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Perspective

Sialidase Inhibitors with Different Mechanisms

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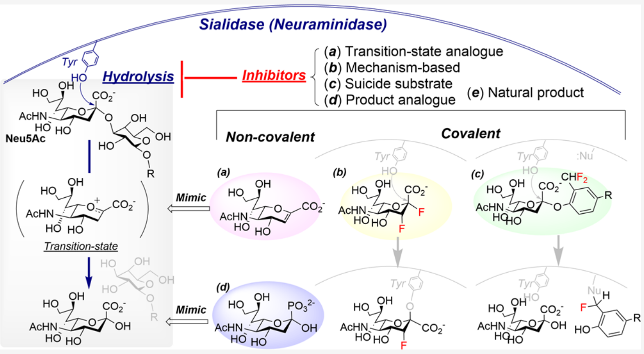
ABSTRACT: Sialidases, or neuraminidases, are enzymes that catalyze the hydrolysis of sialic acid (Sia)-containing molecules, mostly removal of the terminal Sia (desialylation). By desialylation, sialidase can modulate the functionality of the target compound and is thus often involved in biological pathways. Inhibition of sialidases with inhibitors is an important approach for understanding sialidase function and the underlying mechanisms and could serve as a therapeutic approach as well. Transition-state analogues, such as anti-influenza drugs oseltamivir and zanamivir, are major sialidase inhibitors. In addition, difluoro-sialic acids were developed as mechanism-based sialidase inhibitors. Further, fluorinated quinone methide-based suicide substrates were reported. Sialidase product analogue inhibitors were also explored. Finally, natural products have shown competitive inhibiton against viral, bacterial, and human sialidases. This Perspective describes sialidase inhibitors with different mechanisms and their activities and future potential, which include transition-state analogue inhibitors, mechanism-based inhibitors, suicide substrate inhibitors, product analogue inhibitors, and natural product inhibitors.



1. INTRODUCTION

1.1. Sialic Acids, Sialylation, and Desialylation.

Sialic acids (Sias) are carboxylic acid-containing 9-carbon monosaccharides that often present at the terminus of glycan structures of glycoproteins and glycolipids.[1] Sias are attached to either galactose (Gal), *N*-acetyl galactosamine (GalNAc) unit via *α*2,3- or *α*2,6-linkage, or Sias via *α*2,8(9)-linkage, which is known as sialoform.[2] *N*-acetylneuraminic acid (Neu5Ac) is the most abundant form of Sias found in mammalian cells, which also exhibits remarkable structural diversity, with more than 50 different derivatives identified in nature.[2] These structural variations can occur as a variety of O-substitutions (acetylation, lactylation, sulfation, and methylation) at the C-4, C-7, C-8, and C-9 position, or as *N*-acetyl, *N*-glycolyl, or hydroxy at the C-5 position (Figure 1). As terminal carbohydrates with a negative charge, Sias could (i) exert physicochemical effects on the glycoconjugates to which they are attached, (ii) serve as recognition sites, or (iii) mask recognition sites of the glycoconjugates, and are therefore involved in various biological processes.[1−4] The levels and linkages of Sias, known as sialylation status, is a delicate balance between sialylation (attachment of Sias) and desialylation (removal of Sias), which is maintained by sialyltransferases and sialidases, respectively.[3,4]

Desialylation by sialidase alters the functionality of Sia-containing glycoconjugates and is thus often involved in a variety of biological processes.[5] First, desialylation reduces the charge of the glycoconjugate and the entire cell surface, as Sia is a highly electronegative carbohydrate. Second, desialylation unmasks the glycoconjugate's binding site for its molecular ligand of the partner, thus affecting the glycoprotein's function as an enzyme or receptor. Further, desialylation affects the target glycoprotein's folding, thus regulating its interaction with other molecules accordingly. Several biological processes have been

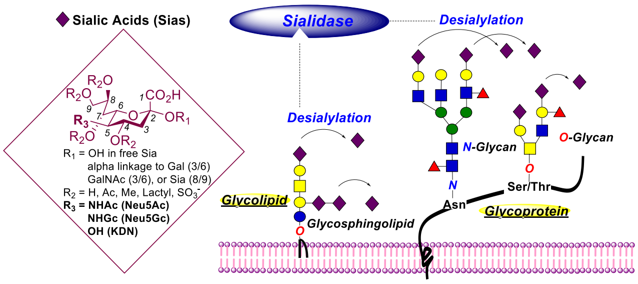


Figure 1. Sialic acids (Sias) and their linkages, cell surface sialylation and desialylation by sialidases.

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Table 1. Classification of Sialidases

	Exosialidase					
	Hydrolytic sialidases	Trans-sialidases	Anhydro-sialidases	Endosialidase	CAZy families	Sia-linkage specificity
Human sialidase	×				GH33	α2,3-, α2,6-, α2,8-Sia
Bacterial sialidase	×	×	×	×	GH33, GH156	α2,3-, α2,6-, α2,8-Sia
Viral sialidase	×			×	GH34, GH58, GH83	α2,3-, α2,6-Sia
Protozoa sialidase	×	×			GH33	α2,3-Sia

Table 2. Mammalian Sialidases in Different Subcellular Locations and Their Target Proteins

	Neu1	Neu2	Neu3	Neu4
Subcellular localization	Lysosome	Cytosol	Cell surface	Lysosome mitochondria endoplasmic reticulum
Relocation	Cell surface[28−52]	Cell surface[53−55]	ND[a]	Cell surface[56,57]
GenBank no.	AAB96774.1	CAB41449.1	AAE69072.1	AAH95117.1
Expression level[b]	1	0.01%−0.03%	5%−10%	5%−10%
Targets[c]	ApoB100[58]	ATG5[90]	ApoB100[58]	ND[a]
	CD5[29]		CD18 (ItGB2)[59−62]	
	CD18 (ItGB2)[59−62]		CD42b (GPlbα)[64,65]	
	CD31 (PECAM1)[60,63]		EGFR[30,78,79]	
	CD36[60]		TGF-β/LAP[91]	
	CD42b (GPlbα)[64,65]			
	CD44[66−68]			
	CD54 (ICAM1)[61]			
	CD64 (FCγR)[69]			
	CD104 (ITGB4)[70]			
	CD107a/b (LAMP-1, LAMP-2)[71,72]			
	CD140 (PDGFR)[73]			
	CD220 (IR)[74−77]			
	CD221 (IGF-1R)[73,74]			
	EGFR[30,78,79]			
	HGFR/Met[80]			
	MMP9[30,32,33,47,60,78−81]			
	MUC1[78,82−85]			
	TLR2[34,60]			
	TLR3[34]			
	TLR4[32,34−36,47,86−88]			
	TLR7[81]			
	TLR9[81]			
	TrKA[33,89]			

[a]ND: no data available. [b]Referred from ref 9. [c]Modified from ref 92. Abbreviations: ApoB100, Apolipoprotein B100; ATG5, Autophagy related 5; CD protein, cluster of differentiation protein; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; IR, insulin receptor; LAP: latency-associated peptide; MMP9, matrix metallopeptidase 9; *MUC1*, mucin 1; TGF-β, transforming growth factor beta; TLR, toll-like receptor; *TrkA, Tropomyosin receptor kinase A*.

clarified through desialylation that controls target glycoproteins' function on the cell surface and their downstream signaling.[5−8] Overall, desialylation could alter the active and inactive state of a protein and attenuate or augment its function in either physiological or pathological processes.[9]

1.2. Sialidases (Neuraminidases) in General. Sialidases (neuraminidases) catalyze the hydrolysis of Sia-containing substrates in either an exo fashion, where the terminal Sia is cleaved, or an endo fashion, where cleavage occurs within oligo-/polysialic acids. Most sialidases are exosialidases (EC 3.2.1.18) that hydrolyze the sialyl substrates with terminal Sias, but fewer are endosialidases (EC 3.2.1.129). On the basis of the catalytic mechanisms, there are three kinds of exosialidases: (i) hydrolytic sialidases, (ii) trans-sialidases, and (iii) anhydrosialidases (intramolecular trans-sialidases, EC 4.2.2.15). Hydrolytic sialidases release free Sias from oligosaccharides, glycolipids, and glycoproteins.[10] In the presence of asialo-substrates, trans-sialidases transfer Sias from sialoglycoconjugates to acceptor molecules.[11] Anhydrosialidases release 2,7-anhydro-α-*N*-acetylneuraminic acid (2,7-anhydro-Neu5Ac) from sialoglycoconjugates.[12,13] Hydrolytic sialidases are generally active against α2,3-, α2,6-, and α2,8-linked substrates but with different preferences. While anhydrosialidases are specific for α2,3-linked substrates.

On the basis of the primary sequence similarity (carbohydrate active enzyme (CAZy) database, available at http://www.cazy.org), sialidases are also classified into five glycoside hydrolase (GH) families: 33, 34, 58, 83, and 156 (Table 1). The family GH33 includes human sialidase and bacterial hydrolytic neuraminidases, trans-sialidases, and anhydrosialidases. The family GH34 includes exclusively viral sialidases from influenza A and B viruses. The family GH58 comprises bacteriophage endosialidases, which are viral sialidases and infect nonhuman hosts. The GH83 family contains viral sialidases of the Paramyxoviridae family, which exhibits both neuraminidase and hemagglutinin activities and infects humans.[14,15] The family GH156 is an exo-sialidase, identified recently from a freshwater hot spring environment.[16] This enzyme hydrolyzes a variety of Sia-containing glycosides, typically α2,3-, α2,6-linked.[16]

There are three sources for the sialidases in the human body, which are (i) directly produced by the body, (ii) supplied by microbial cells as part of natural microflora of the human body, and (iii) through infections, in which the pathogens bring their own sialidases for infection and replication. Human sialidases play important roles in human health, but their overexpression, activation, and mutations cause disorders and diseases. For example, increased expression of Neu1 was confirmed in human pulmonary airway epithelial and microvascular endothelial cells and fibroblasts, which is relevant to the lung pathologies.[17] Mutations of lysosomal sialidase Neu1 cause the lysosomal storage disorder, a fulminant disease called sialidosis, which often develops before birth.[18,19] Sialidases produced from the human gut microbiome may have a beneficial effect on humans, but some bacterial sialidases play pathogenic roles. For example, sialidase of intestinal microbiota targets the intestinal mucin glycoconjugates and plays a regulatory function of physiological and pathological pathways.[20] *Clostridium perfringens* sialidases could cause numerous diseases in humans and animals.[21] Therefore, better understanding of sialidases produced from different organisms and viruses will facilitate clarification of pathological mechanisms and development of effective treatments for certain diseases.

Mammalian Sialidases. Mammalian sialidases are *exo*-sialidases catalyzing the hydrolysis of sialyl substrates and belong to the GH33 family. No mammalian sialidases have been found with trans-sialidase or anhydrosialidase activities to date. On the basis of their subcellular and tissue localization, mammalian sialidases are further classified into Neu1 (localized predominantly in lysosomes), Neu2 (cytosol), Neu3 (plasma membranes), and Neu4 (lysosomes or mitochondria and endoplasmic reticulum) sialidases (Table 2).[22−25] The expression levels of the four mammalian sialidases are different. Neu1 is more highly expressed than Neu3 and Neu4, while Neu2 is less expressed in human tissues.[26] These four sialidases also differ in substrate specificity, enzymatic properties, and sensitivity and relocation in response to cellular stimuli, suggesting different physiological and pathological roles they play.[6,27] Neu1 is typically located in the lysosome, where it associates with its chaperone/transport protein, protective protein/cathepsin A (PPCA) and β-galactosidase, and is involved in the metabolism of sialyglycans.[28] In addition, Neu1 could relocate to the plasma membrane upon stimulation.[28−52] Neu2[53−55] and Neu4[56,57] are also found on the cell surface. Cell surface sialidases act as structural and functional modulators of various extracellular soluble and membrane-bound molecules in a variety of cell types (Table 2).[32,38,39] Therefore, cell surface sialidases play very important roles in receptor activation and signaling pathways and could serve as potential therapeutic targets as well.

Although mammalian sialidases have different substrate specificities and properties, they share a common genomic organization.[93,94] However, the overall amino acid identity of Neu1 compared to the other mammalian sialidases is about 19−24%, whereas Neu2, Neu3, and Neu4 show 34−40% homology to each other.[6] X-ray structures were reported for human Neu2 in free form and in a complex with 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA) inhibitor.[95] The 3D structure of Neu2 is often used for the homology model of Neu1, Neu3, and Neu4, as there is no 3D structure reported for any of them.[96] The main reason for the poor characterization the mammalian sialidases could be due to their membrane-bound structure.[96] More detailed information on the subcellular distribution, substrate specificity, catalytic properties, and amino acid homologies of the four mammalian sialidases can be found in recent review articles.[5−7,92,97−99] Despite the accumulating data, molecular mechanisms underlying mammalian sialidases involvement in cellular phenomena have yet to be fully elucidated.

Microbial Sialidases in the Human Body. Bacterial sialidases are found in the human gastrointestinal (GI) tract, respiratory tract, oral cavity, and reproductive tract.[100] Bacteria bind to the host cells through modification of cell surface glycans by their hydrolytic sialidases.[101] For example, human pathogens *Clostridium (C) perfringens*[102] and *Vibrio cholerae*[103] use their hydrolytic sialidases for mucosal infections. In the human GI tract, several bacterial commensals and pathogens have been identified to have sialidase activity.[104−109] Respiratory tract bacteria, specifically *Streptococcus (S) pneumoniae*,[110] *S. intermedius*,[111] and *Haemophilus influenzae* also have their own sialidases.[112] In the oral cavity, hydrolytic sialidase-producing bacteria was also confirmed, namely *S. oralis*,[113] *S. sanguinis*, *S. intermedius*, *S. mitis*,[114] *Porphyromonas gingivalis*,[115] *Actinomyces oris*,[116] *Tannerella forsythia*,[117] and *Treponema denticola*.[118]

Influenza A and B viruses belong to the Orthomyxoviridae family (GH34) and produce their own neuraminidase with hydrolytic activity.[119] To date, 11 types of neuraminidase (N1−N11) have been identified in influenza A virus and one neuraminidase in the influenza B virus.[119] Only N1 and N2 types of neuraminidases are related to human infections. The N1 type contains avian, swine, and human lineages, while N2 contains avian and human lineages.[119] Paramyxovirus produces hemagglutinin-neuraminidase (HN) proteins, which belong to the GH83 family of viral sialidases, and are responsible for viral attachment and interaction with the fusion protein in viral infection.[14,15]

Trypanosoma (T) cruzi, *T. brucei*, and *T. rangeli* protozoa species also produce their own sialidases. *T. cruzi* and *T. brucei* sialidases are characterized with trans-sialidase activity, while the *T. rangeli* sialidase is strictly a hydrolytic sialidase.[120−122] *T. cruzi* trans-sialidases are more efficient for transferring Sia from the α-sialylglycoside donor to the β-galactopyranosyl unit in the acceptor than catalyzing the sialoside hydrolytic reaction.[123] Therefore, *T. cruzi* trans-sialidase has been used for the synthesis of sialylated oligosaccharides.[119] In the absence of a proper acceptor, trans-sialidases catalyze sialoside hydrolysis with retention of the configuration.[124]

Overall, sialidases catalyze the cleavage of terminal Sias (desialylation), which modulate the functionality of the Sia-containing molecules, and thus are often involved in physiological and pathological processes. Imbalance in the sialidase activity could cause diseases such as cancer, diabetes, heart disease, or neurodegenerative disorders.[7,92] Therefore, sialidases could serve as potential therapeutic targets. Suitable sialidase inhibitors could be used as effective drugs for these diseases depending on which sialidase needs to be inhibited.[125]

1.3. Catalytic Mechanism of Sialidases.

As mentioned previously, exosialidases are responsible for the hydrolysis of sialyl linkages in oligosaccharides, glycoproteins, and glycolipids. The catalytic mechanism of sialidases has been an important research subject. Several mechanisms have been discovered related to a specific group of sialidases.[126,127] Typically, enzymatic hydrolysis of the glycosidic bond proceeds with either net retention or inversion of the anomeric configuration. Retention of the anomeric configuration is completed in two

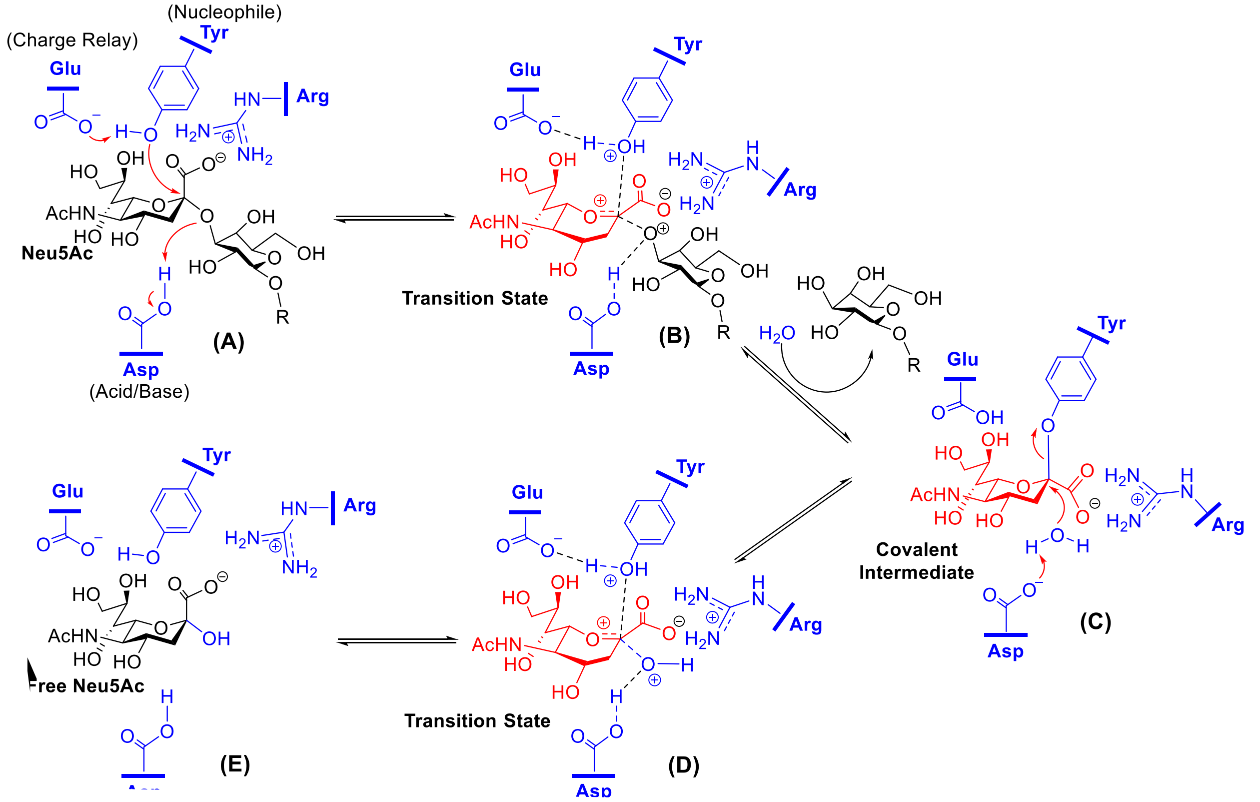


Figure 2. Catalytic mechanism of the hydrolytic sialidase with net retention of the anomeric configuration. (A) First step, Tyr residue acts as a nucleophile to attach the anomeric center (C-2), (B) semiplanar oxocarbenium transition state formation with the adjacent carbohydrate attached, (C) covalent intermediate that is bound to the active site, (D) another semiplanar oxocarbenium transition state formation with a water molecule attached, and (E) finally, release of free Sia as an α-anomer.

inverting steps, a double displacement mechanism, in which the catalytic residues act as the acid/base and nucleophile, respectively. Inversion of the anomeric configuration is completed in a single-step mechanism, in which the substrate and a water molecule are bound simultaneously. The GH33, GH34, and GH83 families of sialidases are *exo*-sialidases, and all perform hydrolysis with net retention of the anomeric configuration, which is completed via the formation and subsequent breakdown of a covalent intermediate to a conserved tyrosine active center.[126,127] The GH58 family sialidase is an *endo*-polysialidase that acts with the inversion of the anomeric configuration.[128] Interestingly, the newly identified GH156 family is an *exo*-sialidase but acts with inversion of the anomeric configuration of the released free Sia from a variety of α2−3- and α2−6-linked sialosides.[16,129]

Sialidases act with either retention or inversion of the anomeric configuration, depending on their intramolecular rearrangement. A number of studies on family GH33 sialidases have demonstrated that these enzymes operate through a two-step, double-displacement mechanism similar to the majority of retaining glycosidases but, involving the participation of a tyrosine residue as the catalytic nucleophile to form a covalent aryl-glycoside intermediate.[130−132] Generally, a nucleophile pair of Tyr/Glu, acid/base aspartate, and the arginine triad are essential residues involved in the mechanism of catalytic cleavage among all types of sialidases. Initially, the positively charged arginines in the catalytic pocket are involved in the coordination of substrates by surrounding the negatively charged carboxylate group of Sia (Figure 2A). Meanwhile, the Tyr residue acts as a nucleophile to attach the anomeric center (C-2), which is assisted by the base Glu to enhance its nucleophilicity, yielding a semiplanar oxocarbenium transition state with the adjacent carbohydrate attached (Figure 2B) and then leading to the formation of an intermediate that is covalently bound to the active site (Figure 2C). Next, the water molecule activated by the Asp residue attacks the anomeric C-2 center in a trans addition to form another semiplanar oxocarbenium transition state with a water molecule attached (Figure 2D). In the final step, free Sia, as an α-anomer, is released from the active site of the sialidase (Figure 2E), completing the hydrolysis with retention of the anomeric configuration. Kinetic isotope effect (KIE) measurements with isotopically labeled natural substrate analogues were used to characterize the transition states of sialidase-catalyzed hydrolysis reactions, which have a pyranosyl ring in the 4H5 half-chair conformation coupled with the adjacent carbohydrate.[133,134]

Mammalian, bacteria, viruses, and fungi sialidases have different primary sequences but share a common catalytic domain.[135] They all form a covalent intermediate with the substrates initially, but the later steps of the catalytic mechanisms for the various sialidases are different. Detailed mechanisms and structural features of different types of sialidases can be found in comprehensive reviews and monographs.[102,136−142]

2. DISCUSSIONS

Sialidases or neuraminidases cause the desialylation of Sia-containing oligosaccharides or glycoconjugates in both physiological and pathological pathways and thus play key roles in

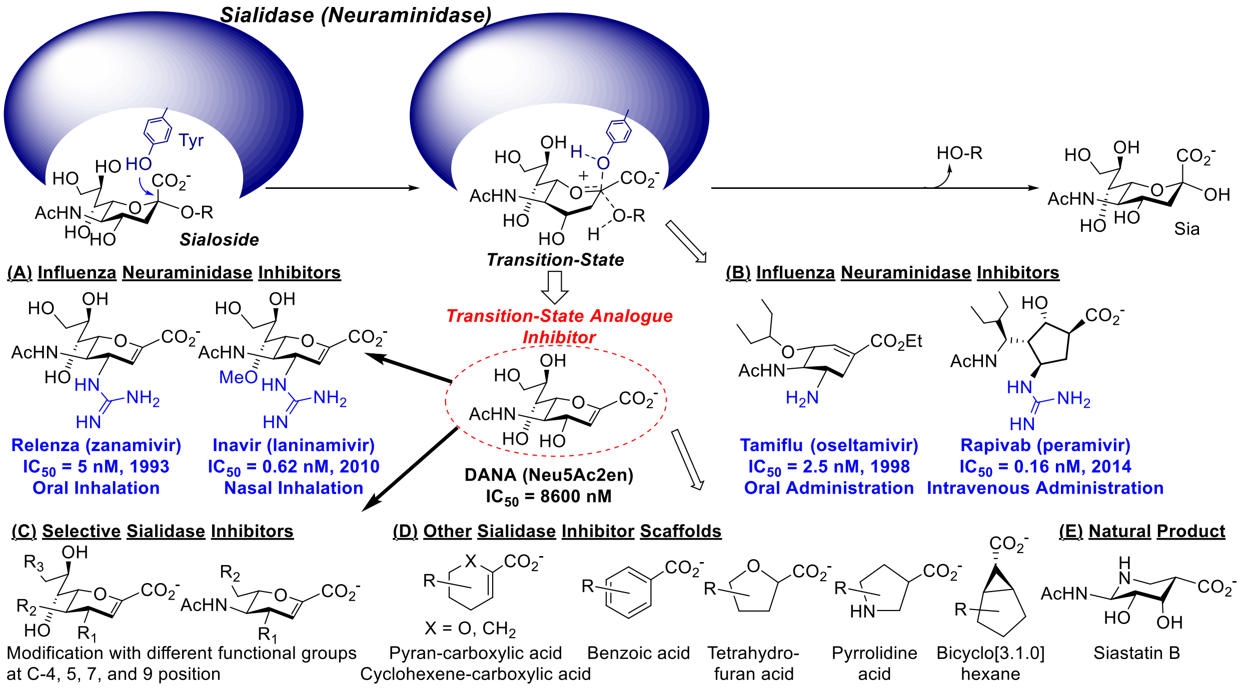


Figure 3. Transition-state analogue sialidase inhibitors in different scaffolds. IC$_{50}$ values are for influenza viral NA inhibition. (A) Influenza neuraminidase inhibitors with pyran scaffold, (B) influenza neuraminidase inhibitors with carbocyclic scaffold, (C) selective sialidase inhibitors by modifying C-4, -5, -7, and -9 positions, and (D) sialidase inhibitors with other kinds of scaffolds.

health and disease.[5,9] Sialidase inhibitors have been developed and used for studying the sialidase function and related biological mechanisms and disease processes and could serve as drugs for sialidase-related diseases, such as viral infection.[7] Inhibition of viral neuraminidase activity is a practical approach for the treatment of influenza infection.[143] Tamiflu (oseltamivir) and Relenza (Zanamivir) are potent inhibitors of influenza virus neuraminidase and have been used for the treatment of influenza A and B for decades.[143,144] Selective inhibitors against bacterial sialidases have been extensively explored for antibacterial action.[145] Inhibitors of human sialidases are recognized as important tools for studying the biological functions of human sialidases and regulating the related biological processes.[97,146] Several selective human sialidase inhibitors have been developed and have shown therapeutic potential for diseases such as inflammation, diabetes, atherosclerosis, fibrosis, neurodegenerative diseases, and cardiovascular diseases.[147] Each type of sialidase inhibitor shows unique activity and potential for different applications. There has been a large number of sialidase inhibitors reported so far, and this Perspective will not describe them all in detail. Instead, it describes the sialidase inhibitors based on their inhibition mechanisms, including (i) transition-state analogue inhibitors, (ii) mechanism-based inhibitors, (iii) suicide substrate inhibitors, (iv) product analogue inhibitors, and (v) natural product inhibitors. More detailed information about specific sialidase inhibitors from the past decade can be found in comprehensive reviews.[97,144−149]

2.1. Transition-State Analogue Sialidase Inhibitors.

Transition-state analogues have been widely used as potent enzyme inhibitors by blocking the active site of the enzyme.[150] They are based on the theory that the enzyme binds the substrate at the transition state with extraordinary affinity. If an inhibitor mimics the transition state structure, it should have high affinity to the target enzyme and could serve as highly potent and specific drugs. In many cases, sialidase inhibitors are proposed to mimic the transition state formed during the sialoside hydrolysis. DANA (Neu5Ac2en) was the first transition-state analogue sialidase inhibitor (Figure 3), which mimics the oxocarbenium ion-like transition state and exhibits moderate inhibitory activity toward influenza viral neuraminidases with K_i values in the micromolar range.[151] In addition, DANA is a product of sialidase-catalyzed hydrolysis reactions. *Streptococcus pneumoniae* sialidase SpNanC specifically hydrolyzes α2,3-linked sialosides and generates the transition-state analogue inhibitor DANA.[152] Also, influenza B virus neuraminidase could catalyze the formation of DANA.[153] Later, DANA was used in the structure-based drug design of the anti-influenza drug zanamivir (Relenza, GlaxoSmithKline) by the substitution of the 4-hydroxyl moiety with a guanidino group in the 1990s (Figure 3A).[154] On the other hand, oseltamivir, which has a carbocyclic scaffold with a 3-pentyl ether side chain as a transition-state analogue, was developed as a potent anti-influenza drug (Figure 3B).[155] The 3-pentyl ether is in lieu of the glycerol side chain in Sia, to render hydrophobic interactions with the Glu276, Ala246, Arg224, and Ile222 residues in the NA active site.[156] Since then, DANA has been used as the model compound for developing selective sialidase inhibitors by modification with different functional groups at C-4, C-5, C-7, and C-9 positions (Figure 3C). In addition, substituted pyran-carboxylic acids, cyclohexene-carboxylic acids, benzoic acids, tetrahydrofuran acids, pyrrolidine acids, and bicyclo[3.1.0]-hexane scaffolds were also developed as transition-state analogue sialidase inhibitors (Figure 3D). Natural products such as Siastatin B (Figure 3E) isolated from a *Streptomyces* strain, resemble the transition state and inhibit sialidases from various microorganisms, animal tissues, and viruses.[157] This section describes the transition-state analogue inhibitors against viral, bacterial, and human sialidases, respectively.

Transition-state Analogue Influenza Virus Neuraminidase (NA) Inhibitors. Influenza virus neuraminidase (NA) becomes a

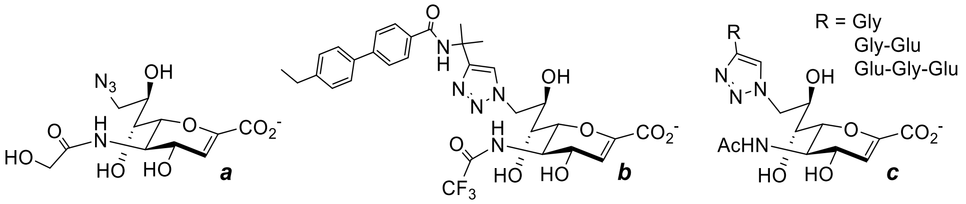
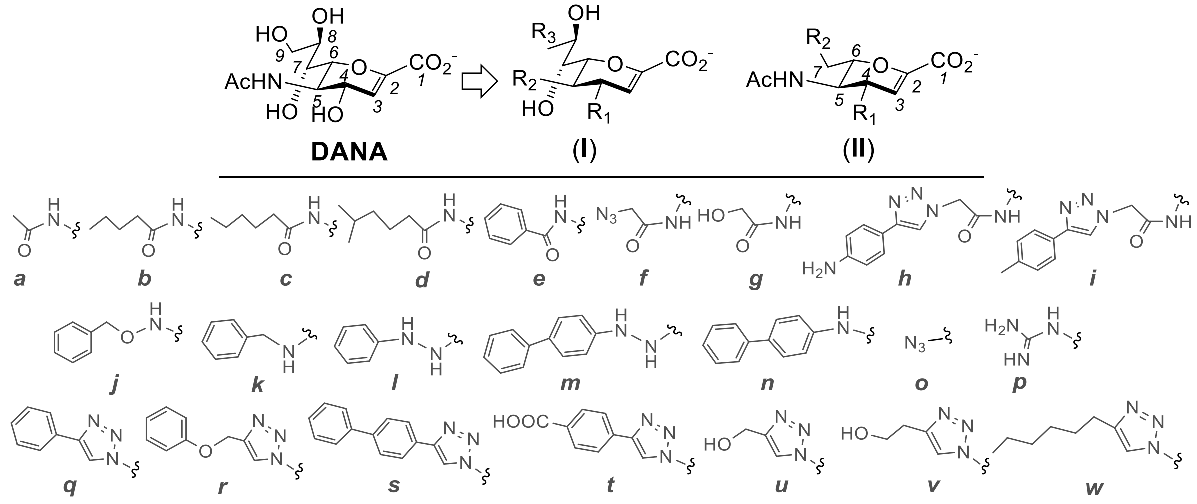


Figure 4. Transition-state analogue bacterial sialidase inhibitors with modification at C-9 and C-5 position of DANA. (a) Neu5Gc9N$_3$2en, (b) 9-Triazole-linked and 5-*N*-trifluoroacetyl derivative of DANA, and (c) 9-triazole-linked peptide derivatives of DANA.

Table 3. Transition-State Analogue Human Sialidase Inhibitors[a]



position	functional group	human sialidase selectivity	other sialidase selectivity	reference
(**I**)-C-4 (R_1)	*o*	Neu2 > Neu3	ND*	184
	p	Neu3 > Neu2	influenza virus	181
(**I**)-C-4/5 (R_1/R_2)	*p* and *d*	Neu2 > Neu1 > Neu4	ND	182
(**I**)-C-4/9 (R_1/R_3)	*p* and *s*	Neu3	ND	181
	p and *t*	Neu3	ND	181
(**I**)-C-5 (R_2)	*b*	Neu1 > Neu3	ND	179,182
	c	Neu1	ND	182
	e	Neu1	ND	182
	h	Neu2	ND	182
	i	Neu2	ND	182
(**I**)-C-5/9 (R_2/R_3)	*b* and *b*	Neu1 > Neu2	ND	182
	b and *a*	Neu1 > Neu2	ND	182
	g and *o*	Neu2	*V. Cholerare*	174
	f and *o*	Neu2	*V. Cholerare*	174
(**I**)-C-9 (R_3)	*b*	Neu1 > Neu3	ND	182
	c	Neu1 > Neu3	ND	182
	e	Neu1	ND	180
	q	Neu3	ND	181
	r	Neu3	ND	183
	s	Neu3	ND	181
	u	Neu4	ND	184
	v	Neu4	ND	184
	w	Neu3	ND	181
(**II**)-C-7 (R_2)	*k*	Neu2	ND	180
	m	Neu2 > Neu3	ND	180
	n	Neu2 = Neu3	ND	180
(**II**)-C-4/7 (R_1/R_2)	*o* and *j*	Neu3	ND	180,185
	o and *l*	Neu2 = Neu3	ND	180
	o and *n*	Neu2 = Neu3	ND	180

[a] ND: no data available.

primary drug target for the prophylaxis and treatment of influenza infections. Influenza virus NA inhibitors are the most successfully studied sialidase inhibitors.[143] DANA was the first influenza virus NA inhibitor reported as a transition state analogue of the enzymatic hydrolysis of the flu receptor sialoside (Figure 3).[151] While DANA shows moderate inhibitory activity, it has been used as a lead for the discovery of potent influenza virus NA inhibitors. Of these, zanamivir, oseltamivir, laninamivir, and peramivir have been developed for the treatment and prophylaxis of human influenza viral infection (Figure 3A).[158−165] *Zanamivir* (4-guanidino-Neu5Ac2en) is an analogue of DANA, in which a positively charged guanidino group was introduced to replace the hydroxyl group at C-4 position. This modification resulted in a significant increase in binding affinity to NA. *Laninamivir* is structurally similar to zanamivir, but has methylation of the C-7 hydroxyl group. Both laninamivir and zanamivir have the pyran scaffold. *Oseltamivir carboxylate* was designed with the aim of simplifying synthesis, while also improving bioavailability. Specifically, a carbocyclic scaffold is used instead of the pyran of zanamivir and DANA. Also, the 3-pentyl ether side chain replaces the hydrophilic glycerol side chain and the amino group replaces the guanidino group. Unlike zanamivir, oseltamivir relies on strong hydrophobic interactions rather than polar interactions (Figure 3B). In addition, carbocyclic compounds resemble the oxo-carbenium transition state intermediate more closely[166] and bind the target NA more tightly than the pyran derivatives.[155,167] As a result, the carbocyclic analogue of DANA has doubled the potency of DANA.[168] *Peramivir* is a cyclopentane derivative, making it structurally unique among the other approved NA inhibitors. Still, it has the functional groups, a guanidino moiety of zanamivir and a hydrophobic side chain of oseltamivir. Several other scaffolds have also been explored, such as benzoic acid, tetrahydrofuran acid, pyrrolidine acid, spiro compound, and bicyclo[3.1.0]hexane, but none of them produce the expected antiviral infection *in vivo* (Figure 3D).[169] However, most of NA inhibitors developed so far are derivatives of commercial drugs. More detailed information about influenza virus NA inhibitors can be found in recent comprehensive reviews.[143,170,171]

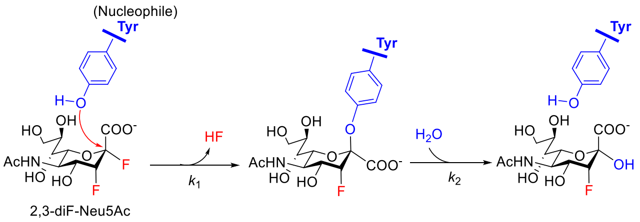
Transition-State Analogue Bacterial Sialidase Inhibitors. Pathogenic bacterial species, such as *Vibrio (V) cholerae* (causes cholera), *S. neumoniae* (causes otitis media in children), and Gram-positive anaerobic bacteria *Clostridium perfringens* (causes gas gangrene disease), utilize their own sialidases for pathogenicity.[172] *C. perfringens* is pathogenic to humans and livestock and often causes gangrene, necrotizing enterocolitis, and food poisoning worldwide.[173] DANA has been used as the template for developing *C. perfringens* and *V. cholera* sialidase inhibitors. Modification with the azido group at the C-9 or C-5 position of DANA increases its selectivity for bacterial sialidases over human sialidases. For example, Neu5Gc9N$_3$2en (Figure 4a) was identified as a selective inhibitor against *V. cholerae sialidase.*[174] It was suggested that the hydrophobic group at the C-9 position of DANA would interact hydrophobically with the target loop moiety of the enzyme. 9-Triazole-linked and 5-*N*-trifluoroacetyl derivatives of DANA transition state analogue (Figure 4b) were reported as selective inhibitors against *V. Cholerae* sialidase.[175] In addition, 9-triazole-linked peptide derivatives of DANA transition state analogues (Figure 4c) selectively inhibited *V. cholerae* and *A. ureafaciens* sialidases.[176] The *in vivo* activities and therapeutic applications of these compounds deserve further investigation.

Transition-State Analogue Human Sialidase Inhibitors. Human sialidases catalyze the removal of Sia residues from glycoproteins and glycolipids. Four human sialidases (Neu1−4, belonging to the family GH33) have been identified and were found to be involved in atherosclerosis, cancer, diabetes, and neurodegenerative diseases.[77,177,178] These four isoenzymes vary in their tissue expression, subcellular location, and substrate specificity (Table 2); however, their precise biochemical roles in different biological processes have not been fully investigated. Selective sialidase inhibitors are important tools for studying the biological functions of human sialidases and elucidating their roles in the regulation of glycoconjugates. They are also expected to serve as potent drugs for human sialidase-related diseases. In the past, there had been no commercially available human sialidase inhibitors. Bacterial or viral sialidase inhibitors were often used to study human sialidases, but typically show broad or weak activity.[167,168] There remains a high demand for potent and selective human sialidase inhibitors for biological studies of the role of human sialidase isoenzymes.

C-9 Pentylamido derivative of DANA is the first selective inhibitor of human sialidases reported, which has a micromolar IC$_{50}$ against Neu1 over the other isoenzymes.[179] Pioneered by Cairo's group, several selective inhibitors of human sialidases have been developed based on the DANA scaffold (Figure 4C).[146] These selective inhibitors of human Neu1, Neu2, Neu3, and Neu4 isoenzymes were developed by modifying DANA at the C-4, C-5, C-7, and C-9 position and combining these modifications.[174,179−185] Mostly, amide formation, oxime, or hydrazide formation and click chemistry were used for these modifications. The major DANA-based transition-state analogue human sialidase inhibitors are summarized in Table 3. Most recently, Bourguet et al. extensively described the structures and stereoselective inhibitors of human sialidases.[97] Based on the already known inhibitors of human sialidases, a structure−activity relationship at C-4, C-5, C-7, and C-9 position is discussed in detail for the development of potent and selective inhibitors.[97] More detailed information about these selective human sialidase inhibitors can be found in recent comprehensive reviews.[143,170,171] These sialidase inhibitors represent useful tools for elucidating the roles of human sialidases in health and disease. Their *in vivo* activities and therapeutic applications deserve further investigation.

2.2. Mechanism-Based Sialidase Inhibitors - 2,3-Difluoro-*N*-Acteylneuraminic Acid Derivatives. Carbohydrate fluorination, in which fluorine is used to replace a hydroxyl group, has been widely used for studying glycan-protein interactions[186] and developing carbohydrate-based drugs.[186,187] Earlier studies demonstrated that C-3-fluorinated *N*-acetylneuraminic acid worked as a competitive inhibitor for bacterial and viral sialidases.[188] In addition, C-3-fluorinated sialosides were reported to inhibit *C. perfringens* bacterial sialidase[189] and the activities of both hemagglutinins and neuraminidases of the influenza virus.[189,190] A number of studies have demonstrated that family GH33 (CAZy) sialidases catalyze the hydrolysis in a two-step, double-displacement mechanism, in which a tyrosine residue serves as the catalytic nucleophile to form a unique covalent aryl-glycoside intermediate.[191,192] 2,3-Difluoro-*N*-acetylneuraminic acid (2,3-diF-Neu5Ac) was developed as a probe to confirm the covalent aryl-glycoside intermediate in *T. cruzi* trans-sialidase, where it attenuates glycosylation (k_1) and deglycosylation (k_2) rates in the catalytic cycle of the sialidases (Scheme 1).[193,194] Specifically, the 3-F inductively destabilizes the formation of a positive charge during

Scheme 1. Sialidase-Catalyzed Reaction with Mechanism-Based Inhibitor 2,3-diF-Neu5Ac in Glycosylation (k_1) and Deglycosylation (k_2) Steps

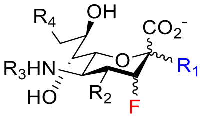


the transition states, thereby reducing the rates of glycosylation (k_1) and deglycosylation (k_2) (Scheme 1). However, the introduction of an anomeric fluorine, a good "leaving group", could counteract the rate-retarding C3−F's effect during glycosylation and increase k_1 only. Another study obtained the cocrystal structure of a covalent intermediate complex at 1.2 Å resolution by cocrystallizing *C. perfringens* NanI sialidase with 2,3-diF-Neu5Ac.[195] These demonstrate that 2,3-diF-Neu5Ac functions as a covalent inhibitor of sialidase, which is also called a mechanism-based inhibitor. $2\alpha,3_{ax}$-diF-Neu5Ac was also demonstrated as a covalent influenza virus NA inhibitor, and the covalent adduct formed between the hydroxyl group of Tyr406 of NA and $2\alpha,3_{ax}$-diF-Neu5Ac.[196] Therefore, mechanism-based inhibitors are useful tools to trap and probe reaction intermediates in enzymatic reactions and also the active sites of the enzymes.

2,3-diF-Sias have the potential to be developed into therapeutics as a novel class of sialidase inhibitors. It was reported that inactivation of *T. cruzi* trans-sialidase by 2,3-diF-Neu5Ac requires very high concentrations of inhibitor (5 mM), which was considered to be largely attributable to the rapid turnover of the covalent intermediate (high k_2). The stability (half-life) of the covalent intermediate is the key to the inhibitory properties of this class of compound.[191] Toward this aim, Hader et al. investigated the contribution by each hydroxyl group of Neu5Ac toward intermediate stabilization of sialidase-catalyzed hydrolysis.[197] So far, several difluoro-Sias were investigated against some parasite trans-sialidases,[131,192,198] bacterial sialidases,[195,199] influenza A viral neuraminidases,[196,200] and human cytosolic sialidase human Neu2 as well (Table 4).[199] 2(*equatorial*), 3(*equatorial*)-DiF-fluoro-Sia with a C4-guanidinium group showed superior *in vitro* anti-influenza A virus efficacy compared to its C4-ammonium or its 2(*equatorial*), 3(*axial*)-diF-Sia counterparts, which is comparable to zanamivir.[200]

Drug resistance has increased drastically to the current anti influenza therapy. Hence, it is urgent to develop potent broad-spectrum antiviral agents that can overcome viral resistance and treat a variety of viral infections. Mechanism-based covalent neuraminidase inhibitors, such as difluoro-Sia,[200] have the potential to achieve both goals. However, possible nonspecific covalent bond formation with other biomolecules could cause side effects and toxicity. In particular, $3F_{ax}$-Neu5Ac could be converted to the corresponding CMP-Sias donor substrate *in vivo*, which could shut down the synthesis of sialylated glycan epitopes. Paulson and co-workers reported the peracetylated analog (P-$3F_{ax}$-Neu5Ac) as a cell-permeable specific inhibitor of the sialyltransferases.[201] This compound has well-known nephrotoxicity, which is a major barrier to its potential therapeutic use.[202] Li et al. explored the selectivity of 2,3-difluoro-Sia by modifications at C-5 and/or C-9 position as well as varying C-3 fluorine stereochemistry (axial or equatorial).[189] As a result, compounds with an axial fluorine at the C-3 position showed better inhibition (up to 100-fold) against bacterial sialidases compared to their C-3 equatorial counterparts. In addition, 9-azido-modified 2,3-diF-Neu5Ac showed increased inhibitory activity against bacterial sialidases; however, C-5-modification showed reduced inhibitory activity. Specifically, 9-azido-9-deoxy-2(equatorial),3(axial)-diF-Neu5Ac (2*e*3*a*DF-Neu5Ac9N_3) was developed as an effective inhibitor with a long effective duration selectively against *C. perfringens* (CpNanI) and *V. cholerae* sialidases (Table 4).[199] Overall, increasing the specificity of binding to the target sites and reducing off-target toxicity are key factors for developing covalent sialidase inhibitors as potential drugs.

Table 4. Mechanism-Based Sialidase Inhibitors[a]



2-position (R1)	3-position (F)	other position 4-R_2	 5-R_3	 9-R_4	sialidase selectivity	ref
F_{ax}	F_{eq}	OH	Ac	OH	ND	188
-OPhNO_2(eq)	F_{ax}	OH	Ac	OH	influenza bacterial	189
-ODSPE(eq)	F_{ax}	OH	Ac	OH	influenza	189, 190
F_{eq}	F_{eq}	OH	Ac	OH	influenza	196, 200
					bacterial	195, 199
					protozoa	192, 198
					hNeu2	199
	F_{eq}	OH	Ac	N_3	hNeu2	199
	F_{eq}	OH	Gc	OH	hNeu2	199
F_{eq}	F_{ax}	OH	Ac	OH	bacterial	199
	F_{ax}	OH	Ac	N_3	bacterial	199
	F_{ax}	guanidino	Ac	OH	influenza	200

[a] ND: data not available; ax, axial; eq, equatorial.

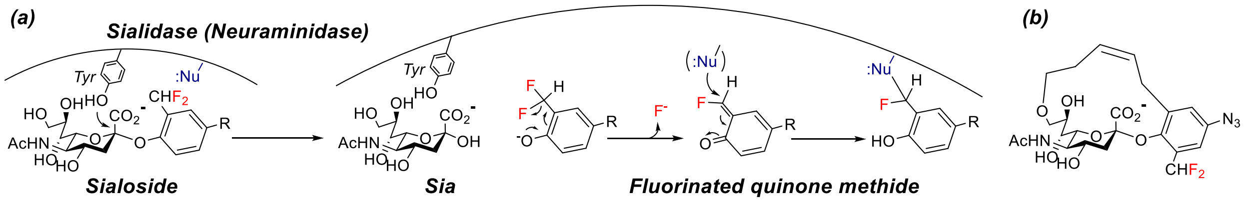


Figure 5. (a) Fluorinated quinone methide-based suicide substrate sialidase inhibitors and their covalent inhibition mechanism and (b) macrocycle-based suicide substrate sialidase inhibitor.

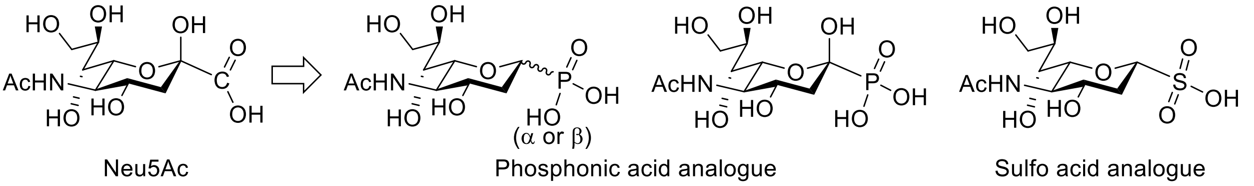


Figure 6. Phosphonic acid and sulfo acid analogues of Sia as product analogue sialidase inhibitors.

2.3. Suicide Substrate Inhibitors of Sialidase: Fluorinated Quinone Methide-Based Inhibitors.

Suicide substrate inhibitors are a class of irreversible inhibitors that react with the enzyme residues through its reactive moieties generated during enzymatic reaction. 2-Difluoromethylphenyl glycosides were first reported as suicide substrate inhibitors of glycosidases in 1990 by Danzin et al., also called mechanism-based inhibitors.[203] Specifically, the phenol aglycone is hydrolytically released by its target enzyme and subsequently transformed into fluorinated quinone methide. This aglycone is a highly reactive electrophilic species that could form a covalent bond with the nucleophilic amino acid residue of its target enzyme and irreversibly inhibits the activity of the enzyme (Figure 5a). Several suicide substrate inhibitors have been developed to glycosidases, including galactosidases[204,205] and *N*-acetyl glucosaminidase.[206] Later, 2-difluoromethyl-4-nitrophenyl glycoside of *α*Neu5Ac was reported as an irreversible inhibitor of *trans*-sialidase with an IC_{50} of 0.6 mM.[207] This suicide substrate inhibitor prevents *T. cruzi* infection of mammalian cells and could serve as a lead compound for developing chemotherapeutics against Chagas disease. Kai et al. made a library of 2-difluoromethylphenyl-sialosides and identified a potent and selective inhibitor for *V. cholerae* and Neu2 sialidase (Figure 5a).[208]

The activation efficiencies of suicide substrate inhibitors depend on the k_{cat} of their target enzymes, and thus, they are called k_{cat} inhibitors.[209] Nevertheless, the inhibition efficiencies of this type of inhibitor depend on three factors: (i) the activations of trifluoromethylphenol groups to form respective reactive quinone methides, (ii) subsequent reaction with amino acid residues at the sialidase active site, and (iii) the diffusion of the difluoromethylphenol and reactive quinone group from the cavity of the active site of the sialidase.[210,211] To overcome this diffusion problem, Kai et al. designed a macrocycle-based suicide substrate sialidase inhibitor by adding a covalent bond between the Sia and aglycone moiety (Figure 5b).[212] By tethering with Sia, the difluoromethylphenol-type aglycone moiety of this inhibitor could stay within the active site of the sialidase after enzymatic cleavage of the sialoside bond and could form a covalent bond with a nucleophilic amino acid side chain of the sialidase. Inhibition assays for various sialidases showed that the irreversible inhibition of this macrocyclic compound depends on the k_{cat} of the sialidase. Those sialidases with small k_{cat} values (influenza viruses, *Clostridium*, *Trypanosoma cruzi*, and Neu2) were inhibited irreversibly, while those with high k_{cat} values (*S. typhimurium* neuraminidase) were not affected by the inhibitor.[208] Overall, suicide substrate sialidase inhibitors can be a versatile tool to elucidate the catalytic mechanism of a target enzyme and serve as drug candidates for certain sialidase targets and diseases.

2.4. Product Analogue Sialidase Inhibitors.

Feedback inhibition is a normal biochemical process to control enzymatic reactions. In this process, the final product inhibits the enzyme and stops the reactions. Therefore, product analogues had been explored for developing novel enzyme inhibitors, which can be used to regulate enzyme activity and study enzyme function as well. Free Neu5Ac is a weak inhibitor of sialidases.[213] Several Sia analogues that mimic the free Sia product structure and its enzyme binding features were developed as sialidase inhibitors and are discussed in this section.

Phosphonic Acid and Sulfo Acid Analogues of Sia. Sialidase active sites contain the triarginyl cluster, which are highly conserved across all known sialidases and could form strong electrostatic interactions with the anomeric carboxyl group of Sias and sialidase inhibitors.[214] For example, in the case of influenza A NA, this triarginyl cluster consists of Arg118, Arg292, and Arg371.[196,215] In the case of *C. perfringens* NanI sialidase, the arginine triad consists of Arg266, Arg555, and Arg615. Replacement of the carboxyl group with a phosphono group was proposed to improve the sialidase inhibitory activity, and therefore, phosphonic acid analogues of Sia were developed as sialidase inhibitors (Figure 6a).[216−219] Obviously, the stronger electrostatic interactions of the anomeric phosphono with the conserved active site NA triarginyl cluster contribute to the increased activity. Interestingly, DANA phosphonates with a C-4-amino/guanidino group were also reported, which showed more potent activity against the NAs of avian and human influenza viruses, including the oseltamivir-resistant strains.[220]

Neu5Ac-derived compounds bearing an anomeric sulfo functional group were also reported as a sialidase inhibitor (Figure 6b).[221] For example, 2-decarboxy-2-deoxy-2-sulfo-*N*-acetylneuraminic acid was reported as a potent inhibitor of avian-origin H5N1 neuraminidase and drug-resistant His275-Tyr NA as compared to the transition state analogue DANA. The sulfo-Neu5Ac analogue was demonstrated as a more potent inhibitor of influenza NA (up to 40-fold) and bacterial sialidase

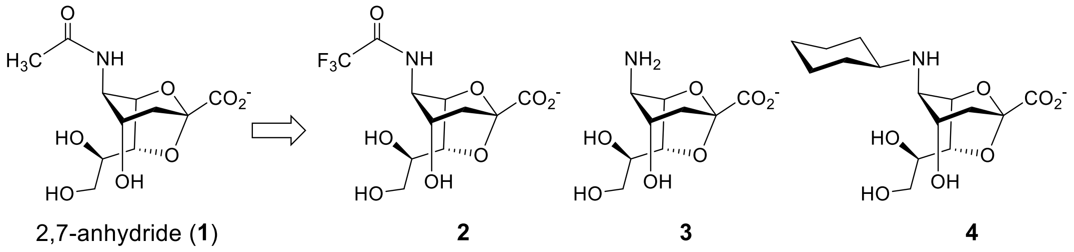


Figure 7. Structures of 2,7-anhydro-Neu5Ac (**1**) and its derivatives **2**−**4**.[229]

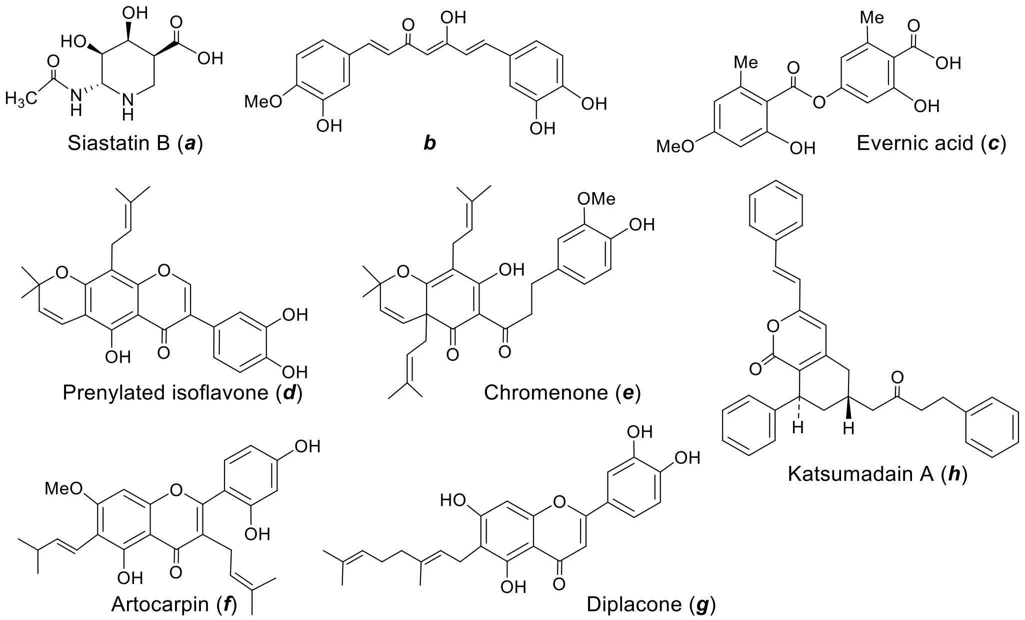


Figure 8. Natural product bacterial sialidase inhibitors. (a) siastatin B, (b) 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one, (c) evernic acid, (d) prenylated isoflavone, (e) chromenone, (f) artocarpin, (g) diplacone, and (h) katsumadain A.

(up to 8.5-fold) relative to the corresponding anomeric phosphonic acids. The anomeric sulfo functional group could enhance electrostatic interactions with the triarginyl cluster. In addition, it serves as a strong electron withdrawing group that could destabilize oxocarbenium ion formation. These results confirm that anomeric sulfo modification offers another type of sialidase inhibitor, its sialidase selectivity deserves further investigation.

*2,7-Anhydro-**N**-acetylneuraminic Acid (2,7-Anhydro-Neu5Ac) Derivatives.* 2,7-Anhydro-*N*-acetylneuraminic acid (2,7-anhydro-Neu5Ac, **1**) (Figure 7) was initially found in rat urine[222] and human wet cerumen[223] as another kind of free Sia. It was found to be the product from the hydrolysis of sialosides catalyzed by intramolecular *trans*-sialidase (IT-sialidase L) from *Macrobdella decora* (leech),[224,225] Gram-positive human pathogenic bacterium *S. pneumoniae*,[226] and Gram-positive human gut commensal *Ruminoccocus (R) gnavus*.[227] It was shown that 2,7-anhydro-Neu5Ac serves as a sole carbon source for the growth of *R. gnavus* in the Sia-rich host gut environment.[228] Interestingly, a recent study indicates that 2,7-anhydro-Neu5Ac derivatives were selective sialidase inhibitors against *S. pneumoniae* sialidases SpNanB and SpNanC.[229] On the basis of crystal structure analysis, several 2,7-anhydro-Neu5Ac derivatives were designed, synthesized, and tested for inhibitory activities against several GH33 family sialidases (Figure 7). 2,7-Anhydro-Neu5TFA (**2**) showed some inhibitory activity against SpNanA, SpNanB, AuSialidase, and VcSialidase. 2,7-Anhydro-Neu5Cyclohexyl (**4**) showed noticeable inhibitory activity against SpNanA, SpNanB, and SpNanC. This study demonstrated an effective product analogue strategy for exploring potential selective inhibitors of intramolecular *trans*-sialidases.

2.5. Natural Products as Sialidase Inhibitors. The development of novel sialidase inhibitors has been largely based on synthetic compounds. Natural products provide diverse chemical scaffolds for drug discovery. A variety of natural compounds have been screened for antisialidase activity, specifically, diarylheptanoid katsumadain A, flavonoids artocarpin, apigenin, luteolin, gossypetin, oligostilbenes viniferin C and pedicularioside, and phenylpropanoid crenatoside. Among these natural compounds, artocarpin, kaempferol, and quercetin analogues were found to be the most potent sialidase inhibitors.[148] This section summarizes major natural product sialidase inhibitors.

Natural Product Viral Sialidase Inhibitors. In the past, various natural compounds have been studied for influenza neuraminidase inhibition. A 2012 review illuminated the research efforts of the first decade of the 21st century (2000−2011), focusing on the structure and influenza neuraminidase inhibition activity of natural products.[148] Approximately 150 natural product compounds were tested for their influenza neuraminidase-inhibiting potential during this period. Among those, flavonoids and (oligo)stilbenes were the most prominent scaffolds. A 2019 review updated recent discoveries of natural products as neuraminidase inhibitors by highlighting their

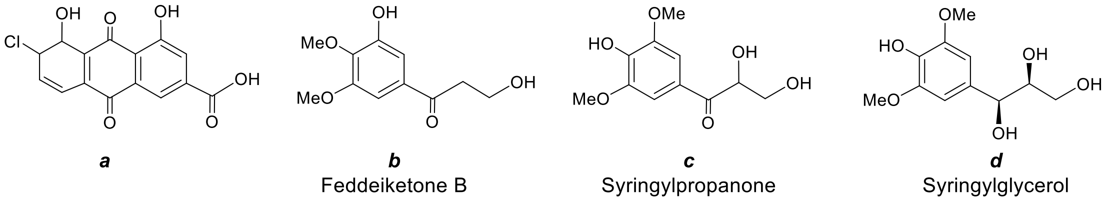


Figure 9. Natural product protozoan sialidase inhibitor, (a) 6-chloro-9,10-dihydro-4,5,7-trihydroxy-9,10-dioxo-2-anthracenecarboxylic acid and human sialidase inhibitors, (b) Feddeiketone B, (c) 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-L-propanone, and (d) syringylglycerol.

structure, function, and inhibition mechanism.[149] About 267 plant secondary metabolites were tested from 2011 to 2017 for their neuraminidase inhibition activity. More detailed information about various natural neuraminidase inhibitors and neuraminidase inhibition assays can be found in these two comprehensive reviews.[148,149] Overall, natural compounds may serve as good lead structures for the discovery and development of potent viral neuraminidase inhibitors.

Natural Product Bacterial Sialidase Inhibitors. Bacterial sialidases play important roles in the pathogenesis of bacterial infection. Various natural compounds were tested for bacterial sialidase inhibition as well. Siastatin B (SB) was initially reported as an inhibitor of *Streptomyces* sialidase.[157] SB has a 6-acetamido-3-piperidine carboxylate structure, which is similar with *N*-acetylneuraminic acid (Figure 8a). SB also shows inhibitory activity against *C. perfringens* sialidase activity.[21,230,231] A recent study demonstrated that SB reduced the growth and survival rate of strain F4969 in the presence of Caco-2 cells.[232] A curcumin derivative, 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl) hepta-1,4,6-trien-3-one (Figure 8b), was reported to inhibit *S. pneumoniae* NanA, *V. cholerae*, and *C. perfringens* sialidases.[233] Another research indicated that the flavonoid diplacone showed inhibitory activity against *C. perfringens* sialidases (*Cp-NanI*).[234] In addition, prenylated isoflavone[235] and chromenone derivatives[236] obtained from *Flemingia philippinensis* exhibit significant inhibition against bacterial sialidase. Park et al. reported phenolic metabolite Evernic acid (Figure 8c), isolated from the methanol extract of *Usnea longissimi*, displayed dose-dependent inhibition against bacterial sialidase.[237] Interestingly, artocarpin and katsumadain A show inhibitory activity against both influenza and *S. pneumoniae* sialidases.[238] Other curcumin and flavanoid derivatives were explored as bacterial sialidase inhibitors.[233,234,239] Overall, natural products provide an alternative resource for the development of new bacterial sialidase inhibitors.

Natural Product Protozoa Sialidase Inhibitors. The protozoan *T. cruzi* trans-sialidase (TcTS) is an attractive drug target for Chagas' disease. Therefore, TcTS inhibitors could be used as therapeutics for the treatment of Chagas' disease. DANA shows an IC_{50} value of several hundred micromolar against TcTS. Flavonoid and anthraquinone derivatives show strong inhibitory activity against TcTS.[240] Specifically, 6-chloro-9,10-dihydro-4,5,7-trihydroxy-9,10-dioxo-2-anthracenecarboxylic acid (Figure 9a) was reported as a specific TcTS inhibitor with a IC50 value of 0.58 μM.[240] The structure−activity relationship (SAR) analysis of the flavonoids revealed that apigenin has the minimal structure necessary for inhibition and may serve as a lead for drug discovery against Chagas' disease.[240]

Human Sialidase Natural Product Inhibitors. Natural compounds have been explored for human sialidase inhibition in recent studies. Albrecht et al. described the identification and evaluation of human Neu1 inhibitor extracted from *Olyra latifolia* L.[241] Specifically, Feddeiketone B (Figure 9b), 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-L-propanone (Figure 9c), and syringylglycerol (Figure 9d) show inhibition effects on the cell membrane Neu1 sialidase activity. These compounds present structural similarities with DANA, and further investigation may be valuable for elucidating the biological functions of human sialidase and exploring potent human sialidase inhibitors.

3. SUMMARY AND FUTURE PERSPECTIVE

Sialidases (neuraminidases) catalyze the removal of Sia residue from sialoglycans and modulate their biological activity and thus are involved in numerous physiological and pathological processes. Expression of sialidases has been confirmed in a variety of organisms and viruses. In humans, four subtypes of sialidases (Neu1−4) have been identified; however, their functions have not been fully clarified. Sialidase inhibitors are highly needed for analysis of the precise function of sialidases and related physiological and pathological processes and development of novel drugs against specific sialidase. Over the past decades, sialidase inhibitors have received a great deal of interest, and various sialidase inhibitors have been developed and even used as drugs, such as anti-influenza drugs. Most sialidase inhibitors are developed by mimicking the transition state of sialidase-catalyzed hydrolysis reactions. In addition, mechanism-based inhibitors, suicide substrate inhibitors, and product analogue inhibitors have been developed. Various natural products have also been extensively isolated and tested for influenza virus, bacterial, and human sialidase inhibition. Nature provides an abundance of structurally diverse chemical scaffolds for lead structures in drug development. It is expected that new pharmacophore models from natural products will be identified, which will provide insights into the sialidase binding site, therefore helping develop selective and potent inhibitors of each sialidase isoenzyme.

Influenza virus neuraminidase inhibitors (oseltamivir, zanamivir, laninamivir, and peramivir) have been widely used in the treatment of influenza infection. However, the new strains of influenza virus are becoming resistant to current neuraminidase inhibitors, presenting serious threats to public health. Therefore, new neuraminidase inhibitors against drug-resistant influenza strains are in high demand. Bacterial pathogens produce sialidases for invasion, infection, and replication. Secondary pneumococcal infections cause severe complications in influenza patients. Therefore, development of inhibitors against both viral and bacterial sialidases could be of great interest. Dual inhibitors acting on both neuraminidases of *S. pneumoniae* and the influenza virus were demonstrated recently.[238,242,243] In addition, it was observed that viral neuraminidase inhibitor oseltamivir has neuropsychiatric side effects.[244] Therefore, for development of inhibitors of sialidases produced by pathogens, it is essential to test their activity on human sialidases and to know if they have side effects *in vivo*.

The surface of an influenza virus particle holds about 50 tetrameric neuraminidase spikes,[245] each spike is a homotetrameric enzyme that could bind four sialosides.[246] Therefore, multivalent influenza virus neuraminidase inhibitors have been proposed and tested.[247] To date, dimeric, trimeric, tetrameric, and polymeric zanamivir derivatives linked through the C-7 hydroxyl group were prepared and showed outstanding antiviral potency. Previous multivalent influenza virus neuraminidase inhibitors were summarized in a 2007 review paper.[247] Since then, several multivalent influenza virus neuraminidase inhibitors have been reported.[248−250] Conjugation of the transition-state analogue DANA to polymeric scaffolds, on the other hand, produces highly potent inhibitors of bacterial sialidases.[251] More than 4 orders of magnitude are added to the inhibitory potency of each clustered DANA for *S. pneumoniae* or *B. thetaiotaomicron* sialidases. This extends the multivalent concept to this important class of bacterial sialidases. This multivalent inhibition strategy provides interesting perspectives for other sialidase families, such as parasitic or human sialidases.

Human sialidases (Neu1−4) play important roles in many physiological processes but are also involved in numerous diseases and disorders.[7] Therefore, human sialidases are promising pharmacological targets. Selective inhibitors of individual human sialidases are essential for a specific disease. Structure-based drug design is highly expected. Except for Neu2 sialidase, there are no 3D structure reported for Neu1, Neu3, and Neu4, which makes selective inhibitor design more difficult. Protein homology modeling based on the crystal structure of the Neu2 enzyme is used for human sialidase inhibitor development.[95] It was found that the binding mode of the glycerol group of DANA is different between human, viral, and bacterial sialidases.[240] This difference may play a role in substrate specificity and provides a new insight for designing selective sialidase inhibitors.

The overexpression and activation of Neu1 cause disorders and thus are of interest for regulation. On the other hand, cell surface relocation of Neu1 has been confirmed in different cell types including immune cells, where it could regulate the sialylation of several receptors and subsequent signaling pathways.[28−52] The extensive review by Pshezhetsky et al. describes the key pathways in which desialylation of cell surface receptors by Neu1 modulates cellular signaling and molecular targeting.[5] Therefore, regulation of Neu1 on the cell surface is highly desired. In other words, the cell surface Neu1 selective inhibitor is required to specifically regulate its activity on the cell surface and subsequent signaling pathways. Interestingly, natural product feddeiketone B, 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-L-propanone, and syringylglycerol (Figure 9) show inhibitory effects against Neu1-mediated sialidase activity at the plasma membrane.[149] Further investigation of these compounds against Neu1 on the live cell surface is expected as they may be valuable for elucidating the biological functions of cell surface Neu1 and useful for regulating its subsequent signaling pathways. However, special attention should be paid when using natural compounds that contain catechol and quinone since they are known to contribute to false positives as pan-assay interference compounds (PAINs).[252,253] They are known to interfere with bioassays via different mechanisms. For example, catechols can chelate metals and is reactive in the oxidized form to nucleophilic amino acids in proteins, such as cysteine and lysine.[252]

Overall, sialidases are involved in numerous physiological and pathological processes and thus are potential therapeutic targets. Development of sialidase inhibitors is important for studying the functions of sialidases and developing therapeutic drugs, but only a few selective inhibitors of sialidase have been developed. New selective sialidase inhibitors with novel scaffolds and new mechanisms of inhibition are highly expected. The important research areas to develop selective sialidase inhibitors are to (i) understand the mechanism of action, (ii) define cellular location of action, and (iii) identify the active site of each sialidase. In particular, determining the 3D structure of a sialidase is fundamental for understanding its function and properties and designing selective inhibitors. We expect that this Perspective gives an in-depth insight into several aspects of inhibitory mechanisms of sialidases and their inhibitors. Additionally, providing ample references about sialidase inhibitors with different mechanisms may be helpful for the ongoing study of sialidases and development of therapeutic agents.

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Xue-Long Sun received a Ph.D. in Pharmaceutical Chemistry from Kitasato University School of Pharmacy (Japan) in 1997. Then, he had worked on synthesis of sialidase and sialyltransferase inhibitors at RIKEN Institute (Japan) as a basic science special research fellow (Drs. Chi-Huey Wong, Osamu Kanie, and Yukishige Ito) and antithrombotic biomaterials at Emory University School of Medicine as a NIH-funded research fellow (Dr. Elliot L. Chaikof). He started his academic career at Emory University School of Medicine (2002−2006) and has moved to Cleveland State University as a Full Professor of Medicinal Chemistry (primary) and Biomedical Engineering (secondary) now. His group is working on Sia-focused glycomics, chemical glycobiology and medicinal chemistry, glycopolymers, and glyco-liposomes-based biomaterials. Furthermore, he has been working recombinant thrombomodulin expression and glyco-engineering research.

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■ ABBREVIATIONS USED

2,7-anhydro-Neu5Ac, 2,7-Anhydro-*N*-acetylneuraminic acid; ApoB100, Apolipoprotein B100; ATG5, Autophagy related 5; ax, axial; CAZy, carbohydrate active enzyme; CD protein, cluster of differentiation protein; 2,3-diF-Neu5Ac, 2,3-difluoro-*N*-acetylneuraminic acid; EGFR, epidermal growth factor receptor, eq: equatorial, Gal: galactose, GalNAc: *N*-acetyl galactosamine, GH, glycoside hydrolase; GI, gastrointestinal; HGF, hepatocyte growth factor; HN, hemagglutinin-neuraminidase; IR, insulin receptor; LAP, latency-associated peptide; MMP9, matrix metallopeptidase 9; MUC1, mucin 1; NA, neuraminidase; Neu5Ac, *N*-acetylneuraminic acid; Neu5Ac2en2, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid; PPCA, protective protein/cathepsin A; Sias, sialic acids; SB, siastatin B; TGF-*b*, transforming growth factor beta; TLR, toll-like receptor; TrkA, tropomyosin receptor kinase A.

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