

Organic & Biomolecular Chemistry

This article is part of the

OBC 10th anniversary
themed issue

All articles in this issue will be gathered together
online at

www.rsc.org/OBC10



Cite this: *Org. Biomol. Chem.*, 2012, **10**, 6112

www.rsc.org/obc

PAPER

Synthesis of selective inhibitors against *V. cholerae* sialidase and human cytosolic sialidase NEU2†‡

Zahra Khedri,§ Yanhong Li,§ Hongzhi Cao,¶ Jingyao Qu, Hai Yu, Musleh M. Muthana and Xi Chen*

Received 16th February 2012, Accepted 8th May 2012

DOI: 10.1039/c2ob25335f

Sialidases or neuraminidases catalyze the hydrolysis of terminal sialic acid residues from sialyl oligosaccharides and glycoconjugates. Despite successes in developing potent inhibitors specifically against influenza virus neuraminidases, the progress in designing and synthesizing selective inhibitors against bacterial and human sialidases has been slow. Guided by sialidase substrate specificity studies and sialidase crystal structural analysis, a number of 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA or Neu5Ac2en) analogues with modifications at C9 or at both C5 and C9 were synthesized. Inhibition studies of various bacterial sialidases and human cytosolic sialidase NEU2 revealed that Neu5Gc9N₃2en and Neu5AcN₃9N₃2en are selective inhibitors against *V. cholerae* sialidase and human NEU2, respectively.

Introduction

Sialidases or neuraminidases (EC 3.2.1.18) are common exoglycosidases (EC 3.2.1.18) that catalyze the cleavage of terminal sialic acid residues from sialyl oligosaccharides, glycolipids, and glycoproteins. They are expressed by various organisms varying from bacteria and viruses to fungi, protozoa, animals, and humans.^{1,2} Endo-sialidases (EC 3.2.1.129) that catalyze the hydrolysis of internal sialyl glycosidic bonds have also been reported.³ Based on their protein sequence similarities, all exo-sialidases known to date are mainly grouped into three glycoside hydrolase families (GH33, GH34, and GH83) in the Carbohydrate Active enZyme (CAZy) database (<http://www.cazy.org>).⁴ Bacterial, mammalian, and protozoan sialidases as well as trans-sialidases (EC 2.4.1.-) are grouped into the GH33 family. Neuraminidases from influenza A and B viruses belonging to GH34 and other viral neuraminidases are grouped into GH83. In addition, a mouse klotho in GH1 has been found to have sialidase activity.^{5,6} Several sialyltransferases in CAZy database, such as glycosyltransferase families GT42⁷ and GT80^{8–10} have also been shown to possess sialidase and/or trans-sialidase activities.^{11,12} Despite their primary sequence differences, bacterial,

viral, and human sialidases share a catalytic domain with a common canonical six-bladed β-propeller fold.¹³

Inhibitors specifically against neuraminidases of influenza viruses have been developed and applied efficiently for the treatment of flu.¹⁴ However, the emergence of drug-resistant viral strains and the increasing threat of viral pandemics¹⁵ urge the discovery of new neuraminidase inhibitors. Due to their important nutritional and pathogenic roles, bacterial sialidases, including those from *Vibrio cholerae* (causes cholera),¹⁶ *Streptococcus pneumoniae* (causes Otitis media in children),¹⁷ gram positive anaerobic bacteria *Clostridium perfringens* (causes gas gangrene disease), and *Salmonella typhimurium* LT2 (causes gastroenteritis)^{18,19} are being considered as potential drug targets. Human sialidases, including lysosomal sialidase NEU1, cytosolic sialidase NEU2, and membrane-associated sialidase NEU3 and NEU4²⁰ have been shown to be related to a number of diseases such as cancer and type I and II sialidosis.^{21,22} They are currently being studied as important targets for inhibitor design.

The strategy of protein crystal structure-based rational design of sialidase inhibitors has been validated by the success of designing influenza virus neuraminidase inhibitors^{23–25} as clinically effective anti-influenza drugs [*e.g.*, Zanamivir (Relenza)¹⁴ and Oseltamivir (Tamiflu)²⁶]. The development of effective and selective inhibitors against bacterial and human sialidases has been less successful despite the fact that an increasing number of bacterial sialidase crystal structures are becoming available. A number of derivatives of the common sialidase transition state analogue 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en or DANA, **1**) have been synthesized as potential inhibitors against human NEU1,²⁷ NEU2,²⁸ and NEU3,^{20,29,30} as well as bacterial (*e.g.* *V. cholerae*^{31,32} and *C. perfringens*³²) sialidases. However, the inhibitory efficiency of the obtained

Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616, USA. E-mail: chen@chem.ucdavis.edu; Fax: +1-5307528995; Tel: +1-5307546037

† This article is part of the Organic & Biomolecular Chemistry 10th Anniversary issue.

‡ Electronic supplementary information (ESI) available: ¹H and ¹³C NMR spectra for compound 4–8. See DOI: 10.1039/c2ob25335f

§ These authors contributed equally.

¶ Current address: National Glycoengineering Research Center, Shandong University, Jinan, Shandong 250012, China.

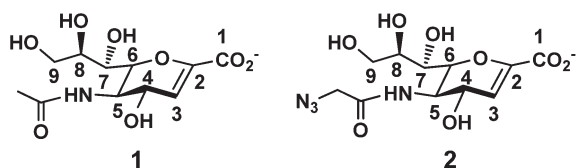


Fig. 1 Structures of Neu5Ac2en **1** and Neu5AcN₃2en **2**.

inhibitors was usually similar to or even lower than Neu5Ac2en (**1**) (Fig. 1).

Recently, conformational flexible loops close to the catalytic site of sialidases were used as potential targets for inhibitor design.^{13,33,34} Based on information gained from mechanism-based labelling, a potent inhibitor selective against *V. cholerae* sialidase with a K_i of 73 nM was identified by screening of a focused library.³⁵

We have shown previously that results from sialidase substrate specificity studies can be used to guide the design of selective sialidase inhibitors.¹¹ In this strategy, α 2–3- and α 2–6-linked sialyl galactosides containing different sialic acid forms or analogues are synthesized and used as sialidase substrates. The structural features of sialic acid forms in substrates selectively cleaved by certain sialidases can be introduced to Neu5Ac2en to obtain inhibitors that are selective against target sialidases. Indeed, sialidase substrate specificity studies using α 2–3- and α 2–6-linked sialyl galactosides with C5-modified sialic acid residues^{11,36} helped to identify Neu5AcN₃2en (**2**) (a C5-derivative of Neu5Ac2en) (Fig. 1) as a selective inhibitor against human NEU2.¹¹ Herein, we explore the application of the strategy in designing additional selective sialidase inhibitors.

Results and discussion

Designing of sialidase inhibitors

To identify the logical positions for systematic modifications, the structures of sialidase-bound inhibitor Neu5Ac2en (a common transition-state analogue) in the reported crystal structures of bacterial and human sialidases were compared. Fig. 2 shows that in addition to the *N*-acetyl group at C5, two significant differences of sialidase-bound Neu5Ac2en are the positions of C9 and the orientations of the hydroxyl groups attached to C9 (Fig. 2).^{13,37–42} Therefore, modifications at C9 or at both C9 and C5 will most likely provide selectivity for Neu5Ac2en-based sialidase inhibitors.

Our recent substrate specificity studies of bacterial sialidases from *C. perfringens*, *V. cholerae*, *S. typhimurium*, and *S. pneumoniae* as well as human NEU2 using thirty α 2–3/6-linked sialosides containing C9-modified sialic acid⁴³ indicate that α 2–3-linked sialoside containing Neu5Ac9N₃ (Neu5Ac9N₃ α 2–3Gal β pNP) is a similarly good substrate as the sialoside containing non-modified Neu5Ac (Neu5Ac α 2–3Gal β pNP) for bacterial sialidases. In contrast, α 2–3-sialoside containing Neu5Gc9N₃ (Neu5Gc9N₃ α 2–3Gal β pNP) is a similarly good or better substrate than sialoside containing non-modified Neu5Ac (Neu5Ac α 2–3Gal β pNP) for human NEU2 and *V. cholerae* sialidase, but not for other bacterial sialidases tested including

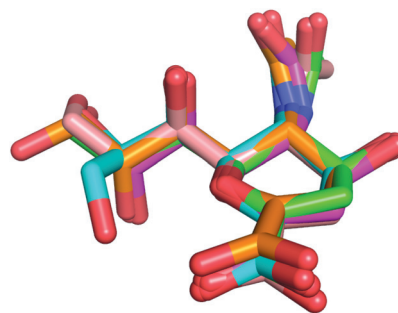


Fig. 2 Overlay of sialidase-bound Neu5Ac2en structures in the reported crystal structures of sialidases^{11,36–41} from *S. pneumoniae* (NanB, carbons are shown in cyan), *V. cholerae* (carbons are shown in light pink), *C. perfringens* (NanI, carbons are shown in orange), *S. typhimurium* (carbons are shown in magenta), and human NEU2 (carbons are shown in green). Nitrogen atoms are shown in blue and oxygen atoms are shown in red. Neu5Ac2en structures are manually overlaid in PyMOL to allow the maximum overlap of the ring and C1 carboxyl of Neu5Ac2en.

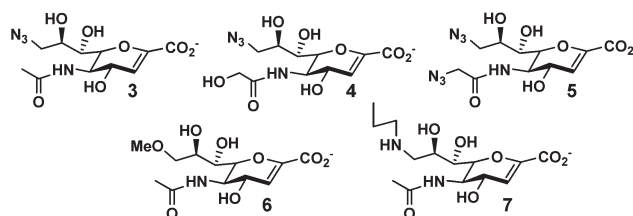


Fig. 3 Structures of Neu5Ac9N₃2en **3**, Neu5Gc9N₃2en **4**, Neu5AcN₃9N₃2en **5**, Neu5Ac9Me2en **6**, and Neu5Ac9NPro2en **7**.

C. perfringens, *S. typhimurium*, and *S. pneumoniae* sialidases. Therefore, we hypothesized that Neu5Ac9N₃2en (**3**) would be a similarly good inhibitor as Neu5Ac2en against *C. perfringens*, *V. cholerae*, *S. typhimurium*, and *S. pneumoniae* bacterial sialidases but will be a weaker inhibitor, compared to Neu5Ac2en, for human NEU2. In addition, Neu5Gc9N₃2en (**4**) would be a similarly good inhibitor as Neu5Ac2en against human NEU2 and *V. cholerae* sialidase, but a worse inhibitor against *C. perfringens*, *S. typhimurium*, and *S. pneumoniae* sialidases. Furthermore, as we demonstrated before,¹¹ azido modification at C5-NHAc group on Neu5Ac2en provided an improved and selective inhibitor against human NEU2. Therefore, we predicted that combining the features of azido modifications at both C5-NHAc group and C9 of Neu5Ac2en will result in a selective inhibitor (Neu5AcN₃9N₃2en, **5**) against human NEU2. Also, Neu5Ac9Me2en (**6**) with a methoxy at C9 of Neu5Ac2en may be a better or a similarly good inhibitor compared to Neu5Ac2en against *S. typhimurium* or *S. pneumoniae* sialidase based on our substrate specificity tests. To test these hypotheses, we synthesized a library of Neu5Ac2en analogues with modifications at C9 and both C5 and C9, including Neu5Ac9N₃2en (**3**), Neu5Gc9N₃2en (**4**), Neu5AcN₃9N₃2en (**5**), and Neu5Ac9Me2en (**6**) (Fig. 3). In addition, a Neu5Ac2en derivative with a C9-propylamine modification (Neu5Ac9NPro2en, **7**) was synthesized to test the effect of a larger C9-group in sialidase inhibitory activities.

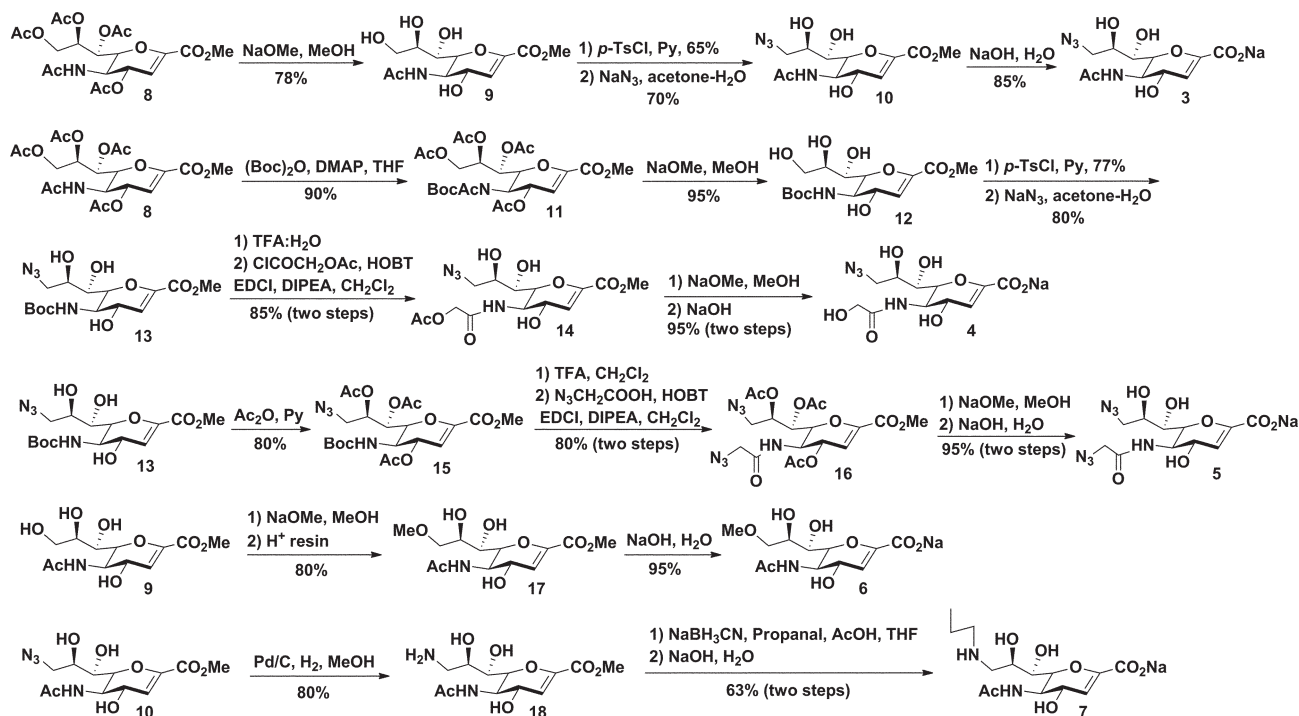
Synthesis of sialidase inhibitors

Compounds 9-azido-9-deoxy-Neu5Ac2en (Neu5Ac9N₃2en, **3**), 9-azido-9-deoxy-Neu5Gc2en (Neu5Gc9N₃2en, **4**), 9-azido-5-(2-azidoacetamido)-5,9-dideoxy-Neu5Ac2en (Neu5AcN₃9N₃2en, **5**), 9-*O*-methyl-Neu5Ac2en (Neu5Ac9OMe2en, **6**), and 9-amino-propyl-5,9-dideoxy-Neu5Ac2en (Neu5Ac9NPro2en, **7**) were synthesized as shown in Scheme 1. Compound Neu5Ac9N₃2en (**3**) was generated using a reported method with optimization.^{27,29} Neu5Gc9N₃2en (**4**) was synthesized from compound **8** by treating it with Boc₂O⁴⁴ to produce *N*-acetyl-*N*-Boc protected product **11**, which was deacetylated under Zemplén condition to form **12**. Selective tosylation of the primary hydroxyl group of **12** followed by azidation provided intermediate **13**. After removal of the Boc group of **13** with TFA in water, the obtained amine was treated with acetoxyacetyl chloride in the presence of base DIPEA and coupling reagents HOBT and EDCI in CH₂Cl₂ followed by deprotection produced the desired compound Neu5Gc9N₃2en (**4**) in a high yield. To synthesize Neu5AcN₃9N₃2en (**5**), compound **13** was acetylated and the Boc protecting group was removed by treatment with TFA in CH₂Cl₂. Coupling of the obtained amine with N₃CH₂COOH in the presence of EDCI–HOBT afforded compound **16** in a good yield. Deprotection of **16** followed by hydrolysis in aqueous NaOH afforded the desired product Neu5AcN₃9N₃2en (**5**). Compound Neu5Ac9OMe2en (**6**) was obtained by base (NaOH in H₂O)-catalyzed hydrolysis of compound **17** which was formed from intermediate **9** by gradual addition of a solution of NaOMe in MeOH followed by neutralization with Dowex (H⁺) resin. To prepare Neu5AcN₃9NPro2en (**7**), compound **10** was hydrogenated under atmospheric pressure to give amine **18**. Reductive amination of **18** with propanal and subsequent demethylation provided the desired product **7** in a moderate yield.

Inhibition studies of bacterial sialidases and human NEU2

The IC₅₀ values of the C9- as well as both C9- and C5-modified Neu5Ac2en derivatives **3–7** synthesized above were determined using human NEU2 and four commercially available bacterial sialidases from *Salmonella typhimurium* (Prozyme), *Clostridium perfringens* (Sigma), *Vibrio cholerae* (Sigma), and *Streptococcus pneumoniae* (NanB, Prozyme). Inhibition studies were conducted using Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP as sialidase substrate. As shown in Table 1, Neu5Ac9N₃2en (**3**) was a similarly effective inhibitor as Neu5Ac2en (**1**) against α 2–3-specific *S. typhimurium* and *S. pneumoniae* sialidases as predicted. However, unlike the prediction, Neu5Ac9N₃2en (**3**) was 3-fold less efficient than Neu5Ac2en (**1**) against *C. perfringens* sialidase when either Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP was used as the substrate. For *V. cholerae* sialidase and human NEU2, Neu5Ac9N₃2en (**3**) was 2.3-fold or 2.4-fold less efficient than Neu5Ac2en (**1**) when Neu5Ac α 2–3Gal β pNP was used as the substrate, while the IC₅₀ values of these two inhibitors were similar when Neu5Ac α 2–6Gal β pNP was used as the substrate. Neu5Ac9N₃2en (**3**) along with a series of C9-triazole derivatives of Neu5Ac2en have been tested previously as inhibitors against human NEU3.²⁹ Neu5Ac9N₃2en (**3**) showed less inhibition activity against NEU3 than Neu5Ac2en (**1**). Nevertheless, slightly improved activity was observed using phenyl-, hexyl-, and phenoxyethyl-C9-triazole Neu5Ac2en derivatives (IC₅₀ values were 20, 23, and 45 μ M, respectively).²⁹

As predicted from substrate specificity studies,⁴³ Neu5Gc9N₃2en (**4**) was a weaker inhibitor than Neu5Ac2en (**1**) against *C. perfringens*, *S. typhimurium*, and *S. pneumoniae* sialidases. However, in disagreement with the prediction, Neu5Gc9N₃2en (**4**) was also a weaker inhibitor than Neu5Ac2en (**1**) against



Scheme 1 Synthesis of Neu5Ac2en derivatives.

Table 1 IC₅₀ values of potential inhibitors against bacterial and human sialidases using either Neu5Acα2–3GalβpNP or Neu5Acα2–6GalβpNP as sialidase substrate

Sialidases	IC ₅₀ (μM) with Neu5Acα2–3GalβpNP as sialidase substrate					
	Neu5Ac2en (1)	Neu5Ac9N ₃ 2en (3)	Neu5Gc9N ₃ 2en (4)	Neu5AcN ₃ 9N ₃ 2en (5)	Neu5Ac9Me2en (6)	Neu5Ac9NPro2en (7)
<i>C. perfringens</i>	20 ± 1	59 ± 5	(1.9 ± 0.4) × 10 ³	(1.6 ± 0.2) × 10 ³	(4.6 ± 0.6) × 10 ²	>1 × 10 ³
<i>V. cholerae</i>	8.6 ± 1.0 ^a	20 ± 1	18 ± 3	(1.4 ± 0.1) × 10 ²	(1.3 ± 0.3) × 10 ²	>1 × 10 ³
<i>S. typhimurium</i>	(2.0 ± 0.1) × 10 ^{2a}	(2.1 ± 0.1) × 10 ²	>1 × 10 ⁴	(1.6 ± 0.4) × 10 ⁴	(1.3 ± 0.2) × 10 ²	(2.3 ± 0.3) × 10 ³
<i>S. pneumoniae</i>	(3.7 ± 0.6) × 10 ³	(3.3 ± 0.4) × 10 ³	>1 × 10 ⁴	>1 × 10 ³	(3.0 ± 0.6) × 10 ³	(5.4 ± 0.6) × 10 ³
NEU2	18 ± 1 ^a	43 ± 2	(1.5 ± 0.3) × 10 ²	13 ± 4	(1.1 ± 0.1) × 10 ³	>1 × 10 ⁴
IC ₅₀ (μM) with Neu5Acα2–6GalβpNP as sialidase substrate						
<i>C. perfringens</i>	13 ± 1	40 ± 4	(1.4 ± 0.4) × 10 ³	(1.2 ± 0.2) × 10 ³	(3.1 ± 0.6) × 10 ²	>1 × 10 ³
<i>V. cholerae</i>	10 ± 1 ^a	8.7 ± 1.0	13 ± 2	(1.1 ± 0.2) × 10 ²	(1.2 ± 0.3) × 10 ²	>1 × 10 ³
NEU2	32 ± 6 ^a	31 ± 6	(1.9 ± 0.7) × 10 ²	13 ± 3	(4.1 ± 0.6) × 10 ²	(2.4 ± 0.8) × 10 ³

^a Data are from ref. 11.

NEU2 and *V. cholerae* sialidase. Although with a mildly decreased IC₅₀ value compared to Neu5Ac2en (1) in inhibiting *V. cholerae* sialidase, Neu5Gc9N₃2en (4) was shown to be a selective inhibitor against this enzyme.

Also as expected, Neu5AcN₃9N₃2en (5) was a selective inhibitor against human NEU2 with a slightly improved IC₅₀ compared to Neu5Ac2en (1) when either Neu5Acα2–3GalβpNP or Neu5Acα2–6GalβpNP was used as the substrate. The inhibitory efficiency was comparable to other reported human sialidase inhibitors.^{27–29} These results indicate that Neu5AcN₃9N₃-containing sialosides are most likely suitable substrates for human NEU2. A previous report using a library of octyl sialyllactosides with modifications at the Neu5Ac residue as sialidase substrates also showed that Neu5Ac with an azide at C5 or C9 could be cleaved by human membrane-associated sialidases NEU3.²⁹

Neu5Ac9OMe2en (6) and Neu5AcN₃9NPro2en (7) with a methoxy and a propylamine substitution, respectively, at C9 of Neu5Ac2en (1) were shown to be weak inhibitors against all five sialidases tested. The inhibitory activities of similar compounds as 7 with amide-linked C9-modified Neu5Ac2en analogues were studied against NEU1–NEU4.²⁷ All the compounds were found to be poor inhibitors of the NEU2–NEU4. However, selective inhibitors with slightly improved inhibition compared to Neu5Ac2en were observed for those containing a methyl, propyl, butyl, or phenyl group.

Inhibition studies of compounds 1, 3, 4, 5, 6, and 7 against β-galactosidase were also carried out. Using 1 mM of GalβpNP as the substrate for β-galactosidase, no inhibitory effect was observed for any of the compounds with a final concentration of 100 μM or 1 mM (data not shown). This confirms that the selective inhibitory activities of Neu5Gc9N₃2en (4) and Neu5AcN₃9N₃2en (5) against *V. cholerae* sialidase and human NEU2, respectively, are not due to inhibition of the compounds against β-galactosidase in the assays.

Docking studies of compounds 3, 4, and 5 in the crystal structures of human NEU2 and *V. cholerae* sialidase

To help the understanding of the selectivity of Neu5Gc9N₃2en (4) and Neu5AcN₃9N₃2en (5) in inhibiting *V. cholerae* sialidase

and human NEU2, respectively, the structure of Neu5Ac9N₃2en (3), Neu5Gc9N₃2en (4), or Neu5AcN₃9N₃2en (5) was docked into the active site of human NEU2 or *V. cholerae* sialidase using docking program FRED. Most docking programs are usually effective but highly inconsistent, while the pose of the ligand structure is predictable and reproducible and the virtual screening is accurate using FRED.⁴⁵ Three structure-based scoring functions PLP (Piecewise Linear Potential),⁴⁶ chemgauss3,⁴⁷ and Oechemscore,⁴⁸ implemented in the program FRED were used to optimize the multiple docked poses. The best scoring pose was selected as the final position of the ligand in the crystal of protein.

As shown in Fig. 4A3 and 4A5, the C9-azido group in Neu5Ac9N₃2en (3) and Neu5AcN₃9N₃2en (5) fits well in the deep pocket of human NEU2 and points out towards the neighbouring cavity close to C9 of the inhibitors. However, replacing the C5-*N*-acetyl group in Neu5Ac9N₃2en (3) by the *N*-glycolyl group in Neu5Gc9N₃2en (4) causes a 180° flipping of the inhibitor (Fig. 4A4 and S1‡). This may be due to the less favorable interaction between the inner neutral cavity (mainly composed of Met85, Ile103, Ile105, and Ala158) and the *N*-glycolyl. The bigger outside positive cavity (mainly contributed by Arg83) may allow more favorable interaction with the *N*-glycolyl group¹¹ (Fig. S1‡). Switching the positions of C5 and C9 groups may lead to lower inhibition efficiency of Neu5Gc9N₃2en (4) against human NEU2. Compared to Neu5Ac9N₃2en (3) in Fig. 4A3 (IC₅₀ = 43 ± 2 μM when Neu5Acα2–3GalβpNP was used as sialidase substrate and IC₅₀ = 31 ± 6 μM when Neu5Acα2–6GalβpNP was used as sialidase substrate), Neu5AcN₃9N₃2en (5) with an N₃-substitution at C5-*N*-acetyl group in Fig. 4A5 fits the binding pocket of NEU2 better, which explains an improved IC₅₀ value for human NEU2 by Neu5AcN₃9N₃2en (5) (IC₅₀ = 13 ± 4 μM when Neu5Acα2–3GalβpNP was used as sialidase substrate and IC₅₀ = 13 ± 3 μM when Neu5Acα2–6GalβpNP was used as sialidase substrate).

For *V. cholerae* sialidase, both Neu5Ac9N₃2en (3) and Neu5Gc9N₃2en (4) fit the substrate binding pocket of the enzyme similarly well (Fig. 4B3 and 4B4), which explains similar IC₅₀ values of these two inhibitors. In comparison, the extra N₃-substitution at C5-*N*-acetyl group in Neu5AcN₃9N₃2en (5)

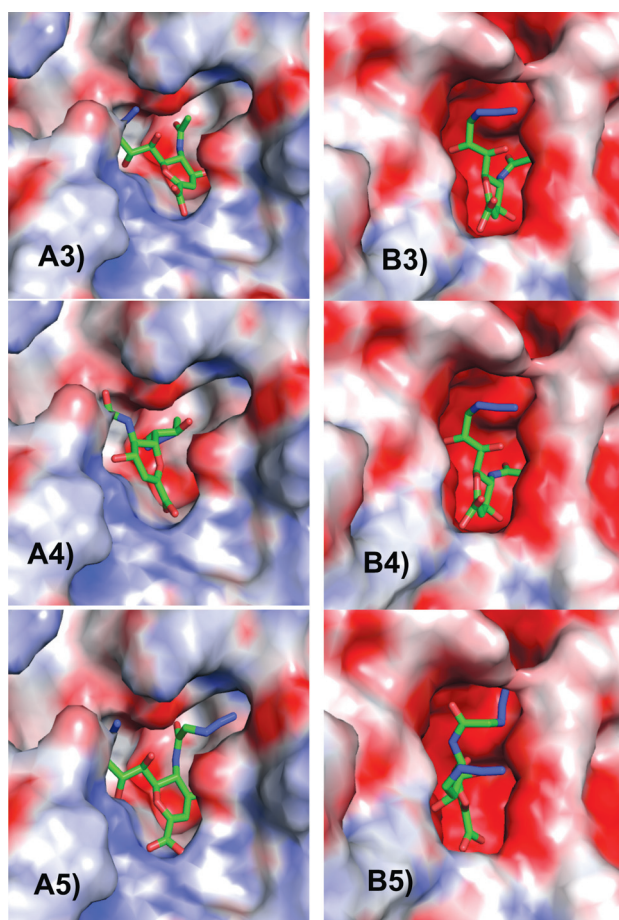


Fig. 4 Docking studies of Neu5Ac9N₃2en (**3**), Neu5Gc9N₃2en (**4**), and Neu5AcN₃9N₃2en (**5**) in the crystal structures of human NEU2 (**A**) and *V. cholerae* sialidase (**B**). The surface is coloured according to the electrostatic potential. Positive potentials are shown in blue, negative potentials are in red, and neutral are in white.

causes flipping and ill fitting of the inhibitor (Fig. 4B5 and S1†), which increases the IC₅₀ values for about 10-fold when either Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP was used as sialidase substrate.

Conclusions

In conclusion, guided by sialidase substrate specificity studies and sialidase crystal structural analysis, several C9- or both C9- and C5-modified Neu5Ac2en analogues were synthesized and their inhibitory activities were tested. Neu5Gc9N₃2en (**4**) and Neu5AcN₃9N₃2en (**5**) were identified as selective inhibitors against *V. cholerae* sialidase and human NEU2, respectively, using Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP as the substrate for human cytosolic sialidase NEU2 and four commercially available bacterial sialidases. Nevertheless, their IC₅₀ values were not improved compared to Neu5Ac2en. Docking of Neu5Ac9N₃2en (**3**), Neu5Gc9N₃2en (**4**), or Neu5AcN₃9N₃2en (**5**) to the crystal structures of human NEU2 and *V. cholerae* sialidase helped to illustrate the selectivity of sialidase inhibitors **4** and **5**. Therefore, substrate specificity study of various

sialidases is a valuable tool for identifying unique structural features of selective inhibitors. However, drastic improvement of inhibitory activity of bacterial and human sialidases will require more significant structural changes which could be facilitated by screening of focused compound libraries.

Experimental

Materials

All chemicals were obtained from commercial suppliers and used without further purification unless otherwise noted. Anhydrous solvents were used to carry out organic reactions under inert argon or nitrogen environment. Neu5Ac α 2–3Gal β pNP and Neu5Ac α 2–6Gal β pNP were synthesized as reported previously.⁴⁹ ¹H NMR (400 and 600 MHz) and ¹³C NMR (151 MHz) spectra were recorded on a Varian Inova 400 and 600 MHz spectrometer. High resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the University of California, Davis. Optical rotation values were recorded on an Autopol IV Automatic polarimeter at 589 nm wavelength. Infrared spectra were recorded on a PerkinElmer Spectrum 100 ATR-FTIR. Silica gel 60 Å (200–425 mesh, Fisher Chemical) was used for flash column chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF₂₅₄ (Sorbent technologies) using anisaldehyde sugar stain for detection. *Clostridium perfringens* sialidase (type VI), *Vibrio cholerae* sialidase (type III), and β -galactosidase from *Aspergillus oryzae* were purchased from Sigma. *Salmonella typhimurium* sialidase and *Streptococcus pneumoniae* sialidase NanB were bought from Prozyme. All of these enzymes were used without further purification. NEU2 was expressed in *E. coli* and purified as described previously.¹⁷ 384-Well plates for sialidase assays were from Fisher Biotech.

Chemical synthesis of sialidase inhibitors

5-Acetamido-2,6-anhydro-9-azido-3,5,9-trideoxy-D-glycero-D-galacto-non-2-enonic acid (Neu5Ac9N₃2en, **3).** To a suspension of **8**⁵⁰ (1.12 g, 2.36 mmol) in dry MeOH (15 mL) was added a catalytic amount of NaOMe and the mixture was stirred at room temperature for 3 h. The reaction was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 8 : 1 : 0.5) to afford **9** (0.56 g, 78%). ¹H NMR (600 MHz, D₂O) δ 6.04 (s, 1H), 4.50 (d, J = 9.0 Hz, 1H), 4.27 (d, J = 11.4 Hz, 1H), 4.08 (t, J = 9.6 Hz, 1H), 3.91–3.85 (m, 2H), 3.81 (s, 3H), 3.66–3.63 (m, 2H), 2.06 (s, 3H); ¹³C NMR (151 MHz, D₂O) δ 177.56, 167.08, 146.16, 115.30, 78.83, 72.67, 70.74, 69.79, 65.69, 55.64, 52.30, 24.82.

A stirred solution of **9** (0.56 g, 1.83 mmol) in anhydrous pyridine (10 mL) was treated with a solution of *p*-toluenesulfonyl chloride (0.52 g, 2.75 mmol) at 0 °C and the mixture was stirred overnight at room temperature. The reaction was stopped by adding MeOH (2 mL) and concentrated. The compound was purified by column chromatography (EtOAc–MeOH = 9 : 1) to give 9-OTs Neu5Ac2en (0.54 g, 65%) which was then dissolved in water (10 mL) and acetone (10 mL) containing NaN₃ (0.76 g, 11.75 mmol) and stirred at 70 °C overnight. The mixture was

concentrated and purified by silica gel flash column chromatography (EtOAc–MeOH = 9.5 : 0.5) to give product **10** (0.27 g, 70%). ¹H NMR (600 MHz, D₂O) δ 6.04 (s, 1H), 4.51 (d, *J* = 9.1 Hz, 1H), 4.27 (d, *J* = 8.4 Hz, 1H), 4.08–4.07 (m, 2H), 3.81 (s, 3H), 3.65–3.63 (m, 2H), 3.51 (s, 1H), 2.06 (s, 3H); ¹³C NMR (151 MHz, D₂O) δ 177.56, 167.08, 146.16, 115.30, 78.83, 72.67, 70.74, 69.79, 65.69, 55.64, 52.30, 24.82.

Compound **10** (0.27 g, 0.82 mmol) was dissolved in an aqueous solution of NaOH (10 mL, 0.1 M) and stirred at room temperature for 1 h. The reaction was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified using silica gel column chromatography (EtOAc–MeOH–H₂O = 8 : 3 : 0.5) to give final product **3** (0.22 g, 85%). [α]₂₄^D = +39.00 (*c* = 1.0 H₂O). ν_{\max} (film)/cm⁻¹ 3205 (m, broad, OH, NH, C–H alkene, COOH), 2101 (s, N₃), 1646 (s, CO carboxylic acid), 1557 (m, CO amide, CC alkene), 1145 (s, C–N). ¹H NMR (600 MHz, D₂O) δ 5.67 (s, 1H, H-3), 4.41 (d, *J* = 8.4 Hz, 1H, H-6), 4.15 (d, *J* = 10.8 Hz, 1H, H-4), 4.03–3.97 (m, 2H, H-5 and H-7), 3.58–3.54 (m, 2H, H-8 and H-9), 3.44 (dd, *J* = 12.6, 5.4 Hz, 1H, H-9'), 2.00 (s, 3H, NHCOMe); ¹³C NMR (151 MHz, D₂O) δ 174.83 (COONa), 169.16 (NHCOMe), 147.39 (C-2), 108.51 (C-3), 75.32 (C-6), 68.69 (2C, C-7 and C-8), 67.63 (C-4), 53.81 (C-5), 49.92 (C-9), 22.26 (NHCOMe). HRMS (ESI) calculated for C₁₁H₁₆N₄NaO₇ (M + H) 339.0917, found 339.0910.

2,6-Anhydro-9-azido-3,5,9-trideoxy-5-(2-hydroxyacetamido)-D-glycero-D-galacto-non-2-enonic acid (Neu5Ge9N₃2en, 4). To a solution of compound **8** (2.47 g, 5.21 mmol) in dry THF (70 mL) was added (Boc)₂O (4.55 g, 20.86 mmol) and 4-dimethylaminopyridine (DMAP) (0.19 g, 1.56 mmol) and the mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature, diluted with CH₂Cl₂, washed with HCl (0.5 M), water, saturated NaHCO₃, and water. The solution was dried, concentrated, and purified by flash column chromatography (EtOAc–hexane = 3 : 7) to afford **11** (2.69 g, 90%). ¹H NMR (600 MHz, CDCl₃) δ 5.99 (s, 1H), 5.87 (d, *J* = 2.4 Hz, 1H), 5.25–4.60 (m, 5H), 4.12–4.04 (m, 2H), 3.75 (s, 3H), 2.32 (s, 3H), 2.01–1.95 (m, 12H), 1.54 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 170.75, 170.31, 169.93, 161.93, 146.46, 109.25, 85.38, 71.10, 67.63, 66.97, 62.03, 52.73, 50.55, 28.08, 20.95.

Compound **11** (2.69 g, 4.69 mmol) was dissolved in dry MeOH (30 mL) containing a catalytic amount of NaOMe. The mixture was stirred at room temperature for 2 h and neutralized using Dowex (H⁺) resin. The resulting suspension was filtrated and concentrated. Silica gel column chromatography (EtOAc–MeOH = 9.5 : 0.5) of the crude product afforded product **12** (1.62 g, 95%). ¹H NMR (600 MHz, CD₃OD) δ 5.91 (d, *J* = 1.8 Hz, 1H), 4.35 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.11 (d, *J* = 10.8 Hz, 1H), 3.89–3.82 (m, 2H), 3.76 (s, 3H), 3.70–3.62 (m, 3H), 1.45 (s, 9H); ¹³C NMR (151 MHz, CD₃OD) δ 164.41, 159.32, 145.13, 113.68, 81.01, 78.60, 71.11, 70.19, 67.94, 64.98, 52.75, 52.52, 28.69.

A stirred solution of **12** (1.62 g, 4.46 mmol) in anhydrous pyridine (50 mL) was treated with a solution of *p*-toluenesulfonyl chloride (2.55 g, 13.37 mmol) at 0 °C and the mixture was stirred overnight at room temperature. The reaction was stopped by adding MeOH (3 mL) and concentrated. Purification of the residue by flash column chromatography (EtOAc–hexane = 7 : 3) afforded 9-tosylated product (1.77 g, 77%) which was then

dissolved in water (15 mL) and acetone (15 mL) containing NaN₃ (2.22 g, 34.20 mmol) and stirred at 70 °C overnight. The mixture was concentrated and purified by silica gel flash column chromatography (EtOAc–hexane = 7 : 3) to give compound **13** (1.06 g, 80%). ¹H NMR (600 MHz, CD₃OD) δ 5.83 (d, *J* = 3.0 Hz, 1H), 4.28 (dd, *J* = 9.0, 3.0 Hz, 1H), 4.01–3.95 (m, 2H), 3.68 (s, 3H), 3.59 (dd, *J* = 10.8, 8.4 Hz, 1H), 3.55 (d, *J* = 9.0 Hz, 1H), 3.46 (dd, *J* = 12.6, 2.4 Hz, 1H), 3.35 (dd, *J* = 13.2, 6.0 Hz, 1H), 1.38 (s, 9H); ¹³C NMR (151 MHz, CD₃OD) δ 164.31, 159.35, 144.99, 113.82, 81.10, 78.33, 70.53, 70.08, 67.79, 55.67, 52.81, 52.49, 28.65. Compound **13** (1.06 g, 2.73 mmol) was dissolved in a mixture of CH₂Cl₂ (30 mL) and an aqueous solution of trifluoroacetic acid (90%, 10 mL) and stirred at room temperature for 30 min. The mixture was concentrated, co-evaporated with toluene, and dried. To a solution of the resulted mixture in CH₂Cl₂ (20 mL) was added hydroxybenzotriazole (HOBt) (0.44 g, 1.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.51 g, 1.2 mmol), and *N,N*-diisopropylethylamine (DIPEA) (0.57 mL, 1.2 mmol) at 0 °C. Acetoxyacetyl chloride (0.23 mL, 2.12 mmol) was added drop-wisely and the reaction mixture was stirred at room temperature for 2.5 h. The reaction solution was concentrated and diluted with CH₂Cl₂, washed with water, saturated NaHCO₃, and water then dried and concentrated. Silica gel column chromatography (EtOAc–MeOH–H₂O = 9 : 2 : 0.5) afforded compound **14** (0.91 g, 85%). To a suspension of **14** (0.91 g, 2.32 mmol) in dry MeOH (10 mL) was added NaOMe (40 mg) and the mixture was stirred for 1 h at room temperature. Water was then added to the solution and pH was adjusted to about 9 by adding NaOMe. The reaction mixture was stirred at room temperature for 1 h. The solution was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 7.5 : 3 : 0.5) to afford product **4** (0.77 g, 95%). [α]₂₄^D = +16.50 (*c* = 1.0 H₂O). ν_{\max} (film)/cm⁻¹ 3184 and 3058 (s, broad, OH, NH, C–H alkene, COOH), 2101 (s, N₃), 1665 (s, CO carboxylic acid), 1570 (s, CO amide, CC alkene), 1153 (s, C–N). ¹H NMR (400 MHz, D₂O) δ 5.69 (d, *J* = 1.6 Hz, 1H, H-3), 4.55 (dd, *J* = 6.4, 1.6 Hz, 1H, H-6), 4.31 (d, *J* = 7.2 Hz, 1H, H-4), 4.15–4.09 (m, 4H, NHCO–CH₂OH, H-5, and H-7), 3.66–3.59 (m, 2H, H-9 and H-8), 3.50 (dd, *J* = 8.8, 4.0 Hz, 1H, H-9'); ¹³C NMR (151 MHz, D₂O) *d* 175.75 (COONa), 165.58 (NHCOCH₂OH), 143.72 (C-2), 112.86 (C-3), 75.78 (C-6), 68.87 (C-7), 68.47 (C-8), 67.19 (C-4), 61.18 (NHCOCH₂OH), 53.81 (C-5), 49.37 (C-9). HRMS (ESI) calculated for C₁₁H₁₆N₄NaO₈ (M + H) 355.0866, found 355.0858.

2,6-Anhydro-9-azido-5-(2-azidoacetamido)-3,5,9-trideoxy-D-glycero-D-galacto-non-2-enonic acid (Neu5AcN₃9N₃2en, 5). To a solution of compound **13** (0.21 g, 0.56 mmol) in pyridine (10 mL) was added acetic anhydride (0.7 mL) at 0 °C and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was purified using silica gel column chromatography (EtOAc–hexane = 3 : 7) to give compound **15** (0.22 g, 80%). ¹H NMR (600 MHz, CDCl₃) δ 5.93 (d, *J* = 4.2 Hz, 1H), 5.53–5.48 (m, 1H), 5.20–5.17 (m, 1H), 4.79 (d, *J* = 15.6 Hz, 1H), 4.29 (dd, *J* = 15.0, 4.2 Hz, 1H), 4.10–4.01 (m, 1H), 3.89 (dd, *J* = 20.4, 3.6 Hz, 1H), 3.78 (s, 3H), 3.45 (dd, *J* = 20.4, 12.0 Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.37 (s, 9H); ¹³C NMR

(151 MHz, CDCl₃) δ 170.97, 170.57, 169.96, 161.72, 155.13, 145.10, 108.64, 80.51, 77.36, 72.93, 69.21, 68.34, 52.71, 50.29, 47.93, 28.20, 20.99, 20.91, 20.79.

To a solution of compound **15** (0.22 g, 0.43 mmol) in CH₂Cl₂ (6 mL) was added 90% aqueous trifluoroacetic acid (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min. It was then concentrated, co-evaporated with toluene, and dried under vacuum. To the resulting ammonium salt in CH₂Cl₂ (3 mL) were added N₃CH₂COOH (52 μ L, 0.51 mmol), HOBT (0.08 g, 0.51 mmol), EDCI (0.10 g, 0.51 mmol), and DIPEA (0.22 mL, 1.29 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 2 h. The mixture was diluted with CH₂Cl₂, washed with water, saturated NaHCO₃, and water then dried and concentrated. Silica gel column chromatography (EtOAc–hexane = 4 : 6) afforded compound **16** (0.17 g, 80%). ¹H NMR (600 MHz, CDCl₃) δ 6.79 (d, J = 9.6 Hz, 1H), 5.93 (d, J = 1.8 Hz, 1H), 5.63 (dd, J = 8.4, 3.0 Hz, 1H), 5.47 (t, J = 3.0 Hz, 1H), 5.17–5.15 (m, 1H), 4.46–4.39 (m, 2H), 3.93 (dd, J = 13.2, 1.8 Hz, 1H), 3.83 (d, J = 5.4 Hz, 2 H), 3.79 (s, 3H), 3.46 (dd, J = 13.8, 8.4 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 171.28, 170.97, 170.50, 167.54, 161.54, 145.32, 108.68, 77.17, 73.41, 68.75, 68.03, 52.78, 52.61, 50.20, 46.41, 20.88.

To a suspension of **16** (0.17 g, 0.35 mmol) in dry MeOH (10 mL) was added NaOMe (40 mg) and the mixture was stirred for 2 h at room temperature. After adding water to the reaction mixture, the pH was adjusted to 9 and the solution was stirred for 1 h at room temperature. The reaction was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 8 : 3 : 0.5) to afford **5** (0.12 g, 95%). [α]_D²⁴ = +35.50 (c = 1.04 H₂O). ν_{\max} (film)/cm⁻¹ 3293 (s, broad, OH, NH, C–H alkene, COOH), 2109 (s, N₃), 1596 (s, CO carboxylic acid), 1542 (s, CO amide, CC alkene), 1072 (s, C–N). ¹H NMR (600 MHz, D₂O) δ 5.68 (d, J = 1.8 Hz, 1H, H-3), 4.50 (dd, J = 8.4, 3.0 Hz, 1H, H-6), 4.28 (d, J = 11.4 Hz, 1H, H-4), 4.14–4.07 (m, 4H, NHCOCH₂N₃, H-5, and H-7), 3.63 (dd, J = 13.2, 2.4 Hz, 1H, H-9), 3.59 (d, J = 9.6 Hz, 1H, H-8), 3.49 (dd, J = 13.2, 6.0 Hz, 1H, H-9'); ¹³C NMR (151 MHz, D₂O) δ 171.08 (COONa), 169.65 (NHCOCH₂N₃), 148.04 (C-2), 107.91 (C-3), 75.03 (C-6), 68.76 (C-2, C-7 and C-8), 67.65 (C-4), 53.86 (NHCOCH₂N₃), 52.15 (C-5), 50.13 (C-9). HRMS (ESI) calculated for C₁₁H₁₅N₇NaO₇ (M + H) 380.0931, found 380.0924.

5-Acetamido-2,6-anhydro-3,5-dideoxy-9-O-methyl-D-glycero-D-galacto-non-2-enonic acid (Neu5Ac9Me2en, 6). A solution of NaOMe (0.17 g, 3.14 mmol) in MeOH (5 mL) was prepared. A 2 mL portion of the prepared solution was added to compound **9** (0.22 g, 0.48 mmol) and stirred at room temperature for 20 min. The second portion of NaOMe in MeOH (2 mL) was added to the reaction mixture and stirred at room temperature for 30 min. Dowex (H⁺) resin was added to the solution to adjust the pH to about 2 and the mixture was stirred at room temperature for 2 h. The reaction was neutralized using Et₃N, filtrated, and washed with MeOH and H₂O. The concentrated crude product was purified by silica gel flash column chromatography (EtOAc–MeOH = 9 : 1) to afford compound **17** (0.12 g, 80%). ¹H NMR (600 MHz, D₂O) δ 6.01 (d, J = 2.4 Hz, 1H), 4.47 (dd, J = 8.4, 2.4 Hz, 1H), 4.25 (d, J = 10.8 Hz, 1H), 4.06 (dd, J = 10.8,

9.0 Hz, 1H), 3.99–3.97 (m, 1H), 3.79 (s, 3H), 3.69 (dd, J = 10.8, 2.4 Hz, 1H), 3.61 (d, J = 9.0 Hz, 1H), 3.55 (dd, J = 10.8, 6.0 Hz, 1H), 3.69 (s, 3H), 2.03 (s, 3H); ¹³C NMR (151 MHz, D₂O) δ 174.79, 164.40, 143.51, 112.69, 76.12, 73.75, 68.39, 68.09, 67.26, 58.65, 53.04, 49.67, 22.23.

Compound **17** (0.12 g, 0.37 mmol) was dissolved in an aqueous solution of NaOH (10 mL, 0.05 M) and stirred at room temperature for 30 min. The solution was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 8 : 3 : 0.7) to afford product **6** (107 mg, 95%). [α]_D²⁴ = +25.30 (c = 1.0 H₂O). ν_{\max} (film)/cm⁻¹ 3278 (s, broad, OH, NH, C–H alkene, COOH), 1590 (s, broad, CO carboxylic acid, CO amide, CC alkene), 1092 (s, C–N). ¹H NMR (600 MHz, D₂O) δ 5.69 (d, J = 2.4 Hz, 1H, H-3), 4.45 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 4.20 (d, J = 11.4 Hz, 1H, H-4), 4.07–4.03 (m, 2H, H-5 and H-7), 3.73 (dd, J = 12.0, 2.4 Hz, 1H, H-9), 3.61–3.57 (m, 2H, H-8 and H-9'), 3.40 (s, 3H, OMe), 2.06 (s, 3H, NHCOMe); ¹³C NMR (151 MHz, D₂O) δ 174.85 (COONa), 169.72 (NHCOMe), 148.01 (C-2), 107.91 (C-3), 75.40 (C-6), 73.91 (C-9), 68.41 (C-7), 68.28 (C-8), 67.79 (C-4), 58.66 (OMe), 50.01 (C-5), 22.30 (NHCOMe). HRMS (ESI) calculated for C₁₂H₁₉NNaO₈ (M + H) 328.1008, found 328.1005.

5-Acetamido-9-aminopropyl-2,6-anhydro-3,5,9-trideoxy-D-glycero-D-galacto-non-2-enonic acid (Neu5Ac9NPro2en, 7). Compound **10** (0.33 g, 1.0 mmol) was dissolved in MeOH (20 mL) and Pd–C (100 mg) was added. The resulting mixture was hydrogenated for 1.5 h with stirring under H₂ at atmospheric pressure and then the catalyst was removed by filtration over Celite and washed with MeOH and water. The concentrated product was purified by flash column chromatography (EtOAc–MeOH–H₂O = 5 : 4 : 3) to afford **18** (0.26 g, 80%). To compound **18** (42 mg, 0.14 mmol) in dry THF (15 mL) was added NaBH₃CN (52 mg, 0.83 mmol), acetic acid to adjust pH to about 5, and propanal (50 μ L, 0.7 mmol). The mixture was stirred at room temperature overnight. The mixture was concentrated and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 5 : 3 : 1) to afford the target compound intermediate (33.5 mg, 70%) which was then dissolved in an aqueous solution of NaOH (10 mL, 0.05 M) and stirred at room temperature for 30 min. The solution was neutralized with Dowex (H⁺) resin, filtrated, concentrated, and purified by silica gel flash column chromatography (EtOAc–MeOH–H₂O = 5 : 3 : 1) to afford product **7** (30.8 mg, 90%). [α]_D²⁴ = +33.20 (c = 1.0 H₂O). ν_{\max} (film)/cm⁻¹ 3255 (s, broad, OH, NH, C–H alkene, COOH), 1583 (s, broad, CO carboxylic acid, CO amide, CC alkene), 1148 (s, C–N). ¹H NMR (600 MHz, D₂O) δ 5.70 (d, J = 1.8 Hz, 1H, H-3), 4.48 (dd, J = 9.0, 2.4 Hz, 1H, H-6), 4.33 (t, J = 9.0 Hz, 1H, H-4), 4.21 (d, J = 10.8 Hz, 1H, H-5), 4.05–4.02 (m, 1H, H-7), 3.57–3.54 (m, 2H, H-8 and H-9), 3.21–3.17 (m, 3H, CH₂-1 propyl, H-9'), 2.07 (s, 3H, NHCOMe), 1.78–1.72 (m, 2H, CH₂-2 propyl), 0.97 (t, J = 7.8 Hz, 3H, CH₃ propyl); ¹³C NMR (151 MHz, D₂O) δ 175.07 (COOH), 169.77 (NHCOMe), 148.06 (C-2), 108.08 (C-3), 75.20 (C-6), 70.57 (C-7), 67.61 (C-8), 64.52 (C-4), 56.17 (C-5), 55.39 (C-9), 50.00 (CH₂-1 propyl), 22.26 (NHCOMe), 16.92 (CH₂-2 propyl), 10.31 (CH₃ propyl). HRMS (ESI) calculated for C₁₄H₂₄N₂O₇ (M + H) 333.1662, found 333.1670.

Inhibition assays of bacteria sialidases and human NEU2

All sialidase assays were carried out at 37 °C in duplicates in 384-well plates in a final volume of 20 μ L containing Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP (0.3 mM), and the β -galactosidase (12 μ g, 126 mU) with or without inhibitors. The amount of the β -galactosidase required to completely hydrolyze the Gal β pNP within the time frame of the assay was pre-determined and confirmed by assays with Gal β pNP (0.3 mM). The assay conditions for various sialidases were as follows: *C. perfringens* sialidase (1.6 mU), MES buffer (100 mM, pH 5.0); *V. cholerae* sialidase (0.5 mU), NaCl (150 mM), CaCl₂ (10 mM), and sodium acetate buffer (100 mM, pH 5.5); *S. typhimurium* sialidase (0.6 mU), NaCl (100 mM) and sodium acetate buffer (100 mM, pH 5.5); *S. pneumoniae* sialidase NanB (0.2 mU), sodium acetate buffer (100 mM, pH 6.0); NEU2 (6 μ g for Neu5Ac α 2–3Gal β pNP and 9.6 μ g for Neu5Ac α 2–6Gal β pNP), MES buffer (100 mM, pH 5.0). The reactions were carried out for 30 min. The assay was stopped by adding CAPS buffer (*N*-cyclohexyl-3-aminopropane sulfonic acid, 40 μ L, 0.5 M, pH 10.5). The amount of the *para*-nitrophenolate formed was determined by measuring the $A_{405\text{ nm}}$ of the reaction mixtures using a microplate reader. Reactions without inhibitors were used as controls.

IC₅₀ values were obtained by varying the concentrations of inhibitors from 0 to 50 mM (twelve concentrations of each inhibitor were used to determine an IC₅₀) with a fixed concentration (0.3 mM) of Neu5Ac α 2–3Gal β pNP or Neu5Ac α 2–6Gal β pNP. Typical concentration–response plots were obtained from the average values of duplicate assay results. The values of IC₅₀ were obtained by fitting the averages to the Michaelis–Menten equation using Grafit 5.0.

Docking studies

The monomeric structure of NEU2 was modelled based on the dimeric structure of NEU2 (pdb: 1VCU).¹³ Neu5Ac9N₃2en (**3**), Neu5Gc9N₃2en (**4**), or Neu5AcN₃9N₃2en (**5**) was docked into the active sites of human NEU2 and *Vibrio cholerae* sialidase (pdb: 1W00), respectively, using molecular building and optimization softwares Gaussview 5.0 and Gaussview 09W and docking software OpenEye. The designation of the protein active site was performed using Fred Receptor 2.2.5. Docking the molecule into the designated active site was achieved using Fred as well as 3D displaying and analyzing software Vida 4.0.0. The electrostatic potential maps were built by PyMOL.

Acknowledgements

This work was supported by NIH grant 1R43AI092874. X.C. is a Camille Dreyfus Teacher-Scholar and a UC-Davis Chancellor's Fellow.

Notes and references

- G. Taylor, *Curr. Opin. Struct. Biol.*, 1996, **6**, 830.
- A. Buschiazio and P. M. Alzari, *Curr. Opin. Chem. Biol.*, 2008, **12**, 565.
- K. Stummeyer, A. Dickmanns, M. Muhlenhoff, R. Gerardy-Schahn and R. Ficner, *Nat. Struct. Mol. Biol.*, 2005, **12**, 90.
- B. L. Cantarel, P. M. Coutinho, C. Rancurel, T. Bernard, V. Lombard and B. Henrissat, *Nucleic Acids Res.*, 2009, **37**, D233.
- M. Kuro-o, *Biochim. Biophys. Acta*, 2009, **1790**, 1049.
- T. C. Camilli, M. Xu, M. P. O'Connell, B. Chien, B. P. Frank, S. Subaran, F. E. Indig, P. J. Morin, S. M. Hewitt and A. T. Weeraratna, *Pigm. Cell Melanoma Res.*, 2011, **24**, 175.
- J. Cheng, H. Yu, K. Lau, S. Huang, H. A. Chokhawala, Y. Li, V. K. Tiwari and X. Chen, *Glycobiology*, 2008, **18**, 686.
- J. Cheng, S. Huang, H. Yu, Y. Li, K. Lau and X. Chen, *Glycobiology*, 2010, **20**, 260.
- H. Yu, H. Chokhawala, R. Karpel, H. Yu, B. Wu, J. Zhang, Y. Zhang, Q. Jia and X. Chen, *J. Am. Chem. Soc.*, 2005, **127**, 17618.
- T. Yamamoto, Y. Hamada, M. Ichikawa, H. Kajiwara, T. Mine, H. Tsukamoto and Y. Takakura, *Glycobiology*, 2007, **17**, 1167.
- Y. Li, H. Cao, H. Yu, Y. Chen, K. Lau, J. Qu, V. Thon, G. Sugiarto and X. Chen, *Mol. Biosyst.*, 2011, **7**, 1060.
- X. Chen and A. Varki, *ACS Chem. Biol.*, 2010, **5**, 163.
- L. M. Chavas, C. Tringali, P. Fusi, B. Venerando, G. Tettamanti, R. Kato, E. Monti and S. Wakatsuki, *J. Biol. Chem.*, 2005, **280**, 469.
- M. Vonitzstein, W. Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. V. Phan, M. L. Smythe, H. F. White, S. W. Oliver, P. M. Colman, J. N. Varghese, D. M. Ryan, J. M. Woods, R. C. Bethell, V. J. Hotham, J. M. Cameron and C. R. Penn, *Nature*, 1993, **363**, 418.
- M. von Itzstein, *Nat. Rev. Drug Discovery*, 2007, **6**, 967.
- M. C. Mann, R. J. Thomson, J. C. Dyason, S. McAtamney and M. von Itzstein, *Bioorg. Med. Chem.*, 2006, **14**, 1518.
- W. F. Diven, W. J. Doyle and B. Vietmeier, *Ann. Oto. Rhinol. Laryngol.*, 1988, **97**, 6.
- M. Kawano, H. Bando, T. Yuasa, K. Kondo, M. Tsurudome, H. Komada, M. Nishio and Y. Ito, *Virology*, 1990, **174**, 308.
- T. Corfield, *Glycobiology*, 1992, **2**, 509–521.
- A. Albohy, M. D. Li, R. B. Zheng, C. Zou and C. W. Cairo, *Glycobiology*, 2010, **20**, 1127.
- E. Monti, M. T. Bassi, R. Bresciani, S. Civini, G. L. Croci, N. Papini, M. Riboni, G. Zanchetti, A. Ballabio, A. Preti, G. Tettamanti, B. Venerando and G. Borsani, *Genomics*, 2004, **83**, 445.
- A. Caciotti, M. Di Rocco, M. Filocamo, S. Grossi, F. Traverso, A. d'Azzo, C. Cavicchi, A. Messeri, R. Guerrini, E. Zammarchi, M. A. Donati and A. Morrone, *J. Neurol.*, 2009, **256**, 1911.
- P. M. Colman, J. N. Varghese and W. G. Laver, *Nature*, 1983, **303**, 41.
- P. M. Mitrasinovic, *Curr. Drug Targets*, 2010, **11**, 315.
- J. N. Varghese, J. L. McKimm-Breschkin, J. B. Caldwell, A. A. Kortt and P. M. Colman, *Proteins: Struct., Funct., Genet.*, 1992, **14**, 327.
- C. U. Kim, W. Lew, M. A. Williams, H. T. Liu, L. J. Zhang, S. Swaminathan, N. Bischofberger, M. S. Chen, D. B. Mendel, C. Y. Tai, W. G. Laver and R. C. Stevens, *J. Am. Chem. Soc.*, 1997, **119**, 681.
- S. Magesh, S. Moriya, T. Suzuki, T. Miyagi, H. Ishida and M. Kiso, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 532.
- S. Magesh, V. Savita, S. Moriya, T. Suzuki, T. Miyagi, H. Ishida and M. Kiso, *Bioorg. Med. Chem.*, 2009, **17**, 4595.
- Y. Zou, A. Albohy, M. Sandbhor and C. W. Cairo, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7529.
- A. Albohy, S. Mohan, R. B. Zheng, B. M. Pinto and C. W. Cairo, *Bioorg. Med. Chem.*, 2011, **19**, 2817.
- M. C. Mann, R. J. Thomson, J. C. Dyason, S. McAtamney and M. von Itzstein, *Bioorg. Med. Chem.*, 2006, **14**, 1518.
- P. H. Nguyen, T. N. Nguyen, K. W. Kang, D. T. Ndinth, J. T. Mbafor, Y. R. Kim and W. K. Oh, *Bioorg. Med. Chem.*, 2010, **18**, 3335.
- H. Hinou and S. Nishimura, *Curr. Top. Med. Chem.*, 2009, **9**, 106.
- R. J. Russell, L. F. Haire, D. J. Stevens, P. J. Collins, Y. P. Lin, G. M. Blackburn, A. J. Hay, S. J. Gamblin and J. J. Skehel, *Nature*, 2006, **443**, 45.
- H. Hinou, R. Miyoshi, Y. Takasu, H. Kai, M. Kuroguchi, S. Arioka, X. D. Gao, N. Miura, N. Fujitani, S. Omoto, T. Yoshinaga, T. Fujiwara, T. Noshi, H. Togame, H. Takemoto and S. Nishimura, *Chem.-Asian J.*, 2011, **6**, 1048.
- H. Cao, Y. Li, K. Lau, S. Muthana, H. Yu, J. Cheng, H. A. Chokhawala, G. Sugiarto, L. Zhang and X. Chen, *Org. Biomol. Chem.*, 2009, **7**, 5137.
- S. Crennell, E. Garman, G. Laver, E. Vimr and G. Taylor, *Structure*, 1994, **2**, 535.
- G. Xu, J. A. Potter, R. J. Russell, M. R. Oggioni, P. W. Andrew and G. L. Taylor, *J. Mol. Biol.*, 2008, **384**, 436.
- G. Xu, X. Li, P. W. Andrew and G. L. Taylor, *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.*, 2008, **64**, 772.

- 40 S. L. Newstead, J. A. Potter, J. C. Wilson, G. Xu, C. H. Chien, A. G. Watts, S. G. Withers and G. L. Taylor, *J. Biol. Chem.*, 2008, **283**, 9080.
- 41 S. J. Crennell, E. F. Garman, C. Philippon, A. Vasella, W. G. Laver, E. R. Vimr and G. L. Taylor, *J. Mol. Biol.*, 1996, **259**, 264.
- 42 X. Xu, X. Zhu, R. A. Dwek, J. Stevens and I. A. Wilson, *J. Virol.*, 2008, **82**, 10493.
- 43 Z. Khedri, M. M. Muthana, Y. Li, S. M. Muthana, H. Yu, H. Cao and X. Chen, *Chem. Commun.*, 2012, **48**, 3357.
- 44 T. Q. Gregar and J. Gervay-Hague, *J. Org. Chem.*, 2004, **69**, 1001.
- 45 M. McGann, *J. Chem. Inf. Model.*, 2011, **51**, 578.
- 46 G. M. Verkhivker, D. Bouzida, D. K. Gehlhaar, P. A. Rejto, S. Arthurs, A. B. Colson, S. T. Freer, V. Larson, B. A. Luty, T. Marrone and P. W. Rose, *J. Comput.-Aided Mol. Des.*, 2000, **14**, 731.
- 47 M. R. McGann, H. R. Almond, A. Nicholls, J. A. Grant and F. K. Brown, *Biopolymers*, 2003, **68**, 76.
- 48 M. D. Eldridge, C. W. Murray, T. R. Auton, G. V. Paolini and R. P. Mee, *J. Comput.-Aided Mol. Des.*, 1997, **11**, 425.
- 49 H. A. Chokhawala, H. Yu and X. Chen, *ChemBioChem*, 2007, **8**, 194.
- 50 T. Ercegovic and G. Magnusson, *J. Org. Chem.*, 1995, **60**, 3378–3384.