydrin allows convenient monitoring of the reaction velocity, leading quantitatively to a degree of 25—30% substitution of $-CO_2C_6H_4NO_2$ groups and their conversion to $-CONHR$ groups

Initial experiments with neoglycoconjugates have revealed that 5—20% molar substitution provides adequate probes for biomedical purposes As shown in Figure 7, the concomitant incorporation of a label like (*N*-biotinyl)hexamethylenediamine (biotin-NH_2) is feasible at room temperature in the presence of triethylamine or diisopropylethylamine Alternatively, phosphatidylethanolamine can be used to provide a hydrophobic anchor for incorporation of the immobilized carbohydrate epitopes into a membrane [18] Only traces of amine impurities, but not water, have to be excluded in the solvents At this stage, the partially *N*-substituted polymer still displays reactive sites Three routes for subsequent treatment generate different types of matrix with distinct hydrodynamic properties (Figure 7) The product of treatment with ethanolamine has an apparent M_r value of 40000 according to analysis by gel filtration and ultrafiltration with filters of increasing exclusion limit with a rather regular spherical conformation of a diameter of 150 Å Conversions of $-CO_2C_6H_4NO_2$ to $-CONH_2$ and $-CO_2H$ cause increases in the apparent M_r values to 50000 and 80000 with enlarged degree of linear extension The pHEAA-type product is generally used for biomedical applications This procedure affords a synthesis of a chemically stable, non-immunogenic and histochemically inert matrix independent of the batch size The product is easily purifiable by gel filtration Condensation of the fully activated polyacrylic acid with aminoalkyl substituents proceeds quantitatively under mild conditions for *e g* carbohydrate ligands and a convenient derivative of a chosen label

4.2 Applications

The labelled neoglycoconjugates can be used for sugar-receptor detection in the same routine procedures as optimized for neoglycoproteins As with that class of tool, increases of the ligand density will not necessarily lead to enhanced binding properties The inhibition of interaction of influenza A virus (one of the causative agents of often epidemic viral flu) with the sialic acid-rich

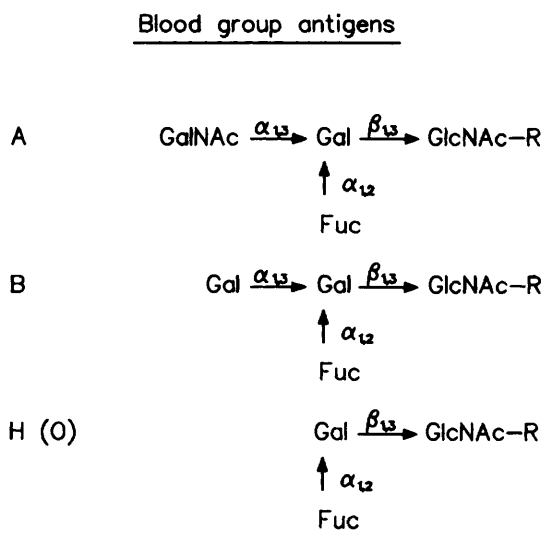


Figure 8 Carbohydrate determinants of the blood group antigens (type 1) of the ABH system (gal D galactose, galNAc *N* acetyl-D-galactosamine, fuc L fucose, glcNAc *N* acetyl-D glucosamine)

glycoprotein fetuin was optimal at a molar conjugation degree of *N*-acetylneuraminic acid of 10% into the activated polymer with significant decreases in inhibitor potency at 5 or 20% [19] This result and corroborating histochemical evidence confirm that it is neither necessary nor desirable to gain a maximal ratio of incorporation

In histochemistry, the labelled marker is used at concentrations of 5—25 μg ml^{-1} with an incubation period of 60—180 min at room temperature in conjunction with commercial ABC kit reagents and the signal-generating enzyme substrates [20] Combined with intentional chemical synthesis, the neoglycoconjugates are being tested to address the issue of functional analysis for carbohydrates by detection of complementary binding sites and correlation of this property with clinical parameters The following example testifies to the importance of this approach Despite their frequent expression in mammalian tissues the physiological significance of the histoblood group antigens of the ABH-system, defined by carbohydrate sequences which are given in Figure 8, is still elusive A discrepancy between our knowledge of the regulation of their enzymatic synthesis and the actual implication(s) of their presence is apparent Hence, respective neoglycoconjugates have been used as tools to localize tissue binding sites in tumour sections, an extensive study already being available for lung tumours [21] When the presence of specific receptors is retrospectively compared with the prognosis of the individual case, the positivity of serial sections for the H- and A-type trisaccharides, but not for the B-type trisaccharide, indicates prolonged patient survival [Figure 9(a–c)]

Interestingly, the differences in staining can also be reflected in quantitative aspects of morphometric parameters (Table 1) The concomitant occurrence of marker binding and of certain characteristics of the syntactic structure analysis or of DNA-related features such as the aneuploidy rate (aberrant nuclear content of chromosomes) should be considered as reasonable phenomenological evidence for functional implications of sugar receptor expression to be pursued by assays in cell biological models In addition to lung carcinoma similar observations of a correlation between the capacity to recognize the H type trisaccharide and morphometric parameters have been observed in prostate cancer [22] The results of this continuing line of research which has been opened by the accessibility of the synthetic tools emphasise why further effort in this area is warranted

Receptor localization and ensuing assays on the cellular level *in vitro* can be conveniently carried out with the neoglycoconjugate An example for a cellular response which is triggered solely by the sugar part, namely lacto-*N*-neofucopentaose III, is the enhanced production of interleukin-10 from B-cells of schistosome-infected mice [23] This potent cytokine is capable of downregulating T helper 1 (T_H1) cells which execute cell-mediated immune responses and produce further signal mediators, namely interferon-γ and

Table 1 Presence of binding sites for synthetic histoblood group trisaccharides in relation to IOD and MST features of the lung carcinomas (mean values)

Feature	Type of blood group trisaccharide					
	A	A$^+$	B	B$^+$	H	H$^+$
Number of cases	(81)	(68)	(67)	(82)	(77)	(72)
IOD features						
Stem Lines	2 7	2 9	3 0	2 7	3 1[a]	2 5[a]
S Phase (%)	12	12	12	12	13[a]	11[a]
Entropy	2 2	2 2	2 2	2 2	2 2	2 2
2CV Std (%)	11	9	10	10	11	9
5C Exc (%)	7 1	7 6	7 6	7 1	8 1[a]	6 5[a]
Structural features (MST)						
Dist Tu Tu (μm)	12	12	12	12	12	12
Dist Pr Pr (μm)	40	38	39	39	39	39
Dist 5C 5C (μm)	25[a]	28[a]	26	27	26	27
Entropy	11	9	10	10	11	9
Current of entropy	29	29	28	30	33[a]	24[a]

Statistically significant ($p<0.05$) abbreviations IOD = integrated optical density 2CV Std = 2CV standard deviation index 5C Exc = aneuploidy related fraction Dist = distance between centres of nuclei of certain cell types (Tu = tumour cell Pr = proliferating tumour cell with IOD value between 2 75 and 3 25 5C = tumor cell nuclei with IOD>5)

interleukin-2 Concomitantly, expansion of T_H2 cells which assist in antibody production is supported This response is supposedly desirable against antigenic pathogens The carbohydrate ligand-induced increase of production of a cytokine can thus explain the obvious shift in the T-cell subsets from cellular to humoral defence mechanisms as a step to clarify its *in vivo* function The approach to link a glycostructure to an inert carrier is likewise helpful to delineate the importance of individual components of rather complex glycosubstances such as the immunoregulatory glycoproteins uromodulin or α_1-acid glycoprotein The separation of N-linked sugar chains from the protein part and the preparation of a reactive substituent by amination of each reducing *N*-acetylglucosamine residue permits the generation of a 'pseudo-glycoprotein' (Figure 10) It is devoid of any interfering influences of the protein part In the illustrated case of α_1-acid glycoprotein the engineered pseudoglycoprotein has revealed that immunomodulatory potency of the complete glycoprotein can be attributed to its sugar part [24] Fractionation of the sugar chains prior to their incorporation into the activated polymer will be instrumental in ascribing distinct functional characteristics to certain glycoelements They can be caused by binding to cell-surface lectins In principle, defined lectin–carbohydrate interactions are capable of eliciting potent immune responses, as documented for plant lectins as a well-established precedent [25] The given examples permit the suggestion that such an immunomodulation warrants further thorough *in vitro* and *in vivo* evaluation to reach a conclusive answer on its potential clinical value

Besides receptor visualization in histology and cytology such soluble polymers are appropriate tools to quantitate specific binding in solid-phase assays The sugar-recognizing protein can be exposed on the surface of polystyrene microtitre plate wells or nitrocellulose sheets Addition of test substances to the assay mixture allows conclusions on their relative affinities to isolated and immobilized sugar receptors *Vice versa*, the neoglycoconjugate can be adsorbed on to a matrix, keeping the receptor in solution so that it is not affected by surface interactions Binding of the polymer to nitrocellulose can be strengthened by intermolecular connections *via* disulfide bridges between SH groups that have been incorporated as cysteamine into the polymer (Figure 11) This type of matrix (a 'pseudo-membrane' surface) establishes, too, a support for assaying ligand-dependent cell adhesion, its favourable properties of reproducibility of surface features and low non-specific adsorption rendering customary use as an alternative to polyacrylamide gels and immobilized neoglycoproteins likely [26]

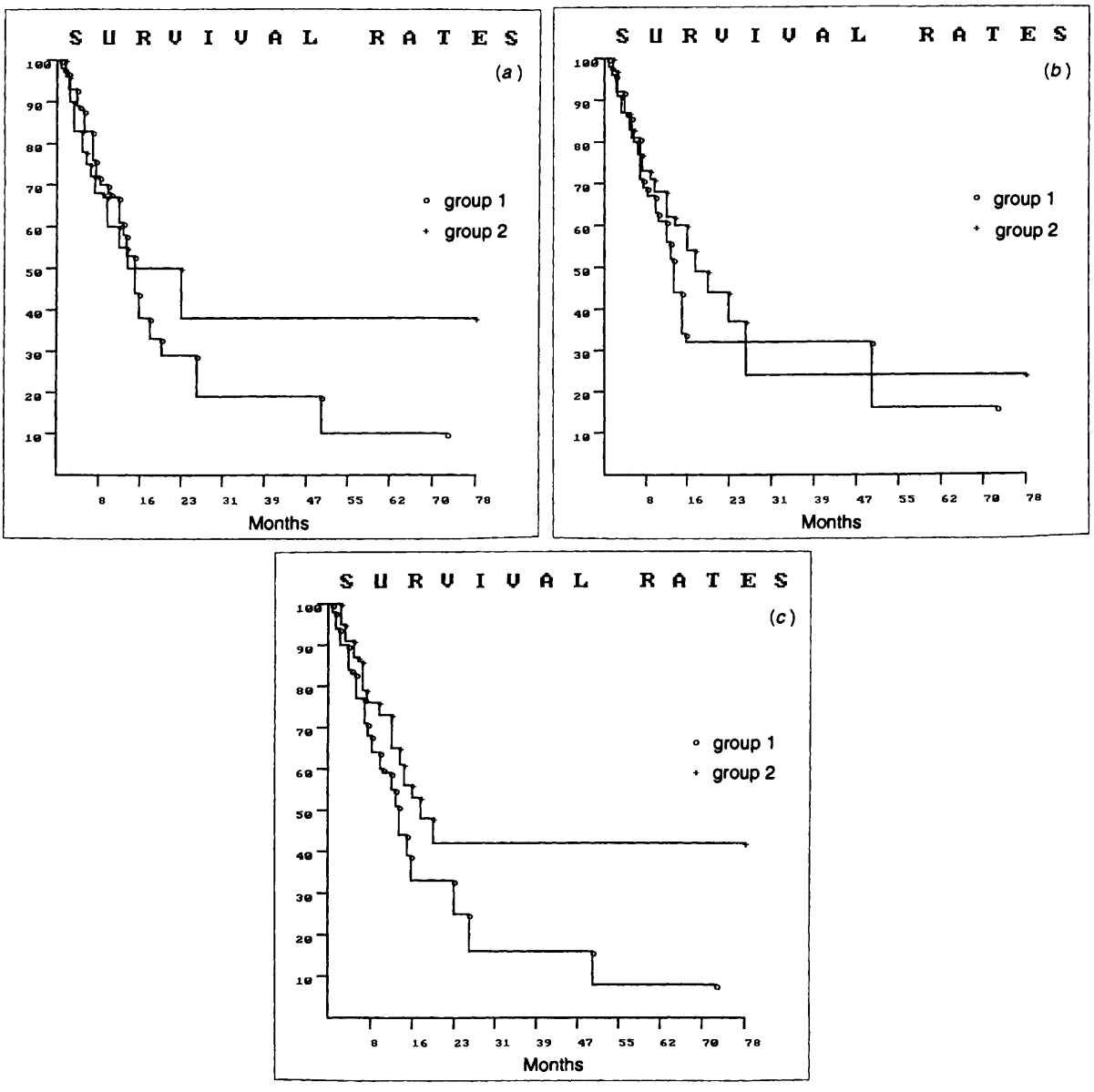


Figure 9 Diagrammatic representation of survival of lung cancer patients after surgery, given as percentage of the total number of patients after distinct intervals and categorized according to the capacity of tumour cells specifically to bind the blood group A- (*a*), B- (*b*) or H-trisaccharide (*c*) The group of positive cases is referred to as group 2 (+), whereas the group with a lack of respective trisaccharide-binding ability is denoted as group 1 (○)

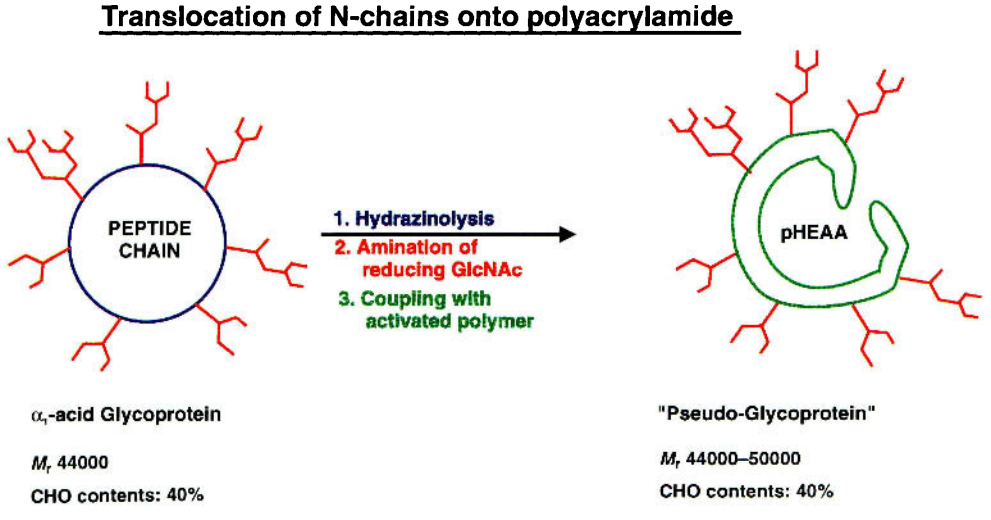


Figure 10 Synthetic strategy to obtain a pseudo-glycoprotein after hydrazinolysis, amination of the generated reducing terminal *N*-acetylglucosamine residue of each resulting oligosaccharide chain in the glycan pool and incorporation of the carbohydrate ligands into the activated carrier Residual reactive sites on the carrier backbone are blocked by treatment with ethanolamine

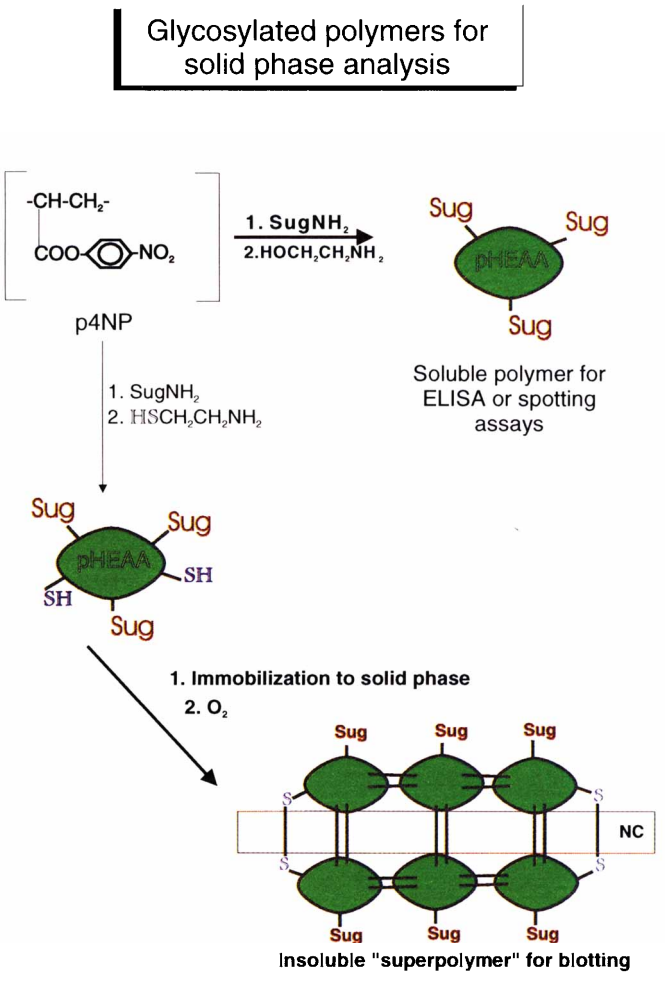


Figure 11 Derivative formation of glycosylated polymers for application in solid-phase assays.

In addition to the detection of sugar receptors like lectins or antibodies, which directly recognize the ligand without subsequent introduction of any structural alterations, neoglycoconjugates can serve as substrates for enzymes of the glycoconjugate metabolism in non-radioactive assays. Stepwise establishment of the complete sugar part of cellular glycoconjugates is facilitated by glycosyltransferases. A prerequisite for the successful establishment of the test system for enzymes of this class is the availability of a receptor (lectin or antibody) which distinguishes the substrate and the expected product. The individual steps of the assay procedure are shown in Figure 12. Tailor-made preparation of the acceptor for the enzymatic transfer of the activated substrate allows the often cumbersome isolation of natural precursors to be avoided. In view of the documented association of alterations in glycosylation with disease processes like inflammation or malignant transformation the outlined test system can be helpful to define the biochemical activity of certain cell types for glycosyltransferase-based diagnostic procedures.[27] By employing 'pseudo-glycoproteins' with glycopeptides a further area of application will be the analysis of peptide motifs, which are required for correct glycosylation, as known from the UDP-*N*-acetylglucosamine: lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase and the UDP-*N*-acetylgalactosamine: glycoprotein hormone *N*-acetylgalactosaminyltransferase.[28]

5 Conclusion and Perspectives

Chemical synthesis provides access to carbohydrate structures for sugar-receptor detection. Moreover, it makes the carrier backbone of a neoglycoconjugate available. Its properties allow *in vivo* applications for blocking distinct lectins to be considered *e.g.* in infectious and inflammatory processes, or drug-carrying neoglycoconjugates to be targeted to certain cell types. In this respect, the synthetic polymer is unmistakably preferable to any protein as a matrix for deliberate substitutions.

Since the conjugation of the ligand to the fully activated polymer requires a nucleophilic compound such as an amino group on the spacer, the poly(4-nitrophenylacrylate) matrix is not confined to accepting carbohydrate moieties. In principle, any potential ligand can be attached to the carrier, if the spacer ends with a nucleophilic group. This process will produce a 'neoliganodoconjugate.' Combined synthetic work and biomedical assay procedures, outlined in this article for glycosciences, can likewise be applied to enhance the knowledge in the field, defined by the nature of the ligand. The given description of the status of use of neoglycoconjugates can thus be considered as an example which may inspire experiments with other biologically active substances.

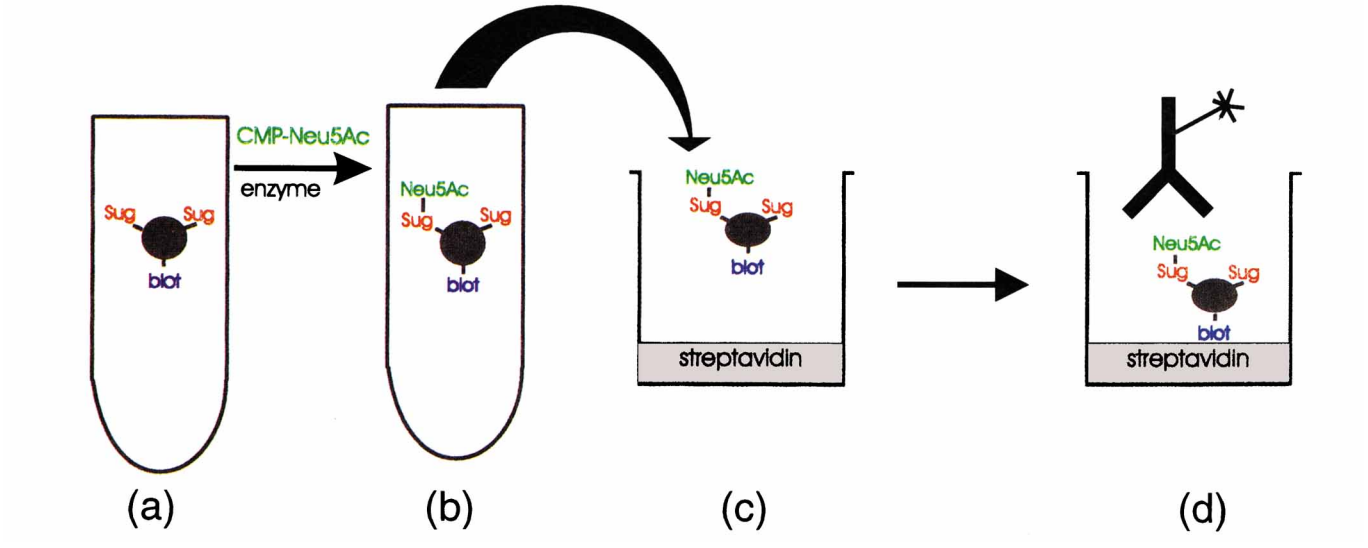


Figure 12 Schematic illustration of a non-radioactive glycosyltransferase assay, using sialyltransferase as example. The appropriate biotinylated neoglycoconjugate (*a*) is exposed to a solution with an unknown quantity of enzymatic activity and the activated sugar unit as substrate (*b*). After termination of the reaction the mixture is transferred to a matrix containing immobilized streptavidin that will bind the biotin-containing neoglycoconjugate (*c*). Addition of a labelled lectin such as commercially available and well-characterized agglutinins from *Limax flavus*, *Maackia amurensis* or *Sambucus nigra* or an antibody with suitable specificity for the expected product and performance of a routine ELISA-type assay will enable determination of extent of product formation (*d*).

Acknowledgements The authors are grateful to R Ohl for excellent processing of the manuscript, Prof J Wittmann for careful reading of the manuscript, and the Dr -M -Scheel-Stiftung fur Krebsforschung for generous financial support

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