Recent Advances in the Synthesis of Sialic Acid Derivatives and Sialylmimetics as Biological Probes

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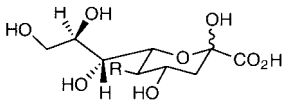
Contents

I. Introduction

There has been intense interest during recent years in identifying the biological functions of carbohydrates. This diverse class of biomolecule is now well recognized as playing significant roles in numerous physiological processes.[1−3] Examples of important functions in which cell-surface glycoconjugates are involved include cell−biomolecule interactions, the masking of receptors by cell-surface glycans, markers in certain cancers, and as ligands for proteins.[1−3] One family of carbohydrates intimately involved in many of these biological processes are the sialic acids. These naturally occurring 2-keto-3-deoxy-nonulosonic acids are a diverse group that are commonly found as the α-ketosidically linked terminal sugar on cell-surface glycoconjugates and are the most abundant terminal sugar on mammalian glycoconjugates.

Sialic acids are acidic 9-carbon sugars (numbering shown in Scheme 1), with 43 different naturally occurring sialic acid derivatives having been reported in nature thus far.[4] The most commonly found derivatives are those derived from 5-acetamido-D-*glycero*-D-*galacto*-2-nonulosonic acid (*N*-acetylneuraminic acid, Neu5Ac, **1**).[4−6] The 5-glycolylamido derivative *N*-glycolylneuraminic acid (Neu5Gc, **2**) and the non-aminated 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN, **3**) are also found in biological systems although they are less common than Neu5Ac.[4−6] Natural derivatives of these sialic acids



1 R = NHAc (Neu5Ac)
2 R = NHGc (Neu5Gc)
3 R = OH (KDN)

typically incorporate acetylation of the hydroxyl groups, most commonly at C-9, although di- and tri-*O*-acetylated derivatives have been reported.[4−6] Other naturally occurring derivatives involve lactoylation or phosphorylation at C-9 and methylation or sulfation at C-8.[4−6] As components of glycoconjugates, sialic acids are found either α(2,3)- or α(2,6)-linked to hexoses (commonly galactose or galactosamine) or α(2,8)-linked to other sialic acids.[1,4,5,7] Linear homopolymers of Neu5Ac and Neu5Gc, either α(2,8)-, α(2,9)-, or alternating α(2,8)-/α(2,9)-linked, have been found in glycoproteins of embryonic neural membranes and neural cell adhesion molecule (NCAM), fish eggs, and certain bacteria.[1,4,5,7−10] *N*-Glycolylneuraminic acid polymers have also been found in sea urchin eggs linked through the glycolyl hydroxyl group resulting in α(2,5)-linkages.[4,5]

Given their position within glycoconjugates, it has become increasingly apparent that sialic acids are intimately involved in a number of important physiological phenomena and disease states.[4−7,11−15] Located at the terminus of numerous cell-surface oligosaccharides, sialic acids are ideally positioned to participate in carbohydrate−protein interactions that mediate recognition phenomena. These include acting as receptors for bacteria and viruses, masking underlying antigenic determinants, mediating cell−cell adhesion via lectins, and enabling cell−cell communication processes.[1−7,11−15] Specific examples of these types of interactions include the binding of influenza virus haemagglutinin to sialic acid prior to viral entry[16] and the interaction of the selectins with sialyl Lewis x during the recruitment of leukocytes in the inflammation process.[1,17,18] The interaction of sialic acids with the immune system highlights the importance of minor structural modifications on the overall physiological response.[19,20] A

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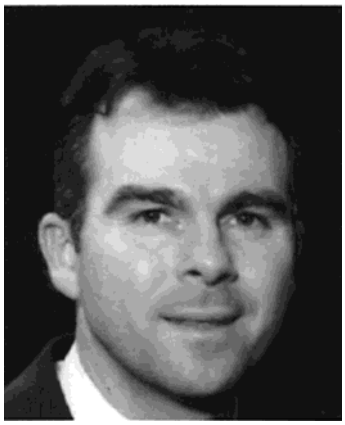
Milton Kiefel completed his Ph.D. in organic chemistry (natural products isolation and total synthesis) at Melbourne University in 1990 before undertaking a postdoctoral position with Professor Gerald Pattenden FRS at the Univeristy of Nottingham (U.K.). He joined Professor von Itzstein's group as a Research Fellow in 1993, within the Department of Medicinal Chemistry at Monash University. In 2000 he moved to Griffith University (Gold Coast campus) to take up a position as Lecturer and Head of Medicinal Chemistry within the Centre for Biomolecular Science and Drug Discovery. His research interests focus on the synthesis of novel sialic acid derivatives and sialylmimetics as probes for sialic acid-recognizing proteins, as well as on the development of novel strategies for the synthesis of carbohydrates of biological interest.



Mark von Itzstein completed his Ph.D. in organic chemistry at Griffith University in 1984 and was awarded an Alexander von Humboldt fellowship to carry out research at the University of Marburg with Professor Manfred Reetz in Germany. His internationally renowned career as a carbohydrate chemist began in the Department of Medicinal Chemistry at Monash University in 1986. One of his research group's major achievements was the design and sythesis of the new anti-influenza drug, Relenza. As a result of this research, he was jointly awarded the prestigious Australia Prize for pharmaceutical design in 1996. In 2000 he returned to Griffith University as the Director of the new Centre for Biomolecular Science and Drug Discovery at Griffith University's Gold Coast campus. The Centre has, as its mission, the task of discovering clinically useful medicines. Mark's research group is particularly interested in the discovery of new generation antibiotics and antivirals and drugs to treat cancer, the complications of diabetes, as well as other conditions. Professor von Itzstein was awarded a von Humboldt Forschungspreis in 2001.

considerable effort has gone into the characterization of sialylglycoconjugates from bacteria[8−10,21] and has resulted in the development of potential vaccines based upon bacterial carbohydrate epitopes.

The growing realization of the significant roles of α-ketosidically linked sialic acids in various disease states and physiological processes has resulted in extensive research into sialic acid chemistry and biochemistry. Several excellent publications over recent years describe the synthesis of both natural and structurally modified sialic acids. The earlier reports by Schauer,[7] Zbiral,[22] DeNinno,[23] Brossmer,[24] and others,[25,26] together with more recent works,[27−30] are an excellent starting point for anyone intending to enter into this field. The interested reader is also encouraged to peruse recent articles describing more broadly some of the pertinent issues relating to carbohydrate chemistry.[31−33] Given the comprehensive nature of these articles, it is not our intention to revisit many of the issues relating to sialic acid chemistry which have already been covered in detail. Rather, this article focuses on the more salient features of the recent advances in sialic acid chemistry, primarily focusing on the last three years. Initially, the synthesis and structural modifications of sialic acids themselves, primarily in relation to *N*-acetylneuraminic acid, will be described. Given their occurrence in natural systems as α-glycosidically linked units, a section will describe recent developments in one of the more difficult aspects of sialic acid chemistry—the formation of specifically α-linked glycosides. The complexity of the chemistry of sialic acids and their derivatives has spawned intense interest in the development of mimetics of sialic acids over recent years. In light of this, the remainder of this article will provide an overview of sialylmimetic chemistry and will highlight the structural diversity that is possible in such sialylmimetics while maintaining excellent mimicry.

II. Structural Modifications of Sialic Acids

By virtue of the polyfunctional nature of sialic acids, much of the chemistry associated with their modification is more complicated than comparable modifications carried out on simple hexoses. In general terms, the synthesis of sialic acids and their derivatives can be conveniently divided into two broad categories, (a) enzymatic or chemoenzymatic methods and (b) purely chemical approaches, each of which will be addressed in turn. It is worth noting that structural modifications have been made at every position within the sialic acid structure.[22,27]

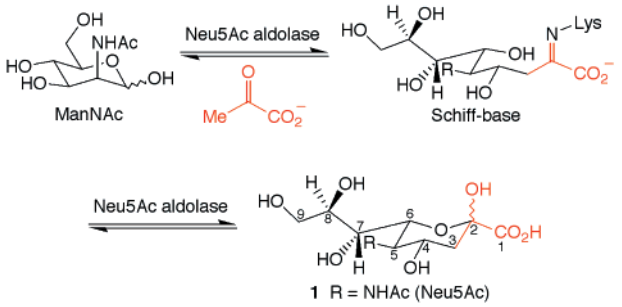
For the purposes of this article, and in keeping with the general state of sialic acid chemistry, the majority of structurally modified sialic acids discussed are derivatives of *N*-acetylneuraminic acid (Neu5Ac, **1**). Where significant work has been carried out on either Neu5Gc (**2**) or KDN (**3**) this will be noted, otherwise it should be taken that the work was done with Neu5Ac. However, for many of the structural modifications presented here for Neu5Ac, it may well be that the same procedure would be equally applicable to the other sialic acids. It must also be remembered that the following sections should not be considered as comprehensive, but rather be viewed as highlighting some of the more interesting or significant contributions to this vast area of research.

A. Enzymatic or Chemoenzymatic Synthesis of Sialic Acids

1. Neu5Ac Aldolase

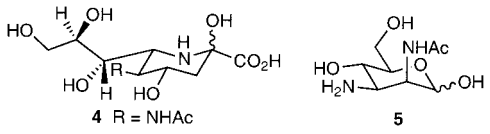
The most common approach to the enzymatic synthesis of Neu5Ac analogues involves the chemical

Scheme 1



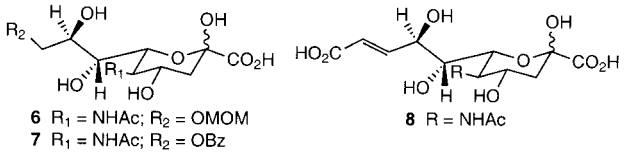
preparation of modified *N*-acetylmannosamine (ManNAc) derivatives and their conversion to the corresponding Neu5Ac derivative using Neu5Ac aldolase (*N*-acylneuraminate pyruvate lyase, EC 4.1.3.3) as depicted in Scheme 1. Both sialic acid synthesis (aldolase activity) and hydrolysis (lyase activity) by this enzyme have been well studied.[34,35] For synthetic purposes, the equilibrium can be shifted to favor the aldol condensation product using an excess of pyruvate.[34] Earlier reports have described in detail the substrate specificity of Neu5Ac aldolase and have demonstrated that C-2 (including OH, leading to KDN derivatives), C-4, and C-6 modified ManNAc derivatives are well tolerated by the enzyme.[27,35,36]

In an attempt to prepare the nitrogen isostere **4**[37] of Neu5Ac, a series of C-3 nitrogen functionalized ManNAc derivatives (e.g., **5**) were prepared.[38] Unfortunately, none of these ManNAc derivatives were found to be substrates for Neu5Ac aldolase, a finding consistent with the reports of others on C-3 substituted ManNAc derivatives.[35]



An interesting use of Neu5Ac aldolase, exploiting both the lyase capability of the enzyme as well as the aldol condensation, involved the incorporation of ^{13}C labels into sialic acid derivatives.[39] A range of C-9, C-8, and C-7 modified Neu5Ac derivatives, prepared through standard chemical methods, were exposed to Neu5Ac aldolase in the presence of ^{13}C-labeled pyruvate, resulting in the incorporation of label at C-3 via a two-step one-pot procedure (Scheme 2). Importantly, the unlabeled pyruvate generated by the decomposition of the initial Neu5Ac substrate was converted into lactic acid (using lactate dehydrogenase, alcohol dehydrogenase, and nucleotide pyrophosphatase) before the addition of the ^{13}C-labeled pyruvate.

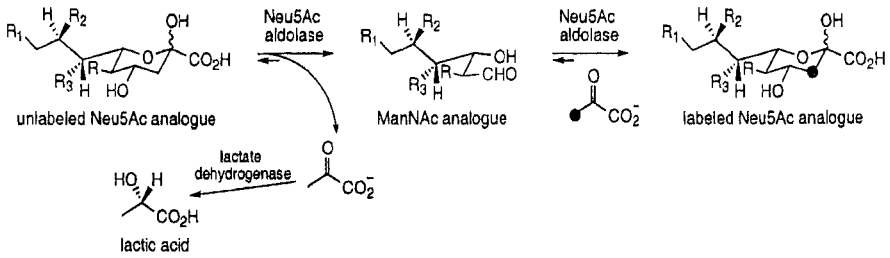
Recently, the crystal structure of Neu5Ac aldolase was described.[40] In addition to the native form being resolved to 1.6 Å, structures of the enzyme complexed with 4-deoxy-Neu5Ac and 4-oxo-Neu5Ac were also determined. As well as the important structural information gained from this investigation, the authors speculate on the mechanistic implications of their findings, particularly with regard to the role of an active site lysine and a strictly conserved tyrosine residue.[40] On the subject of the mechanism of Neu5Ac aldolase, it has been shown that the enzyme is, not unexpectedly, tolerant of simple ether (e.g., **6**) or ester (e.g., **7**) modification at C-9 of Neu5Ac, although the rate of cleavage is reduced when compared to Neu5Ac itself.[41] However, the glycerol chain-extended Neu5Ac derivative **8** was not a substrate for Neu5Ac aldolase and indeed was shown to act as an inhibitor of the lyase.[41] Although the precise nature of the inhibition was not determined, it is plausible that the additional carboxylate group may be interacting with the Schiff base forming active site lysine residue.

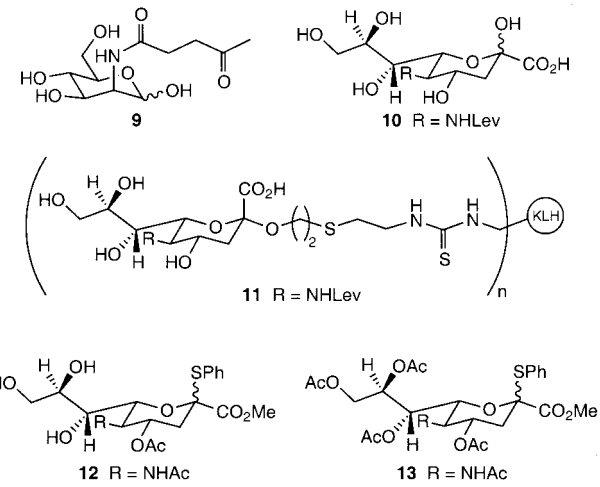


2. Sialic Acid Biosynthetic Enzymes

The mammalian biosynthesis of sialic acids involves the conversion of ManNAc-6-phosphate to Neu5Ac-9-phosphate and then loss of phosphate to give Neu5Ac.[4−7] As with Neu5Ac aldolase, the enzymes involved in this pathway have also been shown to tolerate, to some degree, structurally modified substrates.[4,42] This tolerance extends to the enzymes involved in activation (CMP-Neu5Ac synthase) and transfer (sialyltransferases) of sialic acids to glycoconjugates, as exemplified in some excellent work by the Bertozzi group.[43−45] As way of example, *N*-levulinoylmannosamine (ManLev, **9**) is tolerated by the enzymes involved in the biosynthesis of sialylglycoconjugates. ManLev is readily metabolized by cells in vitro, resulting in the expression of *N*-levulinoylneuraminic acid (**10**) as a component of cell-surface sialoglyconjugates.[43,44] Interestingly, these workers have found that peracetylated ManLev is more bioavailable than **9** itself during this process. The resulting cell-surface glycoconjugates, containing the unnatural sialic acid produced from ManLev,

Scheme 2





have been utilized in immunogenicity studies.[45] This work has shown that rabbits immunized with the unnatural sialic acid conjugate SiaLev-KLH (**11**) produced significant levels of antibodies which recognized SiaLev to the exclusion of natural Neu5Ac.[45] The ability to overcome the issue of immune system tolerance for self-antigens has potentially significant applications in the immunotherapy of cancer, particularly given the interest in the development of anticancer vaccines based upon carbohydrate antigens.[46−49] In this regard, the incubation of *N*-propionylmannosamine in leukemic cells resulted in the expression of α(2,8)-*N*-propionylpolysialic acid on the surface of tumor cells.[50] Since monoclonal antibodies (e.g., mAb 13D9) specifically recognize α(2,8)-*N*-propionylpolysialic acid, the expression of unnatural polysialic acid in this way leads to the possibility of specifically targeting tumor cells.

The enzymes involved in sialylglycoconjugate biosynthesis, most notably CMP-Neu5Ac synthase and the sialyltransferases, have been utilized for the preparation of sialyloligosaccharides with structurally modified Neu5Ac residues. While outside the scope of the present article, the resulting oligosaccharides containing modified sialic acid residues are extremely useful in studies aimed at better understanding issues surrounding the structure−activity relationships of a number of sialic acid-recognizing proteins.

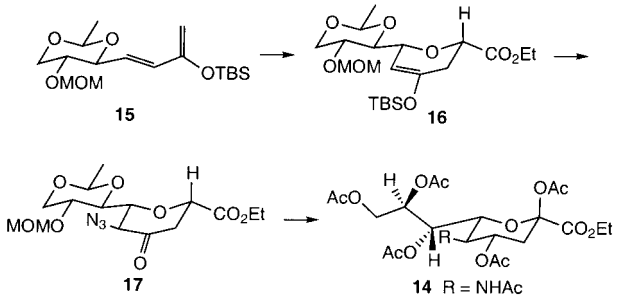
3. Other Enzymes

The chemoenzymatic preparation of the Neu4,5Ac$_2$ derivative **12**, from the fully acetylated derivative **13** using lipase OF (*Candida rugosa*), is noteworthy.[51] The lipase OF only hydrolyzes primary acetyl groups, so this report exemplifies the previously reported[52] ability of acetyl groups to migrate along the glycerol side chain of sialic acids. This "feature" of sialic acids is one of the issues which causes difficulties in the preparation of some structurally modified sialic acids.

B. Chemical Methods for the Synthesis of Sialic Acids

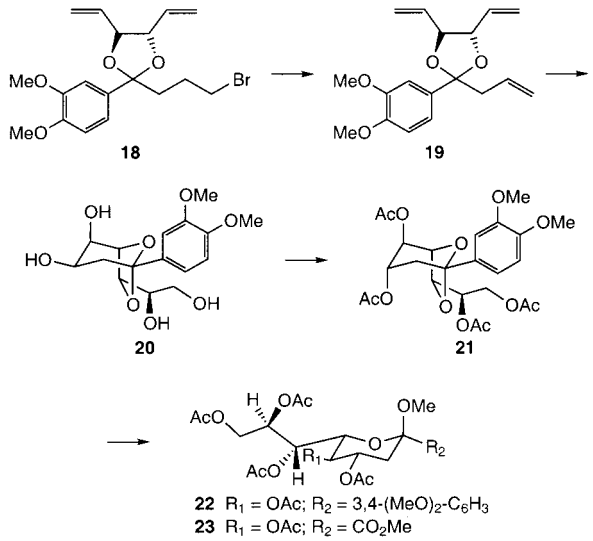
The first chemical synthesis of Neu5Ac (**1**) was reported in 1958 by Cornforth et al.[53] and involved the condensation of *N*-acetylglucosamine with oxobutanedioic acid in the presence of base to give **1** in ∼1% yield. Since that time several improvements have been made to the synthetic method, although the general synthetic strategy is still relevant.[27] KDN (**3**) has also been synthesized, starting from mannose and utilizing such approaches as indium mediated allylation or Wittig chemistry.[27,54,55] The first total synthesis of Neu5Ac (**1**) from noncarbohydrate precursors was reported by Danishefsky et al. in 1988.[56] More recently, the total synthesis of the fully protected Neu5Ac derivative **14** has been described.[57] The key features of this approach are summarized in Scheme 3. The salenCo(II) catalyzed hetero Diels−Alder reaction between the diene **15**, prepared in five steps from D-glucose, and ethyl glyoxylate furnished the cycloaddition product **16** in good yield. Introduction of the requisite amine group at C-5 proved problematic but was eventually achieved by careful exposure of **16** to NaN_3 followed by the addition of ceric ammonium nitrate. The resultant intermediate **17** was then transformed into **14** over several steps involving reduction, protection, oxidation at C-2 using MoO_5·Py·HMPA,[58] and functional group interconversion. The intermediate **17** was also transformed into the corresponding 2-deoxy-Neu5Ac derivative.[57]

Scheme 3



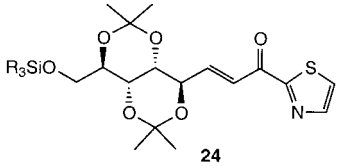
In an elegant and high-yielding formal synthesis of KDN (**3**) from noncarbohydrate precursors, the ketal **18**, which can be obtained in high yield on a 10 g scale, was elaborated to the triene **19** (Scheme 4).[59] Treatment of **19** with Grubbs catalyst resulted in a ring-closing metathesis reaction, and subsequent dihydroxylation provided the tetraol **20**. Importantly,

Scheme 4



due to the constrained nature of **20**, no other stereoisomer was formed during this process. Differential protection of the hydroxyl groups in **20** and subsequent inversion of stereochemistry at that position which would ultimately be C-4 gave the tetraacetate derivative **21**. Exposure of **21** to methanol containing a trace of sulfuric acid gave the methyl glycoside **22**, which was then unmasked (RuO_4, $NaIO_4$) to give the protected KDN derivative **23** in good overall yield (Scheme 4).[59]

In an extension of their earlier work,[55] Dondoni and co-workers have utilized the conjugate addition of benzylamine to the mannose-derived α,β-enone **24** to give predominantly the syn-adduct **25**.[60] The adduct **25** was then converted into the 4-acetamido-4-deoxy-KDN derivative **26**.



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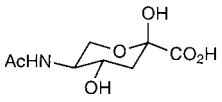
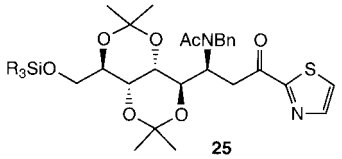


27



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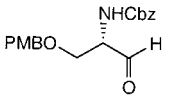

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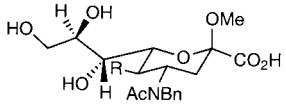
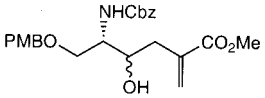


26 R = OH

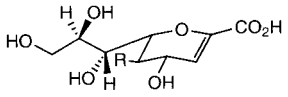


29

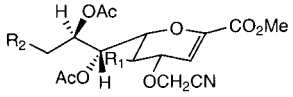
The application of the previously reported[54] indium mediated allylation strategy has been utilized in the synthesis of the six-carbon sialic acid analogue **27**.[61] A chemical approach toward **27** was instigated since the Neu5Ac aldolase catalyzed condensation between pyruvate and the requisite *N*-acetylserine derivative was unlikely to proceed, as aldehydes with fewer than five carbons are generally poor substrates for the enzyme.[35,62] Consequently, exposure of the serine derived aldehyde **28** to methyl 2-(bromomethyl)-acrylate in the presence of indium led to a mixture of adducts **29**. Both diastereomers **29** could be individually converted to the desired glycerol side chain truncated sialic acid analogue **27**.

Aside from the examples mentioned above, generally approaches toward structurally modified Neu5Ac derivatives start with Neu5Ac itself. An excellent article by Zbiral[22] in 1992 provided a detailed overview of the fundamentals of sialic acid chemistry. Since that time, many articles have appeared, with the trend more recently toward articles dealing with structurally modified sialic acid derivatives as probes for specific sialic acid-recognizing proteins (SARPs). For example, the development of the anti-influenza drug Relenza has spawned numerous articles relating to the structural modification of 2-deoxy-2,3-didehydro-sialic acids.[63,64] 2-Deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en, **30**) is considered as a transition-state analogue of the probable intermediate in the sialidase catalyzed cleavage of sialic acid residues from glycoconjugates.[63] This feature of Neu5Ac2en will be discussed further in section IV. As such, Neu5Ac2en (**30**) is an excellent starting point for the development of derivatives with improved inhibitory activity.

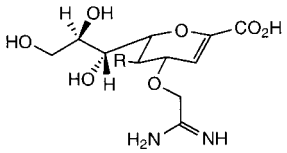
Much of the work surrounding the development of Neu5Ac2en-based inhibitors of sialidases starts with Neu5Ac (**1**) itself and involves introduction of the unsaturation as well as functional group modification. Several articles, including two recent reviews,[63,64] describe much of the previous work toward such compounds which will therefore not be readdressed here. In a recent example of the structural modifications possible within the Neu5Ac2en framework, a series of C-4 and C-9 modified Neu5Ac2en derivatives have been synthesized and evaluated for their inhibition of sialidases.[65] The C-4 cyanomethyl Neu5Ac2en derivatives **31** and **32** were each prepared in a few steps from readily accessible Neu5Ac derivatives. In each instance, the introduction of the



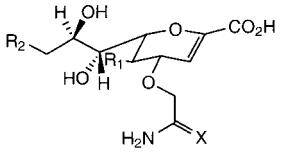
30 R = NHAc



31 R_1 = NHAc; R_2 = OAc
32 R_1 = NHAc; R_2 = N_3



33 R = NHAc

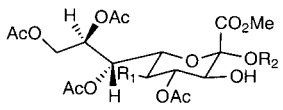


34 R_1 = NHAc; R_2 = N_3; X = O
35 R_1 = R_2 = NHAc; X = S

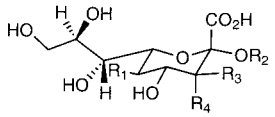
structural modification was carried out on a Neu5Ac derivative, with the 2,3-unsaturation being introduced after initial functionalization.[65] The 2,3-unsaturated derivatives **31** and **32** were then transformed into a series of C-4 substituted (e.g., **33**) or C-4,C-9-disubstituted-Neu5Ac2en derivatives (e.g., **34**) and evaluated for their inhibition of viral (influenza virus) and mammalian (pig liver membrane) sialidases. Although none of the derivatives reported exhibited improved inhibition when compared to Neu5Ac2en itself, compound **35** showed selective inhibition for the viral sialidase while compound **34** was selective for the mammalian sialidase.[65]

The drive to develop specific compounds that would inhibit both the influenza virus adhesion protein haemagglutinin and the sialidase responsible for release of virion progeny[66] led to the synthesis of C-3 modified sialylglycosides.[67] Commencing with Neu5Ac2en, C-3 functionality was introduced by transforming peracetylated Neu5Ac2en into the C-3 hydroxylated derivative **36**, via the corresponding bromohydrin, using known[68] chemistry. Subsequent elaboration of **36** resulted in the synthesis of C-3 modified sialosylglycosides of the general structure **37**. Interestingly, it was found that the C-3 hydroxylated Neu5Ac glycosides (**37**, R_3 = OH, R_4 = H) were more resistant to either acid or sialidase catalyzed

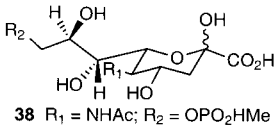
hydrolysis than the parent (3-deoxy) Neu5Ac glycosides.[67] Unfortunately, none of the compounds prepared showed significant improvement in inhibition of either haemagglutinin or sialidase from influenza, although the C-3-*ax*-derivatives (**37**, R_4 = OH or F, R_3 = H) did exhibit specific inhibition of a bacterial sialidase.[67]



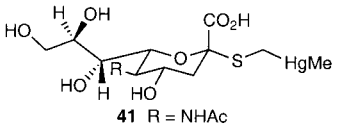
36 R_1 = NHAc; R_2 = 4-NO_2-C_6H_4 or 4-MU



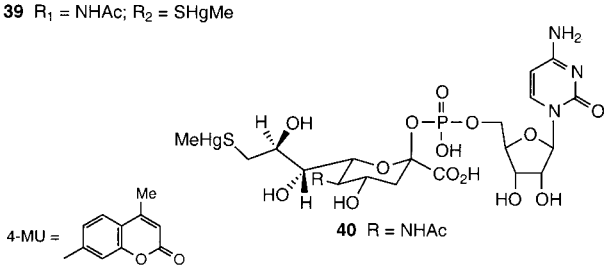
37 R_1 = NHAc; R_2 = 4-NO_2-C_6H_4 or 4-MU



38 R_1 = NHAc; R_2 = OPO_2HMe
39 R_1 = NHAc; R_2 = SHgMe



41 R = NHAc



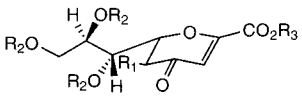
40 R = NHAc

The C-9-phosphono substituted Neu5Ac derivative **38** and the C-9-thiomethyl-mercury-Neu5Ac derivative **39** have both been prepared as possible substrates for enzymatic incorporation into complex sialosylglycosides.[69] Unfortunately, the phosphono derivative **38** was not accepted as a substrate by CMP-Neu5Ac synthase and was also observed to not inhibit the synthase, indicating that it is not recognized by this enzyme.[69] Significantly, the C-9-thiomercury derivative **39** was converted to the desired CMP derivative **40** by reaction with CTP and CMP-Neu5Ac synthase. Compound **39** was also enzymatically transferred into several complex sialosylglycosides, including a C-9-thiomercury-Neu5Ac containing sialyl Lewis x analogue for future use in X-ray crystallographic studies with the selectins.[69] The successful incorporation of mercury into Neu5Ac derivatives has also been reported by us and involved the generation of the thiomercury containing glycoside **41** of Neu5Ac.[70] The value of these types of heavy atom containing Neu5Ac analogues in X-ray crystallographic studies with sialic acid-recognizing proteins remains to be determined.

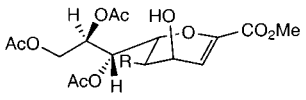
Attempts to synthesize 4-oxo-Neu5Ac2en (**42**) for crystallographic studies with Neu5Ac aldolase found that oxidation of the 4-epi-Neu5Ac2en derivative **43** with chromium-based oxidants gave the expected protected 4-oxo-Neu5Ac2en derivative **44**, but only in modest chemical yield.[71] Interestingly, oxidation of **43** with ruthenium-based oxidants and a large excess of *N*-methylmorpholine *N*-oxide resulted in the exclusive formation of the γ-pyrone derivative **45**.

III. Glycosidations of Sialic Acids

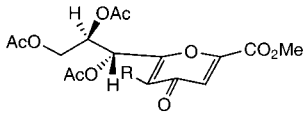
Glycosidations of sialic acids can be conveniently divided into two broad groups, viz: *O*-glycosidations



42 R_1 = NHAc; R_2 = R_3 = H
44 R_1 = NHAc; R_2 = Ac; R_3 = Me



43 R = NHAc

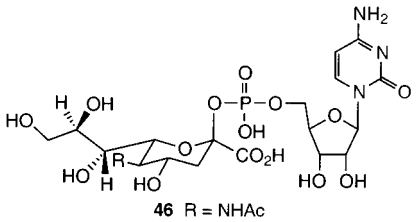


45 R = NHAc

and the formation of sialylglycosides with a linker other than oxygen, namely sulfur, carbon, or nitrogen. The latter examples are typically employed to impart some form of metabolic stability to the resulting sialoside, while sialyl-*O*-glycosides are commonly utilized in studies aimed at understanding the recognition and functional characteristics of sialic acid-recognizing proteins.

A. *O*-Glycosidations of Sialic Acids

The formation of glycosides of sialic acids (sialosides) is one of the biggest challenges in sialic acid chemistry. Aside from CMP-Neu5Ac (**46**), the activated form of Neu5Ac employed by sialyltransferases in the biological construction of complex sialylglycoconjugates, naturally occurring sialic acids are α-ketosidically linked.[1,5,7] The issue of selectively forming

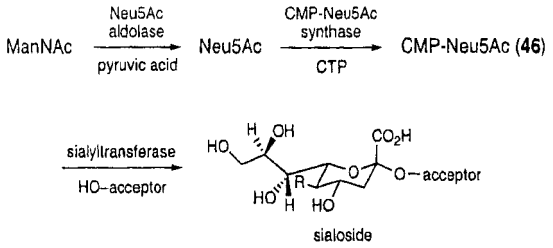


46 R = NHAc

α-linked sialosides by chemical methods is far more complex than glycosidations on simple hexoses, due to the presence of the carboxylate group attached to the anomeric carbon in sialic acids and to the lack of functionality at C-3. The lack of a C-3 substituent removes the ability to use neighboring group participation to direct the stereochemical outcome of the glycoside forming step, while the electron-withdrawing carboxylate group at C-2 makes sialic acid derivatives prone to 2,3-elimination during the glycosidation step. In addition, the anomeric carbon in sialic acids is sterically hindered, resulting in further difficulties with attempts at forming sialosides. These factors have combined to result in the development of some rather unusual anomeric activating groups for the formation of *O*-glycosides of sialic acids.

A recent excellent comprehensive article on *O*-sialylation,[28] together with earlier articles,[22,23,72−75] means that the treatment of the issue here will only be brief. *O*-Sialylation can be divided into either enzymatic or direct chemical methods. Enzymatic approaches toward the formation of *O*-sialosides is typically undertaken because of the need for increased efficiency and the elimination of the need of appropriate protecting group strategies. Enzymatic approaches typically employ sialyltransferases for

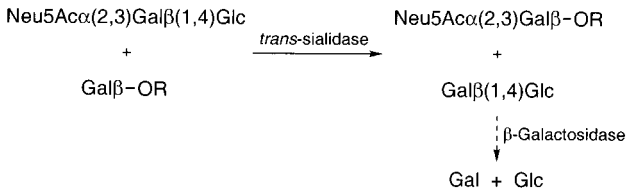
Scheme 5



constructing the glycosidic linkage.[28] CMP-Neu5Ac (**46**) is usually employed as the sialyl donor in these reactions, and the formation of either α(2,6)- or α-(2,3)-linked sialosides is dictated by the choice of sialyltransferase. Importantly in these approaches, CMP-Neu5Ac (**46**) itself can either be obtained chemically or enzymatically by the use of Neu5Ac with CTP and CMP-Neu5Ac synthase.[28,76−78] In addition, Neu5Ac itself can be prepared from the Neu5Ac aldolase catalyzed transformation of ManNAc, resulting in a completely enzymatic synthesis of complex sialosides starting from ManNAc (Scheme 5).[28] The acceptors used in these types of reactions can be structurally modified, with careful choice of sialyltransferase being the key to success given that they have different substrate specificities.[28,79−81] The sialyltransferase catalyzed synthesis of oligosaccharides containing structurally modified Neu5Ac residues can be achieved by utilizing CMP derivatives with functionalized Neu5Ac residues.[82,83] A recent study shows that the α(2,6)-sialyltransferase from rat liver does not tolerate changing the base in CMP-Neu5Ac (**46**) but will readily accept structural modifications at C-5 (e.g., NH_3^+), C-8 (OMe), and C-9 (OPO_3H) of the Neu5Ac residue.[84]

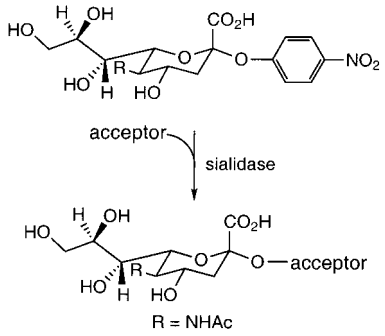
The formation of *O*-sialosides using a *trans*-sialidase has been reported. *trans*-Sialidase enzymes are unique in that they transfer sialic acid residues from one sialoside to another, and therefore they do not require CMP-Neu5Ac (**46**) as the source of sialic acid.[85,86] The specificity of the *trans*-sialidase from *Trypanosoma cruzi* results in the transfer of Neu5Acα(2,3)-Galβ*OR′* to virtually any galactoside acceptor of the structure Galβ*OR″*.[28,85−87] The addition of a β-galactosidase to a *trans*-sialidase catalyzed sialylation reaction using α(2,3)-sialyllactose as the source of Neu5Ac results in improved yields (up to 95%) of sialyl-trisaccharides due to the hydrolysis of the lactose byproduct (Scheme 6).[88]

Scheme 6



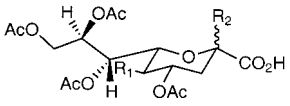
Sialidases (sialic acid glycohydrolases) can also be utilized in the formation of sialosides (Scheme 7).[28,89,90] The nature of the linkage formed in these *trans*-sialylations, either α(2,3) or α(2,6), is controlled by the choice of sialidase. For example, *Vibrio cholerae* and *Clostridium perfringens* sialidases preferentially

Scheme 7



form sialyl-α(2,6)-glycosides, while the sialidases from *Salmonella typhimurium* and Newcastle disease virus form α(2,3)-linked sialosides.[28,90]

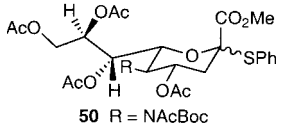
For chemical synthesis of *O*-sialosides, the choice of activating group at C-2 is crucial.[28] Typically, sialosyl phosphites (e.g., **47**),[28,91,92] sialosyl xanthates (e.g., **48**),[28,93,94] and C-2 thio derivatives (e.g., **49**)[28,95,96] are utilized in the chemical glycosidation of sialic acids.[22,27,28] Each of these activated sialosyl donors has their own particular merits in *O*-sialoside formation, and the key in each instance is the choice of reagent for the promotion of the glycosidation.[28]



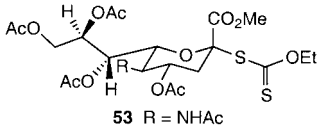
47 R_1 = NHAc; R_2 = $OP(OR')_2$
48 R_1 = NHAc; R_2 = SC(S)OEt
49 R_1 = NHAc; R_2 = Salkyl, Saryl

The comprehensive nature of an excellent recent review on the formation of *O*-sialosides[28] dictates that this article concentrate on just a few examples which are, in our opinion, important recent contributions to this important aspect of sialic acid chemistry. In an attempt to explore the effect of differing levels of protection of a lactosamine acceptor in sialylation reactions, it was found that glycosidation of the sialoside **50** (using *N*-iodosuccinimide/trifluoroacetic acid as promoter) proceeded most efficiently when the lactosamine derivative **51** was employed, to give the corresponding (2,6)-linked sialoside in high yield (79%) but as a mixture of α:β glycosides (ratio ∼1.3:1).[97] Interestingly, the use of fewer protecting groups in the acceptor (e.g., **52**) resulted in the formation of a complex mixture of at least five sialosides without any specific product being favored.[97] In the synthesis of some novel precursors for the solid-phase synthesis of sialylglycopeptides, it was found that the best sialic acid donor for coupling to amino acid derivatives was the sialosyl xanthate derivative **53**.[98] During the course of this work it was also found that alkaline deprotection of the Fmoc group in **54** resulted in the formation of the lactam **55**. This side reaction could be prevented by deprotection of the methyl ester prior to unmasking the amine group.[98]

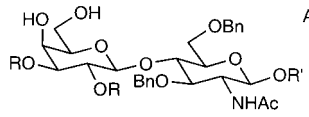
A method for the efficient direct construction of Neu5Acα(2,8)-Neu5Ac glycosides, an important constituent of tumor antigens,[19,99] remains one of the goals of *O*-sialylation. The primary difficulty in



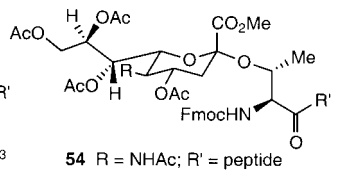
50 R = NAcBoc



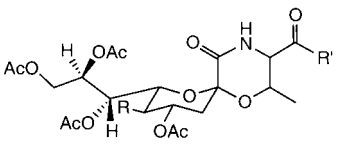
53 R = NHAc



51 R = Bz; R' = $(CH_2)_3NHC(O)CF_3$
52 R = H; R' = $(CH_2)_3NHC(O)CF_3$

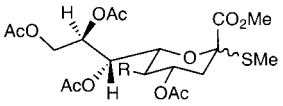


54 R = NHAc; R' = peptide

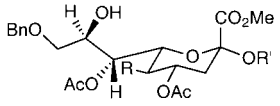


55 R = NHAc; R' = peptide

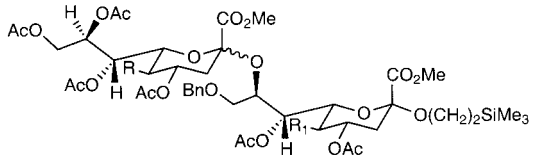
directly forming the α(2,8)-linkage is the lack of nucleophilicity of the C-8 hydroxyl group in the Neu5Ac acceptor. A comparison of the sialosyl donor properties of the Neu5Ac derivatives **56** and **57** revealed that the di-*N*-acetyl derivative **57** is a dramatically better sialosyl donor in glycosidation reactions.[100] Bearing this in mind, the same authors have recently described the synthesis of $Neu5Ac_2$α-(2,8)-linked derivatives such as **58**.[101] Interestingly,



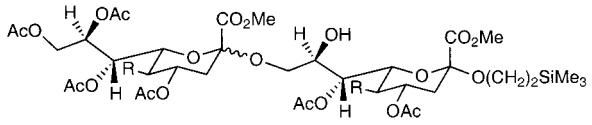
56 R = NHAc
57 R = NAc_2



59 R = NAc_2; R' = $(CH_2)_2SiMe_3$
60 R = NHAc; R' = $(CH_2)_2SiMe_3$



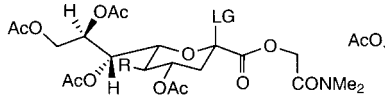
58 R = NAc_2; R_1 = NHAc or NAc_2



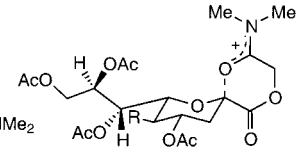
61 R = NAc_2

glycosidation between **57** and the $Neu5Ac_2$ acceptor **59** proceeded more efficiently than the same reaction carried out with the corresponding Neu5Ac acceptor **60**. Of additional note is the yield obtained (98%) for the formation of the $Neu5Ac_2$α(2,9)-$Neu5Ac_2$ disaccharide **61**. While the level of α/*β*-selectivity for these glycosidations is only moderate, this work represents an important advance in this area of difficult chemistry.[101] The use of di-*N*-acetyl sialosyl donors in the formation of α(2,3)-trisaccharides has also been reported to be more efficient than the use of the corresponding Neu5Ac-based sialosyl donor,[102] while trifluoroacetamido sialosyl donors have proved extremely useful in the construction of a range of exclusively α-linked sialosides.[103]

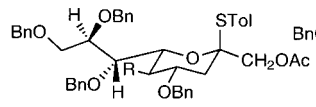
The issue of stereoselectivity of *O*-sialylations is one of the biggest hurdles in chemical glycosidations. Attempts to improve the stereochemical outcome of sialylations has resulted in the development of substituted sialosyl donors. Alteration of the electron-withdrawing effect of the carboxylate group at C-2 has given rise to some success in relation to the stereochemical outcome of *O*-sialylation reactions. In this regard, the incorporation of an *N*,*N*-dimethylglycolamide auxiliary at C-1, producing sialosyl donors such as **62**, caused a significant improvement in the formation of the desired α-ketoside.[104] It is proposed that the *N*,*N*-dimethylglycolamide auxiliary stabilizes the formation of the C-2 oxocarbenium intermediate **63** through neighboring group participation. Importantly, the *N*,*N*-dimethylglycolamide



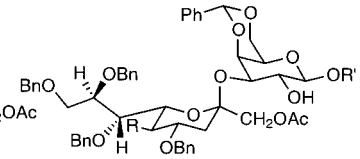
62 R = NHAc



63 R = NHAc



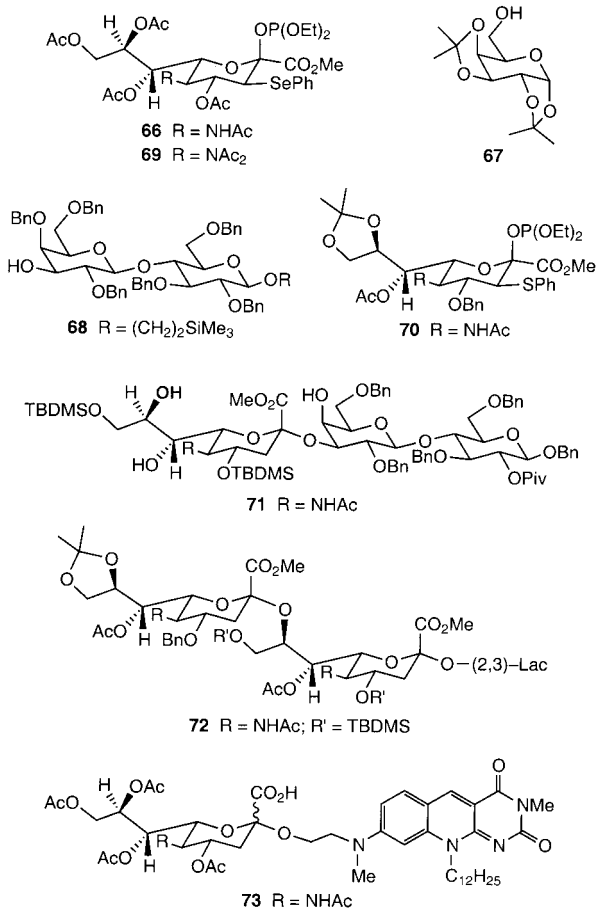
64 R = NHAc



65 R = NHAc; R' = $(CH_2)_5CO_2Me$

auxiliary can be readily introduced by reaction between 2-methanesulfonyloxy-*N*,*N*-dimethylacetamide and the cesium salt of peracetylated Neu5Ac.[104] It has been found that reduction of the carboxyl group of Neu5Ac to a hydroxymethyl group results in compounds, e.g., **64**, which are up to 1000 times more reactive than the corresponding methyl ester sialosyl donors in glycosidation reactions.[105] Interestingly, sialylation between **64** and a galactoside acceptor (DMTST as promoter) resulted in an excellent 95% yield of predominantly the *β*-linked Neu5Ac(2,3)-linked galactoside **65**.[105] This preference for *β*-glycoside formation (*β*:α ratio 15:1) was unexpected. The hydroxymethyl group in the disaccharide **65** can be readily selectively unmasked and oxidized to the requisite carboxylate group.

The use of directing auxiliaries at C-3 of sialosyl donors has been widely reported.[28] While this adds steps to the synthetic sequence, equatorial C-3 substituents assist with the formation of α-sialosides and can also act to reduce 2,3-eliminations, a major side reaction of chemical *O*-sialylation reactions. Ease of installation and subsequent removal are also important considerations with the use of directing auxiliaries at C-3. In this regard, C-3 auxiliaries are usually installed by chemical modification of Neu5Ac2en derivatives.[27,28] In a recent example, the 3-SePh-sialosyl phosphite **66** was utilized in glycosidation reactions with the galactose-based acceptor **67**.[106] The advantage of a selenium-based auxiliary is that it is readily removed under radical conditions (Ph_3SnH, AIBN) in high yield. Unfortunately, the sialosyl donor **66** was found to be inert in attempted

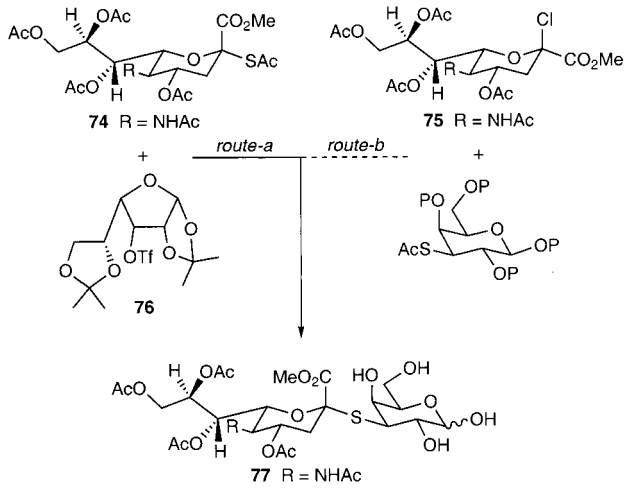


glycosidations with less reactive acceptors such as **68**, presumably due to increased steric crowding about the anomeric center and the weak C−Se bond.[106] Interestingly, use of the C-5 di-*N*-acetyl sialosyl donor **69** failed to show any improved reactivity compared to **66**,[92] unlike those examples cited above. In contrast, the use of a C-3 phenylthio group has resulted in the efficient synthesis of ganglioside GD3.[107] In this instance, the C-3 substituted sialosyl phosphite **70** was found to react with the hindered acceptor **71** (TMSOTf as promoter) to give the α(2,8)-linked tetrasaccharide **72** in 54% yield.[107]

Several reports have appeared describing the incorporation of specific fluorescent labels into sialosides to aid with studies into glycobiological interactions involving various sialic acid-recognizing proteins. The use of biotin is significant because of its excellent affinity for streptavidin and the ease of immobilization onto matrixes. Several biotinylated oligosaccharides have been reported, including biotin linked to Neu5Ac via a UV active spacer.[108] Fluorescence-labeled sialyl Lewis x glycosphingolipids have been prepared to investigate microdomain formation in membranes.[109] The use of fluorescence labeling for diagnostic purposes has been demonstrated with 5-deazaflavin glycosides of Neu5Ac such as **73**, which possess significant antitumor activity against murine leukemia L1210 cells and human oral epidermoid carcinoma KB cells.[110]

It is also worth noting the development of multivalent sialosides as biological probes and potential inhibitors of sialic acid-recognizing proteins. Recently

Scheme 8



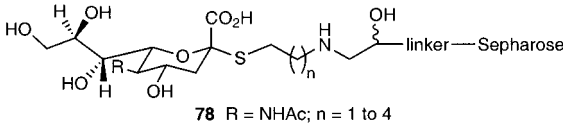
a highly convergent synthesis of a dendrimerized Chitosan-Neu5Ac hybrid has been described.[111] This report follows earlier work by the same group including the preparation of multivalent sialyl Lewis x ligands[112] and hyperbranched sialodendrimers as inhibitors of influenza virus haemagglutinin.[113]

B. Synthesis of Thiosialosides

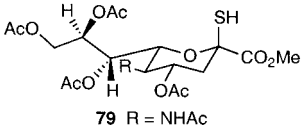
Thioglycosides of sialic acids are not only of interest as glycosyl donors, as shown above, but also as valuable biological probes and potential inhibitors of sialic acid-recognizing proteins. Their value as biological probes stems from their resistance to enzymatic degradation,[114] a fact recently unequivocally shown by NMR studies with thiosialosides in the presence of *Vibrio cholerae* sialidase.[115] Several earlier articles have described in detail approaches toward the synthesis of thiosialosides.[27,75,116−122] The generally adopted method for the synthesis of thiosialosides involves either (a) the selective thiodeacetylation of the anomeric thioacetyl group in **74** and subsequent reaction with an activated sialosyl acceptor[116−120] or (b) the incorporation of the sulfur into the sialosyl acceptor and then coupling with the 2-chloro Neu5Ac derivative **75**.[121,122] The phase transfer catalyzed glycosylation of the 2-chloro-Neu5Ac derivative **75** has also resulted in the formation of thio- and seleno-sialosides.[123] The use of the thioacetyl-Neu5Ac derivative **74** has been successfully employed in the synthesis of a range of simple and complex thiosialosides, while the approach using a thiolated acceptor has proved particularly useful in the synthesis of thiosialosides with a Neu5Acα(2,3)-Gal linkage.[121,122] Thiosialosides with Neu5Acα(2,3)-Gal linkages have attracted considerable attention due to the common occurrence of Neu5Ac-*O*-α(2,3)-Gal linkages in natural oligosaccharides and the consequential desire to prepare thiosialosides as useful biological probes for enzymes that recognize this linkage. Of the two practical approaches toward the synthesis of thiosialosides with Neu5Acα(2,3)-Gal linkages (Scheme 8), that approach which utilizes an activated gulose derivative as the sialosyl acceptor (*route a*) has recently been shown to provide the desired product in excellent yield. Thus, exposure of a mixture of the thioacetyl-Neu5Ac derivative **74** and

the gulose configured triflate **76** to Et_2NH in *N,N*-DMF resulted in the smooth formation of the α(2,3)-linked thiosialoside **77** after partial deprotection.[124]

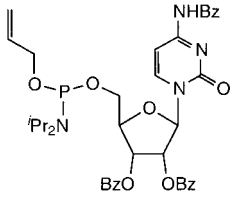
The value of thiosialosides as metabolically stable biological probes has been utilized in the development of novel affinity chromatography matrixes specifically designed for the purification of sialic acid-recognizing proteins.[125,126] In this work, the 2-thioacetyl-Neu5Ac derivative **74** was directly attached to epoxy-activated Sepharose 6B.[125] The resulting matrix has proved valuable in the one-step purification of various sialidases. Similarly, the attachment through the terminal amine unit of a series of thiosialosides with varied aglycon chain lengths (e.g., **78**) to CNBr-activated Sepharose 4B resulted in affinity matrixes suitable for purifying sialidases and sialyltransferases.[126]



78 R = NHAc; n = 1 to 4



79 R = NHAc



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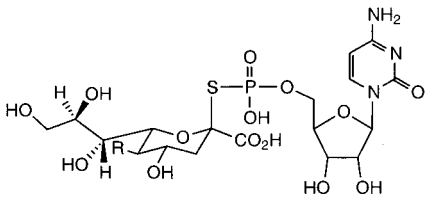
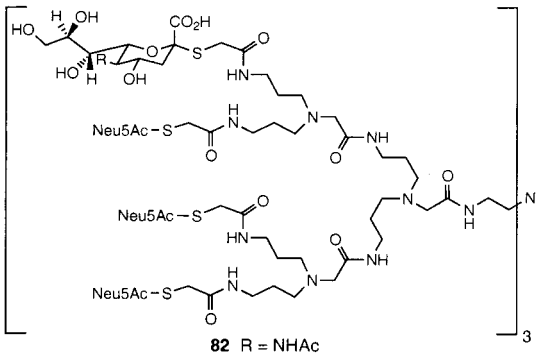


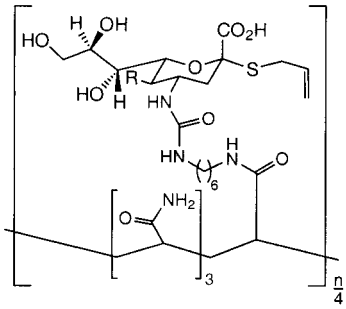
81 R = NHAc

In an important example of the value of thiosialosides as stable biological probes, tetrazole mediated coupling between the 2-thio-Neu5Ac derivative **79** and the cytidine-5′-phosphoramidite **80** gave the thiosialoside analogue **81** of CMP-Neu5Ac after deprotection.[127] In comparison to CMP-Neu5Ac itself, the thioglycoside analogue **81** was found to be hydrolyzed significantly slower (50-fold) in aqueous solution. In studies with the α(2,3)-sialyltransferase from rat liver, it was observed that **81** was a substrate for the enzyme. Not surprisingly however, the thiosialoside **81** was found to be significantly less reactive than the natural substrate CMP-Neu5Ac in the sialyltransferase reaction, although its affinity for the enzyme was only 3-fold lower.[127]

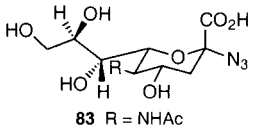
Several examples of multivalent thiosialosides have been reported. Multivalent sialosides are valuable tools as biological probes or potential inhibitors of sialic acid-recognizing proteins, primarily due to the poor affinity (typically millimolar) of monomeric sialosides toward sialic acid-recognizing proteins. A particular example of the value of multivalent sialosides is in the inhibition of influenza virus haemagglutinin, where the haemagglutinin is believed to bind to multiple sialic acid residues on the surface of cells.[128] It has been clearly demonstrated[129,130] that multivalent sialosides such as sialopolymers have increased inhibitory capacity toward influenza virus haemagglutinin when compared to their respective monomeric counterparts. This amplification of carbohydrate−protein binding interactions has prompted considerable interest in the development of novel multivalent sialosides.[131] As way of example, the α-linked multivalent sialoside **82** showed a 182-fold increase in inhibitory activity over the monomeric sialoside **83** used as a standard in the assays.[132] The



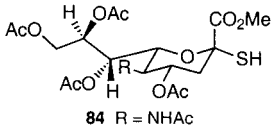
82 R = NHAc



85 R = NHAc



83 R = NHAc

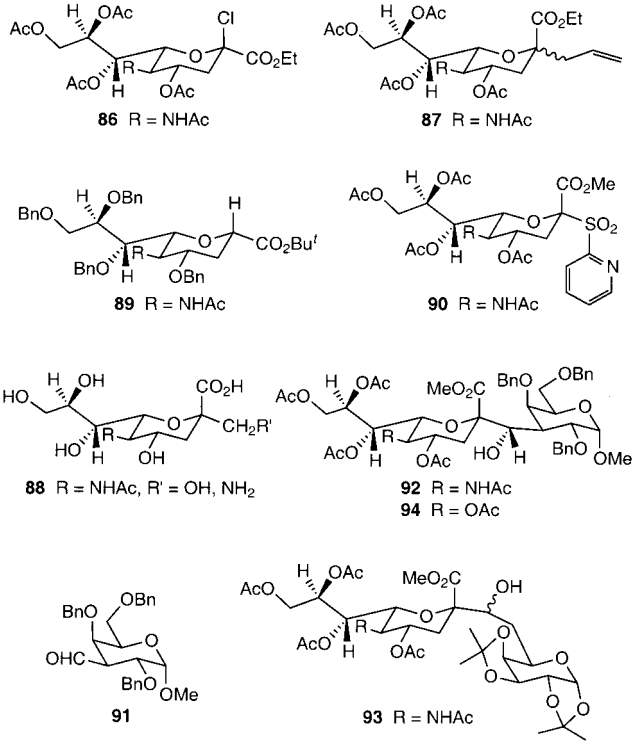


84 R = NHAc

multivalent sialoside **82** was constructed in high yield by reacting the 2-thio-Neu5Ac derivative **84** with the corresponding *N*-chloroacetylated dendrimer in the presence of Et_3N. More recent examples of multivalent thiosialosides include the polymeric 4-*N*-linked thiosialoside **85**, which was shown to inhibit the agglutination of influenza virus to red blood cells with an inhibition constant around 10^{-6} M.[133] Heptavalent thiosialosides of β-cyclodextrins have been prepared in high yield by the Et_2NH mediated coupling between the 2-thioacetyl-Neu5Ac derivative **74** and *N*-chloroacetamido functionalized β-cyclodextrin derivatives with different spacer arms.[134] Such multivalent derivatives exhibit excellent affinity or inhibition of sialic acid-recognizing proteins in vitro.

C. Other Glycosides of Sialic Acids

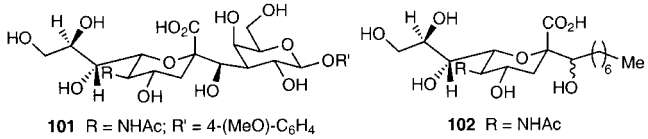
As with thiosialosides, the primary interest in sialyl-*C*-glycosides stems from their metabolic stability. Several articles describe the general principles behind the synthesis of *C*-glycosides,[135−137] and therefore this account will focus primarily on the challenges of introducing a *C*-glycosidic linkage in sialic acids. In one of the first syntheses of a *C*-glycoside of Neu5Ac, the 2-chloro-Neu5Ac derivative **86** was treated with allyl tributyltin in the presence of hexabutylditin to yield a 1:1 anomeric mixture of the *C*-allyl-Neu5Ac derivative **87**.[138] At a similar time,



C-glycosides of Neu5Ac (e.g., **88**) were prepared by hydroxymethylation of the 2-deoxy-Neu5Ac derivative **89**.[139] Since these early reports, several significant advances have been made in this area of sialic acid chemistry, most notably the generation of sialyl-*C*-disaccharides under stereocontrolled conditions. In this regard, treatment of a mixture of the sulfone **90** and the aldehyde **91** under SmI_2 promoted radical conditions resulted in the efficient formation of exclusively the α-linked sialyl-*C*-disaccharide **92**.[140] This same group has also demonstrated that treatment of the 2-chloro-Neu5Ac derivative **86** under similar SmI_2 promoted radical conditions results in the smooth formation of α(2,6)-*C*-linked sialosides such as **93**.[141] The first synthesis of the α(2,3)-linked KDN-*C*-glycoside **94**, as well as an α(2,6)-linked analogue, has also been reported using these conditions.[142] As with the Neu5Ac example cited above, the products in both cases were formed exclusively as the α-glycosides. The Neu5Ac-*C*-glycosidic analogues of gangliosides GM3 and GM4 have also been reported using the SmI_2 promoted coupling of a 2-sulfone-Neu5Ac derivative with 3-formyl-galactosides.[143]

A different approach toward *C*-glycosides of Neu5Ac involves the electrophilic cyclization of the pivotal intermediate **95**, itself constructed by coupling between the lithium anion derived from **96** and the eight-carbon electrophile **97** (Scheme 9).[144] Treatment of **95** with phenylselenyltriflate at low temperature resulted exclusively in a 6-*exo*-trig cyclization to give predominantly the *C*-glycoside **98** after in situ acetylation. Installation of the carboxylate functionality was achieved via the aldehyde **99**, and subsequent introduction of the C-5-acetamido group and deprotection afforded the Neu5Ac-*C*-disaccharide **100**.[144] Importantly, this novel approach allows for the synthesis of KDN-*C*-glycosides and is flexible enough to facilitate the construction of a variety of C-5 functionalized sialyl-*C*-glycosides.

A series of Neu5Ac-*C*-glycosides have been evaluated as inhibitors of the bacterial sialidase from *C. perfringens*.[145] Interestingly, the Neu5Acα(2,3)-*C*-galactoside derivative **101**, an analogue of the natural substrate of the enzyme, exhibited only moderate inhibition ($K_i \sim 10^{-4}$ M) of this sialidase. However, the simple aliphatic *C*-glycoside **102** showed inhibition ($K_i \sim 10^{-6}$ M) of *C. perfringens* sialidase comparable to the transition-state analogue Neu5Ac2en (**30**).[145] Further investigations with these types of

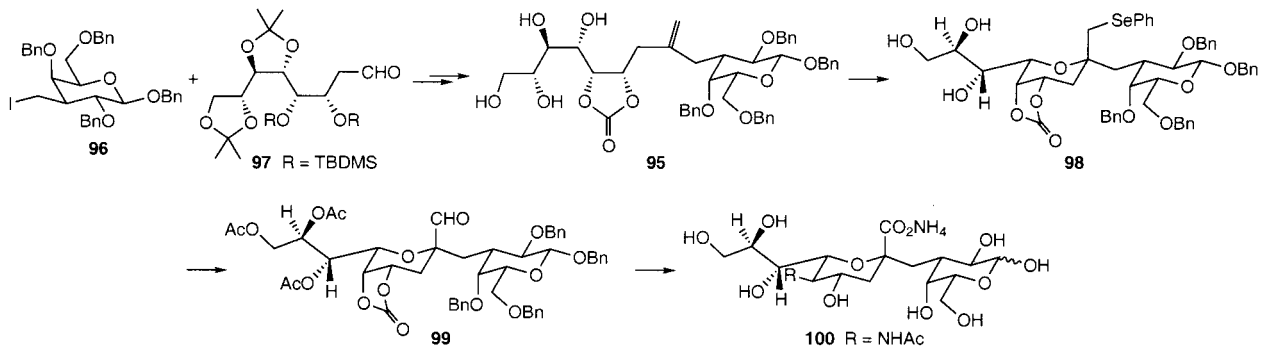


Neu5Ac derivatives will undoubtedly shed more light on these interesting results. Multivalent Neu5Ac-*C*-glycosides have also been shown to inhibit influenza virus haemagglutinin with micromolar inhibition constants.[146] The reported acrylamide copolymers containing *C*-linked Neu5Ac exhibited levels of inhibition comparable to the corresponding *O*-linked copolymers but, importantly, are resistant to the action of any sialidase present.

IV. Sialylmimetics as Biological Probes

The importance of sialic acid containing cell-surface glycoconjugates in several diseases and various biological processes is well documented.[4−7] Central to much of the research aimed at improving our understanding of the precise roles the terminal sialic acid residues play in these important natural phenomena is the development of new methods for the construction of useful biological probes based upon the sia-

Scheme 9



lylglycoconjugates involved in these processes. In the sections above we have presented an overview of the considerable efforts associated with the preparation and analysis of variously modified sialic acid derivatives. Such work may have been intended to identify sialic acid analogues as inhibitors of a particular biological process mediated by sialic acid-recognizing proteins or to develop novel molecules to better understand the structure−activity relationships of such proteins. Unfortunately, and hopefully demonstrated in the previous sections, manipulations involving sialic acid are complicated by the complexity of the chemistry involved.[22,27,28] Over recent years there has been a trend toward the development of glycomimetics, especially with regard to investigating carbohydrate mediated recognition events in biological systems. Glycomimetics can be considered as molecules that contain only those structural features essential for interaction with a given biomolecule. Glycomimetics, particularly those of oligosaccharides, have a number of potential advantages over their parent structures, especially if they are intended to be therapeutic agents. They can be designed such that they are more stable to endogenous degradative enzymes, have increased affinity for the biomolecule under investigation, and can have improved bioavailability and pharmacokinetic profiles. A recent review by Sears and Wong,[17] together with other excellent articles,[147,148] are essential reading for anyone interested in entering this exciting and developing area of glycoscience.

For the purposes of this article, sialylmimetics will be classified into two broad groups. The first section will deal with compounds where the entire sialic acid portion of a molecule of interest has been replaced by only those functional groups involved in important interactions. Such sialylmimetics often bear little or no gross structural resemblance to sialic acid itself. The second section of sialylmimetics will consider analogues of sialic acids, most notably Neu5Ac2en-based, wherein a degree of overall structural similarity to the parent compound remains in the final mimetic. As will become apparent throughout this discussion, the chemistry associated with the synthesis of these sialylmimetics is often much simpler than that required for the parent sialic acid derivative upon which a given mimetic is based.

A. Synthesis of Sialylmimetics

A considerable amount of effort has been devoted to the development of sialylmimetics as inhibitors or biological probes of the selectins. The selectins are a family of cell-surface glycoproteins responsible for the early adhesion events in the recruitment of leukocytes to sites of inflammation.[17,18,149−151] As part of a multistep process, selectins promote the initial tethering and rolling of leukocytes, eventually leading to the migration of leukocytes into the surrounding endothelial tissue. Inappropriate or over-recruitment of leukocytes can have damaging effects, including acute inflammatory diseases such as stroke and reperfusion injury during surgery, and chronic inflammatory diseases such as rheumatoid arthritis and asthma.[17,18] Three selectins (E-, P-, and L-) have been identified, with the letter prefix indicating which type of cell (endothelial, platelet, and leukocyte) they are commonly located upon. E- and P-selectin recognize the cell-surface sialyloligosaccharide sialyl Lewis x (sLe^x), while L-selectin recognizes sulfated oligosaccharides such as heparin and sulfated sLe^x.[17,18,148−158] Figure 1 summarizes the important interactions between sLe^x (or its sulfated derivative) and the selectins based, in part, upon the NMR structure of sLe^x bound to E- and P-selectins,[159] crystallographic data,[160] and structure−activity studies.[17] As can be seen, only five hydroxyl groups and the carboxylate group of Neu5Ac are considered to be involved in important interactions with the selectins.[17,152] The limited number of functional groups considered to be important for interactions with selectins has resulted in the development of some excellent examples of smaller and simpler molecules which have affinity for the selectins comparable to sLe^x itself.[17,30,152,161,162] Given the comprehensive nature of some of these previous articles,[17,30,152] this section will focus primarily on those mimetics of sLe^x that show high affinity for the selectins, as well as some of the more recent articles. We will also attempt to cover the majority of the different structural variants that have emerged as useful mimetics of sLe^x.

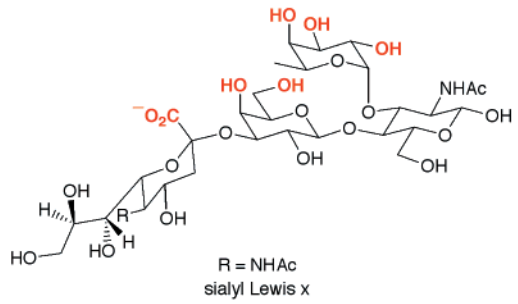
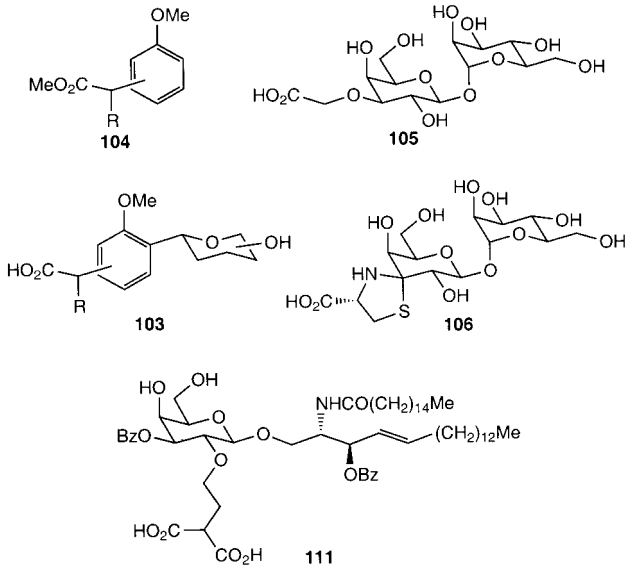


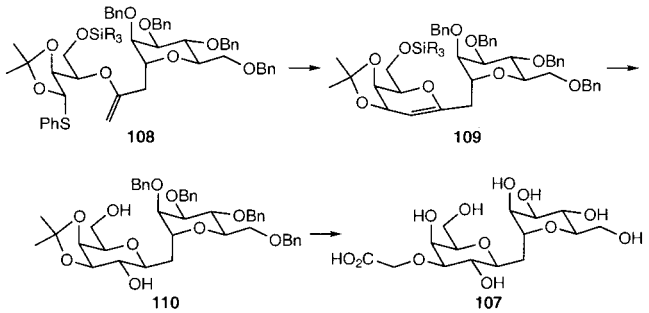
Figure 1. Sialyl Lewis x. Atoms in red are those involved in important interactions with the selectins.

Bearing in mind the structural complexity of sialyl Lewis x (see Figure 1) some of the reported mimetics are comparatively simple molecules. A series of aryl *C*-glycosides of the general structure **103** have been found to be potent inhibitors of L- and P-selectins, with IC_{50} values in the low micromolar range.[163] Compounds of the general structure **103** were synthesized via tin(IV) chloride promoted aryl *C*-glycosidation on **104**, followed by deprotection. As can be clearly seen in **103**, the entire sialic acid portion is replaced by a carboxylate group. This is a common theme among many of the sialylmimetics reported to date and is based upon structure−activity relationships which show that only the carboxylate group of the sialic acid unit in sLe^x is recognized by the selectins.[17,152] The importance of the relative positions of various hydroxyl groups in mimetics such as **103** was studied by altering the carbohydrate bound to the aromatic ring. From the several analogues prepared it was determined that compounds such as **103** with L-fucose attached provided the most potent compounds.[163] These simple sLe^x mimetics are some of the more potent inhibitors of the selectins reported. An earlier report describing the excellent binding affinity (for E-selectin) of the Gal-(1,1)-Man-based



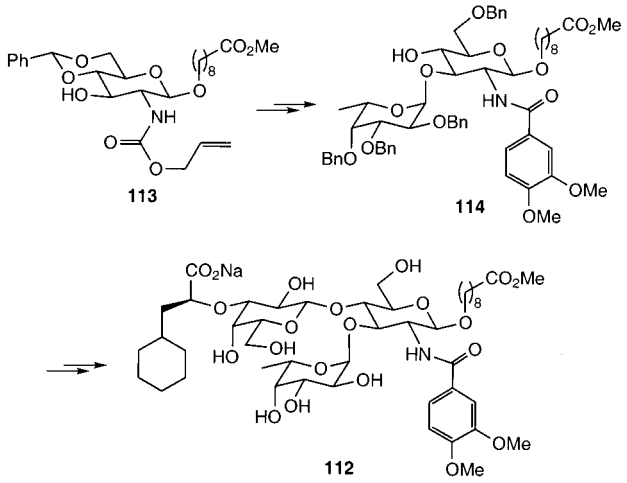
sLe^x mimetic **105**[164] has resulted in the development of more rigid sLe^x mimetics (e.g., **106**) with improved potency toward P-selectin.[165] The fixed orientation of the carboxylate group in **106** was derived from D-cysteine, with the alternative isomer derived from L-cysteine. Although **106** exhibits higher inhibition of P-selectin than its isomer, it is speculated that the increase in potency of **106** compared to **105** is due to the increased hydrophobicity of **106**.[165] The excellent affinity of compounds such as **105** toward the selectins has led to the preparation of the *C*-glycoside analogue **107**.[166] A key feature in the synthesis of the mimetic **107** centered on the cyclization of the enol ether **108**, by treatment with methyl triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine, to give the glycal **109** in high yield as a single stereoisomer (Scheme 10). Hydroboration of **109** and subsequent hydrolysis gave the *C*-glycoside **110** which, after deisopropylidenation and dibutyltin oxide mediated selective alkylation, afforded the mimetic **107** following deprotection.[166]

Scheme 10

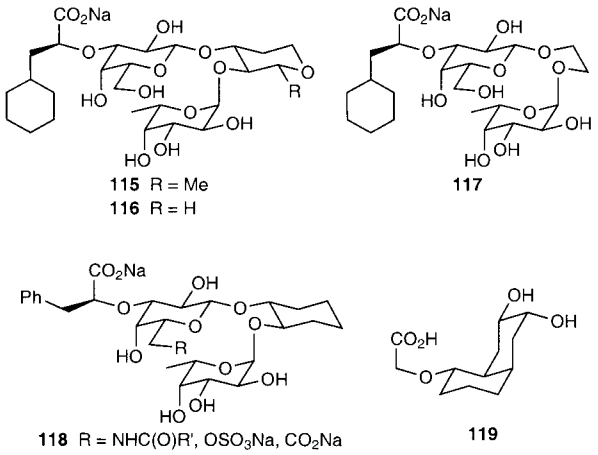


A series of malonate substituted galactosides have been prepared via C-2 alkylation and subsequent malonate introduction on a suitably protected galactoside.[167] Of several C-2 and C-3 malonate substituted compounds prepared, the C-2 substituted galactocerebrosides with a benzoyl group at C-3 (e.g., **111**) were generally the most potent inhibitors of P-selectin.[167] Interestingly, it was found that the anomeric configuration of the galactosides appeared to have little effect on the overall potency, although in vivo studies suggest that α-configured compounds are preferred. A combination of earlier studies with glucosamine modifications of sialyl Lewis x[168] and replacement of sialic acid with simple cyclohexyllactic acid residues[169] has resulted in the development of the trisaccharide-based mimetic **112**.[170] Compound **112** was prepared from the glucosamine derivative **113** via a sequence of glycosidation (with fucose) and functional group conversions to the dimethoxybenzamide derivative **114** (Scheme 11). Subsequent

Scheme 11



glycosidation of **114** (with galactose), dibutyltin oxide alkylation, and deprotection led to the desired mimetic **112**.[170] The sLe^x mimetic **112** was found to be a potent (IC_{50} = 10 μM) E-selectin inhibitor. These same workers have also recently reported the synthesis and E-selectin inhibitory activities of disaccharide-based sLe^x mimetics with either rigid (e.g., **115** and **116**) or flexible (e.g., **117**) spacers between the two sugar units.[171] Of these three compounds, compound **115** showed the most potent E-selectin

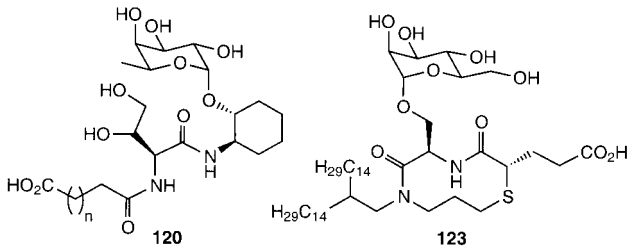


activity, while the flexible sLe^x mimetic **117** exhibited no E-selectin inhibition, even at high concentration. The modification of the C-6 position in the galactose ring of sLex mimetics of the general structure **118** resulted in complete elimination of E-selectin activity (IC_{50} >10 mM), indicating the importance of this group for E-selectin recognition.[172]

Perhaps the most remarkable example of the power of sialylmimetics is the recent report of the *cis*-

decalinic-based sLe^x mimetic **119**.[173] Compound **119** was designed using molecular modeling based upon the key interactions between sLe^x and E-selectin and was synthesized in nine steps from 4-benzyloxycyclohexanone. Interestingly and somewhat surprisingly based on the molecular modeling studies described, both the *cis*- and *trans*-decalinic enantiomers of **119** exhibited similar inhibition of E- and P-selectin, and in each instance the recorded IC_{50} values were comparable to sLe^x itself.[173]

Several other workers have described noncarbohydrate-based mimetics of sialyl Lewis x. Some of these include compounds which can be considered as glycopeptide-based analogues of sLe^x. Early reports by Wong and co-workers described compounds such as **120** with slightly improved binding affinity for E-selectin.[174] Later these same workers described *C*-fucopeptides (e.g., **121**)[175] and macrocyclic derivatives (e.g., **122**)[176] which all showed comparable or better affinity for E- and P-selectin than sLe^x itself. The solid-phase synthesis of the cyclic mimetic **123** of sLe^x resulted in a compound with only moderate affinity for E-selectin.[177] However, compound **123** showed potent inhibitory activity toward P- and L-selectin, indicating the potential importance of the heterocyclic backbone in **123**.[177] The potential to utilize this work in the development of a combinatorial approach toward the synthesis of a library of analogues of **123** should prove valuable in the drive to develop specific inhibitors of the different selectins. Consistent with the findings of others, the recent report on the inhibition of E-selectin by a series of C-6 substituted L-mannosides (e.g., **124**)[178] suggests that hydrophobic interactions are valuable.



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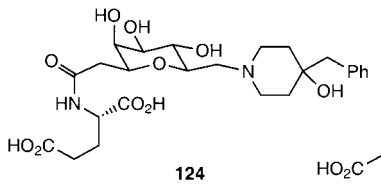

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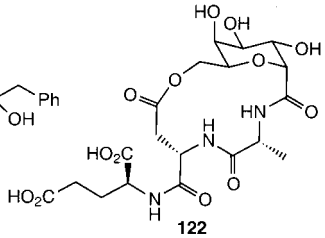

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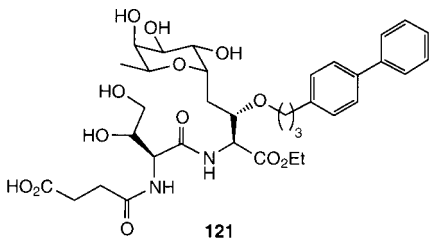
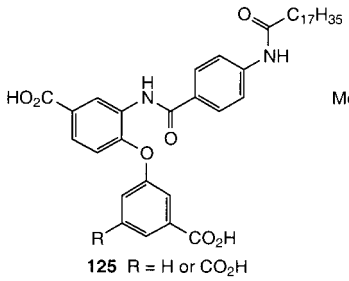
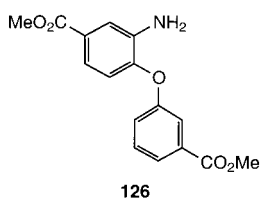


121

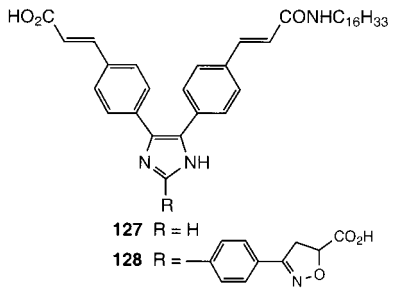
A series of bis-benzoic acid-based compounds (e.g., **125**) have been described for their inhibitory activity toward E-selectin.[179,180] These noncarbohydrate-based sLe^x mimetics were prepared by the condensation of **126** with *p*-nitrobenzoyl chloride followed by hydrogenation and introduction of the hydrophobic amide functionality.[179] The sLe^x mimetic **125** (R = H) shows micromolar affinity for E-selectin, whereas the carboxylate substituted analogue (**125**, $R = CO_2H$) was found to be a poor inhibitor.[179] In a detailed study into the structure−activity relationships of sLe^x mimetics of the general structure **125**, compounds containing different spacer groups, different hydrophobic chains, and altered bis-benzoic acid cores or substitution patterns have been described.[180] The



125 R = H or CO_2H



126



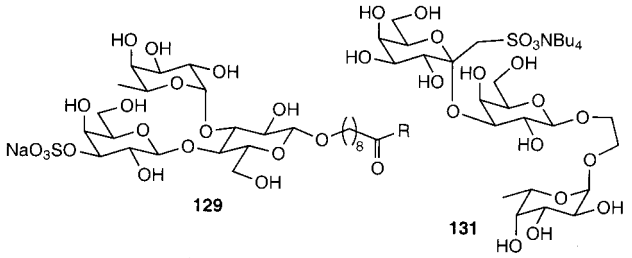
127 R = H

128 R =

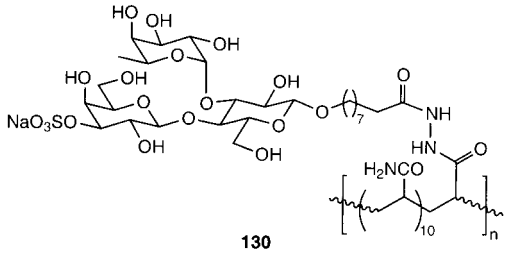
work clearly demonstrates that while all the selectins recognize sialyl Lewis x (or sulfated sLe^x), structural modification of sLe^x mimetics such as **125** has dramatic effects on activity toward specific selectins. This information will no doubt prove extremely valuable in further studies aimed at developing noncarbohydrate-based sLe^x mimetics with highly specific affinity toward individual selectins. The synthesis of several analogues of the imidazole-based sLex mimetic **127**, which has an IC_{50} of 17 μM toward P-selectin, has resulted in the potent P-selectin inhibitor **128** ($IC_{50} = 0.3$ μM).[181]

In addition to sialylmimetics where the entire sialic acid moiety has been replaced by a carboxylate group, several examples of sialylmimetics incorporating other charged groups have been reported. Selectin inhibitors based upon the sulfated trisaccharide **129** have been developed and copolymerized with acrylamide to give water soluble sLe^x mimetics such as **130**.[182] The molecular weight of **130** is reported to be ~150 kDa, and it shows micromolar inhibition of both L- and E-selectin. The acrylamide-based copolymerization of vinyl monomers of α-L-fucose with 6-sulfated galactosides has resulted in terpolymers which show micromolar inhibition of L- and P-selectin.[183] The analogous copolymers formed in the absence of α-L-fucose did not possess inhibitory activity. Compounds where a sulfonomethyl group replaces the sialic acid moiety (e.g., **131**) have been

prepared as sLe^x mimetics, although their affinity for selectins has not yet been described.[184]

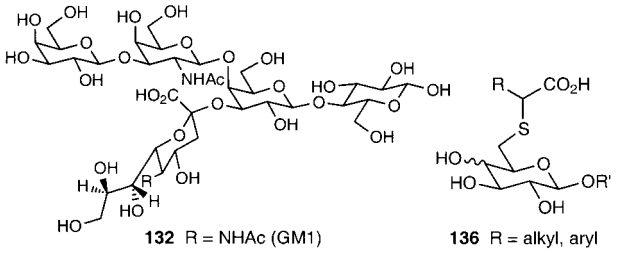


129 131



130

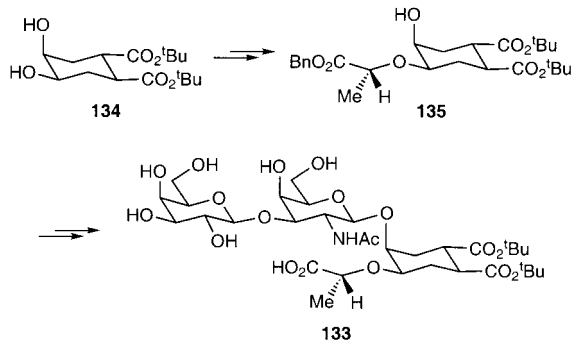
The success with the development of mimetics of sialyl Lewis x has prompted investigations into the development of sialylmimetics for sialic acid-recognizing proteins other than the selectins. The biologically significant interaction of cholera toxin with ganglioside GM1 (**132**) has resulted in the development of sialylmimetics such as **133**.[185] The synthesis



132 R = NHAc (GM1) 136 R = alkyl, aryl

of **133** (Scheme 12) commenced from the enantiomerically pure diol **134** and involved initial dibutyltin oxide mediated O-alkylation to give **135**. Subsequent TMSOTf promoted glycosidation of **135** (with Galβ-(1,3)GalNAc) and deprotection afforded the sialylmimetic **133** which showed high affinity ($K_D = 190\ \mu M$) for cholera toxin.[185] It is worth noting that asialo-GM1 (GM1 with the Neu5Ac moiety removed) exhibited no detectable binding to cholera toxin, indicating the importance of the carboxylate group in sialylmimetics such as **133**.[185] Infection by rotavirus is another example which is believed to involve the

Scheme 12



134 135 133

recognition of sialylglycoconjugates on the host-cell surface.[186] In an attempt to determine the minimal structural requirement for rotaviral adhesion, a series of sialylmimetics of the general structure **136** have been described.[187,188] It was found that while glucose- and galactose-based sialylmimetics (**136**, R′ = Me) showed no inhibition of rotaviral infection, lactose-based sialylmimetics (**136**, R′ = GlcβMe) did exhibit modest inhibition of both animal and human strains of rotavirus.[188]

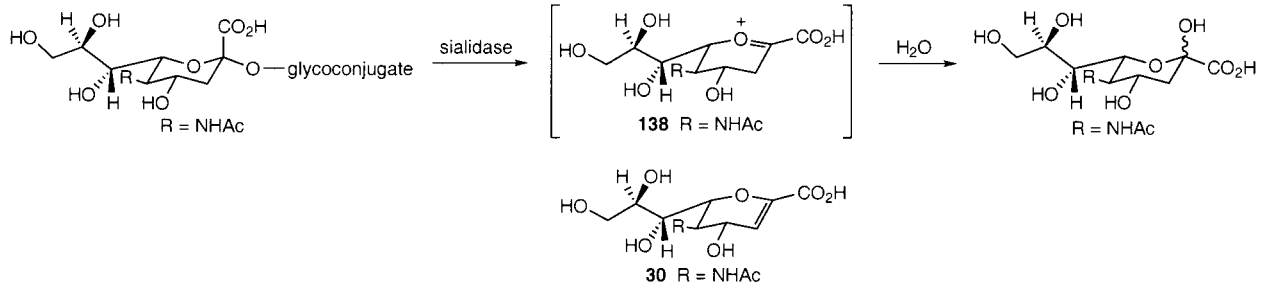
The role of sialyltransferases in the biosynthesis of sialylglycoconjugates, and the realization of the consequent significance of these enzymes in diseases such as inflammation and cancer,[4−7] has prompted considerable interest in the development of sialyltransferase inhibitors. Since sialyltransferases utilize CMP-Neu5Ac (**46**) as their natural substrate, it is not surprising that mimetics of **46** are being investigated as potential inhibitors of this enzyme. The types of mimetics of **46** prepared include structural modifications to both the base and the carbohydrate components, and their inhibition of sialyltransferases has been described.[84,189]

B. Mimetics of Neu5Ac2en

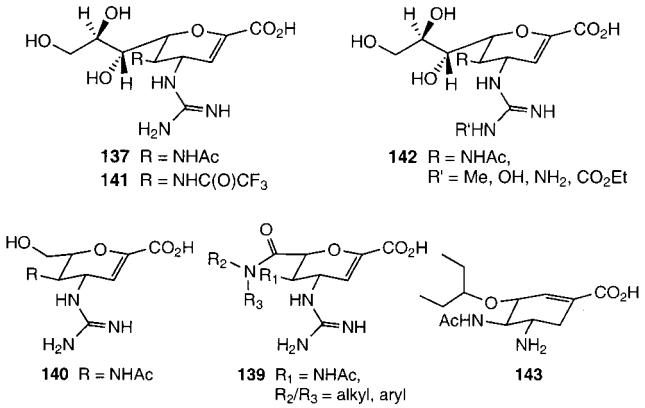
The success of the anti-influenza drug Relenza (**137**)[63,190] has resulted in considerable interest in the development of mimetics of Neu5Ac2en (**30**) as potential inhibitors of sialidases involved in various diseases.[4−7,191,192] As depicted in Scheme 13, sialidases cleave the terminal sialic acid residue from glycoconjugates. A number of diseases (e.g., influenza, cholera, salmonella infection) require the action of a sialidase during the progression of the disease. As can be seen from Scheme 13, Neu5Ac2en (**30**) can be considered as a transition-state analogue of the sialosyl cation **138** formed during the sialidase catalyzed cleavage of sialosides. Importantly, Neu5Ac2en exhibits high levels of inhibition of all sialidases, typically in the micromolar range.[4−7,191,192] Structurally modified Neu5Ac2en derivatives offer the possibility of gaining selectivity for a particular sialidase with potentially higher levels of inhibition than Neu5Ac2en itself.

Relenza (**137**) inhibits influenza virus sialidase selectively ($IC_{50} \sim 10^{-11}$ M) due to interaction of the incorporated guanidino group at C-4 with conserved residues within the active site of the enzyme.[52,176,179] Against other sialidases, Relenza exhibits inhibitory properties similar to or worse than Neu5Ac2en itself, making it an ideal drug.[63,190,193] The potent inhibition of influenza virus sialidase by Relenza (**137**) prompted several investigators to explore analogues of Relenza that possess similar inhibitory activity but which have improved pharmacological properties.[63] Early investigations resulted in the development of glycerol side chain modified compounds such as the carboxamide derivative **139**[194] or the truncated analogue **140**.[195] C-5 functionalized analogues of Relenza like **141**[196] and guanidino modified derivatives such as **142**[197] have also been described. All these analogues

Scheme 13



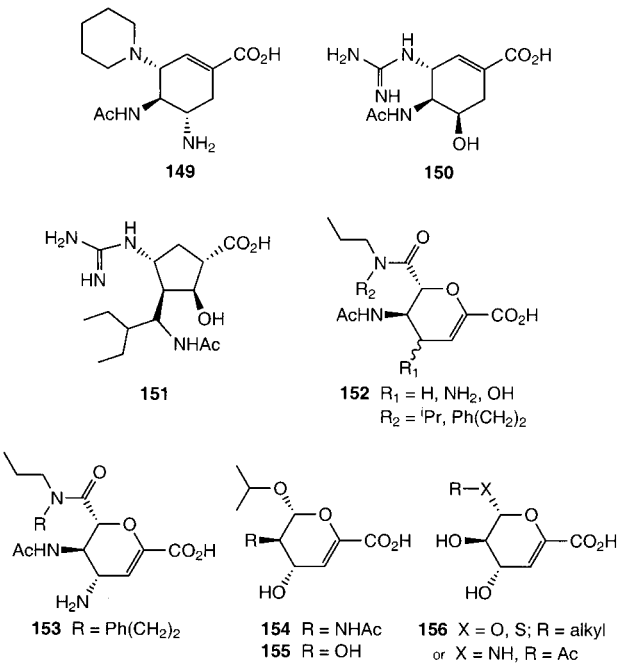
of Relenza failed to show improved inhibition of influenza virus sialidase.[63]



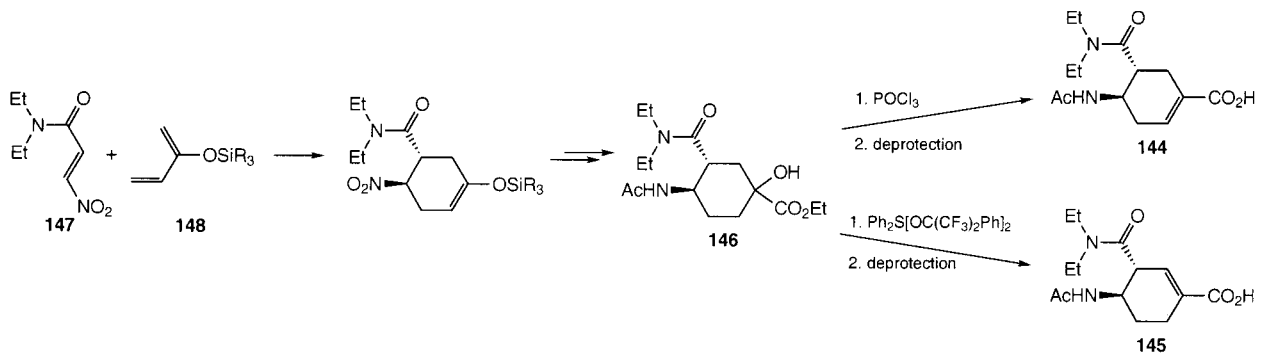
The drive for an anti-influenza agent with improved physiochemical properties has resulted in considerable interest in the development of noncarbohydrate-based mimetics of Relenza.[63] Of the many efforts in this regard during the late 1990s, the most significant discovery came from the Gilead Sciences group with the report of the sialylmimetic **143**.[198,199] It was reasoned that the cyclohexene ring in **143** would adopt a similar conformation to the sialosyl cation transition-state intermediate **138** shown in Scheme 13. Indeed, the carbocyclic analogue **143** exhibits inhibition of influenza virus sialidase comparable to Relenza and is currently marketed, in the form of its orally active ethyl ester prodrug, as Tamiflu.[200]

The discovery that relatively simple carbocyclic compounds such as **143** could exhibit such potent influenza sialidase inhibition has resulted in several reports describing Neu5Ac2en mimetics. The synthesis of the isomeric carbocyclic derivatives **144** and **145** was achieved from the common precursor **146** which itself was prepared via the Diels−Alder reaction of the nitro acrylamide **147** and the diene **148** (Scheme 14).[201] Both **144** and **145** are selective for influenza A sialidase, although only **145** showed significant (IC_{50} ~2×10^{-8} M) inhibition.

Other carbocyclic influenza sialidase inhibitors recently reported include cyclic amine derivatives such as **149**[202] and the guanidino substituted cyclohexene derivative **150**.[203] The Neu5Ac2en mimetic **149** was found to be comparable to **143** with regard to its inhibition of Influenza B virus sialidase, although it was not as potent against influenza A virus.[202] It was observed that as the size of the cyclic amine was increased, or as additional heteroatoms



Scheme 14



or steric constraints were introduced into this moiety, the level of inhibition against both influenza A and B viruses diminished.[202] The five-membered carbocyclic derivative **151** was rationally designed to act as an orally available influenza sialidase inhibitor.[204] In a comprehensive study, compound **151** has been shown to possess comparable sialidase activity to both Relenza (**137**) and **143** against a number of influenza strains.[205] Compound **151** was also shown to have excellent selectivity for influenza sialidase over mammalian, bacterial or other viral sialidases.[205]

Carbohydrate-based Neu5Ac2en mimetics have been described wherein the glycerol side chain of Neu5Ac has been replaced by a carboxamide group (similar to compound **139**). Of the several analogues reported, derivatives of the general structure **152** were found to be quite potent ($IC_{50} \sim 10^{-8}$ M) influenza sialidase inhibitors.[206] Interestingly derivatives such as **152** that contain no substituent at C-4 (R_1 = H) exhibit similar sialidase activity when compared to C-4 substituted Neu5Ac2en mimetics such as **153**. This suggests that the important binding interactions of these compounds, apart from the crucial carboxylate group, are dominated by the hydrophobic carboxamide group at C-6.[206] The synthesis of uronic acid-based mimetics of Neu5Ac2en (e.g., **154**) or KDN2en (e.g., **155**) has been achieved from glucosamine or glucose, respectively.[207] These mimetics of 2,3-didehydro-sialic acids are β-glycosides of uronic acids where the anomeric group of the uronic acid represents the glycerol side chain of the sialic acid (i.e., C-6 of the uronic acid equals C-1 of the sialylmimetic). The replacement of the glycerol side chain with an isopropyl group resulted in compound **154** having comparable or slightly lower activity than Neu5Ac2en against a number of sialidases. Interestingly, the KDN2en mimetic **155** showed only poor inhibition (6% at 1 mM) against the KDN-sialidase from *Crassostrea virginica* when compared to KDN2en itself (95% at 1 mM), suggesting that glycerol side chain modification is not tolerated by this sialidase.[207] Subsequent studies on KDN2en mimetics such as **156**, all prepared from D-glucurono-6,3-lactone,[208] will undoubtedly shed further light on the structure−activity relationships of these sialylmimetics with sialidases.

V. Concluding Remarks

The level of interest in the field of sialic acid chemistry and biochemistry has clearly blossomed over the last 25 years. As we enter the 21st century, we are well placed to build on the significant advances in our knowledge of the biology of the sialic acids, as well as the advances in techniques to enable the synthesis of rationally designed sialic acid derivatives or mimetics. Despite the recent progress in the synthesis of structurally modified sialic acids and complex sialylglycoconjugates, researchers in this field still face considerable challenges. For example, a reliable and efficient method for the chemical synthesis of sialic acid glycosides with a range of acceptors (particularly the formation of α(2,3)-linkages with hindered sialosyl acceptors) is still required if such compounds are to be continually utilized as biological probes for sialic acid-recognizing proteins. Structural modifications have been achieved at every position within the sialic acid structure, although new and improved methods for such manipulations are still being developed. No doubt the coming years will see more research efforts on the development of structurally modified Neu5Gc and KDN derivatives.

The recent interest in the development of sialylmimetics as biological probes and inhibitors of sialic acid-recognizing proteins will undoubtedly contribute to a significant rise in the number of research groups working in this area over the next decade. The excellent progress made thus far in this exciting area of sialic acid chemistry and biology is only the beginning. While numerous challenges remain, it is hoped that further sialylmimetic-based pharmaceutical agents will appear on the market over the next decade.

VI. References

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