Functionalized S-Thio-di- and S-Oligosaccharide Precursors as Templates for Novel $SLe^{x/a}$ Mimetic Antimetastatic Agents

P. Markus Dey[1] and Zbigniew J. Witczak[2]*

[1]*Department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849, USA*

[2]*Department of Pharmaceutical Sciences, Nesbitt School of Pharmacy, Wilkes University, Wilkes-Barre, PA, 18766, USA*

Abstract: Adhesive interactions between molecules expressed on vascular endothelium and circulating tumor cells are key early events in cancer metastasis. Best characterized to date is the selectin family of cell adhesion molecules, which can bind to and stabilize blood-borne cells on organ vasculature, facilitating the cell-cell and cell-substratum interactions leading to tumor seeding and proliferation. Major ligands of E-selectin, the selectin family member expressed on vascular endothelial cells, include sialylated, fucosylated glycans such as Sialyl Lewis type carbohydrate complexes (SLe^x and SLe^a). These carbohydrate antigens are ubiquitously expressed on tumor cells with high metastatic potential, including colon and pancreatic carcinomas, and have been found to selectively and avidly bind E-selectin.

Compounds that prevent E-selectin-$SLe^{x/a}$ binding represent an attractive tool in the prevention of cancer dissemination. Review of preclinical *in vitro* and *in vivo* studies suggest that $SLe^{x/a}$ 'mimetics' may serve as a potent class of anti-metastatic compounds. These agents are designed to outcompete $SLe^{x/a}$ antigens expressed on tumor cell surfaces to prevent initial vascular adhesion. Critical in generating exogenous oligosaccharides as $SLe^{x/a}$ mimetics is the stereoselective joining of specific mono- and di- saccharides that express functional groups integral in E-selectin-$SLe^{x/a}$ binding. Employing sulfur linkages to couple saccharide units enhances the biological stability of these complex carbohydrates. The synthesis of novel *S*-thiodisaccharides and *C*-disaccharides as $SLe^{x/a}$ precursors using the chiral sugars levoglucosenone, isomeric isolevoglucosenone and their functionalized analogs is described. The highly stereoselective functionalization of both enones at the C-4, C-3 and C-2 positions by the set of Michael addition reactions of reactive 1-thiosugars is reviewed. These functionalized S-thio di- and S-oligosaccharide precursors have direct application for use as templates in the synthesis of novel $SLe^{x/a}$ mimetics.

INTRODUCTION

Tumor metastasis represents a major impediment to successful cancer treatment and is associated with poor patient prognosis. While metastasis is a complex multi-step process, abnormal cell-cell recognition between tumor cells and blood vasculature appears obligatory for tumor seeding [1-2]. Adherence of circulating tumor cells to blood vessel walls is an essential event in cancer dissemination [3-4]. Binding of endothelial cell adhesion molecules to carbohydrate antigens expressed on tumor cells is commonly observed with many metastatic cancer cell types preceding tumor intravasion and proliferation [3, 5].

The selectin family of cell adhesion molecules mediates selective adhesive interactions with blood-borne cells. Three major members of the selectin family have been identified: E-selectin, L-selectin, and P-selectin, with their distribution in the body restricted to the leukocyte-vascular system [6]. Selectin members are named according to their initial discovery on cells/tissue, with L-selectin expressed on leukocytes, P-selectin first identified in storage granules of platelets, and E-selectin found on activated vascular endothelial cells.

The extracellular structure of all selectins is composed of an NH_2-terminal lectin-type domain, a single epidermal growth factor-type domain, and varying numbers of complement binding repeats [7-8]. All three selectins have transmembrane domains and variable short cytoplasmic domains containing fewer than 35 amino acids. In general, selectin sequence homology is highly conserved among all species [6].

Selectin adhesion molecules bind via their lectin-type $-NH_2$ terminus to a variety of carbohydrate-decorated cell surface molecules. The expression of specific oligosaccharide structures on either proteins or lipids confers selectin binding affinity [6, 9]. Physiological high-affinity selectin ligands include the glycoproteins P-selectin ligand (PSGL-1), CD24, CD34, glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), and E-selectin ligand (ESL-1) [10-12]. Evidence also exists that selectins can bind heterophilically to one another [6].

*Address correspondence to this author at the Department of Pharmaceutical Sciences, Nesbitt School of Pharmacy, Wilkes University, Wilkes-Barre, PA, 18766, USA; Fax: +1-570-408-7828; e-mail: witczak@wilkes.edu

1389-5575/03 $41.00+.00

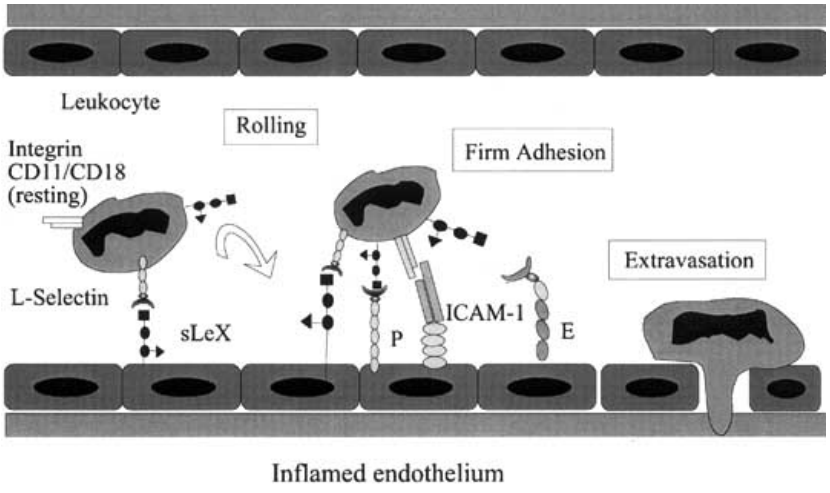

Fig. (1). Mechanism of Selectin-sLe$^{x/a}$-mediated leukocyte extravasation (diapedesis). Constitutively expressed L-selectins on leukocytes can bind to endothelial sLe$^{x/a}$ (or other natural ligands such as sialo mucins), causing slow down and rolling. Alternatively, selectins expressed on vascular endothelium may avidly bind sLe$^{x/a}$ antigens expressed on blood borne cells. Following tissue injury or infection, vascular endothelial selectins are up-regulated (< 10 min) and help firm adhesion of leukocytes through SLe^{x} binding. Further tight adhesion occurs between activated integrins (CD11/CD18) on leukocytes and ICAM-1 activated endothelium. E-Selectins participate in the binding 2-6 h after injury or infection.

Unique to these adhesion molecules is their ability to bind to a variety of molecules that express specific carbohydrate complexes. The ability of selectins, especially E-selectin, to bind to the specific tetrasaccharide Sialyl Lewis antigens SLe^{x} [Neu5Ac*alpha*-2, 3-Gal-*beta*1, 3-(Fuc*alpha*1, 3)-GlcNAc] and SLe^{a} [Neu5-Ac*alpha*-2, 3-Gal*beta*-1, 3-(Fuc-*alpha*1, 4)-GlcNAc] has been well documented [13-14]. These carbohydrate structures are composed of saccharide moieties, which terminate in 3'-fucosyl-N-acetyllactosamine sequences (SLe^{x}) or a 4'-fucosyl isomer that expresses sialic acid 3'-linked to galactose (SLe^{a}).

A major physiological function of the selectin family is the recruitment of leukocytes into sites of inflammation and tissue destruction. SLe$^{x/a}$ -mediated selectin binding governs the initial rolling and capture of blood-borne leukocytes in venules at sites of inflammation [15-16] (See figure 1).

This represents the first step in a cascade of molecular interactions that lead to leukocyte intravasation, enabling the processes of lymphocyte recirculation and leukocyte migration into inflamed tissue [8,17]. The central importance of selectins in these processes has been well documented *in vivo* by the use of adhesion-blocking antibodies as well as by studies on selectin gene-deficient mice [18].

Accumulating evidence reveals that inappropriate E-selectin binding to non-leukocyte cells in the bloodstream may play pathological roles in various disease states, especially tumor dissemination [3,19-20]. Multiple studies reveal that vascular E-selectins can bind circulating tumor cells, tethering these cancer cells to the endothelium [21-23]. In addition, E-selectin molecules are found to be up regulated by cytokines produced in response to tumor-associated immune responses [1]. This cell adhesion molecule-mediated anchorage to organ vessels facilitates a complex cascade of cell-cell and cell-substratum interactions that ultimately lead to tumor cell intravasation and metastasis. Importantly, both peptides and antibodies directed against E-selectin block tumor adhesion *in vitro* and tumor metastasis *in vivo* [24-26].

Increased expression and altered glycosylation of mucin-type glycoproteins on tumor cell surfaces are hallmarks of metastatic cancers [27-28]. Aberrant carbohydrate antigens that are highly expressed on metastatic carcinoma cell lines include the tetrasaccharide SLe^{a} and SLe^{x} epitopes, which serve as ligands for E-selectins expressed on vascular endothelium [2, 29-30]. SLe$^{x/a}$ expression has been strongly correlated with metastatic potential and/or poor prognosis in a number of human carcinomas (See Table 1). In human lung and colon carcinomas, highly metastatic tumor cells express more SLe^{x} on the cell surface and bind more strongly to E-selectin than do their poorly metastatic counterparts [31-32]. Studies have also shown that poorly metastatic carcinomas become highly metastatic after transfection with enzymes that selectively increase SLe^{x} and SLe^{a} expression [33]. The use of antibodies directed against SLe^{x} antigen has been shown to abrogate E-selectin-mediated adhesion to tumor cells [34-37].

Preventing E-selectin-SLe$^{x/a}$ adhesion represents an attractive target in cancer therapeutics [38]. Design of carbohydrate mimetics which compete for E-selectin binding with SLe$^{x/a}$ antigens expressed on tumor cells could inhibit the hematogenous spread of tumor cells and metastasis formation in secondary organs (See figure 2).

Stereo-orientation Requirements of SLe$^{x/a}$ Mimetics

The design of exogenous complex carbohydrates that avidly and selectively bind E-selectin requires in-depth

Table 1. SLex$^{x/a}$ Overexpression and Tumor Metastasis in Human Cancers

Sites of Carcinomas	SLe$^{x/a}$ Expression	Source
Esophageal squamous cell carcinoma	SLe^{a}	Oshiba *et al.*, 2000[39]
Pancreatic cancer cells	SLe^{a}	Nozawa *et al.*, 2000 [40]
Colorectal cancer	SLe^{a} , SLe^{x}	Nakagoe *et al.*, 2000 [41]
gallbladder adrenocarcinoma	SLe^{a}	Kijima *et al.*, 2000 [42]
Differentiated gastric carcinoma	SLe^{x}	Futamura *et al.*, 2000 [43]
Head and Neck tumors	SLe^{a} , SLe^{x}	Renkonen *et al.*, 1999 [44]
Oral squamous cell carcinoma	SLe^{a} , SLe^{x}	Kurahara *et al.*, 1999 [45]
Lung adenocarcinoma cells	SLe^{x}	Martin-Satue *et al.*, 1998 [46]
Pancreatic cancer cells	SLe^{a} , SLe^{x}	Hosono *et al.*, 1998 [47]
Breast carcinoma	SLe^{a} , SLe^{x}	Renkonen *et al.*, 1997 [48]
Renal cell carcinoma	SLe^{a} , SLe^{x}	Muraki *et al.*, 1996 [49]
Pancreatic adrenocarcinoma	SLe^{a}	Kishimoto *et al.*, 1996 [50]
Colon carcinoma	SLe^{x}	Bresalier *et al.*, 1996 [51]
Pancreatic carcinoma	SLe^{a} , SLe^{x}	Iwai *et al.*, 1993 [52]
Colorectal carcinoma	SLe^{x}	Irimura *et al.*, 1993 [53]
Cutaneous squamous cell carcinoma	SLe^{a} , SLe^{x}	Groves *et al.*, 1993 [54]
Colon carcinoma	SLe^{a} , SLe^{x}	Basu *et al.*, 1993 [55]
Urinary bladder cancer	SLe^{x}	Matsusako *et al.*, 1991 [56]
Colon Carcinoma	SLe^{x}	Hoff *et al.*, 1989 [57]

knowledge of the key functional group components and saccharide stereo-orientation associated with SLe$^{x/a}$ binding. Essential for SLe$^{x/a}$ binding to E-selectin is the correct conformation of the tetrasaccharide complex composed, in part, of galactose and fucose units having specific, defined orientations. The three axial hydroxyl units of the fucose unit (10 Å) are thought to participate in the binding process to the adhesion molecule (see figure **3**). Interestingly, while a specific stereo-orientation of the fucose moiety is necessary, replacement of the axial hydroxyls by other groups can maintain or even improve E-selectin binding interactions. It appears that steric conservation of the 10 Å intraatomic distance formed by fucose functional groups, is the critical determinant for E-selectin binding interactions, rather the specific expression of hydroxyl groups. Thus, the biological activity of these functional groups can be replaced with an arabinose moiety or any other open chain precursor having axial stereo-orientation. In addition, any hydrophobic group present in the strategically oriented fucose/arabinose moiety will be directly involved in the potential binding process.

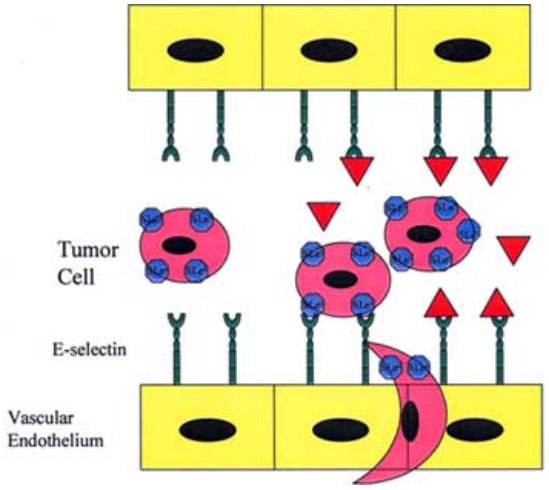

Fig. (2). Figure depicting how SLe$^{x/a}$ mimetics inhibit tumor metastasis. Novel complex compounds (expressed as triangles) bind to E-selectin adhesion molecules expressed on vascular endothelium, competing for E-selectin binding with SLe$^{x/a}$ antigens expressed on circulating tumor cells. Loss of E-selectin-mediated tethering of tumor cells to blood vascular prevents tumor cell intravasation and metastasis.

Thus, new SLe$^{x/a}$ mimetic compounds can be designed and synthesized having stereoselectively-oriented hydrophobic groups. These compounds could be stabilized by strong linkages in the presence of electron withdrawing groups (e.g acetamido carboxyl, etc**),** preserving the preferential biological conformation essential for E-selectin binding.

Review of Published Experimental Design/Procedures

In order to achieve specific SLe$^{x/a}$ -type adhesive interactions, proper synthetic strategies that reproduce the required stereochemistry are critical in constructing SLe$^{x/a}$ mimetics with potential therapeutic applications. As stated previously, SLe$^{x/a}$ mimetics must retain the properly

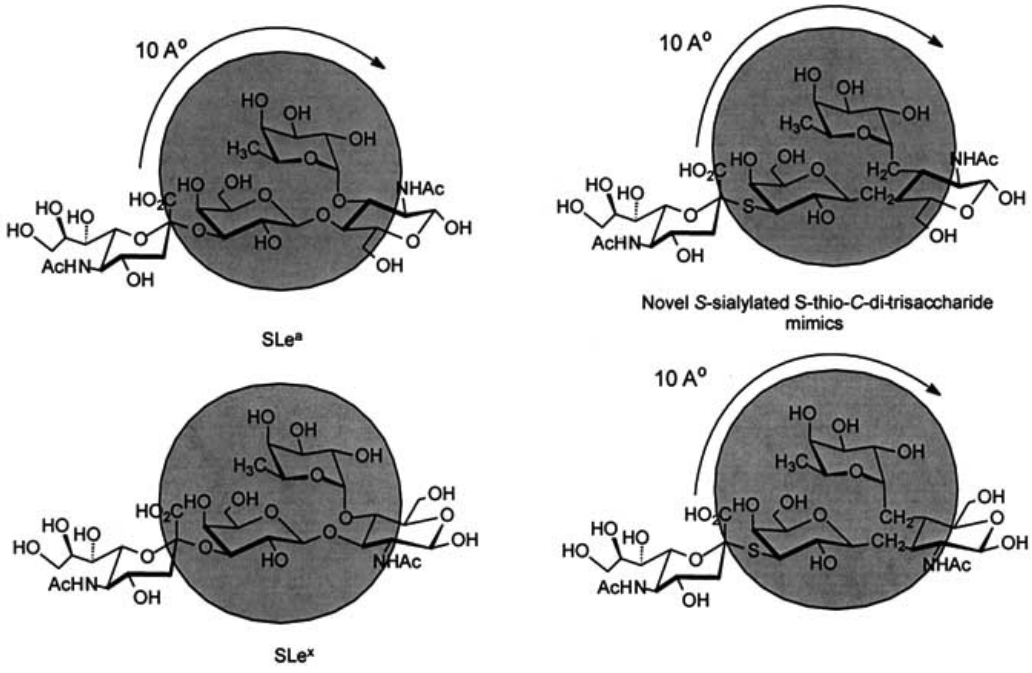

Fig. (3). Functional similarity of binding distances between SLe^a, SLe^x tumor antigens and proposed S-sialylated C-di/tri saccharide SLe^a, SLe^x mimics. Mimicry of the three axial hydroxyl groups of the fucose moiety of SLe^a, SLe^x results in E-selectin binding avidity.

situated D-galactose unit in the carbohydrate sequence of units observed in endogenous $SLe^{x/a}$ in order for hydrogen bond formation and interactions with selectin adhesion molecules (figure 4).

The synthetic strategies involved in the construction of this particular structural target normally require multiple steps of protection/activation, attachment/deprotection of functional groups to generate specific precursors, usually functionalized glucose/galactose derivatives. This highly laborious strategy is often associated with low overall yields, and has previously been explored in a number of past $SLe^{x/a}$ mimetic synthetic approaches [58-61].

General strategies for the attachment of a C-glucosyl residue and stereoselective formation of C-C- bonds have been recently evaluated. The excellent review by Postema [62] describes specific methodologies for the construction of C-C linkages, which are particularly useful under mild reaction conditions. This synthetic strategy cleverly avoids undesirable by-product formation and degradation/isomerization of labile disaccharide/trisaccharide fragments in order to preserve the target molecule's biological activity.

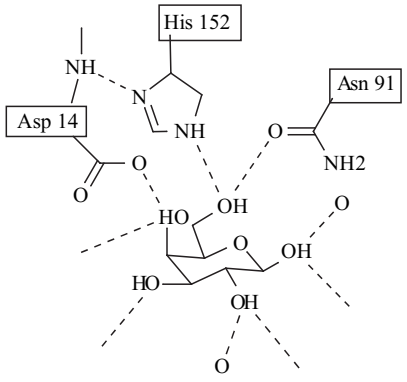

Fig. (4). Intermolecular hydrogen binding in the complex between D-galactose binding bacterial periplasmic protein.

The advantages of using sulfur as opposed to other possible linker molecules, such as oxygen, to improve carbohydrate complex stability has been documented in the literature. Significant differences between oxygen and sulfur linkages have been postulated over the years, particularly with regard to the specific C-S-C bond distances obtained with sulfur. C-S-C bonds create different angles shaping the different conformational characteristics, as compared to oxygen linkages. Moreover, the stronger electronegativity of the sulfur atom in comparison to oxygen contributes to the excellent stability of the S-linkages and furnishes an additional possibility of sulfur oxidation to form highly water-soluble sulfoxides and sulfones. Water solubility of sulfur derivatives is generally higher than their oxygen counterparts, an important advantage over the chemical characteristic of natural oligosaccharides bearing normal glycosidic linkages.

The concurrent introduction of S-linkages into these target molecules, while preserving the required stereosymmetry of functional groups, has proven to be a challenge. A leading example of the introduction of the S-linkage into these target molecules is the Schmidt et. al.

[63] methodology. This procedure utilizes the special complex functionalization *via* a participating thiol group to stereoselectively form multiple S-linkages between all the sugar components as an orthogonal synthesis of a thio-linked SLe^x analog by using known coupling reactions. This particular approach is very labor-intensive and requires multiple protection/deprotection steps. Additionally, a highly specific procedure of preparing strongly reactive leaving group intermediates capable of forming S-linkages stereoselectively is required.

Use of sulfur linkages over oxygen linkages highlights an important difference between the specific biochemical transformations occurring in living cells (plants), producing natural thiosugars, and exogenously synthesized thiosugars. Enzyme-resistant S-linked oligosaccharide derivatives (glucohydrolases) have recently been developed as conventional biological probes. The stability of S-linked complex carbohydrates allows them to have much improved biological activity. These new classes of thiosugars have been generated for use as potential agents with multiple therapeutic mechanisms of action, including as new enzyme inhibitors, and have previously been reviewed by us [64].

New Synthetic Approaches to the Target Precursors

The development of clinically useful $SLe^{x/a}$ mimetics has been hampered by a number of technical and therapeutic concerns. Difficulty finding convenient precursors of strong nucleophiles in the synthesis of 4-C-substituted disaccharide derivatives has been observed, and has contributed to low overall compound yields. Low water solubility observed in previously synthesized *C*-di-and trisaccharides results in the production of agents with limited therapeutic efficacy. Additionally, rapid degradation of oligosaccharide drugs by hydrolysis and endogenous sialidase and fucosidase activity has resulted in the generation of compounds with low half-lives.

Synthesis of Novel S-Thiodisaccharides, C-Disaccharides, and their Oligosaccharide Analogs of $SLe^{x/a}$ by Use of the Chiral Sugar Building Precursor Levoglucosenone, Isomeric Isolevoglucosenone and their Functionalized Analogs

The ability to synthesize compounds from new carbohydrate intermediates called "chiral templates" would likely lead to generation of novel $SLe^{x/a}$ mimetics as potential carbohydrate drug candidates. One of the most useful of these "chiral templates" is levoglucosenone (1,6-anhydro-3, 4-dideoxy-D-*glycero*-hex-3-enopyranos-2-ulose) [65-68]. The rigid 1,6-anhydro-D-sugar ring system of levoglucosenone is highly suitable for the introduction of additional chiralities required for the synthesis of a number of oligosaccharide derivatives with defined stereo-orientations.

The α,β-unsaturated system of levoglucosenone is highly reactive and products of the 1,4-addition are always dominant. Moreover, the Michael-addition reactions of levoglucosenone produce 4-C-substituted derivatives having the **D**-*erythro* configuration in high yields. As a result, the reaction is an easy and attractive approach for the introduction of a C-C bond at *C*-4 of the sugar molecule. Levoglucosenone has been profitably employed in a number of other chemical syntheses, including in the production of (+) multistratin and Prelog-Djerassi lactonic acid [69-70]. Methodologies employing levoglucosenone precursors would have direct application in the synthesis of novel, potent $SLe^{x/a}$ mimetics.

Synthesis of Novel S-Thiodisaccharides, C-Disaccharides and S-C-Trisaccharides

Levoglucosenone's high chemical activity as a α,β-unsaturated ketone and as an excellent acceptor in the Michael-addition reactions with masked carbohydrate carbanions has been previously shown by us [71]. Our work with new carbohydrate nucleophiles from carbohydrate isocyanides [72-73] prompted us to extend the search to other groups of derivatives that would serve as convenient precursors of strong nucleophiles. Particularly, they should possess a functional group at the anomeric center that allows for deprotonation under sufficiently mild conditions to avoid β-elimination. Glycosyl nitromethanes [74] and sulphones [75] are logical candidates. The selection of these nucleophiles was based on the recognized need for derivatives with fully protected anomeric centers, which are resistant to the reaction conditions of carbanion generation, as well as β-elimination.

A highly stereoselective 1,4-addition of glycosyl nitronates [76] under very mild reaction conditions make this approach very advantageous (only two steps) relative to the existing methods for the synthesis of (1-4)-linked C-

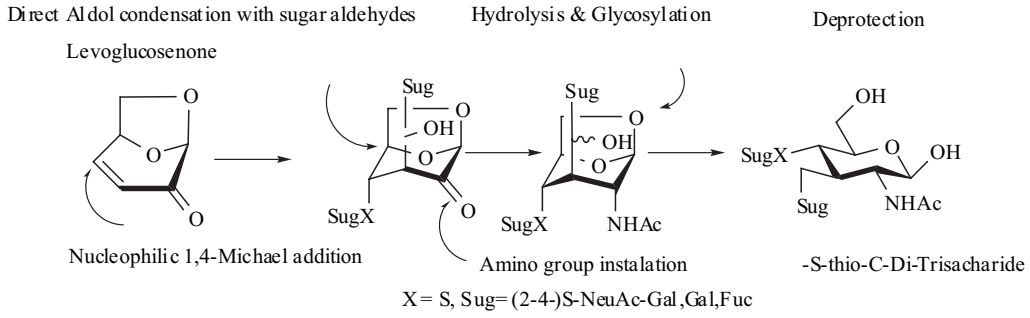

Fig. (5). General methodology of steroselective functionalization of levoglucosenone.

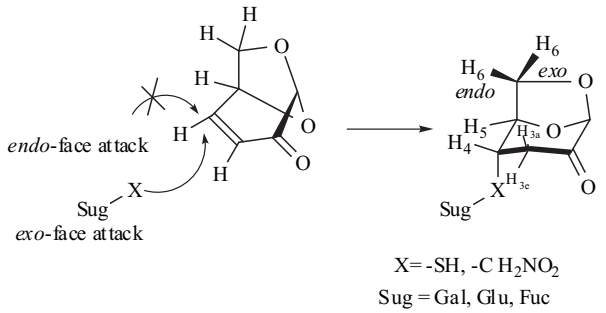

Fig. (6). Preferential exo-face attack of sugar nucleophiles (thiols, glycosyl nitromethanes) with the formation of C-4, 1.4-adducts with axial stereochemistry as proved by measuring the coupling constants $J_{4,3ax}$ and $J_{4,3e}$ in the range of 5.0-7.0Hz and 1.0-1.5Hz, respectively.

disaccharides. The benefit of the stereoselective 1,4-addition is the exclusive production of an *exo*-adduct *via* formation of a 1,4-*C*-linkage from the less hindered face of the levoglucosenone molecule (Figure 6). The correct stereochemistry of the 1,4-adducts can be easily proved by measuring the coupling constants $J_{4,3ax}$ and $J_{4,3e}$, which are in the range 5.0-7.0Hz and 1.0-1.5Hz, respectively.

Synthesis of Novel S-Thiodisaccharides and C-Disaccharides Analogs with Nonhydrolyzable Sulfur or Methylene Bridges

The steric hindrance of the 1,6-anhydro bridge effectively prevents 4-equatorial (*e*) product formation, and only the formation of 4-axial (*a*) (Figure 3) products is observed [77-78]. Consequently, a nucleophilic attack of masked anions will always be directed to the 4-axial position. Our published results [76-78] on the synthesis of S-thiodisaccharides and C-disaccharides clearly confirmed our initial hypothesis of the stereoselectivity of the addition and feasibility of these new methodologies.

This advantageous stereoselective strategy of Michael addition and *C-C* and *C-S* bond formation, exclusively by *exo* attack, has never been proposed as a general synthetic method for these classes of compounds (and Lewis antigen Le^a, *C*-di, and *C*-trisaccharides analogs, in particular). The protocol that was used to synthesize these intermediates is shown in Figure 7. The *C*-disaccharides were produced according to our general methodology utilizing glycosyl nitronates [76] as convenient Michael reaction donors. A one-pot 1,4-Michael addition reaction of glucosyl nitronates to levoglucosenone in the presence of a catalytic amount of tetramethylguanidine (TMG) or tetrabutylammonium fluoride (TBAF), in either acetonitrile or a tetrahydrofuran (THF) solvent, produces target intermediates with $=CH-NO_2$ bridge with required stereochemistry at the *C*-bridge of the *C*-disaccharides. Conventional removal of the nitro group via radical denitrification produces an intermediate C-glycoside, which was independently synthesized via alternate route of coupling a fully benzylated iodo derivative with exocyclic glycal. Subsequent reduction of the keto function at C-2 with L-Selectride in THF solution, followed by the reductive removal of the anomeric hydroxyl group with triethylsilane/boronetherate (Et_3SiH/BF_3Et_2O), produced the intermediate 1,4-*C*-disaccharide in moderate yield. Final deprotection by conventional removal of benzyl group with palladium dioxide $Pd(OH)_2$ in cyclohexane/ethanol solution followed by acetolysis with triethylsilyltriflate in the acetic anhydride $Et_3SiOSO_2CF_3/Ac_2O$ solution produces target heptaacetate derivative of 1,4-*C*-disaccharide.

Stereoselective Functionalization of Both Enones at C-4, C-3 and C-2 by the set of Michael Addition Reactions of Reactive 1-Thiosugars

Synthesis of (1-2)-Thiodisaccharide Precursors

This approach utilizes a convenient functionalization of isomeric isolevoglucosenone *via* the stereoselective Michael

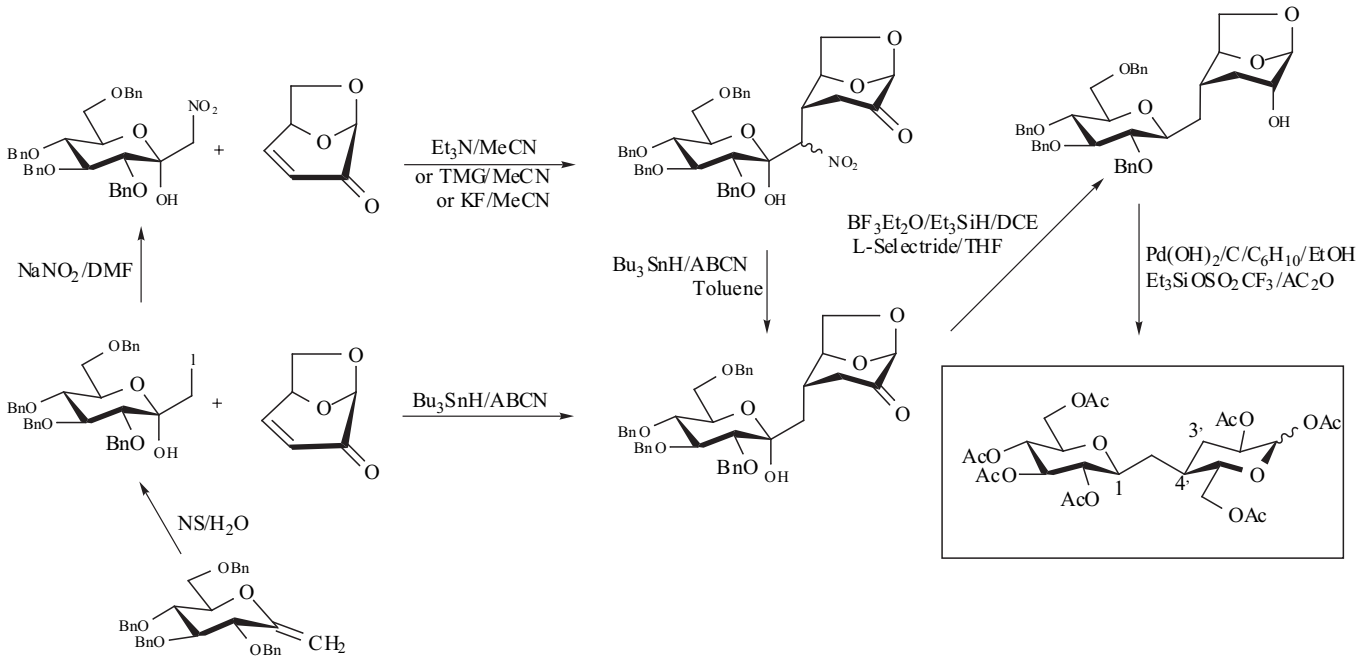

Fig. (7). Synthesis of (1-4)-C-disaccharide from levoglucosenone.

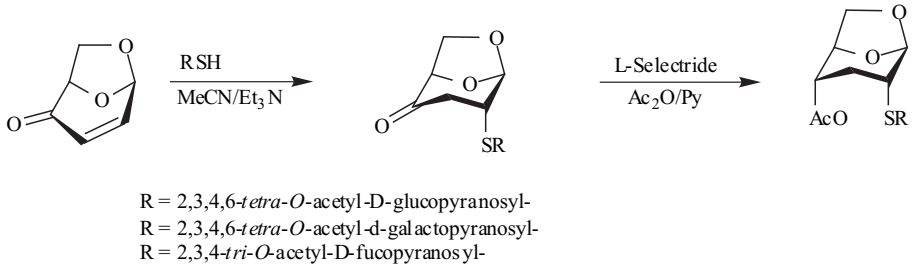

Fig. (8). Functionalization of levoglucosenone as S-thiodisaccharide precursor.

addition reaction of reactive 1-thiosugars to exclusively form thio-bridges at the C-2 position [78]. The reaction proceeds in a polar solvent (preferably acetonitrile) under very mild conditions, and requires participation of a basic catalyst such as triethylamine [78].

As shown in figure 9, deprotection by conventional acetolysis utilizing boron trifluoride etherate ($BF_3{\cdot}Et_2O$) or alternative triethylsilyl triflate/acetic ahydride (Ac_2O) trifluoroacetic acid/ acetic anhydride (TFA/Ac_2O) treatment produces heptaacetates. Subsequent conventional reactive nitroalkanes conveniently produced for levoglucosenone (see figure 10).

Both new nitroalkanes functionalized at the C-4 and C-2 positions are highly reactive toward strong nucleophiles such as 1-thiosugars, serving as convenient precursors in the synthesis of a new family of (1-2)-2-acetamidothiodisaccharides [79].

The reduction of hydroxymethyl nitro group with complex sodium borohydride/cobalt chloride followed acetylation produces 2-acetamido- (1-2)-thiodisaccharides.

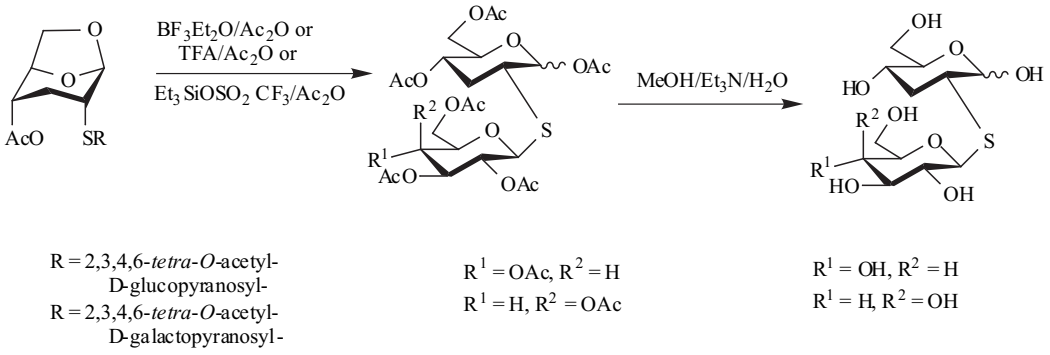

Fig. (9). Synthesis of (1-2) S-thiodisaccharides from levoglucosenone analog.

deacetylation carried out by aqueous methanol solution of catalytical amount of triethylamine produces free *S*-thiodisaccharides in good yield. The (1-2)-S-thiodisaccharides are critically important as convenient chiral building blocks in the production of larger S-oligosaccharides with $SLe^{x/a}$ like conformational shape and stability.

Synthesis of (1-2)-S-2-Acetamido Thiodisaccharides

Addition of the basic (-NHAc) functionality at the C-2 position is achieved by a set of reactions involving very The 2-geminal-acetamido functionality is an additional terminal end molecule for further functionalization of the intermediate precursors into new trisaccharides of the oligosaccharides family of mixed thio-aminosugars.

Conventional deprotection was performed, as depicted in figure 12, utilizing p-toluene sulfonic acid in methanol for cleavage of the 1,6-anhydro bridge and convenient installation of methyl group at the anomeric position [80]. The first representative of this new family of thiodisaccharides is currently under extensive biological evaluation by the authors. Importantly, the basic functional

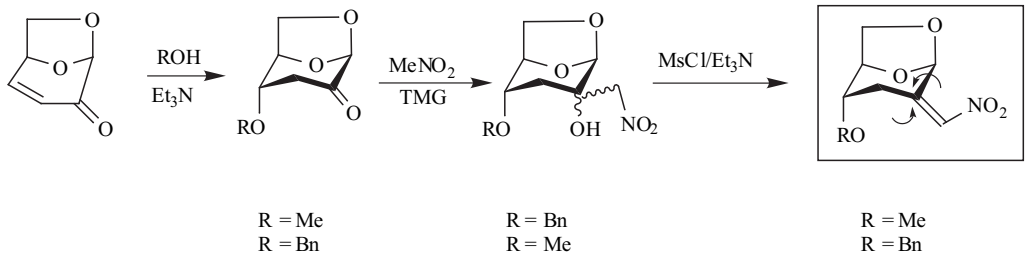

Fig. (10). Synthesis of 2-nitroalkene from levoglucosenone.

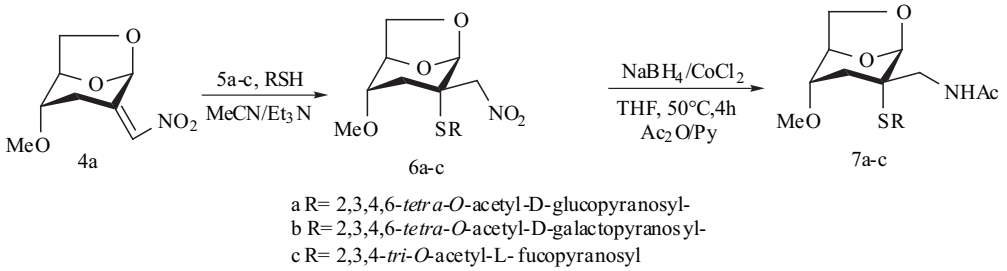

Fig. (11). Functionalization of levoglucosenone at C-2 through addition of sugar moiety and the reduction of C-2 nitro group.

group (-NHAc) at the C-2 geminal position may act as a binding site with a variety of biological receptors, including selectin adhesion molecules. Such disaccharides could be valuable tools to probe the enzyme inhibitory activity of synthesized (1-2)-S-thio-2-acetamidodisaccharides.

Synthesis of (1-3)-S-Thiodisaccharides

This new family of thiodisaccharide precursors was prepared from another analog of levoglucosenone bearing iodine at the C-3 position, [81] which is highly reactive in the S_N2 nucleophilic displacement reaction with reactive 1-thiosugars (see figure 13). Since sulfur is less basic than oxygen and its nucleophilicity much greater, 1-thiolate can be generated and used in the presence of base-sensitive protecting groups. The synthons most utilized in the synthesis of thiooligosaccharides have been the per-acetylated 1-thioglycoses, which must be selectively deacetylated and activated by total deacetylation in a two-step procedure. O-acetylated 1-thioglycoses may also be used after being selectively transformed into-1-thiolates.

The advantage of the stereoselective iodide displacement is the exclusive formation of a S-linkage from the less hindered face of the molecule with inversion of configuration at C-3. The shielding effect of the 1,6-anhydro bridge in the iodo precursor effectively prevents the formation of the 3-axial product, thus yielding only the 3-equatorial product. This equatorial attack of the sulfur nucleophiles was expected [78, 81-82] but a new chiral center at C-3 surprisingly stabilizes the molecule as no epimerization or β-elimination was observed during the coupling reaction. In order to preserve the existing chirality or proceeds the reaction stereoselectively and prevent epimerization or β-elimination of the target molecule, the coupling reaction requires participation of polar solvent system, usually acetonitrile. These generated S-linked thiodisaccharides are again convenient starting templates for additional functionalization via conventional C-2 keto reduction.

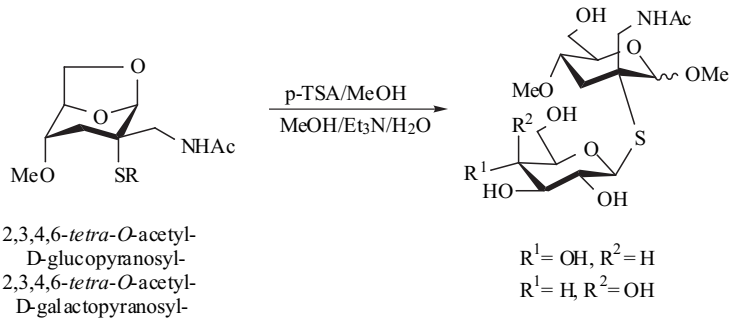

Fig. (12). Deprotection of (1-2)-2-acetamidothiodisaccharides.

Unlike levoglucosenone, where the C-2 keto group reduction is predictably controlled by the 1,6-anhydro bridge, the analogous stereoselective functionalization of C-2 keto group by L-selectride reduction is expected to be

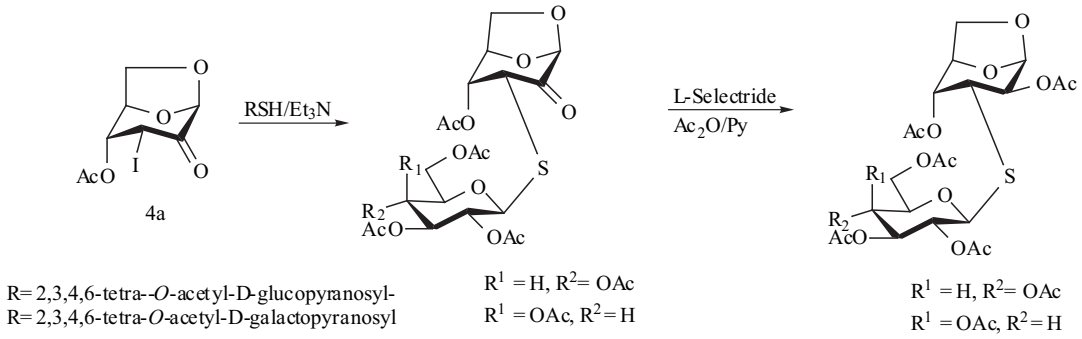

Fig. (13). Synthesis of (1-3)-S-thiodisaccharides from the 2-iodo analog of levoglucosenone.

dominated by the relative steric contribution of the bulky equatorial substituents at the C-3 position, as well as by the 1,6-anhydro bridge. Final deprotection by conventional deacetylation [83-84] produces the target S-disaccharides in 48 % overall yield.

The starting iodo precursor was conveniently synthesized according to our previously published strategy [80], as illustrated in Figure 14.

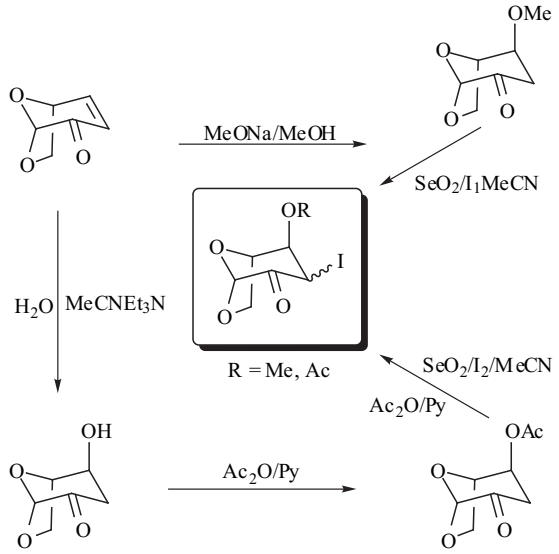

Fig. (14). Synthesis of 3-iodolevoglucosenone derivative as convenient precursor to 1,3-S-dithiosaccharides.

The application of the above iodo precursors in the convenient methodology of introducing S-(1-3)-thio linkages constitutes a new short stereoselective approach to generating (1-3)-3-S-thiodisaccharides. This new class of S-linked thiosugar analogs is highly sought after in order to produce complex carbohydrates with anticancer therapeutic potential, including as specific inhibitors of α-L-fucosidases.

CONCLUSION

The generation of C-C disaccharides and S-thiodisaccharide precursors containing S-linkages at specific positions is critical in the construction of stable, complex oligosaccharide $SLe^{x/a}$ mimetics. These intermediates are designed to produce the active stereo-orientation conformations necessary for biologically relevant interactions required for preferential binding with E-selectin carbohydrate receptors. While multivalency has been shown to be a critical factor in carbohydrate based adhesion phenomena, the design of stable and enzyme-resistant intermediates and/or target molecule with nonhydrolyzable linkages is integral to the potential clinical utility of $SLe^{x/a}$ mimetics [85]. Selective adhesion is also dependent on the polyvalent nature of specific components of complex oligosaccharides. Thus, complex oligosaccharide binding to E-selectin is enhanced by both steric and entropic factors. These general requirements must be followed in order to successfully design and create series of the potential therapeutics mimicking natural molecules with equal or enhanced biological activity.

REFERENCES

[1] Turner, G.A.; Catterall, J.B. *Biochem. Soc. Transact.*, **1997**, *25*, 234.
[2] Tomlinson J.; Wang, J.L.; Barsky, S.H.; Lee, M.C.; Bischoff, J.; Nguyen, M. *Internatl. J. Oncol.,* **2000,** *16*, 347.
[3] Krause, T.; Turner, G.A., *Clin. & Experiment. Metastasis,* **1999,** *17*, 183.
[4] Ohyama, C.; Tsuboi, S.; Fukuda, M. *EMBO J.*, **1999,** *18,*1516**.**
[5] Kim, Y.J.; Borsig, L.; Han, H.L.; Varki, N.M.; Varki, A., *Am. J. Pathol*., **1999,** *155,* 461.
[6] Vestweber, D.; Blanks, J.E. *Physiol. Rev.*, **1999,** *79***,** 181.
[7] Kaltner, H; Stierstorfer, B. *Acta Anatom*., **1998,** *161***,** 162.
[8] Graves B.J.; Crowther, R.L.; Chandran, C.; Rumberger, J.M., Li, S., Huang, K.S., Presky D.H.; Familletti, P.C.; Wolitzky, B.A.; Burns, D.K. *Nature*, **1994,** ***367*****,** 532.
[9] Tyrrell, D.; James, P.; Rao, N.; Foxall, C.; Abbas, S.; Dasgupta, F.; Nashed, M.; Hasegawa, A.; Kiso, M.; Asa, D. *Proc. Natl. Acad. Sci. USA*, **1991,** *88*, 10372.
[10] Nelson, R.M.; Dolich, S.; Aruffo, A.; Cecconi, O.; Bevilacqua, M.P. *J. Clin. Invest.*, **1993,** *91***,** 1157.
[11] Vestweber, D. *J. Cell Biochem*., **1996,** *61*, 585.
[12] Levinovitz, A.; Muhlhoff, J.; Isenmann, S.; Vestweber, D. *J. Cell Biol*., **1993,** *121,* 449.
[13] Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed B. *Science*, **1990,** *250* 1132.
[14] Phillips, M.L.; Nudelman, E.; Gaeta, F.C.; Perez, M.; Singhal, A.K; Hakomori, S.; Paulson, J.C. *Science*, **1990,** *250***,** 1130.
[15] Lawrence, M.B.; Springer, T.A. *J. Immunol*., **1993,** *151*, 6338**.**
[16] Lawrence M.B.; Springer, T.A. *Cell*, **1993,** *65,* 859.
[17] von Andrian, U.H.; Chambers, J.D.; Berg, E.L.; Michie, S.A.; Brown, D.A.; Karolak, D.; Ramezani, L.; Berger, E.M.; Arfors, K.E.; Butcher, E.C. *Blood*, **1993**, 82, 182.
[18] Jung, U.; Ley, K. *J. Immunol.,* **1999**, *162,* 6755.
[19] Kim, Y.J.; Borsig, L.; Varki, N,M.; Varki, A. *Proc. Natl. Acad. Sci. USA*, **1998**, *95,* 9325.
[20] McEver, R.P. *Glycoconjugate J*., **1997,** *14*, 585.
[21] Sato, M.; Narita, T.; Kimura, N.; Zenita. K.; Hashimoto, T.; Manabe, T.; Kannagi, R. *Anticancer Res.,* **1997**, *17*, 3505.
[22] Nakamori, S.; Kameyama, M.; Imaoka, S.; Furukawa, H.; Ishikawa, O.; Sasaki, Y.; Izumi Y.; Irimura, T. *Diseases Colon & Rectum*, **1997**, *4*, 420.
[23] Bresalier, R.S.; Byrd, J.C.; Brodt, P.; Ogata, S.; Itzkowitz, S.H.; Yunker, C.K. *Internatl. J. Cancer,* **1998,** *76***,** 556**.**
[24] Fukuda, M.N.; Ohyama, C.; Lowitz, K.; Matsuo, O.; Pasqualini, R.; Ruoslahti, E.; Fukuda, M. *Cancer Res.,* **2000**, *60*, 450.
[25] Lafrenie, R.M.; Gallo, S.; Podor, T.J.; Buchanan, M.R.; Orr, F.W. *Europ. J. Cancer*, **1994**, *30A*, 2151.
[26] Brodt, P.; Fallavollita, L.; Bresalier, R.S.; Meterissian, S.; Norton, C.R.; Wolitzky, B.A. *Internatl. J. Cancer*, **1997,** *71*, 612.
[27] Muramatsu, T. *Glycobiology*, **1993**, *3*, 294.
[28] Fukuda, M.N.; Ohyama, C.; Lowitz, K.; Matsuo, O.; Pasqualini, R.; Ruoslahti, E.; Fukuda, M. *Cancer Res.*, **2000,** *60*, 450**.**
[29] Fukushima, K; Hirota, M.; Terasaki, P.I.; Wakisaka, A.; Togashi, H.; Chia, D.; Suyama, N.; Fukushi, Y.; Nudelman, E.; Hakomori, S. *Cancer Res.*, **1984**, *44*, 5279.
[30] Hoff, S.D.; Matsushita, Y.; Ota, D.M.; Cleary, K.R.; Yamori, T.; Hakomori, S.; Irimura, T. *Cancer Res.*, **1989**, *49*, 6883.
[31] Takada, A.; Ohmori, K.; Yoneda, T.; Tsuyuoka, K.; Hasegawa, A.; Kiso, M.; Kannagi, R. *Cancer Res.*, **1993**, *53*, 354.

[32] Kageshita, T.; Hirai, S.; Kimura, T.; Hanai, N.; Ohta, S.; Ono, T. *Cancer Res.*, **1995**, *55*, 1748**.**

[33] Yamada, N.; Chung, Y.S., Takatsuka, S.; Arimoto, Y.; Sawada, T.; Dohi, T.; Sowa, M. *Brit J. Cancer*, **1997**, *76*, 582.

[34] Zhang, G.J.; Adachi, I. *Internatl. J. Oncol.*, **1999**, *14*, 71.

[35] Saiki, I.; Koike, C.; Obata, A.; Fujii, H.; Murata, J.; Kiso, M.; Hasegawa, A.; Komazawa, H.; Tsukada, H.; Azuma, I.; Okada, S.; Oku, N. *Internatl J. Cancer*, **1996**, *65*, 833.

[36] Dejana, E.; Martin-Padura, I.; Lauri, D.; Bernasconi, S.; Bani, M.R., Garofalo, A.; Giavazzi, R.; Magnani, J.; Mantovani, A.; Menard S. *Laborat. Invest.*, **1992**, *66*, *324.*

[37] Nakamori, S.; Kameyama, M.; Imaoka, S.; Furukawa, H.; Ishikawa, O.; Sasaki, Y.; Izumi, Y.; Irimura, T. *Diseases of the Colon & Rectum*, **1997**, *40*, 420.

[38] Barchi J.J. Jr. *Curr. Pharmaceut. Des.*, **2000**, *6*, 488.; Baisch, G.; Öhrlein, R; Kolbinger, F.; Streiff M. *Bioorg. Medicinal Chem. Lett.*, **1998**, *8*, 1575.;Baisch, G.; Öhrlein, R.; Streiff, M. *Bioorg. Medicinal Chem. Lett.*, **1998**, *8***,** 161.

[39] Oshiba, G.; Kijima, H.; Tanaka, H.; Kenmochi, T.; Himeno, S.; Kise, Y.; Nishi, T.; Chino, O.; Shimada, H.; Abe, Y.; Yamazaki, H.; Nakamura, M.; Ueyama, Y.; Tanaka, M.; Makuuchi, H. *Internatl. J. Oncol.*, **2000**, *17*, *701.*

[40] Nozawa, F.; Hirota, M.; Okabe, A.; Shibata, M.; Iwamura, T.; Haga, Y.; Ogawa, M. *Pancreas*, **2000**, *21*, 392.

[41] Nakagoe, T.; Fukushima, K.; Nanashima, A.; Sawai, T.; Tsuji, T.; Jibiki, M.; Yamaguchi, H.; Yasutake, T.; Ayabe, H.; Matuo, T.; Tagawa, Y.; Arisawa, K. *Canad. J. Gastroenterol.*, **2000**, *14*, *753.*

[42] Kijima, H.; Kashiwagi, H.; Dowaki, S.; Ohtani, Y.; Tobita, K.; Matsubayasi, H.; Ajioka Y.; Watanabe, H.; Tsuchida, T.; Yamazaki, H.; Nakamura, M.; Ueyama, Y.; Tanaka, M.; Makuuchi, H. *Internatl. J. Oncol.*, **2000**, *17*, *55.*

[43] Futamura, N.; Nakamura, S.; Tatematsu, M.; Yamamura, Y.; Kannagi, R.; Hirose, H. *Brit. J. Cancer*, **2000**, *83*, 1681.

[44] Renkonen, J.; Makitie, A.; Paavonen, T.; Renkonen, R. *J. Cancer Res. & Clin. Oncol.*, **1999**, *125*, 569.

[45] Kurahara, S.; Shinohara, M.; Ikebe, T.; Nakamura, S.; Hiraki, A.; Sasaki, M.; Beppu, M.; Shirasuna, K. *Head & Neck.*, **1999**, *21*, 330.

[46] Martin-Satue, M.; Marrugat, R.; Cancelas, J.A.; Blanco, J. *Cancer Res*., **1998**, *58*, 1544.

[47] Hosono, J.; Narita, T.; Kimura, N.; Sato, M.; Nakashio, T.; Kasai, Y.; Nonami, T.; Nakao, A.; Takagi, H.; Kannagi, R. *J. Surg. Oncol.*, **1998,** *67*, 77**.**

[48] Renkonen, J.; Paavonen, T.; Renkonen, R. *Internatl. J. Cancer*, **1997,** *74*, 296.

[49] Muraki, J.; Kobayashi, M.; Sugaya, Y.; Hashimoto, S.; Morita, T.; Kobayashi, Y.; Tokue, A. *Japan. J. Urol.*, **1996,** *87*, *831.*

[50] Kishimoto, T.; Ishikura, H.; Kimura, C.; Takahashi, T.; Kato, H.; Yoshiki, T. *Internatl. J. Cancer*, **1996,** *69*, *290.*

[51] Bresalier, R.S.; Ho, S.B.; Schoeppner, H.L.; Kim, Y.S.; Sleisenger, M.H.; Brodt, P.; Byrd J.C. *Gastroenterol*., **1996**, *110*, 1354.

[52] Iwai, K.; Ishikura, H.; Kaji, M.; Sugiura, H.; Ishizu, A.; Takahashi, C.; Kato, H.; Tanabe, T.; Yoshiki T. *Internatl. J. Cancer*, **1993,** *54*, 942.

[53] Irimura, T.; Nakamori, S.; Matsushita, Y.; Taniuchi, Y.; Todoroki, N.; Tsuji, T.; Izumi, Y.; Kawamura, Y.; Hoff, S.D.; Cleary, K.R. *Sem. Cancer Bio.*, ***1993***, *4*, 319.

[54] Groves, R.W.; Allen, M.H.; Ross, E.L.; Ahsan, G.; Barker, J.N.; MacDonald, D.M. *Am. J. Pathol.*, **1993**, *143*, 1220.

[55] Basu, M.; Basu, S.S.; Li, Z.; Tang, H.; Basu, S. *Ind. J. Biochem. Biophys.*, **1993,** *30*, 324.

[56] Matsusako, T.; Muramatsu, H.; Shirahama, T.; Muramatsu, T.; Ohi, Y. *Biochem. Biophys. Res. Commun.*, **1991**, *181*, 1218.

[57] Hoff, S.D.; Matsushita, Y.; Ota, D.M.; Cleary, K.R.; Yamori, T.; Hakomori, S.; Irimura, T. *Cancer Res.*, **1989,** *49*, 8663.

[58] Boons G-J.; Demczenko, A.V. *Chem Rev.*, **2000**, *100*, 4539.

[59] Angata, T.; Varki, A. *Chem Rev.*, **2002**, *102*, 439.

[60] Zachara, N.E.: Hart, G.W. *Chem Rev.*, **2002**, *102*, 431.

[61] Sears, P.; Wong, C-H.. *Angew Chem Int. Ed.*, **1999**, *38*, 2300.

[62] Liu, L.; McKee, M.; Postema, M.H.D. *Curr. Org. Chem.*, **2001**, *5*, 1133.

[63] Eisele, T.; Toepfer, A.; Kretzschmar, G.; Schmidt, R.R. *Tetrahedron Lett.*, **1996**, *37* 1389.

[64] Witczak, Z.J. *Current Med. Chem.*, **1999**, *6*, 159.

[65] Shafizadeh, F.; Chin P.P.S. *Carbohydr. Res.*, **1976**, *46*, 149-154, ibid. *58*, 79-87, **1977**.

[66] Witczak Z.J., Levoglucosenone and Levoglucosans Chemistry and Applications" Ed. Witczak Z.J., ATL Press Inc. Mt Prospect. Illinois **1994**.

[67] Witczak, Z.J. *Studies Natural Products Chem.*, **1994**, *14*, 267.

[68] Witczak, Z.J.; Mielguj, R.A. *Synlett.*, **1996**, 108.

[69] Mori M.; Chuman T.; Kato K. *Tetrahedron Lett.*, **1982,** *23***,** 4593.; Mori M.; Chuman T.; Kato K. *Carbohydr. Res*., **1984**, *129*, 73.

[70] Witczak, Z. J.; Li, Y. *Tetrahedron Lett.*, **1995,** *36*, 2595.

[71] Witczak, Z. J. *Pure and Appl. Chem.*, **1994**, *66*, 2189.

[72] Witczak, Z. J. *J. Carbohydr.Chem.*, **1984**, *3*, 359.

[73] Witczak, Z. J. *Tetrahedron Lett.*, **1986**, *27*, 155.

[74] Kobertz, W.R.; Bertozzi, C.R.; Bednarski, M.D. *J. Org. Chem.*, **1996**, *61*, 1894.

[75] Davidson, A.H. Hughes, L.R. Qureshi, S.S. Wright, B. *Tetrahedron Lett.*, **1988**, *29*, 6693.

[76] Witczak, Z. J.; Chhabra, R.; Chojnacki, J. *Tetrahedron Lett.*, **1997**, *38*, 2215.

[77] Witczak, Z.J.; Sun, J.; Mielguj, R. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2169.; Witczak, Z. J.; Chhabra, R.; Chen, H.; Xie X-Q. *Carbohydr. Res.*, **1997**, *301*, 167.

[78] Witczak, Z. J.; Chen, H.; Kaplon, P. *Tetrahedron. Asymm*., **2000**, *11*, 519.

[79] Witczak, Z. J.; Chhabra, R.; Boryczewski, D. *J. Carbohydr. Chem.*, **2000**, *19*, 543.

[80] Zhu, Y-H.; Vogel, P. *J. Chem. Soc. Chem. Commun.*, **1999**, 1873.; Zhu, Y-H.; Vogel, P. *Tetrahedron Lett.*, **1998**, *39*, 31.; Zhu, Y-H.; Vogel, P. *J. Org. Chem.*, **1999**. *64*, 666.; Zhu, Y-H.; Demange, R.; Vogel, P. *Tetrahedron Asymm*., **2000**, *11*, 263.

[81] Witczak, Z. J.; Kaplon, P.; Kolodziej, M. *Monatshefte fur Chemie.*, **2002**, *133*, 521; Witczak, Z. J.; Kaplon, P.; Dey, P. M. *Carb. Res.,* **2003**, *338*, 11.

[82] Shafizadeh, F.; Ward, D.D.; Pang, D. *Carbohydr. Res.*, **1982**, *102*, 217.

[83] Yu, K.L.; Fraser-Reid, B. *J.Chem. Soc. Chem. Commun*., **1989**, 1442.

[84] Zottola, M.; Venkateswara Rao, B.; Fraser-Reid, B. *J.Chem. Soc. Chem. Commun.*, **1991**, *14*, 969.

[85] Ohrlein, R. *Mini Review in Medicinal Chem*., **2001**, *1*, 349.