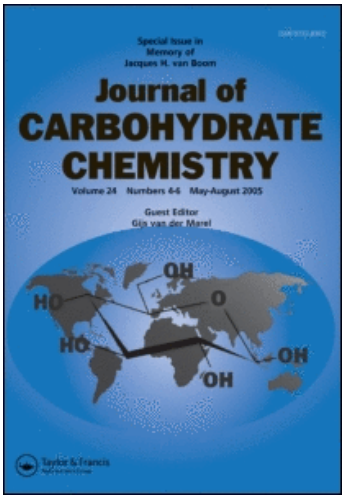


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

DESIGNING NOVEL MULTIVALENT GLYCOTOOLS FOR BIOCHEMICAL INVESTIGATIONS RELATED TO SIALIC ACID

René Roy[a]
[a] University of Ottawa, Ottawa, Canada

Online publication date: 12 March 2002

To cite this Article Roy, René(2002) 'DESIGNING NOVEL MULTIVALENT GLYCOTOOLS FOR BIOCHEMICAL INVESTIGATIONS RELATED TO SIALIC ACID', Journal of Carbohydrate Chemistry, 21: 7, 769 — 798
To link to this Article: DOI: 10.1081/CAR-120016489
URL: http://dx.doi.org/10.1081/CAR-120016489

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF CARBOHYDRATE CHEMISTRY
Vol. 21, Nos. 7–9, pp. 769–798, 2002

DESIGNING NOVEL MULTIVALENT GLYCOTOOLS FOR BIOCHEMICAL INVESTIGATIONS RELATED TO SIALIC ACID*

René Roy

University of Ottawa, Ottawa, Ontario, Canada

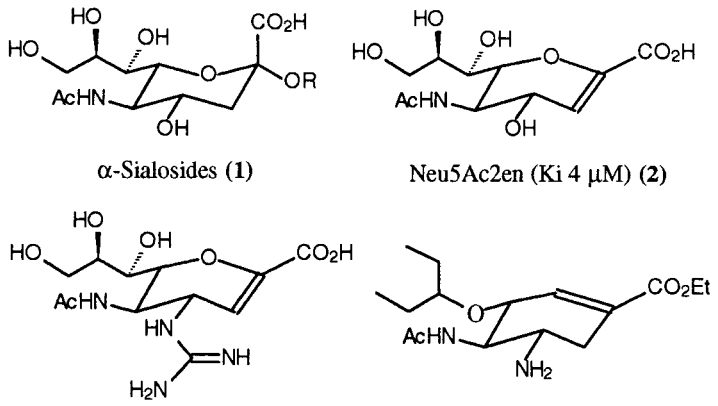
INTRODUCTION

Sialic acids constitute a family of more than 30 neuraminic acid derivatives varying by the nature of their substituents (acetyl, glycolyl, phosphate, sulfate) and by their relative positioning around the pyranose ring.[1] The most common member of this rather unusual nine-carbon amino acid sugar (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid) is represented by *N*-acetylneuraminic acid (Neu5Ac, **1**) (sometime abusively coined sialic acid itself) (Scheme 1). Sialic acids are common mammalian sugars that usually end oligosaccharide sequences of glycolipids (gangliosides), N- and O-linked glycoproteins, and some proteoglycans. They are also found as α-(2-8/9)-linked polysaccharides in encapsulated bacteria. As such, sialic acids are forefront carbohydrate haptens responsible for a wide range of recognition events.[2]

Cell surface sialosides are involved as anchoring motifs for microbial attachment. Various pathogenic agents such as viruses (influenza, coronavirus, Sendai, polyoma, rotaviruses), bacteria and bacterial toxins (*Pseudomonas aeruginosa, Helicobacter pylori, E. coli, Vibrio cholerae, Bordella pertussis*; cholera, tetanus toxins), and parasites (*Streptococcus suis, Plasmodium falciparum, Trypanosoma cruzi*) can adhere to and colonize host tissues after binding to sialosides.[3] It has also been demonstrated that bacterial infections[4] and cancer metastasis[5] can be prevented by blocking receptor sites with high serum carbohydrate concentrations including sialic acid. It is also

*Reprinted from *Glycochemistry: Principles, Synthesis and Applications*; Wang, P.G.; Bertozzi, C.R., Eds.; Marcel Dekker, Inc.: New York, 2001, 277–305.

DOI: 10.1081/CAR-120016489 0732-8303 (Print); 1532-2327 (Online)



Scheme 1.

involved in selectin-mediated cell adhesion related to leukocytes over recruitment to infected or damaged tissues.[6]

Unfortunately, medicinal applications of carbohydrate binding protein inhibitors suffer from major drawbacks. Except for a few marginal cases, carbohydrates show deceptively low binding affinities toward their receptor counterparts.[7] Moreover, simple or more complex oligosaccharides are poorly bioavailable and are rapidly catabolized through various glycosidases. A few strategies are being pursued to overcome these difficulties. Among these, the syntheses of carbohydrate analogs (deoxy, fluoro, epi, etc.), conformationally restricted analogs, and glycomimetics have been more or less successful. One promising example of rational drug design has recently emerged in the field of influenza flu virus neuraminidase inhibitors.[8,9] Compounds that mimic the transition states involved in α-sialoside hydrolyses have surpassed the classical lead Neu5Ac2en (**2**) (K_i 4 μM). Thus, glaxo Wellcome's Zanamivir (4-guanidino-Neu5Ac2en, GG167, **3**) with a K_i of 0.1 nM[8] and Gilead Sciences prodrug GS4104 (**4**, K_i 1 nM)[9] have reached clinical phase II for a long-awaited drug treatment against flu virus infections. GS4104 proved effective against both influenza A and B strains in test animals.

Another useful strategy for the design of potent microbial antiadhesins may rely on the rational syntheses of various multivalent glycoforms that can mimic multiantennary glycoproteins. It can be speculated that common oligosaccharide sequences might express further specificity and enhanced avidity through precise multivalent architectural antigen presentation. Numerous cases exist to point toward multivalency or ''cluster'' effects to ''boost'' carbohydrate-protein bindings.[10] Multivalent macromolecules would be particularly appealing for respiratory, gastrointestinal, and urinary tract infections where bioavailability does not constitute a major problem. Such novel, nonimmunogenic biopolymers may also find applications as carriers for drug delivery and as affinity adsorbents, both in vitro and in vivo, in cases calling for natural killer cell and macrophage activation.

We and others have previously demonstrated that multivalent neoglycoconjugates constitute powerful inhibitors in a number of generally low-affinity carbohydrate–protein binding interactions.[11–16] Potent multivalent neoglycoconjugates have been scaffolded onto a wide range of carriers including polymers,[11] polyamino acids,[12] cyclodextrins,[13] calix[4]arenes,[14] carbohydrates,[15] and dendrimers.[16] This last class of scaffolds is particularly appealing because the effects of size, shape, and valency can be controlled at will.[17] This chapter focuses on ongoing activities regarding the syntheses of multivalent sialosides.

SOURCES OF SIALIC ACID

The cost for commercially available sialic acid has dramatically declined over the last 10 years. Depending on the amount purchased, the cost can be as low as approximately $CDN 20–$CDN 30/g. A practical source, requiring no particular skills, consists of isolating sialic acid through a mild acid hydrolysis of the *Collocalia* mucin isolated from edible bird's nest,[18] which can be purchased from any Chinese grocery [$CDN 100/$CDN 200 g). The mucin is essentially a sialic acid-rich glycoprotein. Thus, 10 g (5% w/w) can be obtained in less than a week. Alternatively, sialic acid can be isolated from egg yolk,[19] from cow's milk,[20] or from the complete acid hydrolysis of colominic acid, a bacterial polysaccharide produced by both *E. coli* K1 and *Neisseria meningitidis* serogroup B or C [α-(2,8/9)]-polysialic acid.[21] It is also possible to produce several discrete oligomers by controlled acid hydrolysis of the polysaccharides.[22] Additionally, it has been feasible to produce the analogous *N*-glycolylneuraminic acid (NAc replaced by $NCOCH_2OH$, Neu5Gc) and their corresponding oligomers by strong base hydrolysis of the *N*-acetyl groups of the polysaccharide (2 M NaOH, 110°C, 7 h); treatment with acetoxyacetyl chloride; followed by de-O-acetylation, and hydrolysis. Alternatively, the de-N-acetylated polysaccharide can be treated with acryloyl chloride and then reductively ozonolyzed.[23] This *N*-glycolylsialic acid analog is the key determinant epitope of the Hanganutziu–Deicher antigen[24] found in some patients who have been treated with animal serum (serum sickness). Moreover, it has been found in large quantity in tumor cells having abnormally high *N*-glycolyl GM3-ganglioside.

Useful Sialic Acid Derivatives as Precursors in Neoglycoconjugate Syntheses

As mentioned above, the sialic acid residue itself constitutes the key immunodominant epitope in several biological interactions. It was therefore appealing to generate a wide range of neoglycoconjugates containing solely sialic acid or its analogous N-glycolylated derivative. Nowadays, sialic acid can be readily prepared as *O*-, *S*-, *N*-, and *C*-glycosides. Stereospecific access to the first three forms can be achieved with complete anomeric stereocontrol. However, great difficulty still attends the synthesis of pure α-linked *C*-sialosides.[25] Fortunately, reports describing the use of samarium diiodide (SmI_2) (Barbier's conditions) seem to open the door toward improved stereocontrol.[26] The following subsections briefly highlight aspects of our approach toward the preparation of suitably functionalized sialosides.

Stereospecific Anomeric Functionalization Under PTC Conditions

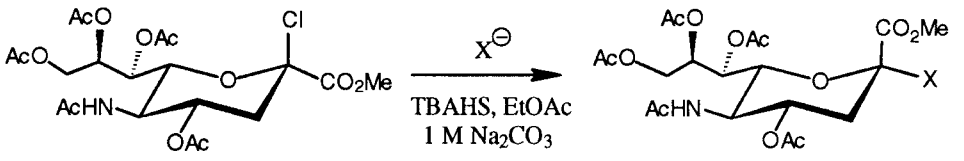
Generally, *N*-acetylneuraminic acid is α-glycosidically linked to D-galactose or to D-*N*-acetylgalactosamine residues through α-(2-3/6) linkages or to another *N*-acetylneuraminic acid residue in α-(2-8/9) linkages.[1] In most cases, terminal Neu5Ac residues constitute the immunodominant epitopes; that is, most binding interactions occur through intrinsic Neu5Ac functionality, with the carboxyl group constituting the key polar group.[27] Based on cumulative observations,[1] initial efforts were centered around the syntheses of α-sialoside derivatives alone.

Anomeric phase transfer catalysis (PTC) has been systematically shown to take place through inversion of configuration through a seemingly S_N2-type mechanism.[28,29] In spite of its quaternary nature and the absence of a potential participating group, acetochloroneuraminic acid (**5**), having an axial β-chloro substituent, has always provided clean and high-yielding anomeric inversion with a wide range of nucleophiles under mild PTC conditions.[29] When tetrabutylammonium hydrogen sulfate (TBAHS) was used as phase transfer catalyst, 1 M sodium carbonate as aqueous phase, and ethyl acetate (or dichloromethane) as organic phase, β-chloride **5** provided exclusively α-sialylated derivatives **6–11** in excellent yields (Scheme 2). The only by-product occasionally formed appeared to be peracetylated Neu5Ac2en (**2**) derivative, resulting from dehydrochlorination through an elimination side reaction (E2).

For instance, treatment of chloride **5** with thioacetic acid, sodium azide, 4-nitrophenol, 4-hydroxybenzaldehyde, 4-nitrothiophenol, or thiophenol afforded derivative **6** (66%),[30] **8** (94%),[31] **9a** (90%),[32] **9b** (65%),[33] **10** (81%),[34] or **11** (80%),[35] respectively. Chemoselective (35) de-S-acetylation of **6** (H_2NNH_2-HOAc, DMF, room temperature <30 min) or NaOMe in methanol at $-40°C$[20] afforded 1-thioderivative **7** in 88% yield. The foregoing α-sialo derivatives were suitably functionalized for coupling to various multivalent carriers (see below).

Using Novel ''Active-Latent'' Glycosylation Strategy to Produce Sialyloligosaccharides

The biological significance of *N*-acetylneuraminic acid containing oligosaccharides has stimulated remarkable progress in devising glycosylation strategies.[36] How-



5

6 X = SAc
7 X = SH
8 X = N_3
9a X = O-Ph-*p*NO_2
9b X = O-Ph-CHO
10 X = S-Ph-*p*NO_2
11 X = SPh

Scheme 2.

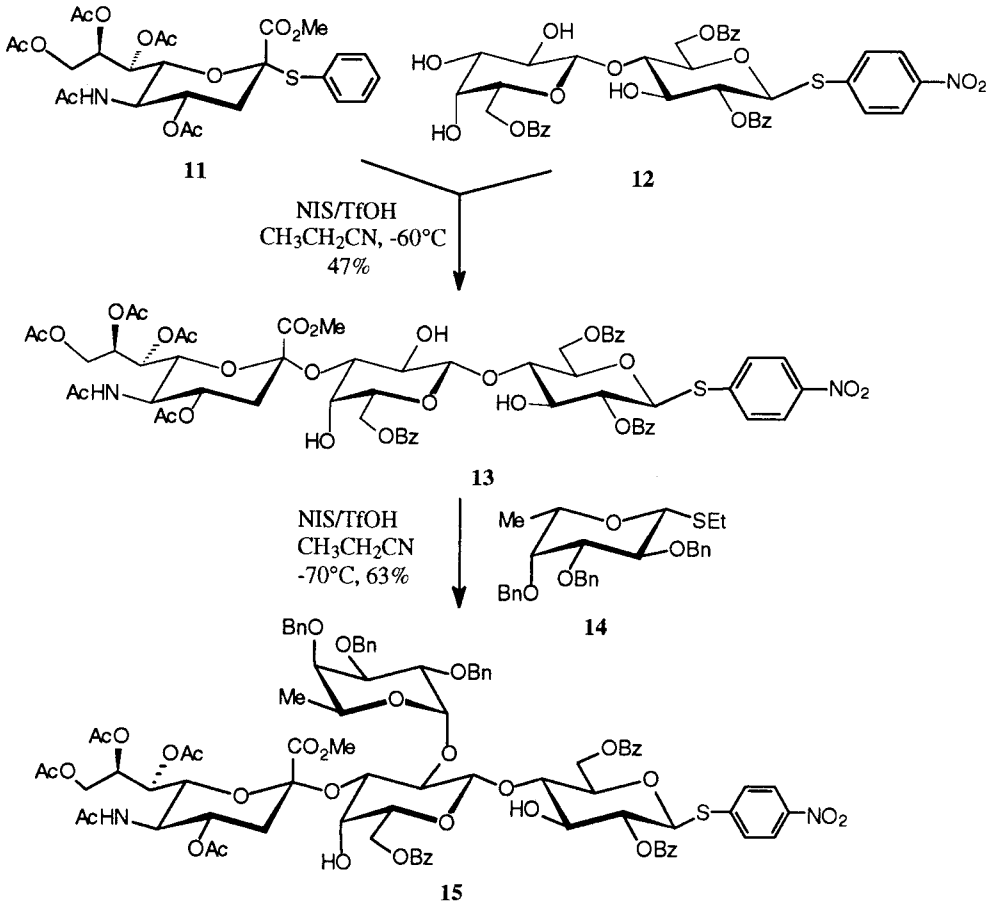
ever, efficient protocols for building sialylated oligosaccharides from monosaccharide components is still a major challenge. Initially, acetochloroneuraminic acid (**5**) has been the glycosyl donor most widely used for the synthesis of α-sialosides, but its efficacy necessitates the use of a temporarily incorporated participating group at C3.[37] Phosphite[38] and *S*-ethyl xanthate[39] derivatives have met some successes, while thioglycosides remain the method of choice.[40] The ''armed'' (ether protecting groups at C2) and ''disarmed'' (ester protecting groups at C2) *n*-pentenyl glycosylation strategy introduced by Madsen and Fraser-Reid[41] has proven to be a powerful tool for complex oligosaccharide syntheses that also is applicable to thioglycosides and few other glycosyl donors (reviewed in Ref. 42). Despite the versatility of this approach, there remains opportunity to further fine-tune the leaving ability of the glycosyl donor and thus to realize a greater potential for oligosaccharide syntheses.

Another versatile strategy, coined the ''active-latent'' glycosylation strategy, in which the reactivity of the carbohydrate units can be directly controlled by the aglycone, together with the differential protection of the glycone itself, enabled the synthesis of various oligosaccharide donors.[34,43–47]

Two realizations were found to be analogous: that the nature of the substituents on the aryl thioglycosides could be used to modulate the nucleophilicity of the anomeric sulfur atom and that protecting groups that could be used as ''armed'' or ''disarmed'' glycosyl donors. Thus an electron-donating group (EDG: e.g., Me, OMe, NHAc, H) placed in the para position of the aryl moiety of an aryl thioglycoside provided ''active'' or ''armed'' glycosyl donors, while electron-withdrawing groups (EWG: e.g., NO_2, Br, Cl) afforded ''latent'' or ''disarmed'' thioglycosyl donors that could be used as building blocks in blockwise oligosaccharide syntheses. ''Latent'' thioglycosyl derivatives, possessing one free hydroxyl group and corresponding to ''temporary inactive'' species, serves as glycosyl acceptors. Their reactivities can be ''turned on'' by transforming their electron-withdrawing thioaryl substituents (e.g., NO_2) into electron-donating groups (e.g., NHAc), thus ''reactivating'' the sulfur atoms toward electrophilic promoters usually employed in glycosylation reactions. Alternatively, the ''active'' thioglycosyl donors should already possess EDG substituents on their aryl moieties. The ''latent'' thioglycosyl acceptors can be glycosylated with ''active'' thioglycosyl donors by using thiophilic promoters. The disaccharide can then be transformed into an ''active'' glycosyl donor by modification of the aryl substituents.

Taking advantage of both ''active-latent'' and ''armed-disarmed'' concepts, it was possible to modulate the reactivities of thioglycosyl donors and acceptors by changing both the nature of the substituents in the para position of the aglycone's thiophenyl group and the protecting groups at the C2 position.[34,43–46] This has expanded the reactivity differences between thioglycosyl donors and acceptors and thus provided improved flexibility for the proper choice of glycosyl donors and acceptors toward building complex sialylated oligosaccharides.

Initially, the method was based on the differential reactivity conferred on each of the partners by the nature of the protecting groups and by the intrinsic nucleophilicity of the thioaryl leaving groups. However, as the method evolved, it became clear that the choice of the promoter was also an important factor to take into consideration. Indeed, it was demonstrated that a ''disarmed'' thioglycoside could be activated in the presence of powerful thiophilic promoters such as *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH), whereas it remained inactivated in the presence of weak thiophilic reagent such as iodonium dicollidine perchlorate or methyl triflate.[43,45]



Scheme 3.

An example of this strategy is illustrated in Scheme 3, which describes the synthesis of a positional isomer of sialyl Lewis^X. For instance, armed glycosyl donor phenyl 2-thio-α-sialoside **11** was chemoselectively activated with NIS/TfOH in the presence of latent 4-nitrophenyl thiolactoside **12** to provide sialyllactose derivative **13** in 47% yield. Then armed-active perbenzylated ethyl thiofucoside **14** was used to further regioselectively glycosylate trisaccharide **13** at O2′ of the galactose residue to give sialylated tetrasaccharide **15** in 63% yield (46). 4-Nitrophenyl sialyl-1-thiolactoside **13** still possesses an aglycone that can be further transformed into an active glycosyl donor ($NO_2 \rightarrow NH_2 \rightarrow NHAc$) or into a reactive acrylamido monomer ($NH—COCH{=}CH_2$) for copolymerization purpose.[48]

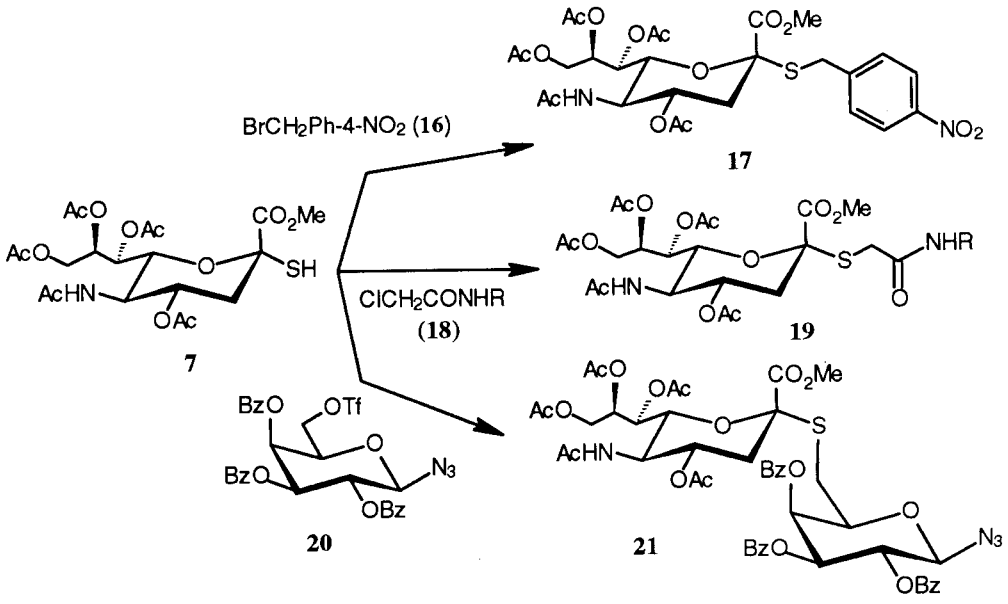
Syntheses of Suitably Functionalized Sialosides

It is clear that multivalent sialosides may offer numerous opportunities for ''medicinal glycobiology.'' They can be used to increase receptor binding interactions

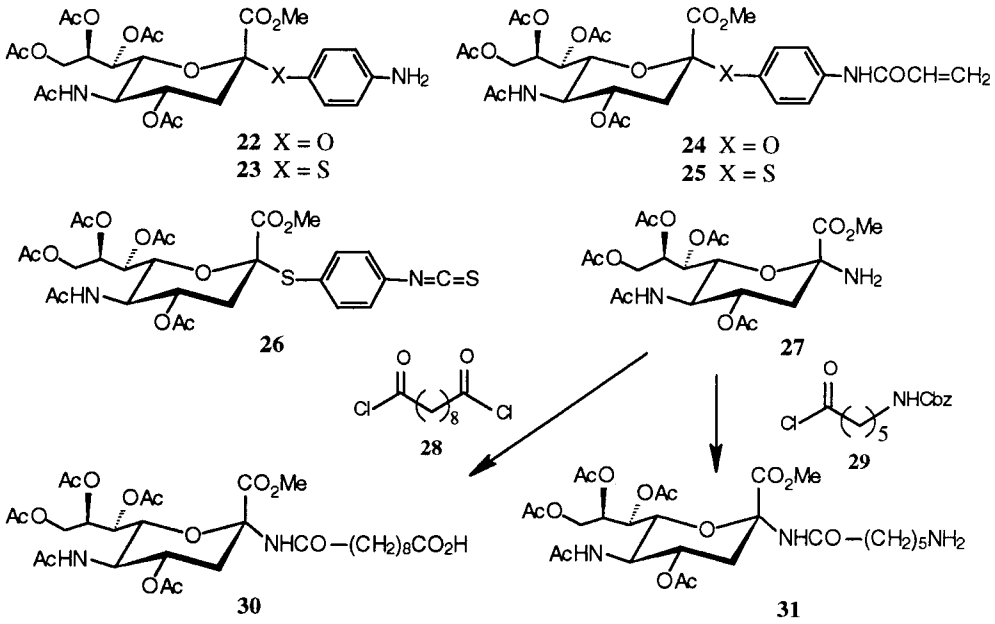
in areas such as flu virus inhibition of hemagglutination, anti-inflammatory agents (selectin antagonists), cancer vaccines and immunodiagnostics, and in treating gastrointestinal infections. Moreover, inasmuch as sialic acid receptor themselves might be organized as clusters, it appeared sound to synthesize multivalent glycoforms varying in molecular weights, shapes, valencies, and geometries to ''scan'' wide ranges of topographical areas. To this end, suitably functionalized *N*-acetylneuraminic acid derivatives were required. A convergent approach in which various sialoside haptens and other carbohydrates of interest could be attached to multivalent scaffolds at a late stage was chosen. This strategy offers the advantage of permitting further optimization of binding interactions where appropriate.

General approaches in which *N*-acetylneuraminic acid derivatives could be used as either electrophiles, nucleophiles, or comonomers were considered. Using derivatives already obtained through PTC (Scheme 2), it was possible to provide the necessary building blocks. Thus, thioacetate **6** was readily de-S-acetylated into potent nucleophile **7** (NaOMe, MeOH, $-40°C$, 30 min, 88%), which had been treated with 4-nitrobenzyl bromide (**16**), a wide variety of *N*-chloroacetamides **18**, or galactosyl derivative **20** (Z. Gan, R. Roy, unpublished results) to afford key precursors **17** (74%), **19**, or **21** (70%), respectively (Scheme 4). *N*-chloroacetamides **18** represent typical examples of multivalent scaffolds onto which were built various *N*-acetylneuraminic acid dendrimers (see below). Multiple chloride substitutions were found to be very efficient and high yielding, together with providing a key ^{1}H NMR signal (δ 4.2 ppm) that was used to evaluate coupling efficiency.

4-Nitrophenyl *O*/*S*-sialosides **9** and **10** were reduced to amine derivatives **22** (HCO_2NH_4, 10% Pd-C, MeOH, reflux) or **23** ($SnCl_2$, EtOH, reflux) which were directly transformed into comonomers **24** or **25** with acryloyl chloride (CH_2═$CHCOCl$, CH_2Cl_2, Et_3N, 86–88%)[49] (Scheme 5). Additionally, 4-aminophenyl 2-thiosialoside



Scheme 4.



Scheme 5.

23 was converted into 4-isothiocyanatophenyl derivative **26** ($CSCl_2$, DIPEA, CH_2Cl_2, 87%) for direct conversion into thiourea-linked PAMAM dendrimers.[50] Because it was also of interest to synthesize glycopeptidomimetics and glycopeptoids (Scheme 4); by either solution or solid phase synthesis, sialosyl azide **8** was reduced into amine **27** (10% Pd-C, MeOH, quant) and treated with sebacoyl chloride (**28**) or with Cbz-protected 6-aminocaproyl chloride **29** to give acid **30**[51] or amine **31**[52] after standard deprotection. Acid **30** was used in solid phase synthesis of hyperbranched dendrimers,[51] while blockwise approach was used to transform amine **31** into oligopeptoids up to an octamer level.[52] With efficient (active-latent) and stereospecific (PTC) methodologies in hand for key building block syntheses, the stage was ready for multivalent sialoside preparations.

GLYCOFORMS, OLIGOPEPTOIDS, AND AMPHIPHILIC NANOSTRUCTURES

As stated, the search for carbohydrate ligands of high affinity and specificity is of prime interest in glycobiology. While classical structure-activity relationships (SARs) have led to the development of only a limited number of effective inhibitors through exhaustive enterprises, the rational design of glycoclusters or glycomimetics spanning cooperative pharmacophores has furthered our fundamental understanding of carbohydrate-protein interactions. Moreover, multivalent glycoforms may assist unraveling cooperative binding interactions as they really appear in in vivo experiments, a situation not always prevailing with isolated protein receptors or their recombinant forms. It is also stimulating to imagine the implications of glycoclusters in signal transduction.

Neoglycoproteins, liposomes, and glycopolymers have been successfully used to demonstrate that multivalency does indeed amplify carbohydrate-protein binding interactions by factors as high as thousands. However, by their very nature, these neoglycoconjugates have ill-defined chemical structures. They are heterogeneous in size and carbohydrate contents. Additionally, neoglycoproteins have been shown to be immunogenic,[53] and the same may hold for high molecular weight glycopolymers, which can mimic repeating units found on bacterial capsular polysaccharides. Thus, while such glycopolymers can demonstrate the role played by multivalency in recognition processes, they fail to allow precise biophysical analyses of the cluster effect. The following paragraphs illustrate our approach toward the rational design of sialic acid containing clusters.

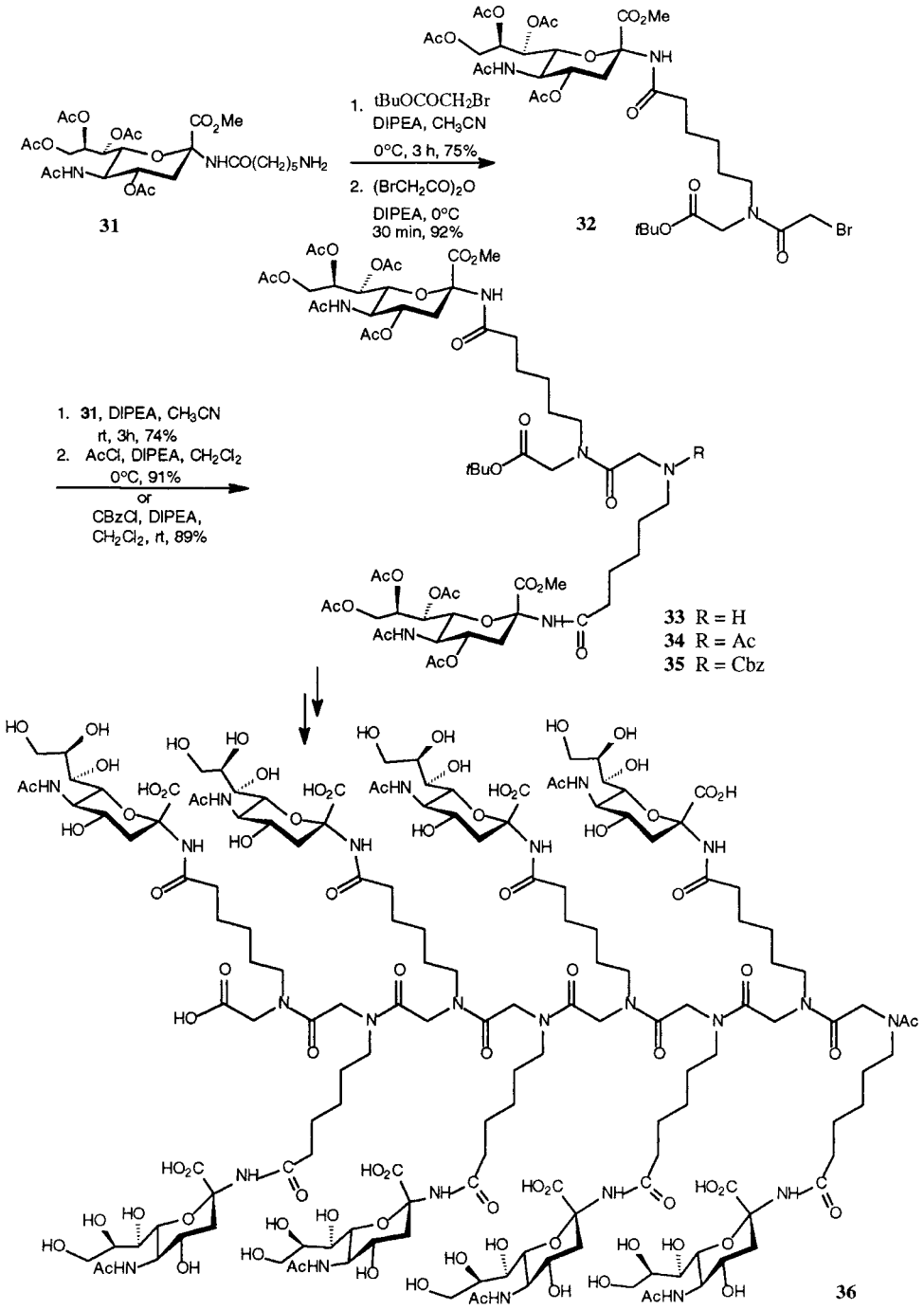
Sialoside clusters, like their other neoglycoconjugate analogs (liposomes, polymers, proteins), have been mainly used as inhibitors of flu virus hemagglutination[54] and as selectin antagonists.[55] Natural tetravalent[56] and semisynthetic divalent[57] sialyloligosaccharides have been prepared by means of chemoenzymatic sialylation of preisolated multiantennary glycans obtained by degradation of glycoproteins or simply by chemical sialylation of tethered glycosides. In recent examples, tetrameric and dimeric sialyl Lewis^X clusters showed inhibitory IC_{50} values of less than 50 nM and 0.15 mM in L-selectin-mediated lymphocyte endothelium interactions, respectively. The enzymatically prepared tetramer was a 60-fold better inhibitor than its corresponding monomer. The description of sialylated clusters was reviewed in 1997[58] and is covered in detail here.

Several pieces of evidence suggest that cooperative binding interactions from small clusters depend on both the overall number of sialic acid ligands and their relative positioning with respect to one another. For instance, we have shown that carbohydrate dimers can be nicely oriented and interspaced with very short spacers to provide efficient ''cross-linkers'' without the need to reach two binding sites from the same lectins.[59] Interestingly, analogous trimers were shown to be less effective on a per-saccharide basis in similar interactions when measured by turbidimetric and inhibition experiments. It was concluded that the ''third'' unbound ligand of a trimer acted counter-productively in the overall binding associations. These observations seem to hold when densely packed glycodendrimers are used as inhibitors (Scheme 6).

As deeper appreciation of multivalent carbohydrate-protein interactions was gained, it became obvious that clusters of various shapes, size, and orientation would provide powerful ligands from which fundamental informations about receptors topography would be obtained. To address these issues, the systematic development of novel multivalent glycoclusters was deemed essential.

Neoglycopeptidomimetics

Solid phase glycopeptide synthesis is now routine operation in laboratories and has been used to prepare multivalent sialyl Lewis^X by chemoenzymatic combination.[60] Such a strategy allows investigators to obtain oligomers of varied valency (up to 8) and interspaced by determined numbers of amino acid residues. While this strategy is conceptually appealing, it still lacks the advantages conferred to peptidomimetics if therapeutically valuable products are foreseen. The design of peptide isosteres would be highly preferable, following common practice in drug develop-



Scheme 6.

ments. The recent development of peptoids derived from simple N-substituted oligoglycines seems to fulfill the required criteria of metabolic stability and synthetic simplicity.[61] Our laboratory has been involved in the design of related N-[62] and O-linked[63] glycopeptoids.

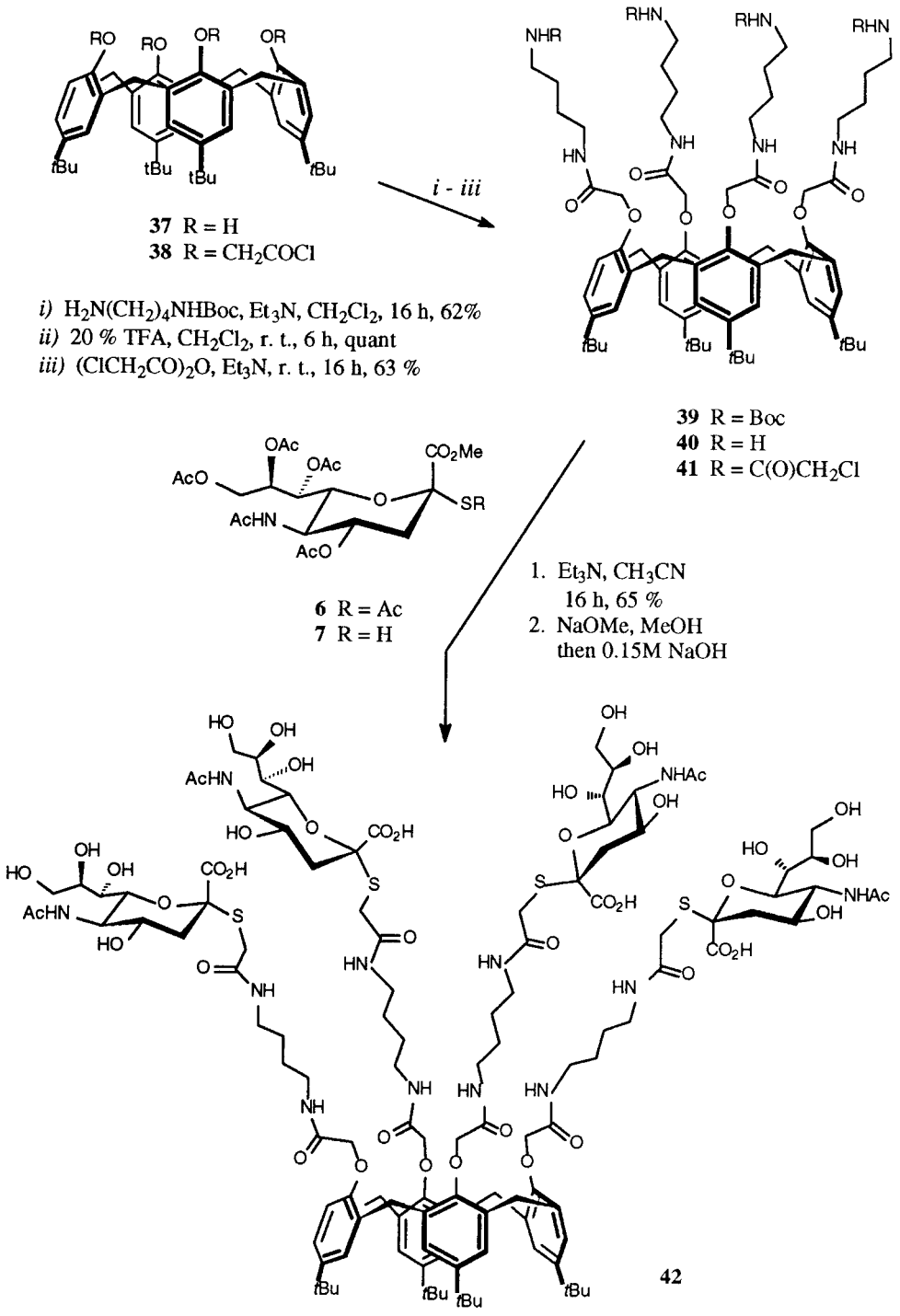
Once model glycopeptoid syntheses had been established, we decided to prepare sialic acid oligopeptoids having controlled distances both between the backbone and between the repeating units.[52] To this end, a convergent blockwise approach with orthogonally protected derivatives was chosen. The following example illustrates the synthesis of linear sialic acid oligomers interspaced by two N-substituted glycine residues onto which was attached a 6-aminocaproic acid spacer (Scheme 6).

α-Sialosyl azide **8** was transformed into Cbz-protected amine derivative, which upon hydrogenolysis afforded amine **31**. A similar strategy has been used by Sabesan[64] to prepare saccharopeptides. This approach allows the preparation of the required α anomer because this configuration is absolutely critical for the sialic acid's biological activity. Amine **31** was then transformed into key building block **32** following initial treatment with *tert*-butylbromoacetate (75%) and N-bromoacetylation with bromoacetic anhydride (92%). This orthogonally protected intermediate **32** can be elongated from either direction depending on the strategy chosen. N-Alkylation of **32** by amine **31** afforded dimer **33** which has been N-acetylated to end group **34** (AcCl, 91%) or to middle group **35** after Cbz protection (89%). Sequential deprotection of *tert*-butyl ester or Cbz group followed by amide coupling (DCC) of the resulting amino acid derivatives afforded tri-, tetra-, hexa-, and octamers such as **36**, after protecting group removal under standard conditions. The resulting ''sialopeptoids'' were thus made available for biological evaluation. Unfortunately, their inhibitory properties against influenza virus hemagglutination were rather negligible compared with dendrimers or polymers (Scheme 6).

Amphiphilic Calix[4]arene Nanostructures

Guided by the need to prepare well-defined clusters having good binding properties in solid phase enzyme immunosorbent assays (ELISA) while having readily exposed carbohydrate ligands, we became interested in synthesizing glycosylated calix[*n*]arene derivatives. In many ways, glycocalix[*n*]arenes, which possess guest-host capabilities that can be used as drug vectors, are structurally related to cyclodextrins. Additionally, they have advantages unsurpassed by the cyclodextrins because they can be readily modified at either ''upper'' or ''lower'' rim.[65] Commercially available *p-tert*-butylcalix[4]arene (**37**) is already equipped with a hydrophobic tail that has been useful in coating polystyrene microtiter plates.[66]

Thus, by freezing **37** in the cone conformation upon treatment with ethyl bromoacetate, ester hydrolysis, and acid chloride formation, known[67] tetraacid chloride **38** was readily made available[68] (Scheme 7). Treatment of **38** with a slight excess of mono-Boc-protected 1,4-butanediamine gave **39** in 62%. Trifluoroacetolysis of the Boc protecting groups resulted in tetramine **40** quantitatively. N-Chloroacetylation ($(ClCH_2CO)_2O$, Et_3N, CH_2Cl_2) gave **41** in 63% yield which, when treated with peracetylated thiosialoside **7** (Et_3N, CH_3CN, N_2, 16 h, room temperature) and protecting



Scheme 7.

group hydrolysis (NaOMe, then NaOH) gave **42** in 65% yield. In spite of having high hydrophobic content, tetrameric thiosialoside **42** was fairly water soluble (4.8 mM, 13 mg/mL). It showed strong binding affinity to the plant lectin wheat germ agglutinin (WGA) in a microtiter plate assay. Moreover, it formed insoluble cross-linked lattices with WGA as demonstrated by turbidimetric experiments. The insoluble complex could be inhibited by monomeric phenylthio α-sialoside **11** (free OH), thus demonstrating the specificity of the binding interaction.

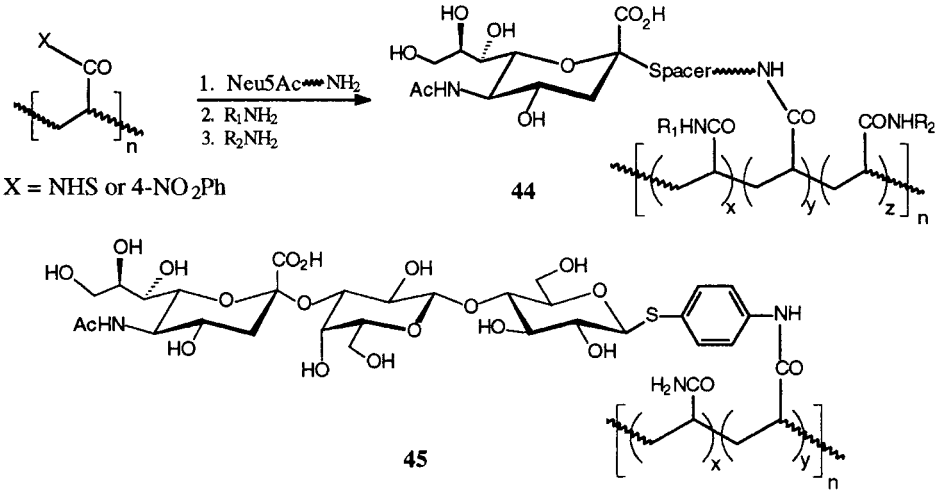
Following several observations leading to the easy formation of well-organized cross-linked lattices with simple clusters,[59,68] including dimers,[69] we became intrigued by the possibility of generating ''sugar rods.'' Several of these molecules could be synthesized via olefin self-metathesis reactions catalyzed by Grubb's catalyst $[(PCy_3)_2Cl_2Ru{=}CHPh]$.[70] Additionally, they can be prepared from α-alkynyl sialosides (Z. Gan, R. Roy, unpublished data) by using palladium(0)-catalyzed cross-coupling chemistry (Sonogashira reaction).[71] These novel derivatives fall outside the scope of this chapter, so their synthesis will be described in due course.

GLYCOPOLYMERS

We introduced the term ''glycopolymer'' to identify water-soluble polymers onto which carbohydrate haptens are covalently appended.[72] In this respect, glycopolymers should differ from pseudopolysaccharides, which refer to chemically modified polysaccharides and to insoluble materials used in affinity chromatography. Curiously, we initially made the first sialylated glycopolymers[73] to screen anti-sialic acid antibodies obtained from sialylated neoglycoproteins.[18,73–75] The original aim was to generate an antigen deprived of cross-reactive hapten (Neu5Ac), which inevitably would have been produced from other protein carriers. It was then quickly realized that these copolymers offered great potential as inhibitors in cell adhesion processes. Patents for a cancer diagnostic kit consisting of a sialylated protein (vaccine) and an ELISA screening antigen (polymer) were filed several years ago.[76] While this activity was ongoing, we reported the inhibitory potential of these novel sialylated copolymers in influenza flu virus inhibition of hemagglutination. These early observations were then similarly made by several other groups.[77,78]

Earlier reviews[10,11,58,79] had described numerous polymerization methods that have been used in synthesizing a wide range of glycopolymers. Amazingly, even though most methodologies can afford better organized copolymers than the one initially used (i.e., by random acrylamide copolymerization or modification), very few alternative methods have been exploited for sialosides. The syntheses and applications of glycopolymers are now covered in several reviews and book chapters,[79–82] and only recent developments are highlighted.

Reducing sugars can be reductively aminated with ammonia or other amines. The resulting amine derivatives can then be transformed into *N*-acrylamide monomers useful in copolymerization strategy. Unfortunately, acrylamide copolymerization affords polymers that may vary greatly in their batch-to-batch molecular weight distributions. An improved protocol consists of synthesizing polyacrylates (**43**) having active ester functionality NHS[83] or $4\text{-}NO_2Ph$[84] (Scheme 8). After aminolysis or hydrolysis, the molecular weight of the resulting polyacrylamides or polyacrylic acids

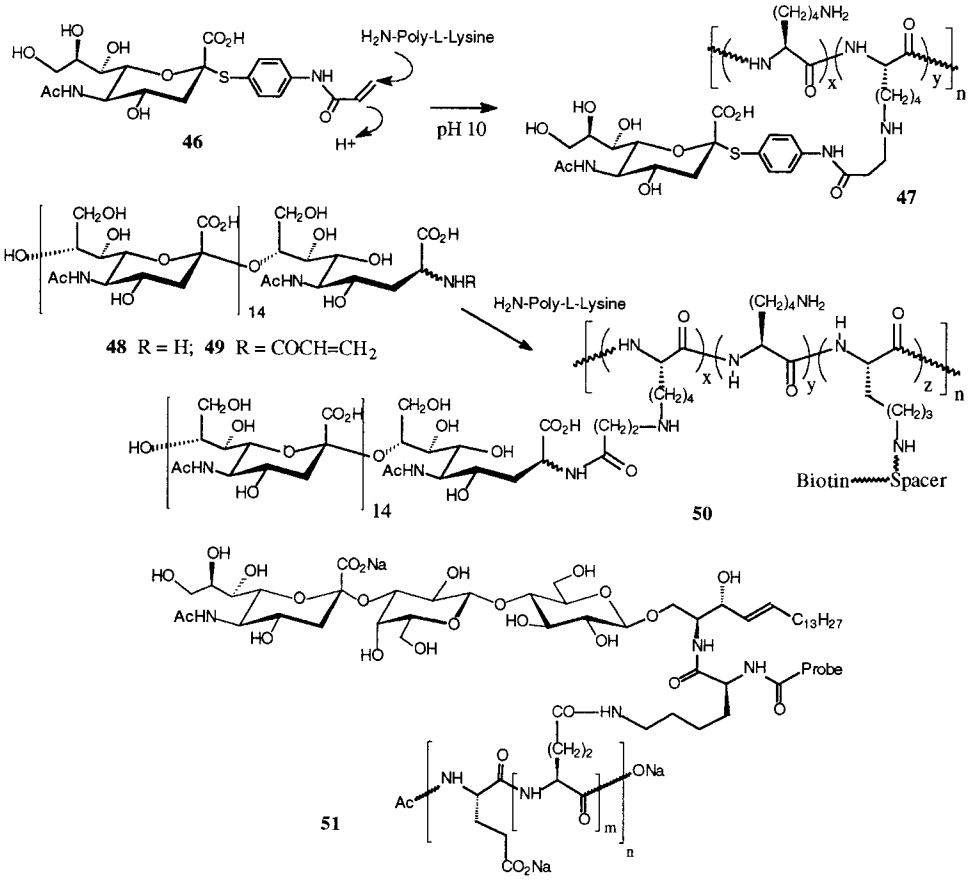


Scheme 8.

can be compared against commercially available polymer standards used in HPLC. This allows the synthesis of reproducible lots of a given copolymer. Once established, the starting polyacrylates **43** can be treated with various amounts of any amine-containing sugars. Quenching the residual reactive esters with amine 1 (R_1NH_2) followed by amine 2 (R_2NH_2), or simply with ammonia or water, afforded copolymers having desired biophysical properties.[77,83] The second and third amines may include probes (e.g., biotin, fluorescamine), lipid groups, other sugars, and peptides. The strategy has also been elegantly used to generate sialopolymer libraries.[85] Sialic acid copolyacrylamides such as **44** were obtained with *O*/*S*-aryl spacers,[32,49] *C*-glycosides,[86] and other related spacers.[78] GM_3-type copolymer **45** was obtained using the foregoing procedure.[48]

Thioaryl sialoside comonomer **46**[49] is easier than *C*-glycoside[25] to produce in a stereocontrolled manner. It is also resistant to sialidases that are simultaneously present on flu virions. Interestingly, it could be directly incorporated onto both poly-L-lysine and proteins by 1,4-conjugate additions (Michael addition) at pH 10 to provide biocompatible random copolymer **47** (Scheme 9).[87] By analogy, reductively aminated α-(2,8)-polysialic acid (colominic acid) **48** can be N-acryloylated into compounds **49**, which undergo 1,4-conjugate addition onto poly-L-lysine to provide copolymer **50**, isolated as its biotin conjugate.[87] In 1998 Wong et al.[88] reported an analogous strategy whereby a lysoganglioside derivative was amidated to poly-L-glutamic acid (DP 540) together with the fluorescent tag 4,4-difluo-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-propionyl group (BODIPY) to give **51**. The copolymer showed picomolar inhibition of H1N1 influenza hemagglutinin that corresponded to an improved binding of 1000-fold over gangliosides GM_3 or lyso-GM_3 and 10^5-fold relative to the monosaccharide sialyl lactose. Lipidlike copolymer **51** is thus as active as a polymerized liposome published in 1993.[89]

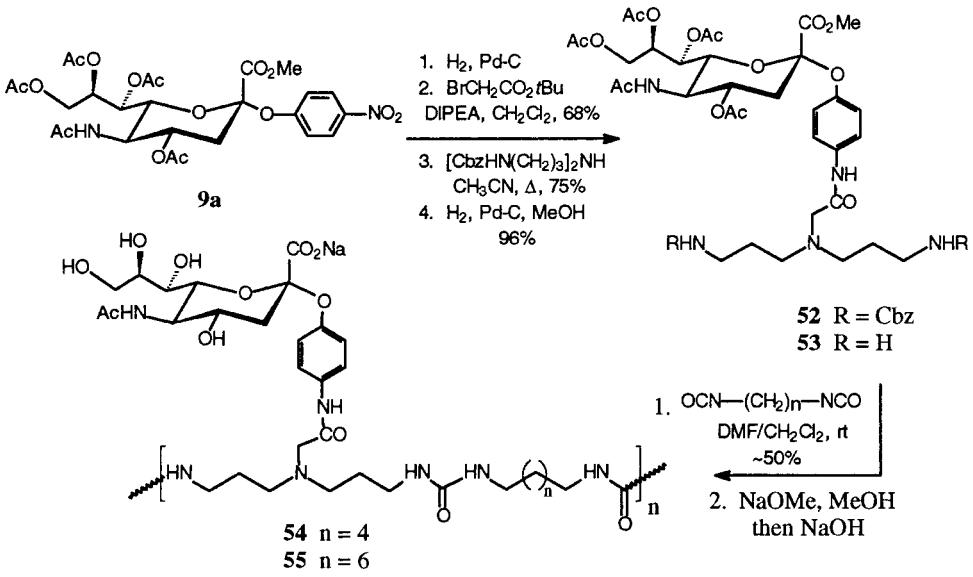
All the strategies discussed so far included sialylated copolymers having randomly distributed sialic acid residues. As these approaches failed to generate regular



Scheme 9.

copolymers onto which the hapten distributions would be interspaced at constant distances, it was deemed necessary to prepare copolymers by addition polymerization.[90] Chapter 8 by Mann and Kiessling offers an alternative strategy for the use of ring-opening metathesis polymerization (ROMP) of norbornene derivatives to generate analogous polymers. The novel strategy described herein depends on a sialic acid monomer (**53**) having two amine groups that can be copolymerized by a reiterative addition process onto a bisisocyanate (Scheme 10) (R. Roy, Y. Makimura, unpublished data). Thus, 4-nitrophenyl sialoside **9a** was reduced and treated with bromoacetyl chloride. After N-alkylation with Cbz-protected 3,3′-iminobis-(propylamine),[91] intermediate **52** was obtained in 75% yield. Hydrogenolysis provided diamine **53** quantitatively. Finally, addition polymerization of **53** with either 1,4-butanediamine or 1,6-hexanediamine bisisocyanates and ester hydrolysis gave low molecular weight copolymers **54** and **55** in $\sim$50% yield, with a degree of polymerization of $\sim$15.

It is worth mentioning that all known glycopolymers have shown strong inhibitory properties when used in conjunction with carbohydrate binding proteins. The



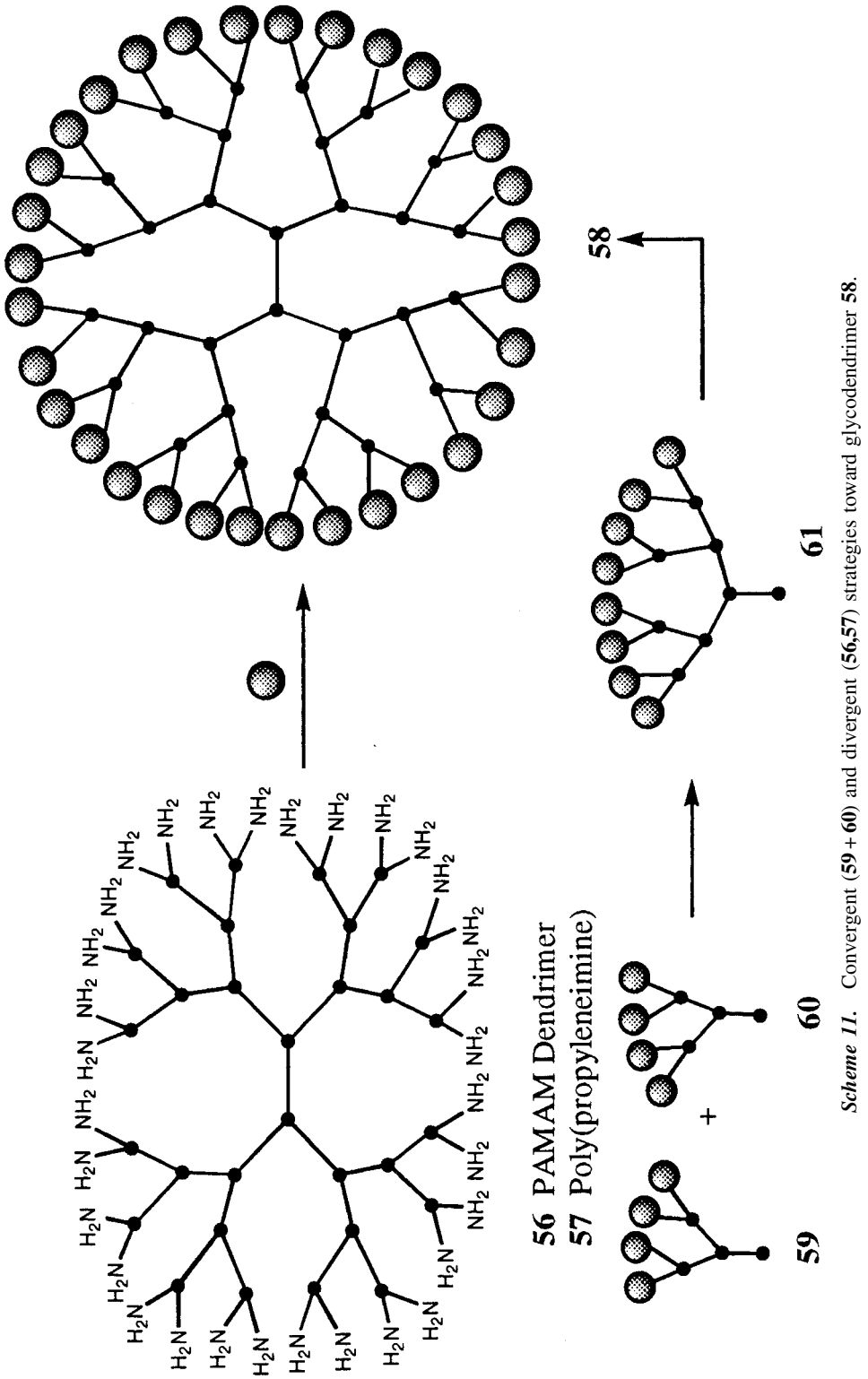
Scheme 10.

exact origins of the increased associative forces conferred on glycopolymers are not clearly understood. Aside from the individual binding site's affinity constants (K_D), which obviously remained unchanged, entropic penalties that occur upon binding are minimized with multivalent ligands. The ligands' local high concentrations are certainly also affecting association/dissociation's kinetics (k_{on}/k_{off}). Some external factors such as steric stabilization have also been invoked to support the observations.[92] It is clear, however, that a closer examination of the active site's topography is a critical factor to be elucidated for the sake of better designing potent ligands/inhibitors. With this criterion in mind, increasing activity is being addressed in attempts to design neoglycoconjugates of intermediate size between small clusters (di-/trimers) and glycopolymers. Glycodendrimers seem to fulfill the foregoing requirement. Each neoglycoconjugate has its own strength and weaknesses and ultimately, it is the targeted application that will dictate which glycoforms will be favorable. Section V provides a brief overview of the progress and limitations encountered during the development of glycodendrimers since their first synthesis in 1993.[93]

GLYCODENDRIMERS

Several reviews cover dendrimer syntheses and applications,[94] but very few describe glycodendrimers as such.[16,52,82,95,96] Dendrimers can basically adopt two shapes: spherical, globular-like structures (**58**) and monodendritic (**59–61**) architectures (Scheme 11). The last family is particularly appealing because it can mimic complex multiantennary glycans found at the tips of natural glycoproteins. Moreover, from cumulative observations, spherical dendrimers, particularly large ones (i.e., $\geq$16–32-

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016



Scheme 11. Convergent (**59** + **60**) and divergent (**56**,**57**) strategies toward glycodendrimer **58**.

mer) have started to show their intrinsic structural limitations; that is, they suffer from severe steric accessibility. This situation is further amplified by the presence of complete bacterial and viral particles wherein the receptors are themselves clustered and congested. Alternatively, they have shown excellent inhibitory properties with soluble or surface-bound lectins and antibodies.

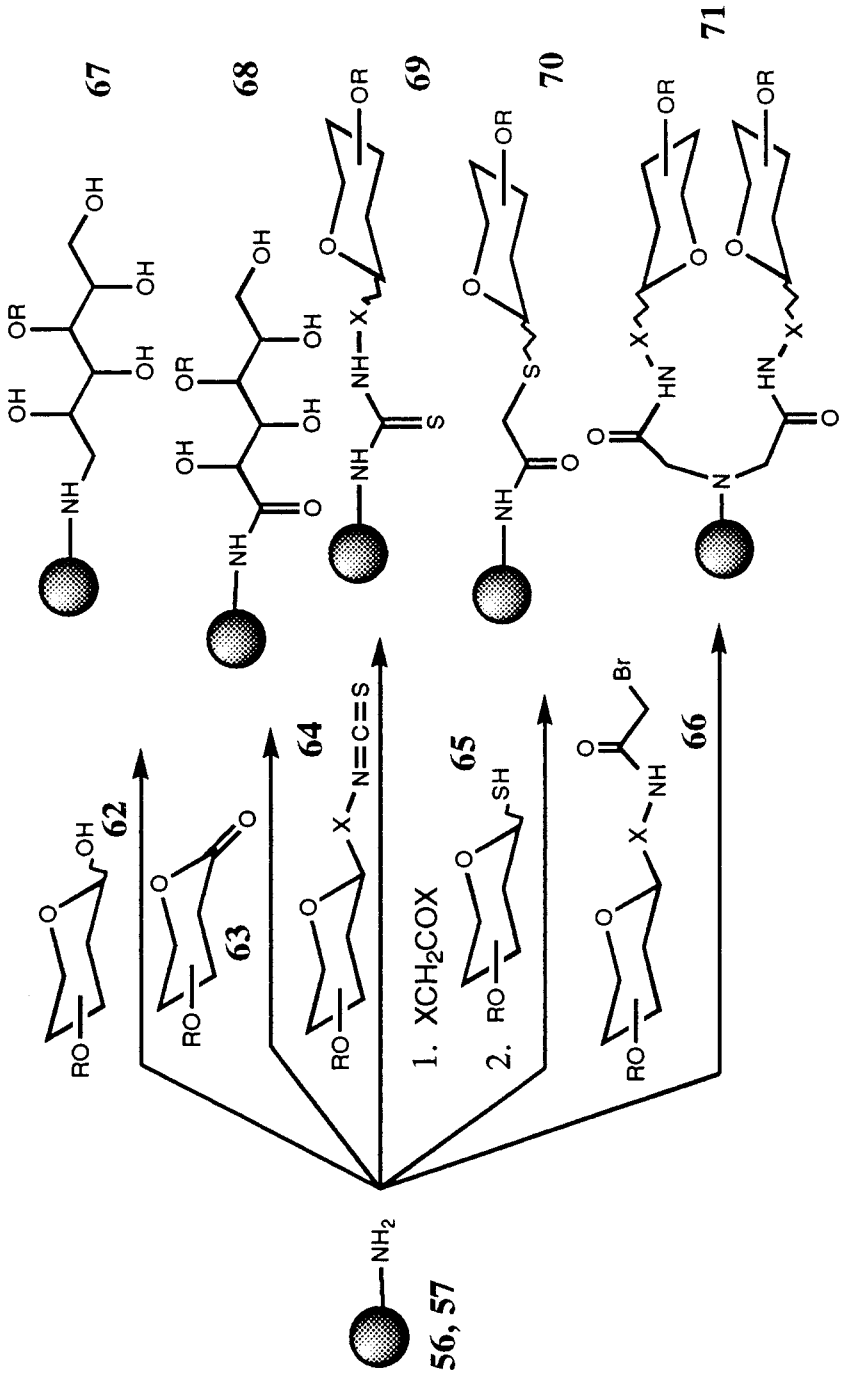
Glycodendrimers can be synthesized by both convergent and divergent strategies. Ideally, they can be simply prepared by conjugation of active carbohydrate derivatives onto preformed dendrimers (Scheme 11). Given the commercial availability of poly(amidoamine) (PAMAM, **56**) and poly(propyleneimine) (Dab, **57**) dendrimers, these amine-ending dendrimers are the most heavily exploited.

Even though dendrimer surfaces can be constructed to exhibit all possible functionalities, amine-terminating groups are synthetically more appealing and have been used most extensively. The potentially useful thiolated dendrimers self-oxidize, while carboxylated dendrimers tend to form intramolecular anhydrides once activated. This last situation may cause defects upon carbohydrate attachment. Although alcohols seem also attractive, a priori, their direct use in glycosylation chemistry is hampered by potentially difficult complete anomeric stereocontrol.

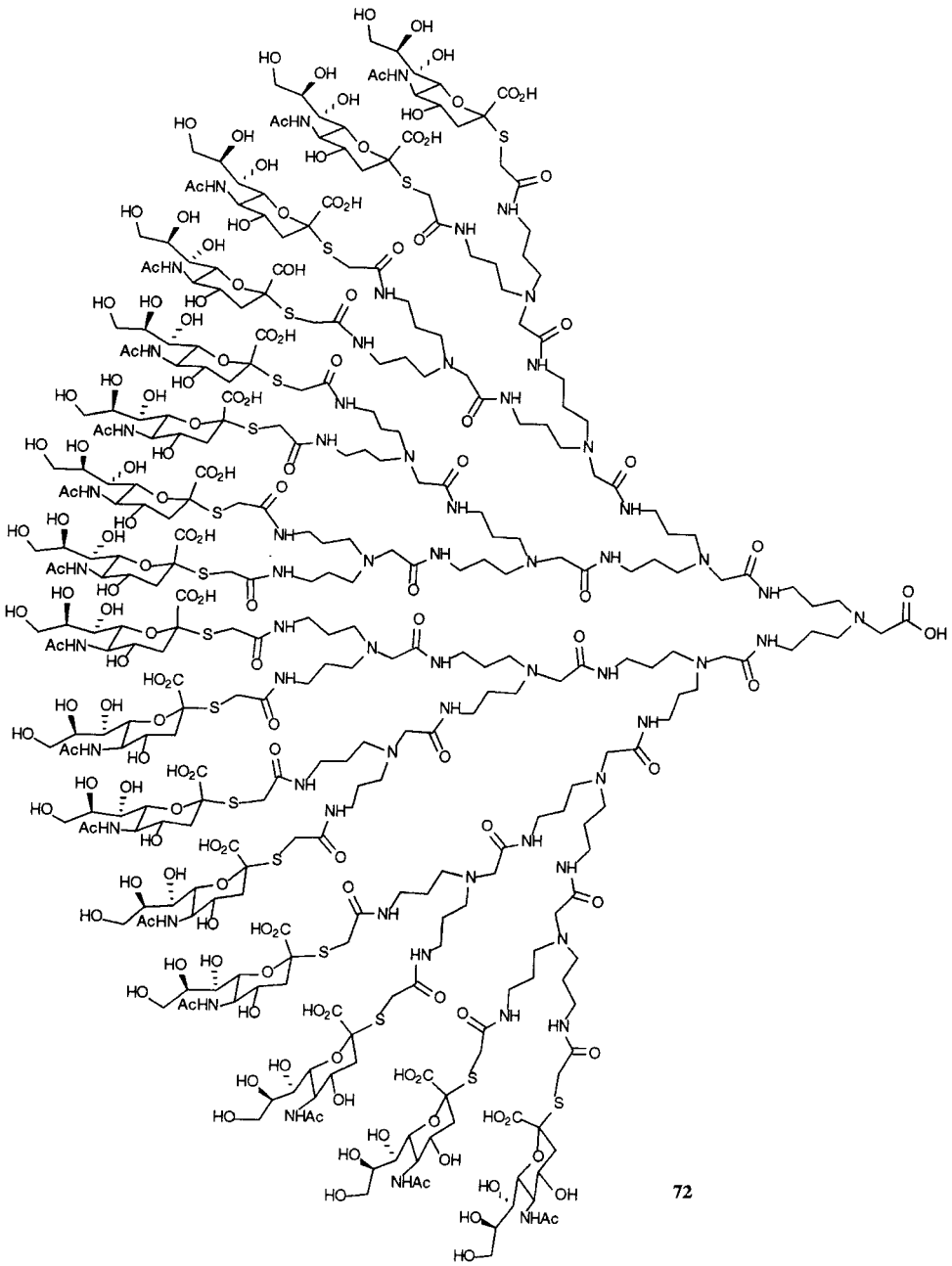
Amine-functionalized dendrimers have been used in several instances. Reducing sugars (**62**) can be directly anchored to PAMAM dendrimers by reductive amination,[97] and sugar lactones (**63**), readily prepared from reducing sugars by oxidation with basic iodine solutions, can be amidated.[98] Aryl[50] or glycosyl[99] isothiocyanato derivatives (**64**) also react rapidly and efficiently with polyaminated dendrimers, even under aqueous conditions.[100] Incorporation of chloro- or bromoacetamido groups onto PAMAM dendrimers [XCH_2COCl or $(ClCH_2CO)_2O$] afforded highly electrophilic species that react readily with thio sugars (**65**) (Scheme 12). The last approach has been successfully applied in double N-alkylation when bromoacylated carbohydrate derivatives (**66**) were used. It simultaneously allowed increasing surface group density.[66,101] These versatile strategies gave to glyco-dendrimers such as **67–71** high-yielding accesses that obviously are applicable to higher oligosaccharides.

Sialylated dendrimers having modest activity in inhibition of hemagglutination of flu viruses have been prepared by using Fmoc-chemistry and L-lysine core in solid phase synthesis reactions.[30,93] Similarly, the foregoing dendrimers, together with analogous peptidomimetic-like dendrimers (**72**) built on 3,3′-iminobispropylamine cores[102,103] showed up to $\sim$32-fold inhibition of binding of human α_1-acid glycoprotein (orosomucoid) to the plant lectin wheat germ agglutinin or the slug lectin from *Limax flavus* (Scheme 13). Similar dendrimers having aryl α-D-mannopyranosides as surface group (L-lysine core) showed $\sim$2000-fold increased inhibition of binding of yeast mannan to concanavalin A or pea lectin.[104] Nanomolar IC_{50}s values have been reported for sialodendrimers **73** obtained by condensation of PAMAM **56** with *p*-isothiocyanatophenyl sialoside **26**[50] (Scheme 14). Additionally, dendritic 3′-sulfo-Lewis^X (Glc) bound to poly-L-lysine backbones inhibited the binding of E-selectin to sialyl-Lewis^X glycolipids 600 times better than the corresponding monomer.[105] The growth valency of the foregoing dendrimers was based on a 2^n progression, where n is the generation (i.e., 2-, 4-, 8-, 16-mer, etc.). It has been deemed of interest to construct dendrimers with a 3^n progression (i.e., 3-, 9-, 27-mer, etc.). To this end, we constructed sialodendrimers (**74**) based on a gallic acid core[106] (Scheme 15).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

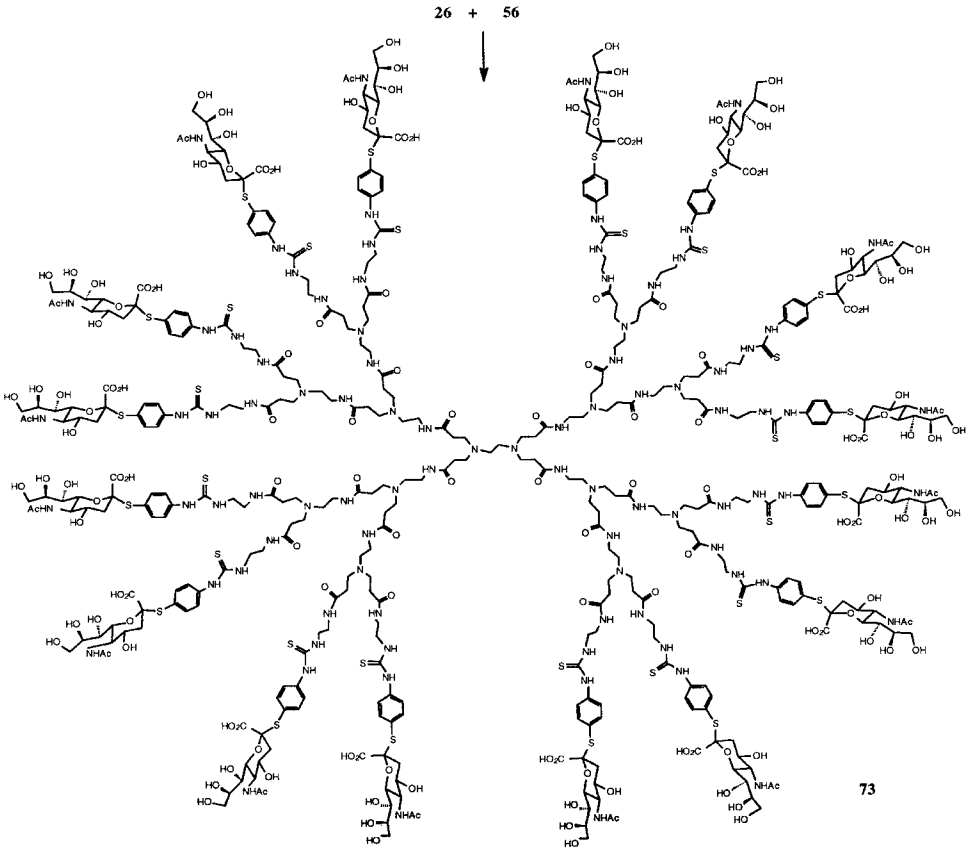


Scheme 12. Divergent construction of glycodendrimers built on polyamine scaffolds using reductive amination (**67**), amide formation (**68**), thiourea bridging with isothiocyanates (**64**), thioethers (**70**), and novel double *N*-alkylation strategy (**71**).



72

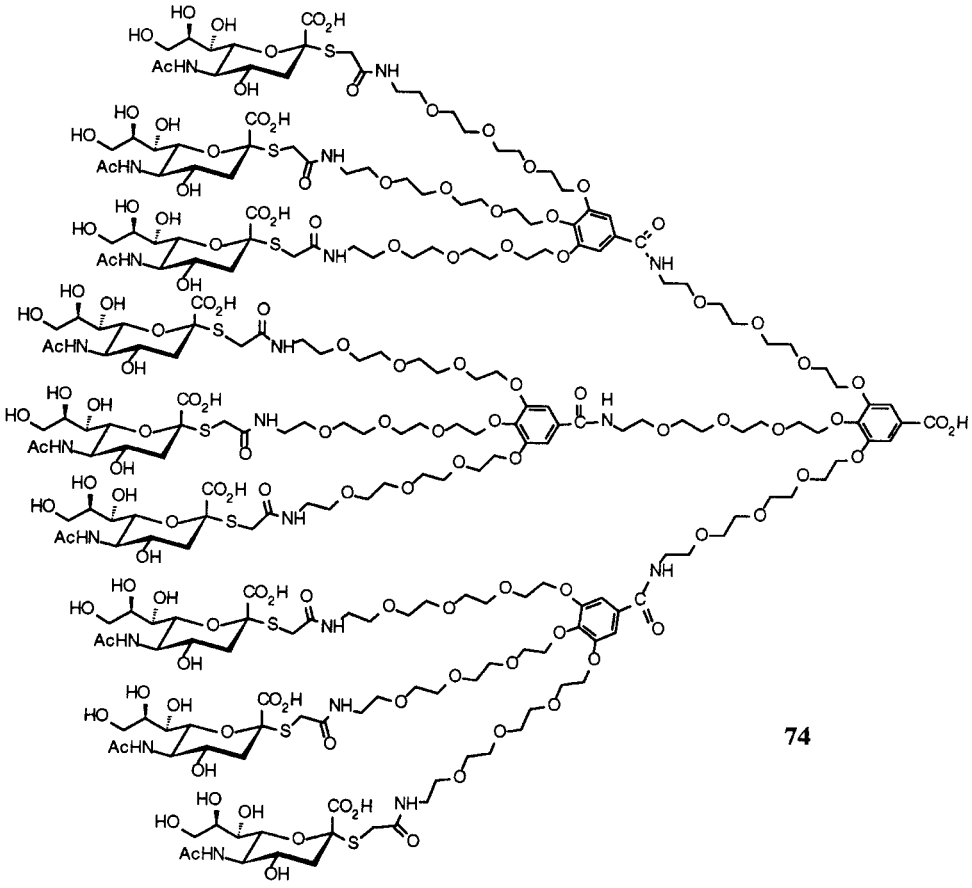
Scheme 13. 16-Mer dendritic sialoside constructed by solid-phase synthesis using 3,3′-iminobis(propylamine) scaffold.[91]



Scheme 14. 16-Mer sialodendrimer synthesized using divergent anchoring of isothiocyanotophenyl sialoside onto PAMAM backbone.

The inhibition of certain interactions by large variations in glycodendrimer efficacy points to the need to design multivalent neoglycoconjugates with defined geometry, valency, and shapes. The seemingly modest results observed with some plant lectins further exemplify the problem encountered in glycobiology as opposed to other drug-protein interactions. A good model to clarify these observations has been recognized with cell adhesion molecules (selectins), where it was established that mediocre in vitro results with sialyllactose do not necessarily translate to poor in vivo experiments.[107] As seen from the synthesis of glycodendrimers derived from the oligosaccharide portion of ganglioside GM_1 (Galβ(1–3)GalNAcβ(1–4)[Neu5Acα(2–3)]Galβ(1–4)Glcβ) built on PAMAM and poly(propyleneimine) dendrimers,[108] the inhibitory properties against cholera toxin B subunit and *E. coli* heat-labile enterotoxin showed IC_{50}s values 15-fold lower than that of GM_1 itself and 1000-fold lower than that of the oligosaccharide.

Just as glycopolymers can be further glycosylated by classical enzymatic reactions via the necessary sugar nucleotides and the corresponding enzymes,[109] glycodendrimers can be treated analogously. In this way, it has been straightforward to



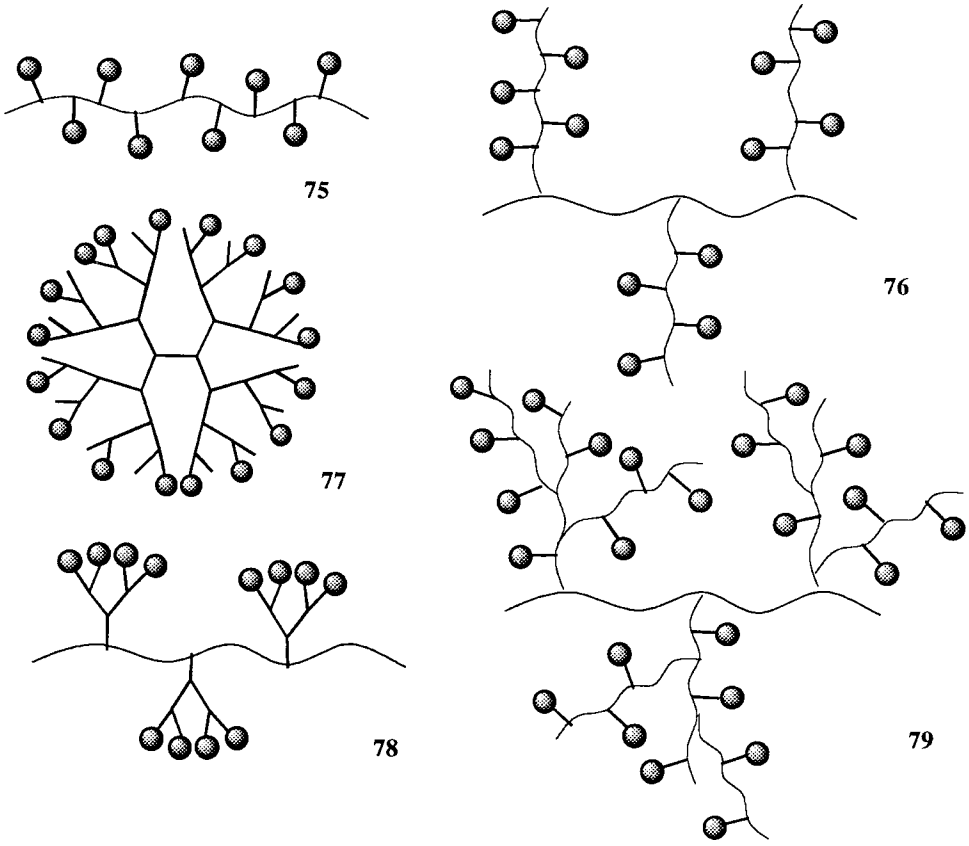
Scheme 15. Dendritic 9-mer sialoside (3^n growth) built on gallic acid core using hydrophilic oligoethyleneglycol spacer.

produce on a dendritic poly-L-lysine core *N*-acetylglucosamine-ending dendrimers sequentially elongated with UDP-galactose, CMP-Neu5Ac, and finally with GDP-fucose and the appropriate glycosyltransferases.[110] Notably, all eight branches of the preformed glycodendrimer could be fully glycosylated to provide octameric sialyl Lewis^X tetrasaccharides in excellent yields.

HYBRID DENDRIMER–POLYMERS

The take-home lesson to date is that several small glycoforms exhibit strong binding affinity. The best examples are derived from the pioneering work of Lee et al.,[111] who showed that *N*-acetylgalactosaminide trimers may be few thousand times better than the monosaccharide in binding to hepatic asialoglycoprotein receptors (ASGRs).[112] There is also a growing body of evidence suggesting that glycoclusters,

when properly designed, showed increased affinity. Given the observations and interpretation by Whitesides et al.[92] that steric stabilization (i.e., wrapping of virion particles by sialopolymers) may be responsible for some of the observed increased affinity, it appeared tempting to combine the best of the accumulated observations to build dendritic-like glycopolymers. Such glycoforms already exist and were described in 1995.[113,114] A fascinating example has been proposed by Fan et al.,[113] who chemoenzymatically synthesized a dendritic mannoside (9-mer) ending with an *N*-acetylglucosaminide acrylamide derivative. The dendrimer was built by the transglycosylation of an asparagine, $Man_9GlcNAc_2Asn$ glycopeptide, obtained by pronase digestion and transferred onto the GlcNAc monomer by means of the endo-*β*-*N*-acetylglucosaminidase isolated from *Arthrobacter protophormiae*. The resulting multiantennary copolymer was a better ligand than soybean agglutinin toward a recombinant rat mannose binding protein (MBP). An analogous strategy has been used by Furuike et al.[114] in the synthesis of an acrylamide-ending *N*-acetyllactosamine trimer that had been copolymerized with acrylamide.



Scheme 16. Schematic representation of novel hyperbranched glycostructures illustrating: regularly interspaced ligands onto poly(ethyleneimine) backbone (**75**), comb-branch (G0) structure (**76**), interspaced spheroidal dendrimer (**77**), rod-shaped, cylindrical polymer with dendritic branch (**78**), and dendrigraft (**79**).

In a recent attempt to generate nontoxic polysialosides that would be powerful influenza flu virus hemagglutinin inhibitors as well as inhibiting infectivity, we synthesized the novel nanostructures shown in Scheme 16.[115] To this end, poly (ethyleneimine) was used as scaffolding for the attachment of 4-isothiocyanatophenyl sialoside (deprotected **26**) to give ''classical'' randomly substituted copolymers such as **75** (Scheme 16). The poly(ethyleneimine) (PEI) backbone was prepared by leaving-ring-opening cationic polymerization of 2-ethyl-2-oxazoline, with methyl tosylate as an initiator and morpholine as a terminator, followed by acid hydrolysis.[116] Alternatively, the secondary amines of the poly(ethyleneimine) were used as initiator for the second-generation cationic polymerization,[117] which after sialoside conjugation gave comb-branch (G0) structures **76**. For comparison purposes, PAMAM dendrimers having partial amine substitutions and interspacing hydroxyl groups[115] were prepared according to our published protocol (Scheme 14) to provide structures such as **77**. Rod-shaped, cylindrical dendrimers synthesized by means of the PAMAM reiterative strategy onto PEI backbone[116] were also used to prepare dendritic copolymers like **78**. Finally, use of hyperbranched PEI/PEI backbones gave dendrigraft structures **79** (PEI G1–G3). Preliminary results showed comb-branched (**76**) and dendrigraft (**79**) to be the most effective sialopolymers made to date (50,000-fold better than the monomer). They also blocked infection of mammalian cells in vitro. The results also varied significantly with various strains of influenza A (H2N2, X-31) and Sendai viruses.

CONCLUSIONS

The construction of neoglycoconjugates has reached an unprecedented level of sophistication and imagination. This is undoubtedly the result of increased interest from the traditional glycobiology community as well as by a wide range of synthetic chemists now entering the field, a trend facilitated by access to modern analytical tools. The degree of refinement in the techniques employed to quantitate polyvalent interactions has also steadily increased over the last few years (reviewed in Ref. 92).

The need for multivalent sialosides is just emerging as their implications in novel biological interactions are being continuously unraveled. Our ignorance about the in vivo receptors' topography and valency requirements is amazingly challenging. It is our opinion that the synthetic demand for glycoclusters, glycopolymers, and hybrid molecules thereof will significantly increase within the next few years. The medicinal chemistry dogma for small-molecule therapeutics will also need to be revisited and adapted to glycopharmaceuticals.

ACKNOWLEDGMENTS

Financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged. I am particularly indebted to my past and present graduate students and postdoctoral fellows who had courageously tackled the chemistry of polysialosides: C. A. Laferrière, D. Zanini, S. J. Meunier, S. Cao, W. K. C. Park, M. Letellier, Q. Wu, S.-N. Wang, Z. Gan, M. Llinares, and U. K. Saha, and F. Hernandez Matéo (visiting professor from Granada). Generous dona-

tion of sialic acid by Dr. M. Kawase from NGK Insulators, Ltd (Handa, Japan) is also acknowledged.

REFERENCES

1. (a) Schauer, R. *Sialic Acids—Chemistry, Metabolism and Functions*; Cell Biology Monographs, Springer-Verlag: Vienna, 1992; Vol. 10. (b) von Itzstein, M.; Kiefel, M.J. In *Carbohydrates in Drug Design*; Witczak, Z.J., Nieforth, K.A., Eds; Marcel Dekker: New York, 1997; 39–82.
2. Varki, A. Glycobiology **1993**, *3*, 97–130.
3. Karlsson, K.A. Curr. Opin. Struct. Biol. **1995**, *5*, 622–635.
4. Lis, H.; Sharon, N. Chem. Rev. **1998**, *98*, 637–674.
5. Beuth, J.; Ko, H.L.; Pulverer, G.; Uhlenbruck, G.; Pichlmaier, H. Glycoconj. J. **1995**, *12*, 1–6.
6. Rosen, S.D.; Bertozzi, C.R. Curr. Opin. Cell Biol. **1994**, *6*, 663–673.
7. Toone, E.J. Curr. Opin. Struct. Biol. **1994**, *4*, 719–728.
8. von Itztein, M.; Wu, W.Y.; Kok, G.B.; Pegg, M.S.; Dyason, J.C.; Jin, B.; Phan, T.V.; Smythe, M.L.; White, H.F.; Oliver, S.W.; Colman, P.M.; Varghese, J.N.; Ryan, D.M.; Wood, J.M.; Bethell, R.C.; Hotham, V.J.; Cameron, J.M.; Penn, C.R. Nature **1993**, *363*, 418–423.
9. Kim, C.U.; Lew, W.; Williams, M.A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M.S.; Mendel, D.B.; Tai, C.Y.; Laver, W.G.; Stevens, R.C. J. Am. Chem. Soc. **1997**, *119*, 681–690.
10. Lee, Y.C.; Lee, R.T. Acc. Chem. Res. **1995**, *28*, 321–327.
11. Roy, R. Trends Glycosci. Glycotechnol. **1996**, *8*, 79–99.
12. (a) Duncan, R.; Kopecek, J. Adv. Polym. Sci. **1984**, *57*, 51–101. (b) Monsigny, M.; Roche, A.C.; Midoux, P.; Mayer, R. Adv. Drug Deliv. Rev. **1994**, *14*, 1–24.
13. García-López, J.J.; Hernández-Matéo, F.; Isac-Gracía, J.; Kim, J.M.; Roy, R.; Santoyo-González, F.; Vargas-Berenguel, A. J. Org. Chem. **1999**, *64*, 522–531, and references cited therein.
14. Marra, A.; Dondoni, A.; Sansone, F. J. Org. Chem. **1996**, *61*, 5111–5158.
15. Dubber, M.; Lindhorst, T.K. Carbohydr. Res. **1998**, *310*, 35–41.
16. Roy, R. Polym. News **1996**, *21*, 226–232.
17. Zanini, D.; Roy, R. *Carbohydrate Mimics: Concepts and Methods*; Chapleur, Y., Ed.; Verlag Chemie: Weinheim, 1998; 385–415.
18. (a) Czarniecki, M.F.; Thornton, E.R. J. Am. Chem. Soc. **1977**, *99*, 8273–8279. (b) Roy, R.; Laferrière, C.A. Can. J. Chem. **1990**, *68*, 2045–2054.
19. Koketsu, M.; Juneja, L.R.; Kawanami, H.; Kim, M.; Yamamoto, T. Glycoconj. J. *9*, 70–74.
20. Veh, R.W.; Michalski, J.C.; Corfield, A.P.; Sander-Wewer, M.; Gies, D.; Schauer, R. J. Chromatogr. **1981**, *212*, 313–322.
21. Jennings, H.J.; Bhattacharjee, A.K. Carbohydr. Res. **1977**, *55*, 105–112.
22. Jennings, H.J.; Roy, R.; Michon, F. J. Immunol. **1985**, *134*, 2651–2657.
23. Roy, R.; Pon, R.A. Glycoconj. J. **1990**, *7*, 3–12.
24. Higashi, H.; Naiki, M.; Matuo, S.; Okouchi, K. Biochem. Biophys. Res. Commun. **1977**, *79*, 388–395.

25. (a) Paulsen, H.; Matschulat, P. Liebigs Ann. Chem. **1991**, 487–495. (b) Nagy, J.O.; Bednarski, M.D. Tetrahedron Lett. **1991**, *32*, 3953–3956.
26. Vlahov, I.R.; Vlahova, P.I.; Linhardt, R.J. J. Am. Chem. Soc. **1997**, *119*, 1480–1481.
27. Lemieux, R.U. Acc. Chem. Res. **1996**, *29*, 373–380.
28. Roy, R. *Handbook of Phase Transfer Catalysis*; Sasson, Y., Neumann, R., Eds.; Blackie Academic & Professional: New York, 1997; 244–275.
29. Roy, R.; Tropper, F.D.; Cao, S.; Kim, J.M. ACS Symp. Ser. **1997**, *659*, 163–180.
30. Roy, R.; Zanini, D.; Meunier, S.J.; Romonowska, A. ACS Symp. Ser. **1994**, *560*, 104–119.
31. Tropper, F.D.; Andersson, F.O.; Braun, S.; Roy, R. Synthesis **1992**, 618–620.
32. Roy, R.; Andersson, F.O.; Harms, G.; Kelm, S.; Schauer, R. Angew. Chem., Int. Ed. Engl. **1992**, *31*, 1478–1481.
33. Roy, R.; Tropper, F.D.; Romanowska, A.; Letellier, M.; Cousineau, L.; Meunier, S.J.; Boratynski, J. Glycoconj. J. **1991**, *8*, 75–81.
34. Cao, S.; Meunier, S.J.; Andersson, F.O.; Letellier, M.; Roy, R. Tetrahedron: Asymmetry **1994**, *5*, 2303–2312.
35. Park, W.K.C.; Meunier, S.J.; Zanini, D.; Roy, R. Carbohydr. Lett. **1995**, *1*, 179–184.
36. (a) Okamoto, K.; Goto, T. Tetrahedron **1990**, *46*, 5835–5857. (b) DeNinno, M.P. Synthesis **1991**, 583–593.
37. (a) Ito, Y.; Ogawa, T. Tetrahedron **1990**, *46*, 89–102. (b) Ercégovic, T.; Magnusson, G. J. Org. Chem. **1995**, *60*, 3378–3384.
38. Martin, T.J.; Schmidt, R.R. Tetrahedron Lett. **1992**, *33*, 6123–6126.
39. Marra, A.; Sinaÿ, P. Carbohydr. Res. **1990**, *195*, 303–308.
40. Hasegawa, A.; Kiso, M. *Carbohydrates. Synthetic Methods and Applications in Medicinal Chemistry*; Ogura, H., Hasegawa, A., Suami, T., Eds.; VCH: Weinheim, 1992; 243–266.
41. Madsen, R.; Fraser-Reid, B. *Modern Methods in Carbohydrate Synthesis*; Khan, S.H., O'Neil, R.A., Eds.; Harwood Academic Publishers: Amsterdam, 1996; 155–170.
42. Boons, G.J. Tetrahedron **1996**, *52*, 1095–1121.
43. Cao, S. ''Active-Latent'' Glycosylation Strategy in Oligosaccharide and Glycoconjugate Syntheses. Ph.D. Dissertation; University of Ottawa: Ottawa, ON, Canada, 1996.
44. Roy, R.; Andersson, F.O.; Letellier, M. Tetrahedron Lett. **1992**, *33*, 6053–6056.
45. Cao, S.; Hernández-Matéo, F.; Roy, R. J. Carbohydr. Chem. **1998**, *17*, 609–631.
46. Cao, S.; Gan, Z.; Roy, R. Carbohydr. Res. **1999**, *318*, 75–81.
47. Raghavan, S.; Kahne, D. J. Am. Chem. Soc. **1993**, *115*, 1580–1581.
48. Cao, S.; Roy, R. Tetrahedron Lett. **1996**, *37*, 3421–3424.
49. Laferrière, C.A.; Roy, R.; Andersson, F.O. Methods Enzymol. **1994**, *242*, 271–280.
50. Zanini, D.; Roy, R. J. Org. Chem. **1998**, *63*, 3486–3491.
51. Llinares, M.; Roy, R. J. Chem. Soc., Chem. Commun. **1997**, 2119–2120.
52. (a) Roy, R. Topics Curr. Chem. **1997**, *187*, 241–274. (b) Saha, U.K.; Kim, J.M.; Roy, R. Syntheses of Glycoforms of Biological Interest. *Proceedings of the Eighth European Carbohydrate Symposium, Spain*, 1995; C IL-5.

53. Roy, R.; Laferrière, C.A.; Pon, R.A.; Gamian, A. Methods Enzymol. **1994**, *247*, 351–361.
54. (a) Glick, G.D.; Knowles, J.R. J. Am. Chem. Soc. **1991**, *113*, 4701–4703. (b) Sabesan, S.; Duus, J.Ø.; Domaille, P.; Kelm, S.; Paulson, J.C. J. Am. Chem. Soc. **1991**, *113*, 5865–5866. (c) Sabesan, S.; Duus, J.Ø; Neira, S.; Domaille, P.; Kelm, S.; Paulson, J.C.; Bock, K. J. Am. Chem. Soc. **1992**, *114*, 8363–8375. (d) DeFrees, S.A.; Kosch, W.; Way, W.; Paulson, J.C.; Sabesan, S.; Halcomb, R.L.; Huang, D.H.; Ichikawa, Y.; Wong, C.H. J. Am. Chem. Soc. **1995**, *117*, 66–79.
55. Simaek, E.E.; McGarvey, G.J.; Jablonowski, J.A.; Wong, C.-H. Chem. Rev. **1998**, *98*, 833–862.
56. Seppo, A.; Turunen, J.P.; Penttilä, L.; Keane, A.; Renkonen, O.; Renkonen, R. Glycobiology **1996**, *6*, 65–71.
57. Maaheimo, H.; Renkonen, R.; Turunen, J.P.; Penttilä, L.; Renkonen, O. Eur. J. Biochem. **1995**, *234*, 616–625.
58. Roy, R. *Carbohydrates in Drug Design*; Witczak, Z.J., Nieforth, K.A., Eds.; Marcel Dekker: New York, 1997; 84–136.
59. (a) Roy, R.; Pagé, D.; Figueroa Perez, S.; Verez Bencomo, V. Glycoconj. J. **1998**, *15*, 251–265. (b) See also: Brewer, C.F. Chemtracts, Biochem. Mol. Biol. **1996**, *6*, 165–179.
60. Unverzagt, C.; Kelm, S.; Paulson, J.C. Carbohydr. Res. **1994**, *251*, 285–301.
61. Zuckermann, R.N.; Martin, E.J.; Spellmeyer, D.C.; Stauber, G.B.; Shoemaker, K.R.; Kerr, J.M.; Figliozzi, G.M.; Goff, D.A.; Siani, M.A.; Simon, R.J.; Banville, S.C.; Brown, E.G.; Wang, L.; Richter, L.S.; Moos, W.H. J. Med. Chem. **1994**, *37*, 2678–2685.
62. (a) Saha, U.K.; Roy, R. Tetrahedron Lett. **1997**, *38*, 7697–7700. (b) Saha, U.K.; Roy, R. J. Chem. Soc., Chem. Commun. **1996**, 201–202. (c) Saha, U.K.; Roy, R. Tetrahedron Lett. **1995**, *36*, 3635–3638. (d) Saha, U.K.; Roy, R. J. Chem. Soc., Chem. Commun. **1995**, 2571–2573.
63. (a) Kim, J.M.; Roy, R. Carbohydr. Res. **1997**, *298*, 173–179. (b) Kim, J.M.; Roy, R. Tetrahedron Lett. **1997**, *38*, 3487–3490. (c) Kim, J.M.; Roy, R. Carbohydr. Lett. **1996**, *1*, 465–468.
64. Sabesan, S. Tetrahedron Lett. **1997**, *38*, 3127–3130.
65. (a) Böhmer, V. Angew. Chem., Int. Ed. Engl. **1995**, *34*, 713–745. (b) Ikeda, A.; Shinkai, S. Chem. Rev. **1997**, *97*, 1713–1734.
66. Roy, R.; Kim, J.M. Angew. Chem., Int. Ed. Engl. **1999**, *38*, 369–372.
67. Arnaud-Neu, F.; Barrett, G.; Cremin, S.; Deasy, M.; Fergusson, G.; Harris, S.J.; Lough, A.J.; Lourdes, G.; McKervey, M.A.; Schwing-Weill, M.J.; Schwinte, P.J. Chem. Soc., Perkin Trans. 2 **1992**, 1119–1125.
68. Meunier, S.J.; Roy, R. Tetrahedron Lett. **1996**, *37*, 5469–5472.
69. Pagé, D.; Roy, R. Biorg. Med. Chem. Lett. *6*, 1765–1770.
70. (a) Dominique, R.; Das, S.K.; Roy, R. J. Chem. Soc., Chem. Commun. **1998**, 2437–2438. (b) Das, S.K.; Dominique, R.; Smith, C.; Nahra, J.; Roy, R. Carbohydr. Lett. **1999**, *3*, 361–368. (c) Hu, Y.J.; Roy, R. Tetrahedron Lett. **1999**, *40*, 3305–3308.
71. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. Tetrahedron Lett. **1975**, 4467–4470. (b) Roy, R.; Das, S.K.; Dominique, R.; Trono, M.C.; Hernández-Matéo, F.; Santoyo-González, F. Pure Appl. Chem. **1999**, 565–571.

72. Roy, R.; Tropper, F.D.; Romanowska, A. Bioconjug. Chem. **1992**, *3*, 256–261.
73. Roy, R.; Laferrière, C.A.; Gamian, A.; Jennings, H.J. J. Carbohydr. Chem. **1987**, *6*, 161–165.
74. Roy, R.; Laferrière, C.A. Carbohydr. Res. **1988**, *177*, C1–C4.
75. Laferrière, C.A. Synthesis of Sialic Acid Antigens. Ph.D. Dissertation; University of Ottawa: Ottawa, Ontario, Canada, 1990.
76. Roy, R.; Laferrière, C.A. U.S. Patents 5,034,516, 1991; 5,192,661, 1993.
77. (a) Gamian, A.; Chomik, M.; Laferrière, C.A.; Roy, R.Can. J. Microbiol. **1991**, *37*, 233–237. (b) Spaltenstein, A.; Whitesides, G.M. J. Am. Chem. Soc. **1991**, *113*, 686–687.
78. (a) Nifant'ev, N.E.; Shashkov, A.S.; Tsvetkov, Y.E.; Tuzikov, A.B.; Abramenko, I.V.; Gluzman, D.F.; Bovin, N.V. ACS Symp. Ser. **1994**, *560*, 267–275. (b) Sparks, M.A.; Williams, K.W.; Whitesides, G.M. J. Med. Chem. **1993**, *36*, 778–783. (c) Nagy, J.O.; Wang, P.; Gilbert, J.H.; Schaefer, M.E.; Hill, T.G.; Callstrom, M.R.; Bednarski, M.D. J. Med. Chem. **1992**, *35*, 4501–4502.
79. Roy, R. *Carbohydrate Chemistry*; Boons, G.J., Ed.; Chapman & Hall: London, 1998; 243–321.
80. Magnusson, G.; Chernyak, A.Y.; Kihlberg, J.; Kononov, L.O. *Neoglycoconjugates: Preparation and Applications*; Lee, Y.C., Lee, R.T., Eds.; Academic Press: San Diego, CA, 1994; 53–143.
81. (a) Bovin, N.V.; Gabius, H.J. Chem. Soc. Rev. **1995**, *24*, 413–421. (b) Bovin, N.V. Glycoconj. J. **1998**, *15*, 431–446.
82. Roy, R. *Modern Methods in Carbohydrate Synthesis*; Khan, S.H., O'Neil, R., Eds.; Harwood Academic Publishers: Amsterdam, 1996; 378–402.
83. (a) Sigal, G.B.; Mammen, M.; Dahmann, G.; Whitesides, G.M. J. Am. Chem. Soc. **1996**, *118*, 3789–3800. (b) Choi, S.K.; Mammen, M.; Whitesides, G.M. Chem. Biol. **1996**, *3*, 97–104.
84. Byramova, N.E.; Mochalova, L.V.; Belyanchikov, J.M.; Matrosovich, M.N.; Bovin, N.V. J. Carbohydr. Chem. **1991**, *10*, 691–700.
85. Choi, S.K.; Mammen, M.; Whitesides, G.M. J. Am. Chem. Soc. **1997**, *119*, 4103–4111.
86. Kingery-Wood, J.E.; Williams, K.W.; Sigal, G.B.; Whitesides, G.M. J. Am. Chem. Soc. **1992**, *114*, 7303–7305.
87. (a) Roy, R.; Laferrière, C.A. J. Chem. Soc., Chem. Commun. **1990**, 1709–1711. (b) Roy, R.; Pon, R.A.; Tropper, F.D.; Andersson, F.O. J. Chem. Soc., Chem. Commun. **1993**, 264-265. (c) Romanowska, A.; Meunier, S.J.; Tropper, F.D.; Laferrière, C.A.; Roy, R. Methods Enzymol. **1994**, *242*, 90–101.
88. Kamitakahara, H.; Suzuki, T.; Nishigori, N.; Suzuki, Y.; Kanie, O.; Wong, C.H. Angew. Chem., Int. Ed. Engl. **1998**, *37*, 1524–1528.
89. Spevak, W.; Nagy, J.O.; Charych, D.H.; Schaefer, M.E.; Gilbert, J.H.; Bednarski, M.D. J. Am. Chem. Soc. **1993**, *115*, 1146–1147.
90. Pagé, D. Effect of Shape, Size, and Valency of Multivalent Mannopyranosides on Their Binding Properties to Phytohemaggutinins. MSc Dissertation; University of Ottawa: Ottawa, Ontario, Canada, 1997.
91. Murahashi, S.I.; Naota, T.; Nakajima, N. Chem. Lett. **1987**, 879–882.
92. Mammen, M.; Choi, S.K.; Whitesides, G.M. Angew. Chem., Int. Ed. Engl. **1998**, *37*, 2754–2794.

93. Roy, R.; Zanini, D.; Meunier, S.J.; Romanowska, A. J. Chem. Soc., Chem. Commun. **1993**, 1869–1872.
94. (a) Chow, H.F.; Mong, T.K.K.; Nongrum, M.F.; Wan, C.W. Tetrahedron **1998**, *54*, 8543–8660. (b) Smith, D.K.; Diederich, F. Chem. Eur. J. **1998**, *4*, 1353–1361. (c) Seebach, D.; Rheimer, P.B.; Greiveldinger, G.; Butz, T.; Sellner, H. Top Curr. Chem. **1998**, *197*, 125–164. (d) Astruc, D. C.R. Acad. Sci. Paris **1996**, *322* (Ser. IIb), 757–766. (e) Ardoin, N.; Astruc, D. Bull. Soc. Chim. Fr. **1995**, *132*, 875–909. (f) Archut, A.; Vögtle, F. Chem. Soc. Rev. **1998**, *27*, 233–240. (g) Zeng, F.; Zimmerman, S.C. Chem. Rev. **1997**, *97*, 1681–1712. (h) Tomalia, A.; Durst, H.D. Top Curr. Chem. **1993**, *165*, 193–313. (i) Newkome, G.R.; Moorefield, C.; Vögtle, F. *Dendritic Molecules: Concepts, Syntheses, Perspectives*, 2nd Ed.; Wiley-VCH: Weinheim, 1998. (j) Fréchet, J.M.J.; Hawker, C.J. Compr. Polym. Sci. **1996**, 140–201.
95. (a) Roy, R. Curr. Opin. Struct. Biol. **1996**, *6*, 692–702. (b) Lindhorst, T.K. Nachr. Chem. Tech. Lab. **1996**, *44*, 1073–1079.
96. (a) Toyokuni, T.; Singhal, A.K. Chem. Soc. Rev. **1995**, 231–242. (b) Jayaraman, N.; Nepogodiev, S.A.; Stoddart, J.F. Chem. Eur. J. **1997**, *3*, 1193–1199.
97. Roy, R.; Thompson, J.; Sashiwa, H.; Das, S.K.; Tripathy, S.; Gabius, H.J. Préparation et Propriétés de Néoglycoconjugués Impliqués lors du Rejet Chronique de Xénotransplantation. In *Proceedings of 67th ACFAS Congress, Ottawa*; 1999.
98. Aoi, K.; Itoh, K.; Okada, M. Macromolecules **1995**, *28*, 5391–5393.
99. (a) Pagé, D.; Aravind, S.; Roy, R. J. Chem. Soc., Chem. Commun. **1996**, 1913–1914. (b) Lindhorst, T.K.; Kieburg, C. Angew. Chem., Int. Ed. Engl. **1996**, *35*, 1953–1956. (c) Pagé, D.; Roy, R. Glycoconj. J. **1997**, *14*, 345–356.
100. Kieburg, C.; Lindhorst, T.K. Tetrahedron Lett. **1997**, *38*, 3885–3888.
101. Roy, R.; Kim, J.M. Polym. Mater. Sci. Eng. **1997**, *77*, 195–196.
102. Zanini, D.; Roy, R. J. Am. Chem. Soc. **1997**, *119*, 2088–2095.
103. Zanini, D.; Roy, R. J. Org. Chem. **1996**, *61*, 7348–7354.
104. Pagé, D.; Zanini, D.; Roy, R. Bioorg. Med. Chem. **1996**, *4*, 1949–1961.
105. (a) Roy, R.; Park, W.K.C.; Zanini, D.; Foxall, C.; Srivastava, O.P. Carbohydr. Lett. **1997**, *2*, 259–266. (b) Roy, R.; Park, W.K.C.; Srivastava, O.P.; Foxall, C. Bioorg. Med. Chem. Lett. **1996**, *6*, 1399–1402.
106. (a) Meunier, S.J.; Wu, Q.; Wang, S.N.; Roy, R. Can. J. Chem. **1997**, *75*, 1472–1482. (b) Roy, R.; Park, W.K.C.; Wu, Q.; Wang, S.N. Tetrahedron Lett. **1995**, *36*, 4377–4380.
107. Mulligan, M.S.; Paulson, J.C.; DeFrees, S.; Zheng, Z.L.; Lowe, J.B.; Ward, P.A. Nature **1993**, *364*, 149–151.
108. Thompson, J.P.; Schengrund, C.L. Glycoconj. J. **1997**, *14*, 745–837.
109. Nishimura, S.I.; Yamada, K. J. Am. Chem. Soc. **1997**, *119*, 10555–10556.
110. Palcic, M.M.; Li, H.; Zanini, D.; Bhella, R.S.; Roy, R.Carbohydr. Res. **1998**, *305*, 433–442. See also: Zanini, D.; Roy, R. Bioconjug. Chem. **1997**, *8*, 187–192.
111. Lee, R.T.; Lee, Y.C. *Neoglycoconjugates: Preparation and Applications*; Lee, Y.C., Lee, R.T., Eds.; Academic Press: San Diego, CA, 1994; 23–50.
112. Lee, Y.C. *Carbohydrate Recognition in Cellular Function*; Bock, G., Harnett, S., Eds.; John Wiley & Sons: New York, 1989; 80–95.

113. Fan, J.Q.; Quensenbery, M.S.; Takegawa, K.; Iwahara, S.; Kondo, A.; Kato, I.; Lee, Y.C. J. Biol. Chem. **1995**, *270*, 17730–17735.
114. Furuike, T.; Nishi, N.; Tokura, S.; Nishimura, S.I. Chem. Lett. **1995**, 823–824.
115. Reuter, J.D.; Myc, A.; Hayes, M.M.; Gan, Z.; Roy, R.; Q., D.; Yin, R.; Piehler, L.T.; Esfand, R.; Tomalia, D.A.; Baker, J.R., Jr. Bioconjug. Chem. **1999**, *10*, 271–278.
116. Yin, R.; Zhu, Y.; Tomalia, D.A. J. Am. Chem. Soc. **1998**, *120*, 2678–2679.
117. Tomalia, D.A.; Hedstrand, D.M.; Ferrito, M.S. Macromolecules **1991**, *24*, 1435–1438.