Inhibitors of Sialyltransferases: Potential Roles in Tumor Growth and Metastasis

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Abstract: For over thirty years it has been evident that there is altered glycosyltransferase activity in neoplastic tissue when compared to healthy tissue. It has also long been speculated that disruption of the neoplastic expression of sialic acid on cellular glycoconjugates, is a valid target in anti-metastatic therapeutic development. Over the years attempts have been made to synthesize inhibitors of sialyltransferases in a effort to assist in the validation or dissolution of these enzymes as potential therapeutic targets.

Keywords: sialyltransferase, inhibitors, metastasis, glycoconjugates.

INTRODUCTION

The glycosylation of cellular proteins and lipids is an integral part of many cellular processes and functions. The structurally complex oligosaccharide side-chains found on cellular proteins and lipids have a tightly regulated spatio-temporal distribution. The cellular machinery required for the production of the vast array of oligosaccharide structures found in human cells involves an estimated 250-300 different enzymes [1]. Carbohydrate side chains cover a wide functional spectrum which includes many of the cellular activities that are essential for the growth, development and survival of an organism [2].

Sialic acid containing glycoconjugates play a vital role in such biomolecular processes as inflammation, embryogenesis, organogenesis, immune defence, migration and homing of leukocytes, metastasis of neoplastic cells, and infection by a variety of pathogens. Considerable research has been undertaken since the late sixties to determine the importance of altered cell surface glycoconjugates and therefore glycosylation patterns, in relation to malignancy and the metastastatic properties of tumor cells. Whilst differences in glycosylation have been noted in tumor cells for over 50 years the exact structural aberrations and the cellular mechanisms underlying these changes have only recently begun coming to light. The relationships between cellular glycosylation patterns and human diseases have been reviewed by a number of authors [3,4,5]. In spite of many contradictory opinions, overall, the indications for the involvement of sialic acid in metastasis and tumorogenicity are positive, but as shown in later studies, they are much more complex than initially thought.

Table 1. Some of the Glycosyltransferase Families, their Donor Substrate and the Anomeric Linkage Formed between the Donor and Acceptor

Galycosyltransferases	Activated Donor	Linkages Formed
Galactosyltransferases	UDP-α-Gal	α 1,2, β 1,3, β 1,4
Fucosyltransferases	GDP-α-Fuc	α 1,2, α 1,3, α 1,4, α 1,6
Mannosyltransferases	GDP-α-Man	α 1,2, α 1,3, α 1,4, α 1,6, β 1,4
N-acetylglucosaminyl transferases	UDP-α-GlcNAc	β 1,2 (core), β 1,4, β 1,6
N-acetylgalactosaminyl transferases	UDP-α-GalNAc	α 1,3, β 1,4
Sialyltransferases	CMP-β NeuNAc	α 2,3, α 2,6, α 2,8

The development of inhibitors of sialyltransferases has the capacity to validate members of this class of enzymes as targets for therapeutic development. Some of the significant recent work in this area has been highlighted by Giannis [6]. As expected, most research appears to focus on developing therapeutics to disrupt metastasis, although as our understanding of the processes these enzymes are involved in expands, further targets are sure to be elucidated.

Biosynthesis of Glycoconjugates

The glycosyltransferase family of proteins encompasses all the enzymes that are involved in the sequential addition

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1389-5575/03 $41.00+.00

of monosaccharides to the growing carbohydrate chain. Glycosyltransferases are generally located in the Golgi apparatus and catalyse the following basic reaction:

Nucleotide activated monosaccharide donor + *n*saccharide acceptor = *n*+ *1*saccharide + nucleotide

Each class of glycosyltransferase exhibits pronounced acceptor substrate and donor specificity and usually catalyses the formation of a specific anomeric linkage between the acceptor and donor, Table (**1**). Glycoconjugates can be further subdivided into O- and N-linked glycans on proteins and glycans linked to lipids. Proteoglycans are a separate category of glycoconjugates where, in general, most of the molecule consists of repeating disaccharide units [7].

The sialyltransferases have special characteristics, which set them apart from other glycosyltransferases. They transfer sialic acids from a nucleoside monophosphate sugar, cytidine monophosphate-β-5-N-Acetylneuraminic acid (CMP-β-Neu5Ac), to the non-reducing termini of acceptor oligosaccharides, as shown in Scheme (**1**). All other glycosyltransferases transfer monosaccharides from nucleoside diphosphate sugars.

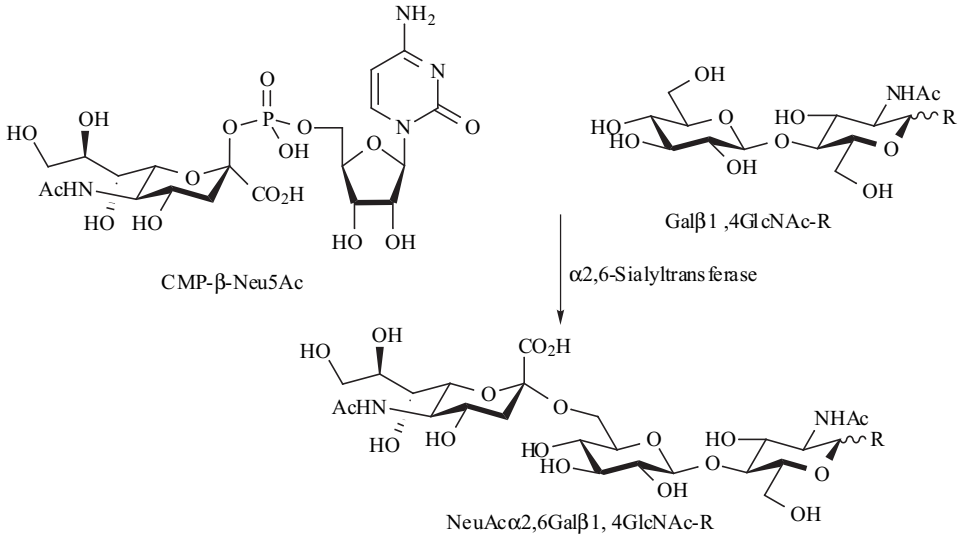
The Sialyltransferase Family

According to the one-gene-one-enzyme hypothesis, there should be one sialyltransferase for each type of sialic acid-oligosaccharide linkage. This includes all classes of complex carbohydrates and all types of glycosidic linkage. Five sialic acid linkages commonly occur in a wide variety of mammalian glycoconjugates - Neu5Acα2->6Gal, Neu5Acα2->3Gal, Neu5Acα2->6GalNAc, Neu5Acα2->6GlcNAc, Neu5Acα2->8Neu5Ac. Hence, there should be at least five specific sialyltransferases to account for all of these structures. However, the substrate specificities of sialyltransferases appear to extend beyond the non-reducing terminal sugar to include larger portions of the structure for acceptor substrate recognition. The terminal disaccharide of the acceptor is the principal determinant of specificity. For example, there are two separate sialyltranferases to catalyse the transfer of sialic acid in an α2,3 linkage to Galβ1-4GlcNAc and Galβ1-3GalNAc, respectively. Thus, different oligosaccharides containing the same sialic acid linkage may require separate sialyltransferases for their synthesis [8]. Specificity and/or affinity may be further influenced by the degree of branching of the oligosaccharide acceptor and the protein to which it is attached [9].

One of the mechanisms by which glycoconjugate sialic acid content is increased in tumor cells, is through the activity of sialyltransferases. The most frequently described change in glycosylation pattern that is associated with cancer is the presence of highly branched, heavily sialylated structures [10,3] The formation of these aberrant structures involves a combination of altered regulation of biosynthesis and often re-expression of fetal glycosidic antigens [11,5]. It is believed that there are at least 20 sialyltransferases responsible for the formation of all of the linkages observed in mammalian systems [12-14,118]. To date, sialyltransferases have been isolated and sequenced from a number of different tissues and a variety of species including human, rat, mouse, chicken and pig [15]. There is little sequence homology across the family with the exception of two relatively large conserved motifs, designated as the Long- and Short-motifs, and a Very Small motif made up of two conserved amino acids separated by four residues [16,17]. The L and S motifs are believed to form the CMP-NeuAc donor binding pocket and part of the acceptor binding site respectively [16,18-21].

Sialyltransferases, Sialic Acid and Cancer

Early studies on the nature of the changes in cell surface glycosylation recognized that sialic acid levels were elevated on malignant cells [10,22-24]. Whilst these studies alluded to some relationship between cell surface sialic acid and malignancy, they did not clearly establish a direct link between sialyltransferase expression and activity, cell surface sialic acid content, and tumor growth and metastasis. In the early 80's, a study employing murine tumor cell lines appeared to establish a positive correlation between cell metastatic potential and sialic acid content [25]. In a range of murine tumor cell lines metastatic capacity was positively



Scheme 1. α2,6-Sialyltransferase catalysed transfer of sialic acid from CMP-β-Neu5Ac to the oligosaccharide, Galβ1-4GlcNAc.

correlated with sialylation of available galactose and *N*-acetylgalactosamine residues on cell-surface glycoconjugates. The results indicated that increased sialylation of the cell surface *may* contribute to metastatic potential by increasing cell adhesiveness, particularly the cell's capacity to aggregate platelets, thereby decreasing the susceptibility of the cells to destruction by host immune mechanisms. It was also proposed that hypersialylation of tumor cell surface could result in larger tumor emboli and increased adherence to vascular endothelium.

Other studies concluded that although there was an increase in sialic acid content in neoplastic tissue, this apparently did not correlate with malignancy. For example, it was shown that some highly invasive hepatomas were determined to have significantly less sialic acid content than less malignant hepatomas [26]. Similarly, the research of Weiss *et al.* [27] seemed to indicate that the presence of sialic acid *per se* does *not* determine blood-born arrest properties. In a number of experiments with metastatic cells, it was determined that there was no difference in potential between cells treated with neuraminidase (to give 30-60% loss of sialic acid) and those untreated [28]. In relation to the inconsistent agreement of these results, it is often very difficult to determine the changes that occur in the *O*-linked and *N*-linked sialic acid containing glycans in malignancy. Often there are subtle shifts in the balance between *O*- and *N*-linked epitopes [29,30].

The possible link between sialyltransferases, cell surface sialic acid and tumor growth and metastasis was initially supported by results from studies using the purported sialyltransferase inhibitor KI-8110, a CMP-sialic acid derivative [31-33,34]. Treatment of human colorectal cancer cell lines with KI-8110 prior to intrasplenic injection into athymic nude mice, significantly reduced the numbers of animals developing hepatic tumors [35,36]. Subsequently it was discovered that KI-8110 does not actually inhibit sialyltransferases, rather it inhibits the CMP-sialic acid transporter thus depleting the available CMP-sialic acid donor in the Golgi. Elsewhere, it was reported that an increase in sialylation on some metastatic cell surfaces resulted in decreased attachment to basement membrane proteins collagen type IV and fibronectin, predisposing the tumor cells to increased mobility and decreased growth control by substratum contact. Enzymatic removal of cell surface sialic acid using neuraminidase subsequently led to an increase in the adhesiveness of highly metastatic cell lines *in vitro* [37]. Whilst not directly providing evidence for the role of sialyltransferases in malignancy, these studies highlighted the complex interrelationship between glycosyltransferase activity, substrate availability and the resultant cellular glycosylation pattern.

More recently the increased use of molecular techniques has begun to tease out the complexities of glycosylation pathways and the regulation and expression of the enzymes involved in these pathways. It is increasingly apparent that disregulations of these pathways are strongly correlated with many pathological conditions, including many types of cancer, Table (**2**), [3,5,38-41].

In an examination of a variety of colorectal adenocarcinomas from 46 patients Lise *et al.* demonstrated that there was a negative correlation between ST6Gal I expression in tumors and a good clinical outcome [43]. They found that moderate and well-differentiated tumors had increased expression of ST6Gal I when compared to poorly differentiated adenocarcinomas. Ito *et al.* demonstrated both up and down regulation in the expression of mRNA for various sialyltransferases in colon cancer tissues, suggesting that complex patterns of mRNA expression could be associated with specific tumor types [52]. Using RT-PCR techniques it has been shown that ST6Gal I and ST3Gal III mRNA expression are elevated in human colorectal tumors [44]. These observations support earlier studies by Dall'Olio *et al.* that examined the activity of various sialyltransferases in human colon cancer cell lines [53-55]. Their studies of colon cancer tissues identified an increased activity of ST6Gal I and a concomitant increase in reactivity with the α2,6-sialyl-linkages specific lectin from Sambucus nigra (SNA) [56]. Analysis of the transcriptional regulation of sialyltransferase expression established that ST6Gal I is regulated by different promoters. The promoters controlling ST6Gal I expression in cancers cells form part of the complex mechanisms associated with aberrant tumor glycosylation. In other experiments, transfection of Fisher rat fibroblasts with the human H-*ras*-1 oncogene results in an increase of cell surface Neu5Acα2-6Galβ1-4GlcNAc epitopes which correlates with an increased level of ST6Gal I activity and endows the cells with a tumorogenic phenotype [57]. All of these observations provide strong evidence for a link between sialyltransferase expression and activity, cell surface sialic acid and tumor progression in human colorectal

Table 2. Summary of Some of the Tumors Where Cell Surface Sialic Acid Correlates with Poor Prognosis

Disease	Phenotype	Enzyme/mechanism	Reference
Colon cancer	↑α2,6 sialic acid	↑ ST6Gal I expression/activity	42-44
Gastric cancers	↑ cell surface Sialic acid	↑ sialyltransferase activity, ↓ glycan branching & chain elongation	45,58,59
Breast cancer	↑α2,6 and α2,3 containing glycans	↑ expression of ST3Gal III and ST6Gal I	46-48
Leukemia	↑sialylation	↑ ST3Gal	49
Ovarian cancer	↑ sialylation	?	39,40
Small cell lung carcinoma	↑ PSA	↑ expression of α2,8-polysialyltransferases	40
Melanoma	↑ Sialic acid containing gangliosides	↑ ST8 Sia I	50,51

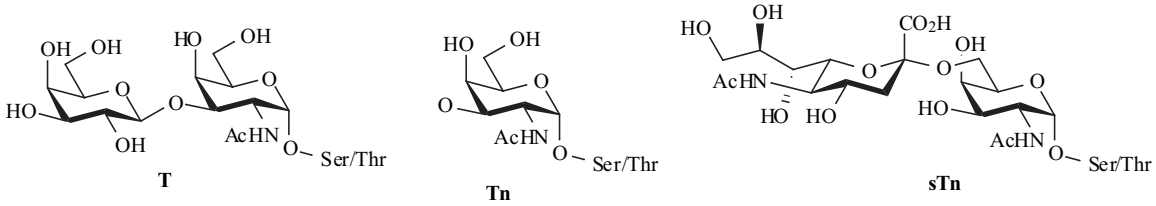


Fig. (1). O-linked glycoconjugate structures known as cancer associated antigens.

cancers. The situation is less well defined in other types of cancers, however there is increasing evidence that various sialo-oligosaccharide structures are involved in tumor growth and metastasis of various tumor types.

Sialo- Oligosaccharide Structures Involved in Cancer

The sialyl-Tn antigen (**sTn**, Neu5Acα2-6GalNAc-Ser/Thr) on mucins has long been regarded as a cancer-associated antigen. Several research groups have identified expression of **sTn** as a poor prognostic factor in various cancers including stomach adenocarcinomas [58,59,45], ovarian cancers [39,40], and breast cancer [30]. Ikehara *et al.* proposed that increased expression of ST6GalNAc I in [64]. Aberrant expression of Lewis type antigens has been reported for cancers including lung [65,66], melanoma [67] and colon cancer [68]. A study of 137 patents that had undergone resection as treatment for gastric cancer, found that patients that were positive for **sLe^a** generally had larger tumors and a poorer overall survival [69]. The same researchers completed a study of 159 primary colorectal cancers and found that the disease free survival rate of patients with **sLe^x** positive tumors was significantly poorer than those with negative **sLe^x** for tumors [70]. In colorectal tumors classified according to their growth pattern as polypoid or non-polypoid, 90.6% of non-polypoid tumors had significant expression of **sLe^x**. Patients with non-polypoid-type carcinomas had a significantly younger age of disease onset, significantly smaller maximal tumor diameter,

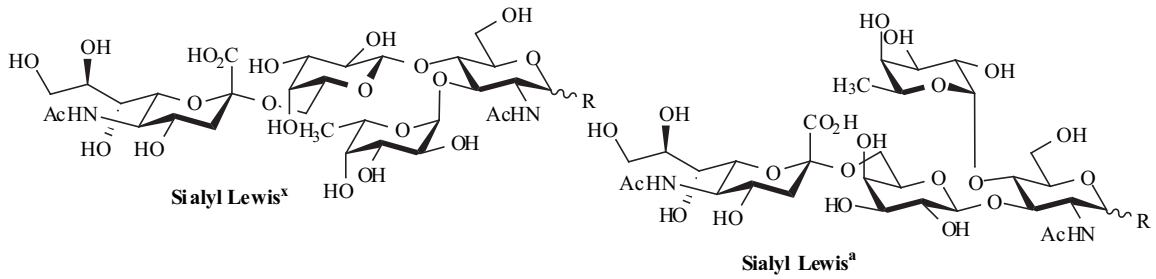


Fig. (2). Cell surface glycoconjugate structures implicated as also having roles in cancer metastasis.

malignant cells results in underglycosylation of mucin molecules as the enzyme effectively competes with other glycosyltransferases for the GlcNAc-protein acceptor, thereby terminating carbohydrate chain elongation [60]. In normal adult colonic epithelia expression of **T**, **Tn** or **sTn**, Fig. (**1**) antigens are undetectable.

However the **T** and **Tn** antigens are weakly detectable in hyperplastic polyps and **T**, **Tn** and **sTn** are relatively strongly expressed in adenomatous polyps and carcinomas [61-63]. In colorectal cancer **sTn** is reported to be expressed by 87% of cases independent of patient age, gender, tumor degree of differentiation or Dukes' stage. Five year survival for **sTn** positive patients is 73% whilst for **sTn** negative patients it is 100% [62].

The sialyl Lewis$^{a/x}$ (**sLe$^{a/x}$**), Fig. (**2**) structures have also been extensively studied in relation to their roles in cancer significantly higher rate of lymph node metastasis and significantly worse prognosis than those with polypoid-type carcinomas [71].

As a result of their critical involvement in inflammation, the roles of **sLe$^{a/x}$** structures has been the subject of intensive study [72,73]. The complex set of interactions between **sLe$^{a/x}$** epitopes and the selectin family of receptors are important in the adhesion of lymphocytes to epithelial cells and subsequent extravasation. It is not clear if similar molecular mechanisms are involved in the adhesion and invasion of tumor cells during the formation of distant metastases.

Polysialic acid (PSA) is a linear polymer of sialic acid residues whose formation is catalysed by the α2,8 polysialyltransferase family of enzymes [74,75]. The PSA has a very restricted expression and distribution in normal

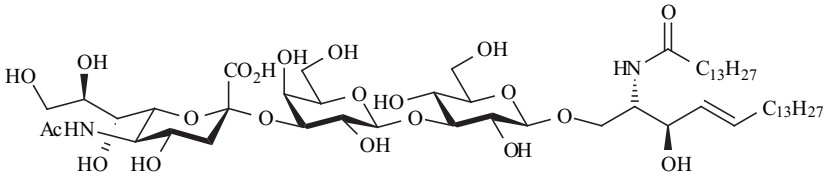


Fig. (3). Ganglioside G_{M3}.

cells and only commonly occurs on N-linked glycans of the neural cell adhesion molecule (N-CAM). PSA-N-CAM is highly developmentally regulated and has a role in maturation of the nervous system [76]. High levels of PSA are re-expressed in tumors of neuroectodermal origin including medulloblastomas and neuroblastomas [77,78]. PSA expression has also been correlated with poor prognosis in small cell lung carcinoma [79]. Similarly, sialylated gangliosides have also been associated with cancers. Ruan *et al.* observe that melanoma cells typically are characterized by high levels of G_{M3} gangliosides, Fig. (**3**), and the ST8Sia I enzyme [50]. In a study designed to evaluate the changes in ganglioside and sialic acid profiles as potential markers for the diagnosis of liver malignancies, Lu *et al.* found that ganglioside content and sialic acid levels in hepatoma tissues were significantly elevated during proliferation and abnormal differentiation [80].

It is increasingly apparent that elevated levels of sialic acid on tumor cells, play a pivotal role in the growth and metastasis of tumors cells. Many of the sialic acid containing glycans that are proliferated in various tumors serve as markers for poor patient prognosis [64].

INHIBITORS OF SIALYLTRANSFERASES

The continuing development of a range of effective sialyltransferase inhibitors is essential for further elucidation of the involvement of sialic acid in the biosynthetic mechanisms that lead to malignant transformation of cells. It is possible that in some cancers, sialyltransferase inhibitors could become part of the therapeutic armory available for the treatment of these diseases.

There are a number of approaches towards inhibition of sialyltransferases, including (i) donor based inhibition, (ii) acceptor based inhibition and (iii) product based inhibition. As will be discussed, there is very little information available on the enzyme's transition state. To date there has been no publication on a crystal structure for any sialyltransferases. There are two common design rationales in the synthesis of sialyltransferase inhibitors. By far the most actively pursued is the synthesis of donor based inhibitiors, which are modeled on the enzyme substrate, cytidine monophosphate-*N*-acetylneuraminic acid (CMP-NeuAc), Fig. (**4**). The alternative is an acceptor-substrate based strategy, with inhibitors modeled on the known range of oligosaccharide acceptors.

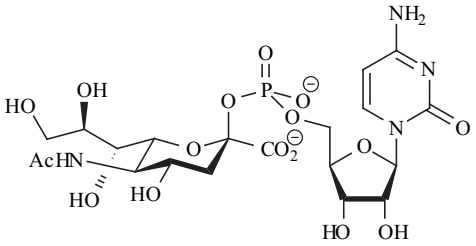


Fig. (4). The sialyltransferase donor, CMP-NeuAc.

Donor Based Inhibitors

Korytnyk and co-workers carried out an analysis of naturally occuring nucleotides and some non-natural derivatives to determine their inhibitory capabilities with human serum sialyltransferase [81]. All of the nucleotides and nucleotide analogues tested were shown to have some inhibitory effect on serum sialyltransferase activity, Table (**3**). There was a relationship established between number of phosphate moieties and strength of inhibitory activity for all types of substrates. Cytidine triphosphate (CTP) was shown to be the most effective [82]. Of other potential non nucleotide based inhibitors screened, neither sialic acid itself, nor the sialyltransferase acceptor galactose, were shown to have any inhibitory influence on the enzyme.

Table 3. Effects of Nucleotides and Nucleotide Analogues on Human Sialyltransferase

Compound	K_i[a]	Type of inhibition[a]
UTP	0.35 mM	Noncompetitive
UDP	2.0 mM	Noncompetitive
UMP	5.67 mM	Noncompetitive
ATP	0.20 mM	Noncompetitive
AMP	7.0 mM	Noncompetitive
CTP	16.0 μM	Competitive
CDP	19.0 μM	Competitive
CMP	50.0 μM	Competitive
Cytidine	-[c]	
5'-F-CMP	70.0 μM	Competitive
[d]ara-CTP	0.50 mM	Competitive
[d]ara-CMP	1.10 mM	Competitive
Cytidine 3':5'-cyclic monophosphoric acid	3.0 mM	Competitive

[a]Type of inhibition and K_i were determined graphically using the method described by Dixon [83] at substrate concentrations of 9.65 and 4.83 μM.
[b]The pH of the assay medium was unaffected by introduction of nucleotides.
[c]K_i and the type of inhibition could not be determined
[d]Structure not represented

Potential sialyltransferase inhibitors were also designed by Korytnyk and co-workers based on the CMP-NeuAc substrate [84,85]. It was speculated that due to the known millimolar inhibitory effects of cytidinemonophosphate (CMP), and the lack of any inhibition by *N*-acetylneuraminic acid, that binding to the enzyme occurs predominantly at the nucleotide portion of the enzyme. Consequently, CMP analogues were synthesised Fig. (**5**) and tested in a enzyme assays with an ectosialyltransferase (cell associated sialyltransferases) and a human serum sialyltransferase. The most powerful inhibitor of those tested was the experiment control inhibitor, CMP itself. The level of inhibitory efficiency by this range of analogues is given in the table below. All compounds were tested as inhibitors of L-1210 leukemic cells with the bis-aldehyde **2** displaying an ID_{50} of 6.5 x $10^{-4}M$. This compound was subsequently tested in a mouse model for anti-leukemic activity. It was shown that there was a significant increase in life span, with toxicity noted at higher concentrations. It was speculated that as well as effecting glycoconjugate biosynthesis, this

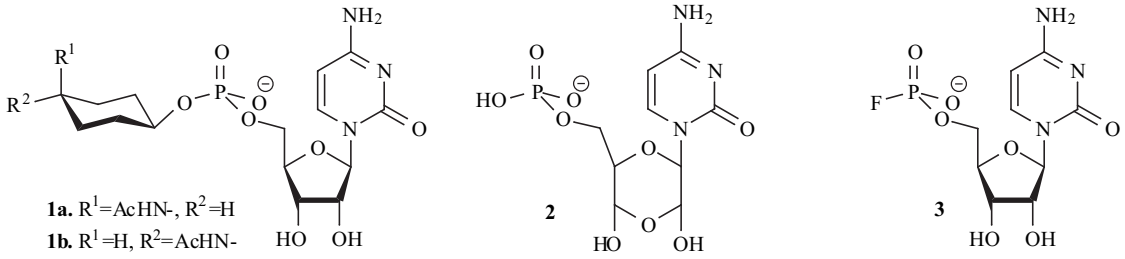


Fig. (5). CMP and CMP analogues for inhibitor studies.

compound may well be influencing DNA biosynthesis and perhaps other metabolic pathways, so its specific effectiveness as a sialyltransferase inhibitor is questionable.

As mentioned ealier, two disaccharides KI-8110 and KI-8115, Fig. (**6**), were shown to decrease the incorporation of sialic acid into glycoconjugates on murine lymphocyte surfaces [86-89]. Sialidase and CMP-NeuAc hydrolase activities were unaffected, as was the incorporation of galactose and N-acetylglucosamine into cell surface glycoconjugates. This led to the conclusion that the disaccharide-nucleosides were acting as sialyltransferase inhibitors, although this assumption later proved to be erroneous. A study of the sialyltransferase inhibitor KI-8110 in conjuction with hepatic macrophages (Kuppfer cells), clarified these observations [90]. The study showed that decreased levels of sialylation of metastatic liver cells from a colorectal carcinoma cells lead to a promotion of Kupffer cell recognition of the metastatic cells.

Inhibition with both KI-8110 and KI-8115 was then presumed to proceed *via* inhibition of the sialyl transport protein. Specifically KI-8110 and KI-8115 were observed to inhibit the transfer of sialic acid to exogenous desialylated glycoproteins or exogenous desialylated glycolipids.

In an effort to regulate the biosynthesis of gangliosides, Hatanaka *et al.* embarked upon the synthesis of a CMP-NeuAc analogue whereby the labile phosphate linkage was replaced with an alkyl chain Fig. (**7**) [91]. The potential inhibitor was tested against G_{M3} and G_{D3} synthetases.

Table 4. Percentage Inhibition of CMP Analogues 1a, 1b, 2, and 3

Compound	Concentration (mM)	Ectosialyltransferase activity %inhibition	Human Serum sialyl-transferase activity % inhibition
CMP	0.125 1.25	18 72	85 100
3 5'F-CMP	0.125 1.25	19 74	76 98
2 Ribodialdehyde-CMP	0.125 1.25	0 46	0 55
1b Cytidine 5'-(trans-4-N-acetylcyclohexyl)phosphate	0.125 1.25	0 15	0 28
1a Cytidine 5'-(cis-4-N-acetylcyclohexyl)phosphate	0.125 1.25	0 2	0 20

Inhibition of both the synthases with analogue **4** was noted at near 10mM concentration.

Although no biological data was published in this communication, the synthesis of the C-sialoside **5** is worthy of note purely due to the interesting potential such a structure provides. An obvious initial modification to make to CMP-NeuAc in an effort to synthesise sialyltransferase inhibitory molecules, is to try and ameliorate the lability of

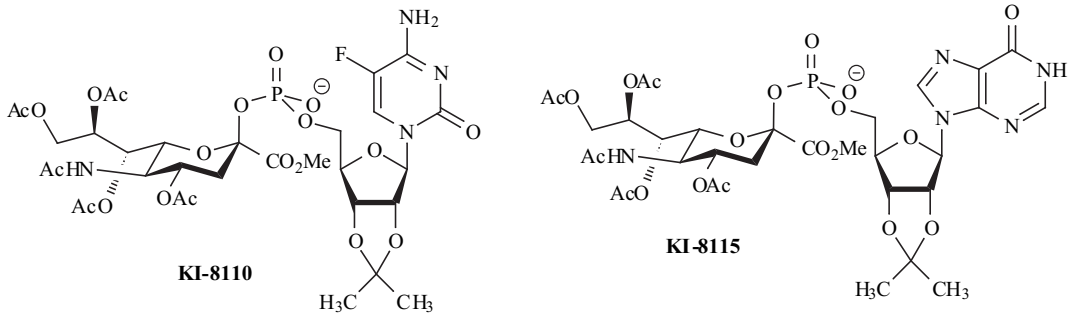


Fig. (6). KI-8110 was shown to be an inhibitor of CMP-NeuAc transport.

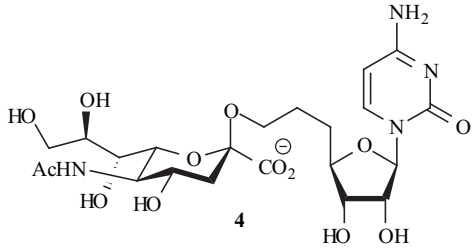


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Fig. (7). Replacement of the phosphate bridge with an alkyl chain ameliorates lability.

the anomeric carbon-phosphate bond. If the chemistry is available to incorporate a C-C bond at the anomeric center in a simple manner, then the opportunity arises to synthesise potential sialyltransferase binding substrates with no enzymatically catalysable lability at the anomeric centre, Fig. (**8**) [92].

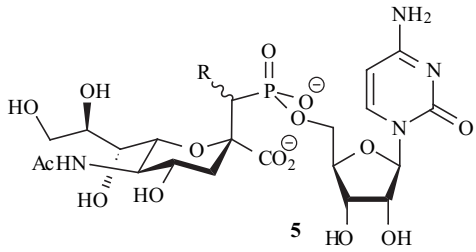

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Fig. (8). The C-glycoside will not be transferred under physiological conditions.

Although the purpose of this next study was to synthesize potential accelerators of sialic acid transfer, some interesting notes of a chemical nature are made which may be of assistance in the design of inhibitors, and provide an interesting comparison to the above article. A synthetic analogue of CMP-NeuAc was prepared, wherein the glycosidic oxygen atom as part of bridging phospho moiety was substituted by a sulphur atom **6**, Fig. (**9**). Initially the analogue was subjected to solvolysis conditions in aqueous solution, and it was found that solvolysis of the synthetic analogue proceeds at a rate almost two orders of magnitude slower than the natural substrate. This compound was then examined in a comparative assay with the natural substrate, with α2,3-sialyltransferase as transferring enzyme and a UV-active lactoside acceptor. Results indicated that there was an apparent three-fold lower binding affinity of the thio-derivative compared to the natural substrate. This essentially negligible discrepancy, was explained as possibly being a result of the difference between C-S and C-O bond lengths. Whilst the synthetic substrate did successfully act as a donor

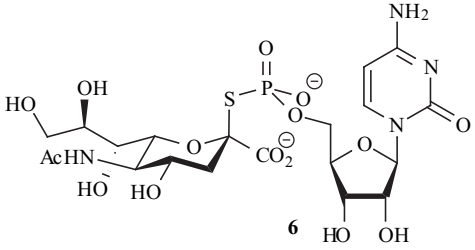

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Fig. (9). Rate of transfer and binding properties significantly altered with replacement of the glycosidic oxygen for a sulphur.

in the transfer of sialic acid to the lactoside acceptor, it was two orders of magnitude less effective [93].

Schmidt also published a brief study detailing a phosphite/phosphonate exchange reaction in the synthesis of substrate inhibitors. The reaction was originally detected as a side reaction in sialylations where low reactivity acceptors were employed [94]. The compounds were assayed against an α2,6-sialyltransferase from rat liver, and were shown to be weak competitive inhibitors in the high micromolar range, with the beta analogue **7** displaying higher enzyme affinity. The results indicated that there is some enzyme tolerance to structural modification at the anomeric centre of the substrate. Analogue **7** [Fig. **10**] showed a K_i=250μM, compared to a K_M=45μM for the natural donor substrate.

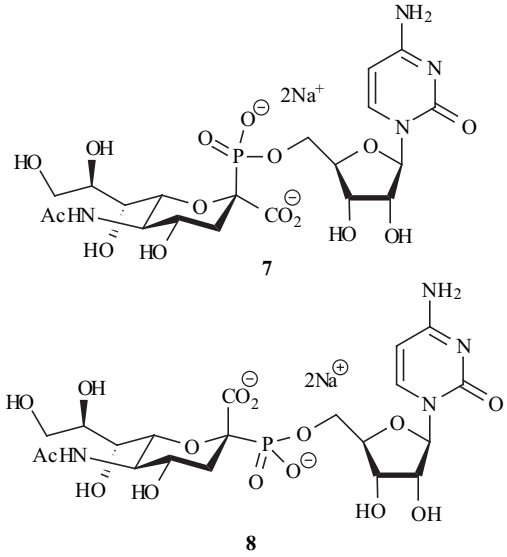

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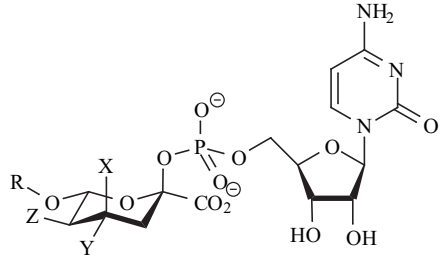
Fig. (10). Two potential donor substrates with the bridge length reduced by one atom.

Schmidt also synthesised potential inhibitors based on CMP-quinic acid, Fig. (**11**) [95] The assays were once again run with an α2,6-sialyltransferase from rat liver. A new assay was developed whereby a UV labelled acceptor was employed. A ratio of products to unreacted starting material was determined by UV-HPLC analysis. By employing an internal standard and using time course experiments, the inhibitory efficiencies of the potential inhibitors could be calculated. The results indicated compound **9** as the most promising lead molecule, Table (**5**).



9: X=Z=OH, Y=R=H,
10: X=R=H, Y=NHAc, Z=OH
11: X=R=H, Y=$NH_3^{\oplus}$, Z=OH

Fig. (11). Exploring potential improvement in binding efficiencies with substitution around a psuedo-sialic acid ring.

Table 5. Inhibiton Constants of CDP and Inhibition Results with Compounds 9-11

Inhibitor	α(2,6)-sialyltransferase	K_i [μM]	Inhibition Mode	Reference
CDP	Bovine Colostrum	10	Competitive	[96]
CDP	Human serum	19	Competitive	[97]
CDP	Rat liver	10 ± 2	Competitive	
9	Rat liver	44 ± 7	Competitive	
10	Rat liver	84 ± 14	Competitive	
11	Rat liver	1400 ± 300	Competitive	

[a]Concentrations of CDP used for determination of K_i: 40μM, 20μM, and 5μM.

[b]Concentrations used for determination of K_i: **9**, 0.5mM, and 0.05mM; **10**, 1mM, 0.5mM and 0.25mM; **11**, 2mm and 0.75mM

Recently, a series of *N*-acetylmannosamines were synthesised in a effort to achieve small molecule inhibitors of polysialic acid (PSA) biosynthesis [98]. Two molecules were tested; a compound referred to as ManProp **12** displayed no activity whilst a compound described as ManBut **13**, Fig. (**12**) was shown to inhibit PSA biosynthesis at a concentration of 1 mM, with inhibition essentially complete at a concentration of 3 mM. Inhibition was determined to occur through a metabolic mechanism whereby ManBut is converted to an unnatural sialic acid derivative which effectively acts as a chain terminator in PSA biosynthesis. It was confirmed that ManBut was not a general inhibitor by analysis of its effects on total cellular sialosides using a periodate-resorcinol assay. The implication of these results is that the enzymes involved in CMP-sialic acid synthesis accept ManBut. What is unclear is which enzyme fails to recognise the substrate after a ManBut moiety has successfully incorporated into the glycoconjugate.

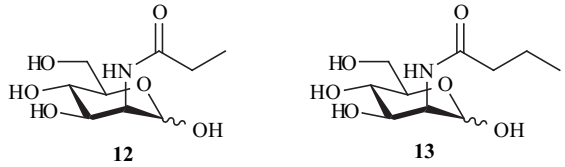


Fig. (12). Small molecule inhibitors accepted by CMP-synthetases.

Development of Transition State Mimetics

Unlike glycosidases, the mechanisms of glycosyltransferases have not received the same degree of scrutiny. Horenstein commented that the main difference between glycosidases and glycosyltransferases is that the former transfers glycons with unactivated leaving groups to water, whilst the latter transfers glycons having an activated leaving group to either a carbohydrate hydroxyl or an amino acid side chain [99]. Horenstein also reported investigations into the mechanism of solvolysis of CMP-NeuAc. To conduct these investigations, a series of ^{2}H-, ^{3}H-, and ^{14}C-substituted CMP-NeuAc isotopomers were synthesised for use in β-^{2}H and ^{14}C kinetic isotope effect (KIE) experiments. An observed primary KIE of 1.030 for solvolysis of CMP-NeuAc, was pivotal in formation of the assumption that the mechanism was not S_N2 in nature. This initial assumption was made on the basis that for an S_N2 mechanism, the KIE would fall between 1.08-1.15. It was proposed that the observed mechanism was consistent with a dissociative pathway. To develop support for an oxocarbenium ion transition state, a correlation with the observed KIE of 1.030 was made to the acid catalysed hydrolysis of glycosides which have primary ^{14}C KIEs of between 1.02 and 1.05, and have a corresponding transition states with oxocarbenium ion character. Secondly, it was hypothesised that since a large secondary ^{14}C KIE of 1.037 at the carboxylate carbon was normal, ie. not due to remote effects, that in proceeding from the ground to transition state, there was likely a substantial loosening of the vibrational environment of the carboxylate group, leading to the postulation that perhaps the carboxylate was becoming more CO_2 like.

The β-dideuterium KIEs were shown to increase from ~1.25 - ~1.354 over a pH range of 4.0 – 6.0. In glycoside hydrolysis, a β-^{2}H KIE arises when the transition state leaving group and nucleophile bond order sum to less than 1, resulting in a positive charge developing at the anomeric carbon. The size of isotope effect is indicative of the magnitude of charge development and the dideuterium KIE is maximised when charge development is complete. Horenstein draws a correlation to an study of aryl sialosides by Sinnot [100], who observed a $(k_H/k_d)_{max}$ between ~1.2-1.4 with predominant protonation of the carboxylate, and a $(k_H/k_d)_{max}$ of 1.098 at pH 6.67 with complete ionisation of the carboxyl group, leading Sinnot to conclude that there might be some kind of participation of the carboxyl group. Thus the KIEs as observed by Horenstein would indicate a largely ionised carboxylate that most likely is not involved in any intramolecular nucleophilic transition pathway.

In summary, the combination of a small primary ^{14}C KIE and a large β-dideuterium KIE were taken by Horenstein to strongly support a transition state structure for CMP-NeuAc solvolysis that is late and without nucleophilic participation of the carboxylate. Horenstein [101] proposed that the carboxylate group could possibly approach co-planarity with the oxocarbenium ion plane minimising orbital overlap and providing a maximisation of opportunity for hyperconjugation. Essentially, directly preceeding the transition state, short-lived oxocarbenium ion intermediates are formed, likely stabilised by intramolecular ion-pairing with the carboxylate group, Fig. (**13**).

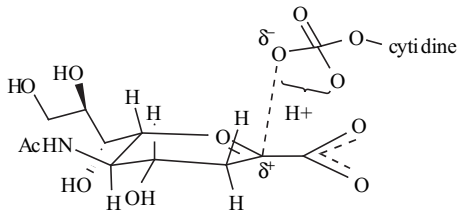
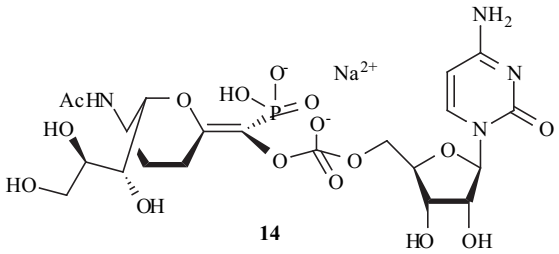


Fig. (13). The Cytidine phosphate leaving group approaches co-planarity with the anomeric carboxyl group which approaches a trigonal configuration.

In an attempt to unmask the mechanism of action of the sialyltransferase, experiments were conducted with an altered nucleotide-sugar donor, UMP-NeuAc [102]. This derivative whilst very similar to CMP-NeuAc has a 30-fold higher K_m than the natural substrate. The k_{cat} is also 5 times lower than that of the natural substrate. By using the increased barrier for the chemical step and the weaker binding of the unnatural analogue, the authors hoped to get full expression of kinetic isotope effects. The values they received agreed well with those from the earlier solvolysis experiments. Similarities were noted between both the β-^{2}H isotope and the primary ^{14}C isotope effects for the sialyltransferase UMP-NeuAc and the solvolysis of CMP-NeuAc. Molecular modelling calculations were found to support protonation by the enzyme of the non-bridging oxygen atom to facilitate glycosyl transfer. The studies were taken to confirm that rat liver α2,6-sialyltransferase has a transition state with a nearly full positive charge, indicating that the cleavage of the CMP group is complete or very nearly so before transfer. The authors suggest that optimal inhibitors of sialyltransferases might be those which promote the enzyme's ability to reach a conformation that reflects the catalytic form.

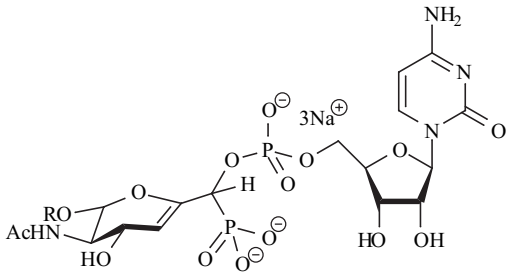
In pursuit of further elucidation of the transition state geometry of sialyltransferases Schmidt *et al.* have achieved some considerable inroads towards possible transition state mimics [103,104]. An inhibitor [Compound **14** (K_i = 40nm)] Fig. (**14**), was synthesised containing, as in CMP-NeuAc, two negative charges separated by five bonds. The analogue [98] also contained trigonal planar geometry at a psuedo-anomeric centre, in keeping with Horenstein's purported transition state.



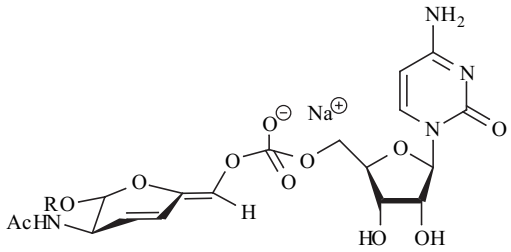
14

Fig. (14). A potent rat α(2,6)sialyltransferase inhibitor.

Recently Schmidt published another study on inhibitors based on glycosides of *N*-acetylglucosamine, Fig. (**15**), Table (**6**) [105]. The purpose of this research was to determine whether certain side-chain modifications could increase the binding affinity. A range of 8 different potential inhibitors, four sets of diasteriomers were synthesised. The conclusions drawn from the results, reinforced earlier conclusions, that (i) planarity at the anomeric carbon, (ii) increased distance between the anomeric carbon and the CMP leaving group, and (iii) the presence of at least two negative charges close to the glycosylation cleavage site, are all required for high affinity to the enzyme.



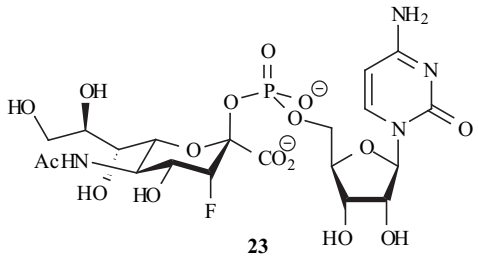
R=phenyl: **15, 16;** R=2-hydroxyethyl: **17, 18**



R=phenyl: **19, 20;** R=2-hydroxyethyl: **21, 22**

Fig. (15). Inhibitors designed from an N-acetylglucosamine starting material showing good efficacy

The following study by Wong *et al.* drew on the conclusion that incorporation of a fluorine to the 3-position of sialic acid provides an effective inhibitor for bacterial and viral sialidases. It was proposed that if the sialyltransferase reaction mechanism passes through a cationic transition state, then a fluorinated CMP-NeuAc derivative would be expected to inhibit sialyltransferases as a non-reactive mechanism based inhibitor. To test this theory, a compound was synthesised and assayed as an inhibitor of α2,6-sialyltransferase (Calbiochem, San Diego, CA). Results indicated that **23**, Fig. (**16**) competed with CMP-NeuAc (K_m = 15μM), with a K_i = 5.7 ± 1.2μM. This result was considered consistent with a transition state structure with considerable oxocarbenium ion characteristic [106].



23

Fig. (16). Altered anomeric electron density is provided by a 3-fluoro substituent.

The basis for the design of inhibitor **24** was the Horenstein model wherein the conjugated carboxylate group of **24** mimics the oxocarbenium ion coplanarity of the transition state, Fig. (**17**). The increased transition state distance between the phosphate oxygen and the anomeric

Table 6. Affinity of CMP-NeuAc (K_M) to α(2,6)-Sialyltransferase of Rat Liver and Inhibition Constants of Inhibitors 15-22

Comp.	K_M (μM)	K_i (μM)	Inhibition mode	K_M/K_i	ref
CMP-NeuAc	46				[95]
15		0.029±0.006	competitive	1580.0	
16		0.69±0.19	competitive	67.0	
17		0.059±0.018	competitive	780.0	
18		0.038±0.009	competitive	1210.0	
19		158±41	competitive	0.3	
20		25±7	competitive	1.8	
21		2.4±0.4	competitive	19.0	
22		3.5±1.4	competitive	13.0	

carbon is purportedly mimicked through the introduction of the bicyclic spacer. The inhibitory effects of the compound were investigated with rat α2,3- and α2,6-sialyltransferases using radiolabeled [9-^{3}H] CMP-NeuAc as the donor substrate. Lactose was used as an acceptor for the α2,3-transferase and LacNAc for the α2,6-sialyltransferase. K_is were estimated to be 10 and 20μM for α2,3- and α2,6-sialyltransferases respectively [107].

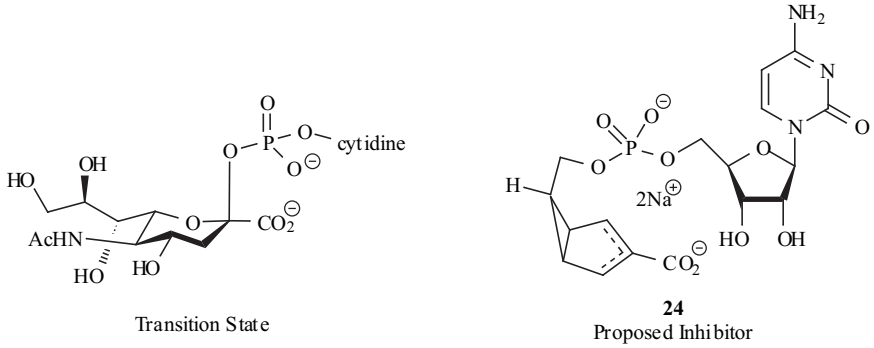


Fig. (17). A proposed CMP-NeuAc transition state and a inhibitor based on this model.

Acceptor Based inhibitors

One consequence of tumorogenic abberation, is that tumor cells synthesise *N*-glycans with increasing numbers of antennae and poly-*N*-acetyllactosamine side chains [108], thus proliferating the number of possible sialylation sites. Although greater enzyme affinity appears available by exploiting the charged characteristics donor substrate, it is less likelyhood of successful inhibition employing this approach. This, he claims, is due to the fact that most sugar nucleotides act as donors for around ten different glycosyltransferases (~20 in the case of sialyltransferases) and so differentiation between glycosyltransferases will be problematic [109]. He also claims, that to a large extent recognition in a glycosyltranferase mediated biotransfer, relies on the acceptor and not the nucleotide portion. Hindsgaul synthesised deoxy acceptors of glycosyltransferases, reasoning that if the acceptor hydroxyl group was required for binding, then the deoxy analogue would not act as an inhibitor, and if the acceptor hydroxyl was not required for binding, then the acceptor deoxy derivatives could act as potential competitive inhibitors,

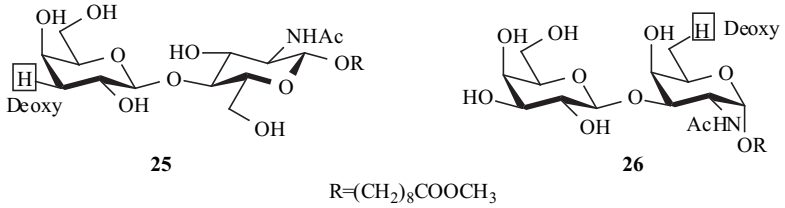


Fig. (18). Deoxy analogues to probe acceptor hydrogen bonding characteristics.

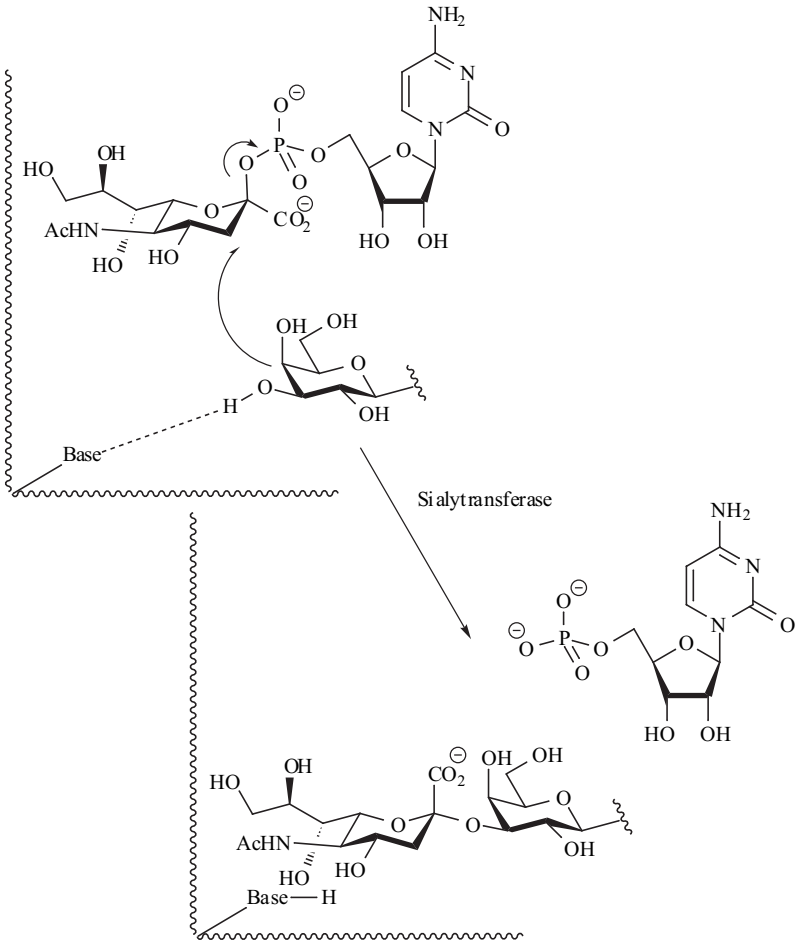


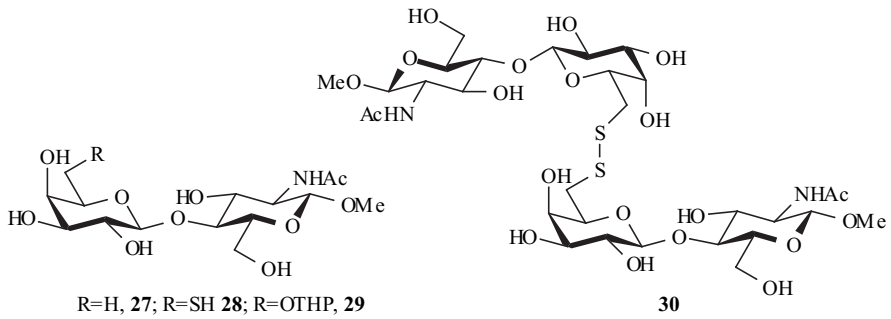
Fig. (19). Nucleophilic sialic acid transfer catalysed by a basic enzyme cavity moiety and an α2,3-sialyltransferase.

Fig. (**18**). Although theoretically sound, the binding affinity of acceptors is weak (low mM range) and you would expect similar weak binding affinity from a deoxy-acceptor derivative.

Results indicated that neither of the above deoxy analogues acted as inhibitors. It was concluded that the reacting acceptor hydroxyl group was required for acceptor recognition and binding to the enzyme. Further, it was proposed that the acceptors bind to the transferase by donating a proton to a basic hydrogen bond acceptor, Fig. (**19**). Hindsgaul commented that if the theory of hydrogen bond formation between acceptor and enzyme was valid, then suicide type inhibitors could be designed based on a covalent linkage formed at this site between enzyme and acceptor.

Acting in part on Hindsgaul's observations, Hashimoto and co-workers [110] embarked upon the synthesis of *N*-acetyllactosamine analogues, as inhibitors of rat liver ST6Gal I. Derivatisation was based on the same principles that Hindsgaul employed, if the 6-deoxy acceptor analogue **27** was a successful inhibitor, then the acceptor hydroxyl group was not required for binding. The 6-deoxy-6-thio acceptor analogue **28** could act either as an acceptor or an inhibitor for the transferase, and the 6-*O*-tetrahydropyran (THP) **29** derivative could explore steric constraints in the acceptor binding region, Fig. (**20**).

The 6-deoxy lactosamine derivative proved to be the most effective inhibitor (0.76mM (mixed inhibition)), having five times the inhibitory activity of the thiol and THP derivative, and 2.5 times the inhibitory activity of the



R=H, **27**; R=SH **28**; R=OTHP, **29** **30**

Fig. (20). Exploring the effects of modifications to the 6'-OH group of sialyltransferase acceptors.

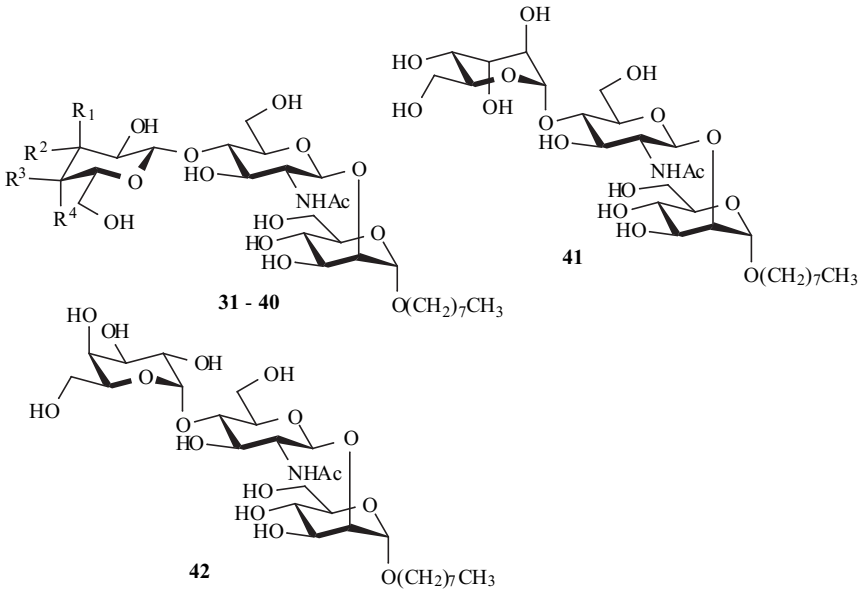


Fig. (21). Modified trisaccharide sialyltransferase acceptors.

lactosamine disulphide dimer **30**. It was interesting to note that those analogues with bulky substituents at the 6-hydroxyl showed no dramatic decrease in activity compared with analogues without bulky substituents, indicating a certain degree of stereochemical latitude around the galactose moiety in the acceptor binding site of the enzyme. This could imply recognition by the enzyme not only of terminal residue of the oligosaccharide chain, but also internal residues. Kinetic results also indicated that donor based inhibitors did not interfere with acceptor inhibition, and that mixed inhibition was occuring in competition studies between the methyl lactosamine and 6-derivatised analogues. The mixed inhibition was speculated to arise from at least two possible lactosamine binding sites present on the enzyme.

Table 7. Compounds Generated for an Acceptor Based Structure/Function Study of Sialyltransferases

Comp.	Structure	R^1	R^2	R^3	R^4
31	β-D-Gal-R	H	OH	H	OH
32	3-deoxy-β-D-Gal-R	H	H	H	OH
33	3-deoxy-3-fluoro-β-D-Gal-R	H	F	H	OH
34	β-D-Gul-R (3-epimer)	OH	H	H	OH
35	3-O-methyl-β-D-Gal-R	H	OMe	H	OH
36	3-amino-3-deoxy-β-D-Gal-R	H	NH_2	H	OH
37	4-deoxy-β-D-Gal-R	H	OH	H	H
38	4-deoxy-4-fluoro-β-D-Gal-R	H	OH	H	F
39	β-D-Glc-R (4-epimer)	H	OH	OH	H
40	4-O-methyl-β-D-Gal-R	H	OH	H	OMe
41	α-L-Alt-R (5-epimer)				
42	β-L-Gal-R (enantiomer)				

Van Dorst *et al.* undertook a study of the acceptor binding pocket of various sialyl-transferases [111]. An examination was made of the acceptor substrate specificity of an α2,6-sialyltransferase from rat liver, a recombinant full-length form of the same enzyme from human liver, and a soluble form of a recombinant α2,3-sialyltransferase. The enzymes were employed in assays with trisaccharide acceptors, with substrates functionalised in the 3 or 4 –OH of the terminal galactosyl moiety, Fig. (**21**), Table (**7**).

Substituents such as fluoro, methoxy and amino groups were employed as well as C-3 and C-4 epimers. Significant decrease in binding efficiency with substrates modified at the galactosyl C-3 or C-4 positions indicated that there are probably hydrogen bonding contributions from these positions. Due to the lower rate of sialylation encountered with 3 or 4 fluoro substituted galactoses, it is suggested that the 3 and 4 –OH are hydrogen bond donating functions, Table (**8**) [112].

Another interesting result was that comparison of the deoxy and fluoro derivatives indicated that there was a higher affinity of the enzyme for the latter.

Another series of acceptor based inhibitor experiments, dealt with the exposure for 24 hours of the sugar analogue GalNAc-α-*O*-benzyl **43** to mucus secreting HT-29 cells, resulting in the inhibition of the Galβ1-3GalNAc α2,3-sialyltransferase. It was proposed that the benzyl glycoside **43** is glycosylated to give Galβ1-3GalNAc-α-*O*-benzyl, which competes with the natural ligand Fig. (**22**). At a concentration of 2mM, mucus secretion was noted to cease altogether. Notably, the secretion was resumed when GalNAc-α-*O*-benzyl was ceased to be administered to the cells. It was postulated that the acceptor substrate could competetively inhibit ST3Gal IV, an enzyme able to transfer CMP-NeuAc to either of the ligands Galβ1-3GalNAc or Galβ1-4GalNAc. Further, it was considered possible that high concentrations of the competitive substrate may decrease the amount of CMP-NeuAc in the golgi lumen, and can therefore compete with other sialyltransferases expressed in HT-29 cells *via* the donor substrate [113,114].

Table 8. Acceptor Based Structure/Function Study with a Range of Sialyltransferases

Comp.	Acceptor	Relative rates of sialylation		
		RL (Rat liver) α-2,6-ST %	HL (Human liver) α-2,6-ST %	RL (Rat liver) α-2,3rST %
31	β-D-Gal-R	100	100	100
32	3-deoxy-β-D-Gal-R	7	13	0
33	3-deoxy-3-fluoro-β-D-Gal-R	2	8	0
34	β-D-Gul-R	1	<1	0
35	3-O-methyl-β-D-Gal-R	1	<1	0
36	3-amino-3-deoxy-β-D-Gal-R	2	<1	0
37	4-deoxy-β-D-Gal-R	40	29	2
38	4-deoxy-4-fluoro-β-D-Gal-R	23	17	7
39	β-D-Glc-R	6	5	0
40	4-O-methyl-β-D-Gal-R	2	2	51
41	α-L-Alt-R	<1	<1	0
42	β-L-Gal-R	0	0	0

It was determined that α-galactosamine benzyl glycoside had a differential effect on the sialylation of apical *versus* basolateral glycoproteins, the former being partially inhibited the latter unaffected.

Further Enzyme Characteristics

This final section deals with studies that although not directly involved in inhibitor synthesis, have to some extent defined characteristics of the CMP-NeuAc binding pocket of sialyltransferase enzymes. Gross *et al.* discussed the kinetic properties of several synthetic 9-substituted sialic acid analogues [115]. Predominantly interested in the incorporation of modified sialic acids to glycoproteins to facilitate resistance to pathogenic infection mechanisms, Gross and co-workers made a study of the binding pocket of a number of sialyltransferases *via* derivatisation of the 9-OH position of CMP-NeuAc The different sialic acids were 9-deoxy 9-amino-NeuAc, 9-deoxy 9-azido-NeuAc, 9-deoxy 9-acetamido-NeuAc, 9-deoxy 9-benzamido-NeuAc, and 9-deoxy 9-hexanoylamido-NeuAc, Fig. (**23**). The amino derivative is reportedly resistant to bacterial, viral and mammalian sialidases and so transfer of this acid to glycoconjugates has ramifications in therapeutic development. The azido allows the opportunity to introduce photoreactive labels, and the different amides can be used to study the spatial characteristics of the enzyme pocket in that region. Many of the derivatives displayed a faster transfer rate than the natural substrate, particularly the azido derivative. The 9-amino derivative was noticable for its slow transfer rate from 50-100 times slower than the most quickly transferred substrates. Of most interest from a transferase inhibitor design point of view, is that all unnatural substrates were accepted by the enzyme.

A continuation in part of the above study, Gross and Brossmer studied effect of transfer rate in regard to substitution at the 5-deoxy 5-amido position of CMP-NeuAc, Fig. (**24**) [116]. This in conjunction with the above study seems to indicate that there is a certain degree of spatial lassitude in the enzyme pocket in regard to

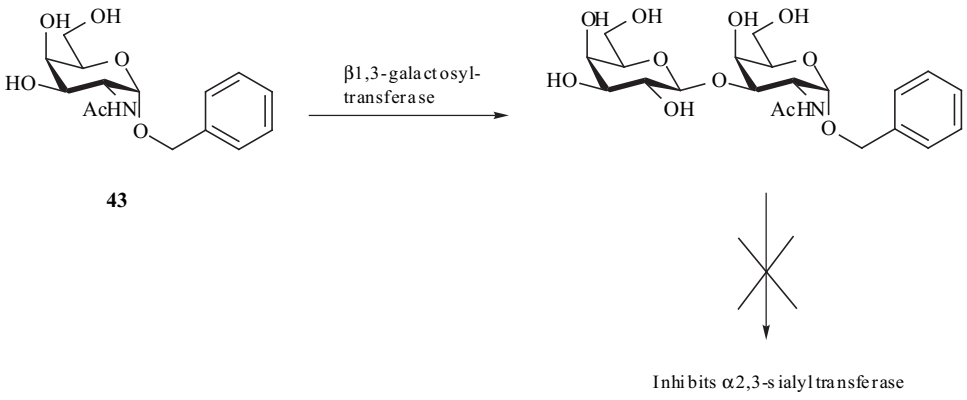


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Fig. (22). A modified N-acetylgalactosamine sialyltransferase inhibitor.

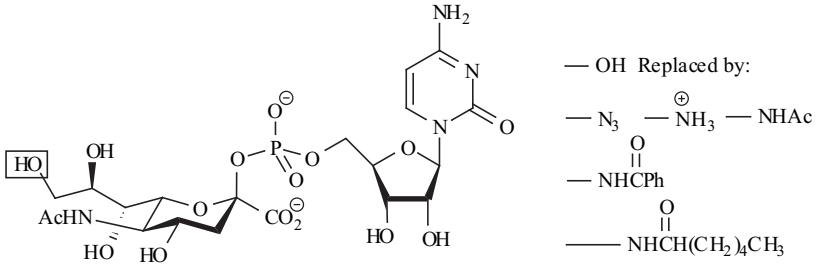


Fig. (23). Exploring the effects on sialic acid transfer by modification to the 9-OH group.

derivatisations made to the sialic acid moiety of CMP-NeuAc. Whether this kind of study can for example, be exploited to assist in the design a range of inhibitors that effectively differentiate between the different sialyltransferases, has yet to be realised.

The authors comment that the acetyl group at C-5 of the sialic acid, represents a critical structural element for enzyme-substrate interactions. Results for the artificial donor substrates described in this paper were relatively consistent across both rat, porcine submaxillary glands and human liver

Derivatisation of the sialic acid moiety of CMP-NeuAc **52** appears to have less effect on the substrate binding than modification or replacement of the base moiety, which appears critical to the binding of substrate analogues to the enzyme. Schmidt and co-workers have also synthesised analogues of the CMP-NeuAc substrate focusing on cytidine mimetics, variation at the C5 *N*-acetyl functionality, and substitution of the polyhydroxy side chain (Fig. (**25**)) [117]. Modifications at either the amino group or the side chain of the sialic acid residue were determined not to be critical to binding of the substrate although substitution of the base

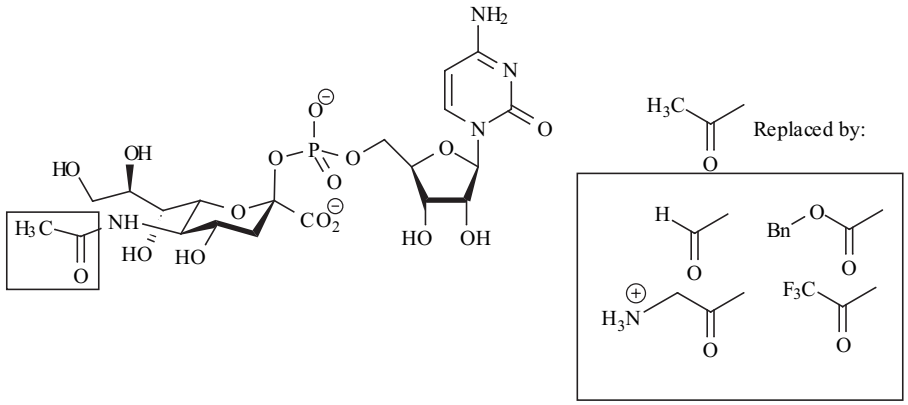


Fig. (24). Exploring the effects on sialic acid transfer by modification to the 5-acetamide function.

α2,6-sialyltransferases, and the synthetic analogues did not differ significantly in rate of transfer from the parent CMP-NeuAc. Interestingly the benzyloxycarbonyl derivative gave ~1.4-1.7 fold increase in the rate of transfer. The structural variation at C-5 had slightly more effect on the α2,3-sialyltransferase from porcine liver (20-50% of the CMP-NeuAc rate). It was commented that in spite of the high degree of sequence homology between rat and human liver α2,6-sialyltransferase, donor substrate affinity and sensitivity towards modification at positions C-5 or C-9 of the NeuAc moiety turned out to be markedly different.

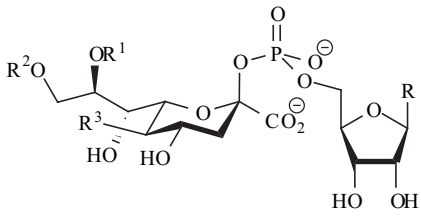


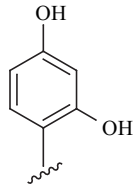
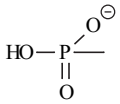
Fig. (25). Effects on transfer with modifications to the cytidine moiety, 5-acetamide, 8-OH and 9-OH functional groups of CMP-NeuAc.

residue was not well tolerated Table (**9**). This research provides a foundation in the examination of potential cytidine isosteres.

CONCLUSION

The various approaches to sialyltrasferase inhibitor design herein reviewed provide some important insights into the donor and acceptor characteristics that are required/tolerated by this family of enzymes. In addition, these studies have provided possible strategies that could be employed in further design of selected inhibitors. Further progress/advances in what is a very fascinating and medically relevant field, would be assisted by a more detailed understanding of the nature of sialyltransferase enzyme pockets. With no crystallographic data, and such a low homology amongst the sialyltransferases, researchers have been challenged in this difficult field of research. Despite these challenges, some excellent results have been obtained, exemplified by the recent synthesis of nanomolar inhibitors. Such inhibitors are expected to significantly increase our understanding of the biological roles played by

Table 9. Rates of Transfer with CMP-NeuAc Analogues (Refer to Structure Fig. (25))

Comp.	R	R^1	R^2	R^3	K_m (μM)
44	OMe	H	H	AcHN-	Not a substrate
45		H	H	AcHN-	Not a substrate
46	Cytidine	H	H	H_3N^+	[a]Substrate
47	Cytidine	H	H	C_4H_9-CO-NH-	50 ± 10
48	Cytidine	H	H	^+H_3N-CH_2-CO-NH-	[a]Substrate
49	Cytidine	H	H	EtO-CO-NH-	31 ± 5
50	Cytidine	Me	H	AcHN-	[a]Substrate
51	Cytidine	H		AcHN-	[a]Substrate
52	Cytidine	H	H	AcHN-	46 ± 5

[a]Sialyltransfer was very slow and therefore the reaction was not quantified.

these enzymes, and affirm this class of enzymes as a genuine therapeutic target. With the excellent research undertaken to date and further progress expected, the demystification of these enzymes, that commenced many years ago, draws closer to completion.

REFERENCES

[1] van den Eijnden, D. H., Joziasse, D. H. *Current Opinion in Structural Biology*, **1993**, *3*, 711-721.
[2] Varki, A. *Glycobiology*, **1993**, *3*, 97-130.
[3] Brockhausen, I., Schutzbach, J., Kuhns, W. *ACTA ANATOMICA* **1998**, *161*, 36-78.
[4] Orntoft, T. F., Vestergaard, E. M. *Electrophoresis,* **1999**, *20*, 362-371.
[5] Dall'Olio, F. *J. Clin. Pathol.: Mol. Pathol.,* **1996**, *49*, M126-M135.
[6] Schroder, P. N., Giannis, A. *Angew. Chem. Int. Ed.*, **1999**, *38*(10), 1379-1380
[7] Fukuda, M., Hindsgaul, O. *Molecular Glycobiology*; IRL Press: Oxford University, **1994**.
[8] Beyer, T. A., Sadler, J. E., Rearick, J. I., Paulson, J. C., Hill, R. L. *Adv. Enzymol. Relat. Areas Mol. Biol.,* **1981**, *52*, 23-175.
[9] Joziasse, D. H., Schiphorst, W. E., Van den Eijnden, D. H., Van Kuik, J. A., Van Halbeek, H., Vliegenthart, J. F. *J. Biol. Chem.,* **1987**, *262*, 2025-2033.
[10] Warren, L., Fuhrer, J. P., Buck, C. A. *Proc. Natl. Acad. Sci. USA,* **1972**, *69*, 1838-1842.
[11] Feizi, T. *Selectins*, **1985**, *314*, 53-57.
[12] Tsuji, S. *J. Biochem. (Tokyo)*, **1996**, *120*, 1-13.
[13] Sasaki, K., Katsutoshi, L. *Trends Glycosci. Glycotechnol.*, **1999**, *8*, 195-215.
[14] Strandberg, Y., In *Institute for Molecular Bioscience*; University of Queensland: Brisbane, **2001**, p 240.
[15] Field, M. C., Wainwright, L. J. *Glycobiology*, **1995**, *5*, 463-472.
[16] Wen, D. X., Livingston, B. D., Medzihradszky, K. F., Kelm, S., Burlingame, A. L., Paulson, J. C. *J. Biol. Chem.*, **1992**, *267*, 21011-21019.
[17] Geremia, R. A., Harduin-Lepers, A., Delannoy, P. *Glycobiology*, **1997**, *7*, v-vii.
[18] Datta, A. K., Sinha, A., Paulson, J. C. *J. Biol. Chem.*, **1998**, *273*, 9608-9614.
[19] Datta, A. K., Chammas, R., Paulson, J. C. *J. Biol. Chem.*, **2001**, *276*, 15200-15207.
[20] Datta, A. K., Paulson, J. C. *J. Biol. Chem.*, **1995**, *270*, 1497-1500.
[21] Datta, A. K., Paulson, J. C. *Indian-J.-Biochem.-Biophys.*, **1997**, *34*, 157-165.
[22] van Beek, W. S., LA; Emmelot, P. *Cancer Res.*, **1973**, *33*, 2913-2922.
[23] Passaniti, A., Hart, G. W. *J. Biol. Chem.*, **1988**, *263*, 7591-7603.
[24] Gessner, P. R., S. Quentmaier, A., Kemmner, W. *Cancer Letters,* **1993**, *75*, 143-149.
[25] Yogeeswaran, G., Salk, P. L. *Serum ST*, **1981**, *212*, 1514-1516.
[26] (i) Morré, D.J., Kloppel, T.M., Merritt, W.D., Keenan T.W., *J. Supramol. Structure*, **1978**, 9, 157-177; (ii) Merritt, W.D., Richardson, C.L., Keenan, T.W., Morré, D.J. *J. Natl. Cancer Inst.*, **1978**, *60*, 1313-1327.
[27] Weiss, L., Fisher, B., Fisher E.R. *Cancer*, **1974**, *34*, 182-183.
[28] Weiss, L. *Journal of the National Cancer Institute*, **1972**, 50, 3-19.
[29] Hakomori, S. *Cancer Res.*, **1996**, *56*, 5309-5318.
[30] Burchell, J. M., Mungul, A., Taylor-Papadimitriou, J. *J. Mammary Gland Biol. Neoplasia*, **2001**, *6*, 355-364.
[31] Kijima-Suda, I., Toyoshima, S., Itoh, M., Furuhata, K., Ogura, H., Osawa, T. *Chem. Pharm. Bull.*, **1985**, *33*, 730-739.
[32] Kijima-Suda, I., Miyamoto, Y., Toyoshima, S., Itoh, M., Osawa, T. *Cancer Res.*, **1986**, *46*, 858-862.
[33] Kijima-Suda, I., Miyazawa, T., Itoh, M., Toyoshima, S., Osawa, T. *Cancer Res.*, **1988**, *48*, 3728-3732.
[34] Harvey, B. E., Toth, C. A., Wagner, H. E., Steele Jr, G. D., Thomas, P. *Cancer Res.,* **1992**, *52*, 1775-1779.
[35] Wagner, H. E., Thomas, P., Wolf, B. C., Rapoza, A., Steele Jr, G. *Archives of Surgery,* **1990**, *125*, 351-354.
[36] Harvey, B. E., Thomas, P. *Biochem. Biophys. Res. Commun.*, **1993**, *190*, 571-575.
[37] Dennis J; Waller C; Timpl R; V, S. *Selectins*, **1982**, *300*, 274-276.
[38] Kim, Y. J., Varki, A. *Glycoconjugate J.*, **1997**, *14*, 569-576.
[39] Davidson, B., Gotlieb, W. H., Ben-Baruch, G., Kopolovic, J., Goldberg, I.;,Nesland, J. M., Berner, A., Bjamer, A., Bryne, M. *Gynecol. Oncol.*, **2000**, *77*, 35-43.

[40] Davidson, B., Berner, A., Nesland, J. M., Risberg, B., Kristensen, G. B., Trope, C. G., Bryne, M. *Hum. Pathol.*, **2000**, *31*, 1081-1087.

[41] Dennis, J. W., Granovsky, M., Warren, C. E. *Bioessays*, **1999**, *21*, 412-421.

[42] Dall'Olio, F., Malagolini, N., di Stefano, G., Minni, F., Marrano, D., Serafini-Cessi, F. *Int. J. Cancer*, **1989**, *44*, 434-439.

[43] Lise, M., Belluco, C., Perera, S. P., Patel, R., Thomas, P., Ganguly, A. *Hybridoma*, **2000**, *19*, 281-286.

[44] Petretti, T., Kemmner, W., Schulze, B., Schlag, P. M. *Gut*, **2000**, *46*, 359-366.

[45] Kakeji, Y., Maehara, Y., Morita, M., Matsukuma, A., Furusawa, M., Takahashi, I., Kusumoto, T., Ohno, S., Sugimachi, K. *British Journal of Cancer*, **1995**, *71*, 191-195.

[46] Recchi, M. A., Harduin Lepers, A., Boilly Marer, Y., Verbert, A., Delannoy, P., *Glycoconj-J.*, **1998**, *15*, 19-27.

[47] Lin, S.;,Kemmner, W., Grigull, S., Schlag, P. *Exp. Cell Res.*, **2002**, *276*, 101-110.

[48] Burchell, J., Poulsom, R., Hanby, A., Whitehouse, C., Cooper, L., Clausen, H., Miles, D., Taylor-Papadimitriou, J. *Glycobiology*, **1999**, *9*, 1307-1311.

[49] Baker, M. A., Taub, R. N., Kanani, A., Brockhausen, I., Hindenburg, A. *Blood*, **1985**, *66*, 1068-1071.

[50] Ruan, S. T., Raj, B. K. M., Lloyd, K. O. *J. Neurochem.*, **1999**, *72*, 514-521.

[51] Haraguchi, M., S. Yamashiro, A. Yamamoto, K. Furukawa, K. Takamiya, K. Lloyd, H. Shiku, Furukawa, K. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 10455-10459.

[52] Ito, H., Hiraiwa, N., Sawadakasugai, M., Akamatsu, S., Tachikawa, T., Kasai, Y., Akiyama, S., Ito, K., Takagi, H., Kannagi, R. *Int. J. Cancer*, **1997**, *71(4)*, 556-564.

[53] Dall'Olio, F., Malagolini, N., Di Stefano, G., Ciambella, M., Serafini-Cessi, F. *Int. J. Cancer*, **1991**, *47*, 291-297.

[54] Dall'Olio, F. M., N., Serafini-Cessi, F. *Int. J. Cancer*, **1992**, *50*, 325-330.

[55] Dall'Olio, F., Trere, D. *ST in tumor tissues/Tumor Specific*, **1993**, *37*, 257-265.

[56] Dall'Olio, F., Chiricolo, M., Ceccarelli, C., Minni, F., Marrano, D., Santini, D. *Int. J. Cancer*, **2000**, *88*, 58-65.

[57] Lemarer, N., Stehelin, D. *Glycobiology*, **1995**, *5*, 219-226.

[58] Werther, J. L., Tatematsu, M., Klein, R., Kurihara, M., Kumagai, K.;,Llorens, P., Neto, J. G., Bodian, C., Pertsemlidis, D., Yamachika, T., Kitou, T., Itzkowitz, S. *Int. J. Cancer*, **1996**, *69*, 193-199.

[59] Ikehara, Y., Kojima, N., Kurosawa, N., Kudo, T., Kono, M., Nishihara, S., Issiki, S., Morozumi, K., Itzkowitz, S., Tsuda, T., Nishimura, S., Tsuji, S., Narimatsu, H. *Glycobiology*, **1999**, *9*, 1213-1224.

[60] Ikehara, Y., Kojima, N., Kurosawa, N., Kudo, T., Kono, M., Nishihara, S., Issiki, S., Morozumi, K., Itzkowitz, S., Tsuda, T., Nishimura, S. I., Tsuji, S., Narimatsu, H. *Glycobiology*, **1999**, *9*, 1213-1224.

[61] Itzkowitz, S. H., Yuan, M., Montgomery, C. K., Kjeldsen, T., Takahashi, H. K., Bigbee, W. L., Kim, Y. S. *Cancer Res.*, **1989**, *49*, 197-204.

[62] Itzkowitz, S. B., E., Kokal, WA, Modin, G, Hakomori, S., Kim, Y.S. *Cancer*, **1990**, *66*, 1960-1966.

[63] Orntoft, T., Harving, N., Langkilde, N. C. *Int. J. Cancer,* **1990**, *45*, 666-672.

[64] Le Pendu, J., Marionneau, S., Cailleau-Thomas, A., Rocher, J., Le Moullac-Vaidye, B., Clement, M. *Apmis*, **2001**, *109*, 9-31.

[65] Kadota, A., Masutani, M., Takei, M., Horie, T. *Int. J. Oncol.*, **1999**, *15*, 1081-1089.

[66] Ogawa, J., Sano, A., Inoue, H., Koide, S. *Ann. Thorac. Surg.*, **1995**, *59*, 412-415.

[67] Ohyama, C., Tsuboi, S., Fukuda, M. *EMBO J.*, **1999**, *18*, 1516-1525.

[68] Satoh, H., Ishikawa, H., Kamma, H., Yamashita, Y. T., Takahashi, H., Ohtsuka, M., Hasegawa, S. *Clinical Cancer Research*, **1997**, *3(4)*, 495-499.

[69] Nakamori, S., Furukawa, H., Hiratsuka, M., Iwanaga, T., Imaoka, S., Ishikawa, O., Kabuto, T., Sasaki, Y., Kameyama, M., Ishiguro, S., Irimura, T. *Journal of Clinical Oncology*, **1997**, *15*, 816-825.

[70] Nakamori, S., Kameyama, M., Imaoka, S., Furukawa, H., Ishikawa, O., Sasaki, Y., Izumi, Y., Irimura, T. *Diseases Of the Colon and Rectum*, **1997**, *40(4)*, 420-431.

[71] Nakagoe, T., Fukushima, K., Nanashima, A., Sawai, T., Tsuji, T., Jibiki, M. A., Yamaguchi, H., Yasutake, T., Ayabe, H., Matuo, T., Tagawa, Y. *J. Gastroenterol. Hepatol.*, **2001**, *16*, 176-183.

[72] Fukuda, M. *Seikagaku*, **2000**, *72*, 269-283.

[73] Akahori, T., Yuzawa, Y., Nishikawa, K., Tamatani, T., Kannagi, R., Miyasaka, M., Okada, H., Hotta, N., Matsuo, S. *J. Immunol.*, **1997**, *158(11)*, 5384-5392.

[74] Hildebrandt, H., Becker, C., Gluer, S., Rosner, H., Gerardy-Schahn, R., Rahmann, H. *Cancer Res.*, **1998**, *58*, 779-784.

[75] Seidenfaden, R., Gerardy-Schahn, R., Hildebrandt, H. *European Journal of Cell Biology*, **2000**, *79*, 680-688.

[76] Bruses, J. L., Rutishauser, U. *Biochimie*, **2001**, *83*, 635-643.

[77] Gluer, S., Zense, M., Radtke, E., von Schweinitz, D. *Langenbeck's Arch. Surg.*, **1998**, *383*, 340-344.

[78] Livingston, B. D., Jacobs, J. L., Glick, M. C., Troy, F. A. *J. Biol. Chem.*, **1988**, *263*, 9443-9448.

[79] Scheidegger, E. P., Sternberg, L. R., Roth, J., Lowe, J. B. *Journal of Biological Chemistry*, **1995**, *270*, 22685-22688.

[80] Lu, C. Q., Lu, J., Wang, B. L., Cui, Y. Z. *Mol. Cell. Biochem.*, **2000**, *207*, 29-33

[81] Klohs, W. D., Bernacki, R. J., Korytnyk, W. *Cancer Research.*, **1979**, *39*, 1231-1236.

[82] (i) Bernacki, R. J. *Eur. J. Biochem.*, **1975**, *58*, 477-481; (ii) Shah, S. N., Raghupathy, E. *Proc. Soc. Exp. Biol. Med.*, **1977**, 1*55*, 516-518; (iii) Kilton, L. J., Maca, R. D. *J. Natl. Cancer. Inst.*, **1977**, *58*, 1479-1481.

[83] Dixon, M. *Biochem. J.*, **1963**, *238*, 1772-1776

[84] Klohs, W. D., Bernacki, R. J., Korytnyk, W. *Cancer Research*, **1979**, *39*, 1231-1236

[85] Korytnyk, W., Angelo, N., Klohs, W., Bernack, R. J. *Eur. J. Med. Chem.*, **1980**, *15*, 77-84.

[86] Toshiaki, O. *Sialic Acids*, **1988**, *Proc. Jpn.-Ger. Symp.,* **1988**, 214-15.

[87] Isao Kijima-Suda, Satoshi Toyoshima, Masayoshi Itoh, Kimio Furuhata, Haruo Ogura, Toshiaki Osawa *Chem. Pharm. Bull.*, **1985**, *33*(2), 730-739.

[88] Haruo Ogura, Kimio Furuhata *Carbohydrate Research*, **1986**, *158*, 37-51.

[89] Petrick, A. T., Meterissian, S., Steele G. (Jr), Thomas, P. *Clin. Exp. Metastasis*, **1994**, *12*, 108-116, Hakomiri, S. *Cancer Res.*, **1985**, *45*, 2405-14, Yogeeswaran, G. *Adv. Cancer Res.*, **1983**, 3*8*, 289-350.

[91] Yasumaru Hatanaka, Makoto Hashimoto, Kazuya I.-P. Jwa Hidari, Yutaka Sanai, Yoshitaka Nagai, Yuichi Kanaoka *Heterocycles*, **1996**, *43*(3), 531-534.

[92] Masakasu Imamura, Hironobu Hashimoto, *Chemistry Letters*, **1996**, 1087-1088.

[93] Cohen, S. B., Halcomb, R. L. *J. Org. Chem.*, **2000**, *65*, 6145-6152.

[94] Muller, B., Martin, T. J., Schaub, C., Schmidt, R. R. *Tetrahedron Letters*, **1998**, *39*, 509-512.

[95] Schaub, C., Muller B., Schmidt. R. R. *Glycoconjugate Journal*, **1998**, 15, 345-354.

[96] Paulson, J.C., Barlow, J.J. *Carbohydrate Research*, **1975**, *43*, 299-304.

[97] Klohs, W.D., Bernacki, R.J., Korytnyk, W. *Cancer Research*, **1987**, *39*, 1231-1238

[98] Mahal, L. K., Charter, N. W., Angata, K., Fukuda, M., Koshland (Jr), D. E., Bertozzi, C. *Science*, **2001**, 294, 380-382.

[100] Ashwell, M., Guo, X, Sinnot, M.L. *J. Am. Chem. Soc.,* **1992**, *114*, 10158-10166.

[101] Horenstein, B .A., Bruner, M. *J. Am. Chem. Soc.*, **1996**, *118*, 10371-10379

[102] Bruner, M., Horenstein, B. A. *Biochemistry*, **2000**, *39*, 2261-2268.

[103] Amann, F., Schaub, C., Muller, B., Schmidt, R. R. *Chem. Eur. J.*, **1998**, *4*(6), 1106-1115.

[104] Muller, B., Schaub, C., Schmidt, R. R. *Angew. Chem. Int. Ed.*, **1998**, *37*(20), 2893-2897.

(105) Schwörer, R., and. Schmidt, R. R. *Journal of the American Chemical Society*, **2002**, *48*(8), 1632-1637.

[106] Burkart, M. D., Vincent, S. P., Wong, C.-H. *Chem. Commun.*, **1999**, 1525-1526.

[107] Hongbin Sun, Jingsong Yang, Amaral, K. E., Horenstein, B. A. *Tetrahedron Letters*, **2001**, *42*, 2451-2453.

[108] Fukuda, M., Hindsgaul, O., *Molecular Glycobiology*, IRL Press at Oxford University: New York, **1994**.

[109] Hindsgaul, O., Kaur, K. J., Srivastava, G., Blaszczyk-Thurin, M., Crawley, S. C., Heerz, L. D., Palcic, M. M. *The Journal of Biological Chemistry*, **1991**, *266*(27), 17858-17862.

[110] Yasuhiro Kajihara, Hisashi Kodama, Tomio Wakabayashi, Kenichi Sato, Hironobu Hashimoto *Carbohydrate Research*, **1993**, *247*, 179-193

[111] van Dorst, J. A. L. M., Kamerling, J. P., Vliegenthart, J. F. G. *Pure and Applied Chem.*, **1997**, *69*(3), 537-542.

[112] van Dorst, J. A. L. M., Tikkanen, J. M., Krezdorn, C. H., Streiff, M. B., Berger, E. G., van Kuik, J. A., Kamerling, J. P., Vliegenthart, J. F. G. *Eur. J. Biochem.*, **1996**, *242*, 674-681.

[113] Huet, G., Hennebicq-Reig, S., de Bolos, C., Ylloa, F., Lesuffleur, T., Barbat, A., Carriere, V., Kim, I., Real, F. X., Delannoy, P., Zweibaum, A. *The Journal of Cell Biology*, **1998**, *141*(6), 1311-1322.

[114 Ulloa, F., Franci, C., Real, F. X. *The Journal of Biological Chemistry*, **2000**, *275*(25), 18785-18793.

[115] Gross, H. J., Rose, U., Krause, J. M., Paulson, J. C., Schmid, K., Feeney, R. E., Brossmer, R. *Biochemistry*, **1989**, *28*, 7386-7392.

[116] Gross, H. J., Brossmer, R. *Glycoconjugate Journal*, **1995**, *12*, 739-746

[117] Oufner, G., Schworer, R., Muller, B., Schmidt, R.R. *Eur. J. Chem.*, **2000**, *8*, 1467-1482.

[118] Takashima, S., Tsuji, S., Tsujimoto, M. *J. Biol. Chem.*, **2002**, *277*(48), 45719-45728.