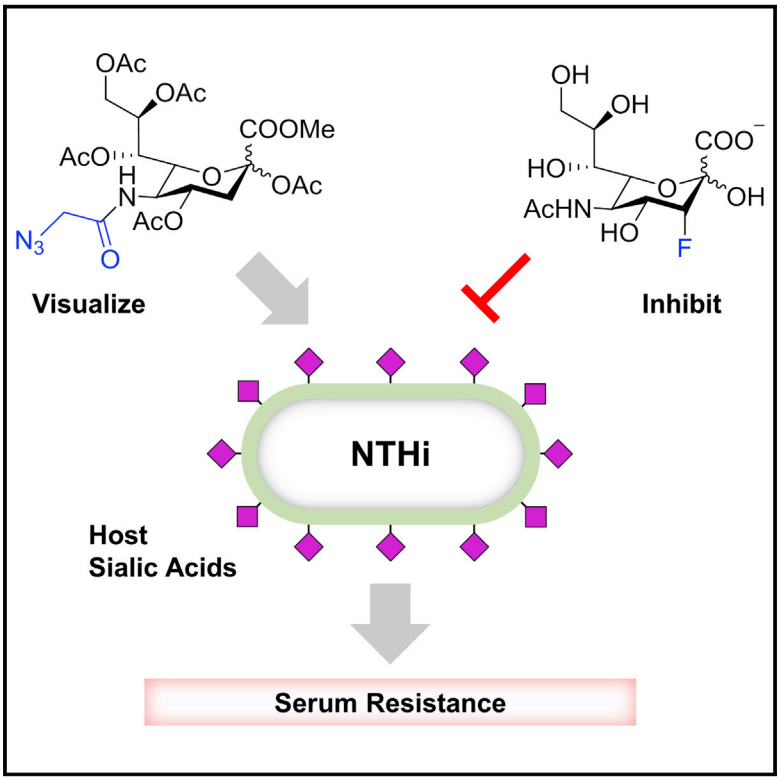
Brief Communication

Cell Chemical Biology

Selective Inhibition of Sialic Acid-Based Molecular Mimicry in *Haemophilus influenzae* Abrogates Serum Resistance

Graphical Abstract



Highlights

- NTHi expresses host sialic acids on its surface to evade the immune system
- Ac_5SiaNAz enables visualization of host sialic acid transfer to NTHi
- SiaNAc-$3F_{ax}$ blocks sialic acid incorporation in NTHi, but not in host cells
- Sialic acid blockade with SiaNAc-$3F_{ax}$ enhances serum-mediated killing of NTHi

Authors

Torben Heise, Jeroen D. Langereis, Emiel Rossing, Marien I. de Jonge, Gosse J. Adema, Christian Büll, Thomas J. Boltje

Correspondence

jeroen.langereis@radboudumc.nl (J.D.L.), t.boltje@science.ru.nl (T.J.B.)

In Brief

Molecular mimicry of non-typeable *Haemophilus influenzae* (NTHi) with host sialic acid sugars mediates resistance to serum killing and increases virulence. Heise et al. have developed sialic acid derivatives that allow either visualization or inhibition of host sialic acid transfer to NTHi, the latter enhancing serum-mediated killing.

Heise et al., 2018, Cell Chemical Biology *25*, 1–7
September 20, 2018
https://doi.org/10.1016/j.chembiol.2018.05.018

CellPress

Please cite this article in press as: Heise et al., Selective Inhibition of Sialic Acid-Based Molecular Mimicry in *Haemophilus influenzae* Abrogates Serum Resistance, Cell Chemical Biology (2018), https://doi.org/10.1016/j.chembiol.2018.05.018

Cell Chemical Biology

Brief Communication

CellPress

Selective Inhibition of Sialic Acid-Based Molecular Mimicry in *Haemophilus influenzae* Abrogates Serum Resistance

Torben Heise,[1,5] Jeroen D. Langereis,[2,3,5,*] Emiel Rossing,[1] Marien I. de Jonge,[2,3] Gosse J. Adema,[4,6] Christian Büll,[4,6] and Thomas J. Boltje[1,7,*]

[1]Cluster of Molecular Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen 6525 AJ, the Netherlands

[2]Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen 6525 GA, the Netherlands

[3]Radboud Centre for Infectious Diseases, Radboudumc, Nijmegen 6525 GA, the Netherlands

[4]Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboudumc, Nijmegen 6525 GA, the Netherlands

[5]These authors contributed equally

[6]Senior author

[7]Lead Contact

*Correspondence: jeroen.langereis@radboudumc.nl (J.D.L.), t.boltje@science.ru.nl (T.J.B.)

https://doi.org/10.1016/j.chembiol.2018.05.018

SUMMARY

Pathogens such as non-typeable *Haemophilus influenzae* (NTHi) evade the immune system by presenting host-derived sialic acids. NTHi cannot synthesize sialic acids and therefore needs to utilize sialic acids originating from host tissue. Here we report sialic acid-based probes to visualize and inhibit the transfer of host sialic acids to NTHi. Inhibition of sialic acid utilization by NTHi enhanced serum-mediated killing. Furthermore, in an *in vitro* model of the human respiratory tract, we demonstrate efficient inhibition of sialic acid transfer from primary human bronchial epithelial cells to NTHi using bioorthogonal chemistry.

INTRODUCTION

Several pathogens that colonize the human host have evolved the ability to engage in ''molecular mimicry,'' the expression of host-like molecular structures at the cell surface. These structures are used by pathogens as a molecular cloak to avoid detection and clearance by the host immune system. A prime example of an opportunistic pathogen that uses molecular mimicry is non-typeable *Haemophilus influenzae* (NTHi). NTHi is a commensal organism in the upper respiratory tract microbiome, which can become an opportunistic pathogen mainly in children and the elderly (Van Eldere et al., 2014; Langereis and de Jonge, 2015). NTHi infections are found in 55%–95% of children suffering from otitis media, an inflammatory disease that affects 65–300 million people globally and is a major cause of hearing loss (Van Eldere et al., 2014). In addition, NTHi is found in up to 87% of patients with acute exacerbation of chronic obstructive pulmonary disease, a leading cause of death worldwide (Van Eldere et al., 2014). The switch of NTHi from a symbiotic colonizing bacterium to an opportunistic pathogen is associated with uptake and expression of host sialic acids. Sialic acids are complex nine-carbon sugars abundantly expressed on human glycoconjugates (Severi et al., 2007) and can be released by the action of sialidases. Unbound sialic acids can be taken up by NTHi using a tripartite ATP-independent periplasmic (TRAP) transporter system (Cross et al., 2003; Vimr et al., 2004) and enzymatically incorporated into the variable outer core of the NTHi lipooligosaccharide (LOS) (Figure 1) (Apicella, 2012). LOS sialylation is an important NTHi virulence factor that confers resistance to serum killing and increases biofilm formation, although the underlying mechanisms are still poorly understood (Hallström and Riesbeck, 2010; Langereis and Hermans, 2013). NTHi strains defective in sialic acid utilization are no longer virulent in animal models for otitis media, making the sialic acid utilization pathway a promising therapeutic target (Jenkins et al., 2010).

Herein we report a sialic acid-based inhibitor (SiaNAc-3F_{ax}) that prevents sialic acid incorporation into NTHi LOS at low micromolar concentrations, which led to enhanced serum-mediated killing of NTHi (Figure 1). Furthermore, we report the use of sialic acid-based probes and bioorthogonal chemistry to visualize the transfer of sialic acids from host to NTHi using a model system of the human airway.

RESULTS AND DISCUSSION

Visualization and Inhibition of NTHi LOS Sialylation

To evaluate the efficacy of sialic acid-based inhibitors on LOS sialylation, we have developed a fast and reliable assay to measure LOS sialylation in NTHi. Sialic acid incorporation into NTHi LOS was visualized using a metabolic sialic acid glycoengineering approach with azidoacetyl (SiaNAz)- and propargyloxycarbonyl (SiaNPoc)-modified sialic acids (Figures 2A and S1A) (Sletten and Bertozzi, 2009; Tra and Dube, 2014; Lantos et al., 2016). NTHi bacteria were grown in sialic acid-deficient medium supplemented with SiaNAz or SiaNPoc and incorporation of these sialic acid analogs into LOS was detected by reacting

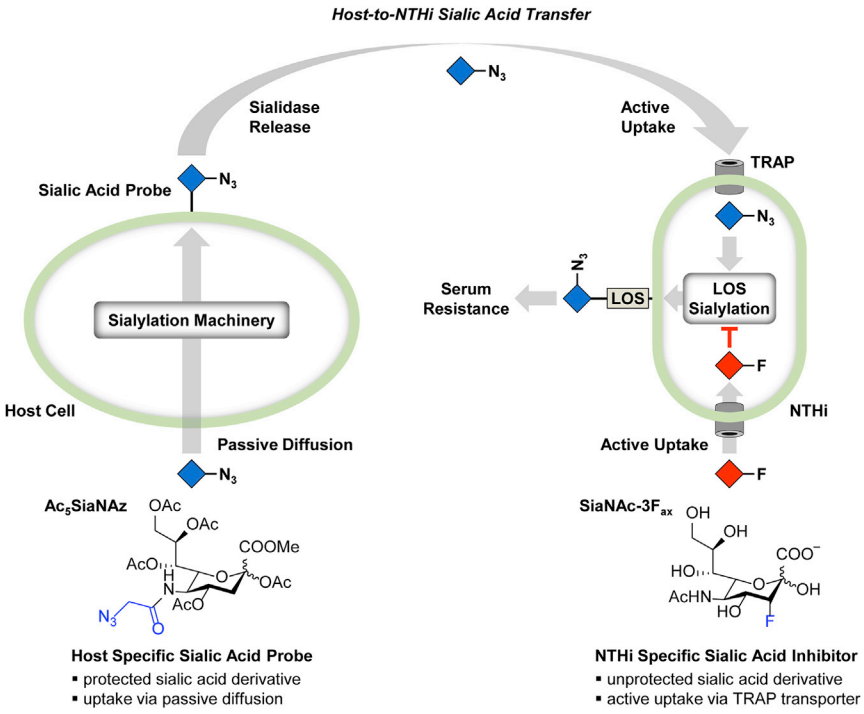


Figure 1. Sialic Acid Utilization by NTHi

Host sialic acids are released by sialidases and taken up by NTHi via active uptake using a tripartite ATP-independent periplasmic (TRAP) transporter system. Incorporation of sialic acids in NTHi LOS leads to resistance to serum-mediated killing.

them to biotin-alkyne or biotin-azide, respectively, using copper-catalyzed alkyne-azide cycloaddition (CuAAC) followed by staining with fluorescent streptavidin (Figure 2A).

Incorporation of SiaNAz and SiaNPoc sialic acid analogs was dose dependent (Figures 2B, S2B, and S2C) and required a functional TRAP sialic transporter, because no labeling was detected for siaP transporter mutant NTHi (ΔsiaP) (Figures S2D and S2E). To assess if passive diffusion of sialic acid analogs over the NTHi membrane was possible, we tested the incorporation of the protected (peracetylated and methyl-ester protected) sialic acid analogs Ac_5SiaNAz and Ac_5SiaNPoc (Büll et al., 2015, 2017; Cheng et al., 2016). Neither Ac_5SiaNAz nor Ac_5SiaNPoc were incorporated in NTHi LOS (Figures 2B and S2F), indicating that they were not able to enter the bacteria via diffusion and also not recognized as substrates for the TRAP system. Vice versa, human THP-1 cells utilized only protected sialic acid analogs for sialoglycan synthesis, but not the non-protected sialic acid analogs (Figure 2C).

Having established a fast, robust, and selective assay to measure NTHi LOS sialylation, we set out to selectively block this process with sialic acid-based inhibitors. Recently it has been shown that protected, fluorinated sialic acid can be taken up by passive diffusion and metabolized into CMP-SiaNAc-$3F_{ax}$, which acts as a competitive inhibitor of sialyltransferases in mammalian cells (Rillahan et al., 2012; Büll et al., 2013; Burkart et al., 1999). We reasoned that non-protected fluorinated sialic acid could be actively taken up through the TRAP transporter system of NTHi and inhibit NTHi sialyltransferases to prevent LOS sialylation. Therefore, a non-protected fluorinated sialic acid analog, SiaNAc-$3F_{ax}$, was synthesized and its effect on NTHi LOS sialylation was assessed (Gantt et al., 1964). NTHi was grown in defined media containing 100 μM SiaNAz and SiaNPoc and increasing concentrations of SiaNAc-$3F_{ax}$ (0.01–100 μM). SiaNAc-$3F_{ax}$ blocked the incorporation of SiaNAz and SiaNPoc in a dose-dependent manner with a half maximal inhibitory concentration (IC_{50}) of 700 nM for SiaNAz and 90 nM for SiaNPoc (Figures 2D and S2A–S2C). Even though SiaNAz was present in a 100-fold access, 1 μM SiaNAc-$3F_{ax}$ showed over 50% reduction of SiaNAz incorporation into LOS (Figure 2D).

In line with the findings obtained with protected SiaNAz and SiaNPoc sialic acid analogs, no inhibition was observed with the protected fluorinated sialic acid (Ac_5SiaNAc-$3F_{ax}$) (Figures 2E and S2D). In human THP-1 cells, however, treatment with protected Ac_5SiaNAc-$3F_{ax}$ effectively decreased sialic acid expression, whereas this was not the case for the non-protected analog SiaNAc-$3F_{ax}$ (Figures S2E and S2F). Importantly, treatment of THP-1 cells with up to 1 mM sialic acid mimetics for 24 or 48 hr did not affect cell viability or proliferation (Figures S2G and S2H).

Inhibition of NTHi LOS Sialylation Increases Serum-Mediated Killing

Having demonstrated that SiaNAc-$3F_{ax}$ potently inhibited NTHi LOS sialylation, we next investigated the functional consequences on serum-mediated killing. NTHi was grown in defined media containing natural sialic acid (SiaNAc) and subjected to a killing assay with pooled normal human serum. With increasing sialic acid concentrations, NTHi acquired resistance to serum-mediated killing (Figure 3A). This process required a functional sialic transporter, because SiaNAc addition had no effect on serum resistance of the ΔsiaP mutant (Figure 3B). No difference in factor H binding to NTHi strain 86-028NP and ΔsiaP mutant grown with or without SiaNAc was detected (Figure S3A), indicating that resistance to serum-mediated killing is independent of factor H, which is consistent with previous findings (Langereis et al., 2014; Figueira et al., 2007). Having established that sialylation increased serum resistance, we investigated whether inhibition of LOS sialylation could mitigate this effect. Therefore, NTHi was grown in defined media containing 1,000 μM SiaNAc as well as increasing concentrations of SiaNAc-$3F_{ax}$ (1–100 μM) and subjected to serum-mediated killing. Increasing concentrations of SiaNAc-$3F_{ax}$ completely abolished sialic acid-mediated serum resistance of NTHi in a dose-dependent manner (Figure 3C), with an IC_{50} of 1.2 μM (Figure 3D). On the other hand, no inhibition was observed with the protected fluorinated sialic acid inhibitor, Ac_5SiaNAc-$3F_{ax}$ (Figure S3B).

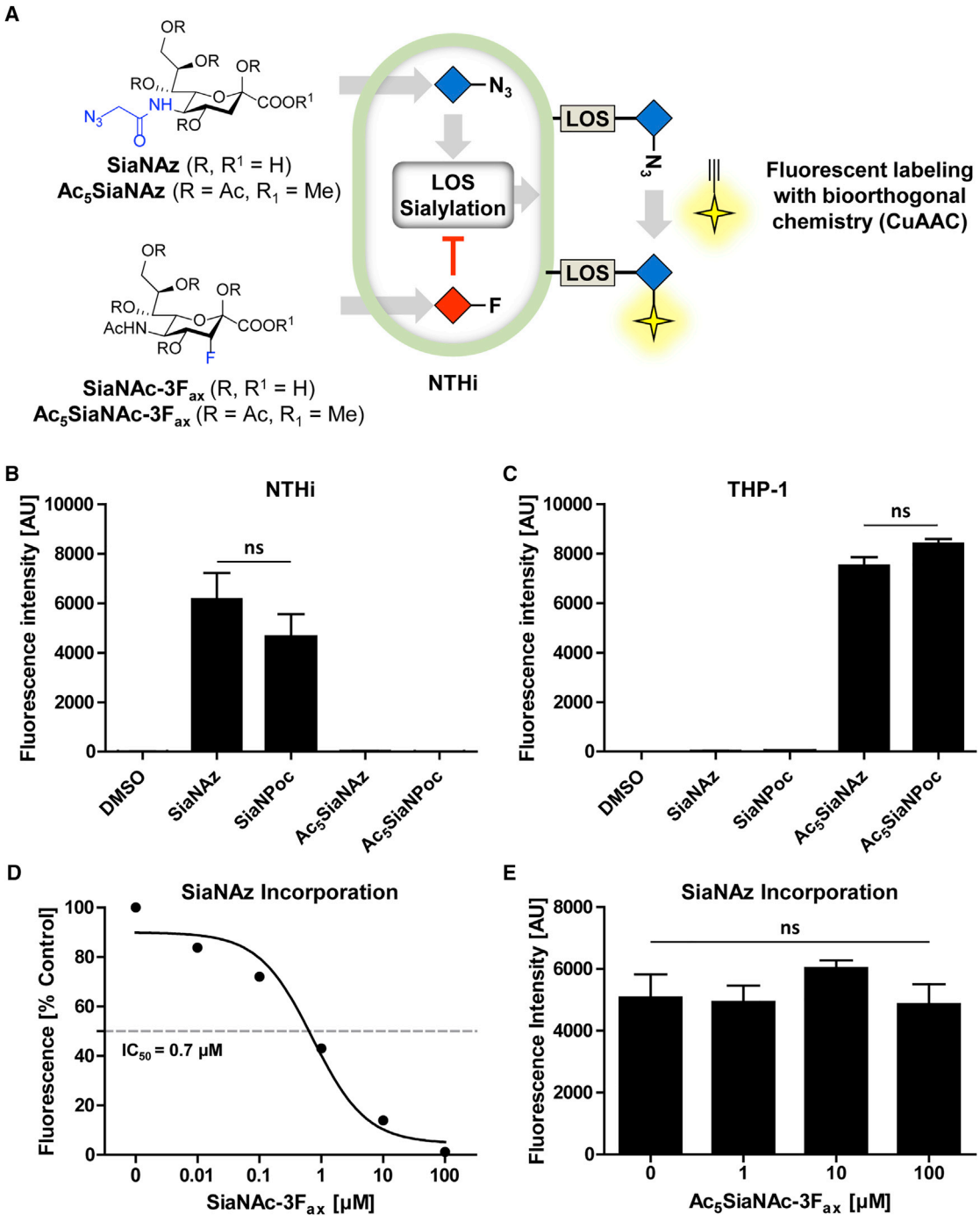


Figure 2. Visualization and Selective Inhibition of Sialylation in NTHi

(A) Schematic presentation of labeling and inhibition of NTHi LOS sialylation with sialic acid mimetics.

(B and C) NTHi 86-028NP bacteria or THP-1 cells were grown in the presence of 100 μM SiaNAz, SiaNPoc, Ac_5SiaNAz, or Ac_5SiaNPoc. Bar diagrams show incorporation of sialic acid analogs in NTHi (B) or THP-1 cells (C) as determined by CuAAC and flow cytometry (n = 4 ± SEM).

(D) IC_{50} curve of the effect of SiaNAc-$3F_{ax}$ on the incorporation of 100 μM SiaNAz by NTHi.

(E) NTHi 86-028NP bacteria were grown in medium with 100 μM SiaNAz and 1, 10, or 100 μM Ac_5SiaNAc-$3F_{ax}$. Incorporation of SiaNAz by NTHi was determined by CuAAC followed by flow cytometric analysis (n = 4 ± SEM). One-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. NS, not significant.

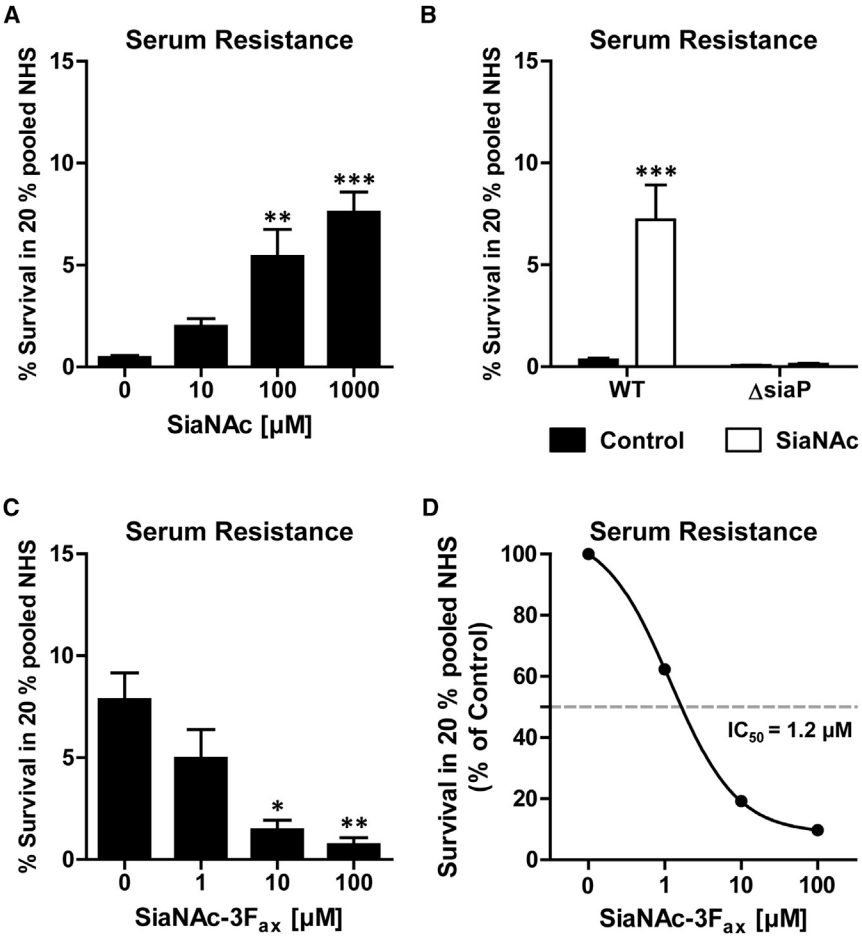


Figure 3. Inhibition of Sialylation Reduces Serum Resistance of NTHi

(A) NTHi 86-028NP bacteria were grown in RPMI-1640 medium with 1, 10, 100, or 1,000 μM SiaNAc, and serum survival in 20% normal human serum (NHS) was determined (n = 3–7 ± SEM).

(B) NTHi 86-028NP wild-type (WT) or Δ*siaP* mutant bacteria were grown in medium supplemented with 1,000 μM SiaNAc, and serum survival was determined (n = 7 ± SEM).

(C) NTHi 86-028NP bacteria were grown with 1,000 μM SiaNAc and 0.01, 0.1, 1, 10, or 100 μM SiaNAc-3F$_{ax}$. Serum survival was determined for 1 hr with 20% pooled NHS (n = 3 ± SEM).

(D) IC$_{50}$ curve of the effect of SiaNAc-3F$_{ax}$ on SiaNAc-mediated serum resistance. One-way ANOVA with Dunnett's multiple comparison test was used for statistical analysis.

*p < 0.05, **p < 0.01, ***p < 0.001.

Visualization and Inhibition of Sialic Acid Transfer from Primary Human Bronchial Epithelial Cells to NTHi

These results demonstrate that SiaNAc-3F$_{ax}$ effectively inhibits LOS sialylation thereby decreasing resistance to serum-mediated killing. However, it remains elusive how sialic acid is released from host tissue and transferred to NTHi. Hence, we constructed a model system of the respiratory tract to study transfer of host sialic acids to NTHi in a physiological setting (Figure 4A). Primary human bronchial epithelial cells (PHBECs), which express mucins at the apical side, were differentiated in a transwell system using basolateral medium supplemented with Ac$_5$SiaNAz or Ac$_5$SiaNPoc, and incorporation of SiaNPoc into secreted glycoproteins released on the apical side of the PHBEC culture was confirmed by western blot analysis (Figure S4A). Noteworthy, only protected sialic acid analogs, but not the non-protected versions, were incorporated into the secreted proteins (Figure S4A). Subsequently, NTHi was grown at the apical side of the transwell for 24 hr, and uptake and incorporation of SiaNAz or SiaNPoc sialic acids into the LOS was determined using CuAAC and flow cytometry. Incorporation of PHBEC-derived azide sialic acids by NTHi was clearly detected when Ac$_5$SiaNAz was added to the PHBEC growth medium at the basolateral side (Figure 4B). Similar results were obtained with PHBECs grown in the presence of Ac$_5$SiaNPoc, albeit to a lower extent even though Ac$_5$SiaNAz and Ac$_5$SiaNPoc are incorporated with comparable efficiency (Figures 2B and 2C). Recently, we showed that Poc, but not azide-modified sialic acids, was largely resistant to cleavage by bacterial sialidases (also known as neuraminidases) (Heise et al., 2017). This difference in sensitivity toward release by sialidase could explain the lower transfer of SiaNPoc compared with SiaNAz.

To confirm the requirement for sialidase activity for sialic acid transfer, sialidase inhibitor N-acetyl-2,3-dehydro-2-deoxy-neuraminic acid (DANA) was added 2 days prior to NTHi infection. Treatment with DANA significantly reduced transfer from host cell sialic acids to NTHi (Figure 4C). Combined with the reduced transfer of SiaNPoc, these data strongly suggest that sialidase activity is required for the transfer of host cell sialic acids to NTHi. So far, sialidase activity by *Haemophilus influenzae* has not been conclusively demonstrated (Apicella, 2012), therefore it is possible that endogenous host sialidases contribute to the release of the sialic acid analogs from host cell glycans.

Next, we determined whether sialic acid transfer could be inhibited with SiaNAc-3F$_{ax}$. NTHi wild-type and ΔsiaP mutant strain were grown at the apical side of the transwell, both with and without the SiaNAc-3F$_{ax}$ inhibitor, for 24 hr, and subsequently the uptake and incorporation of SiaNAz sialic acid analogs into LOS was determined. Clear incorporation of PHBEC-derived SiaNAz sialic acid was detected for NTHi wild-type strain, but not for the ΔsiaP mutant strain (Figure 4D), while treatment with 1 μM SiaNAc-3F$_{ax}$ completely inhibited SiaNAz incorporation into wild-type NTHi. Furthermore, wild-type NTHi grown on PHBECs showed higher survival in serum compared with the siaP mutant, (Figure 4E). Increased survival was also observed for DMSO- and Ac$_5$SiaNAz-treated PHBEC cultures, suggesting that both normal and azide sialic acids mediate NTHi serum

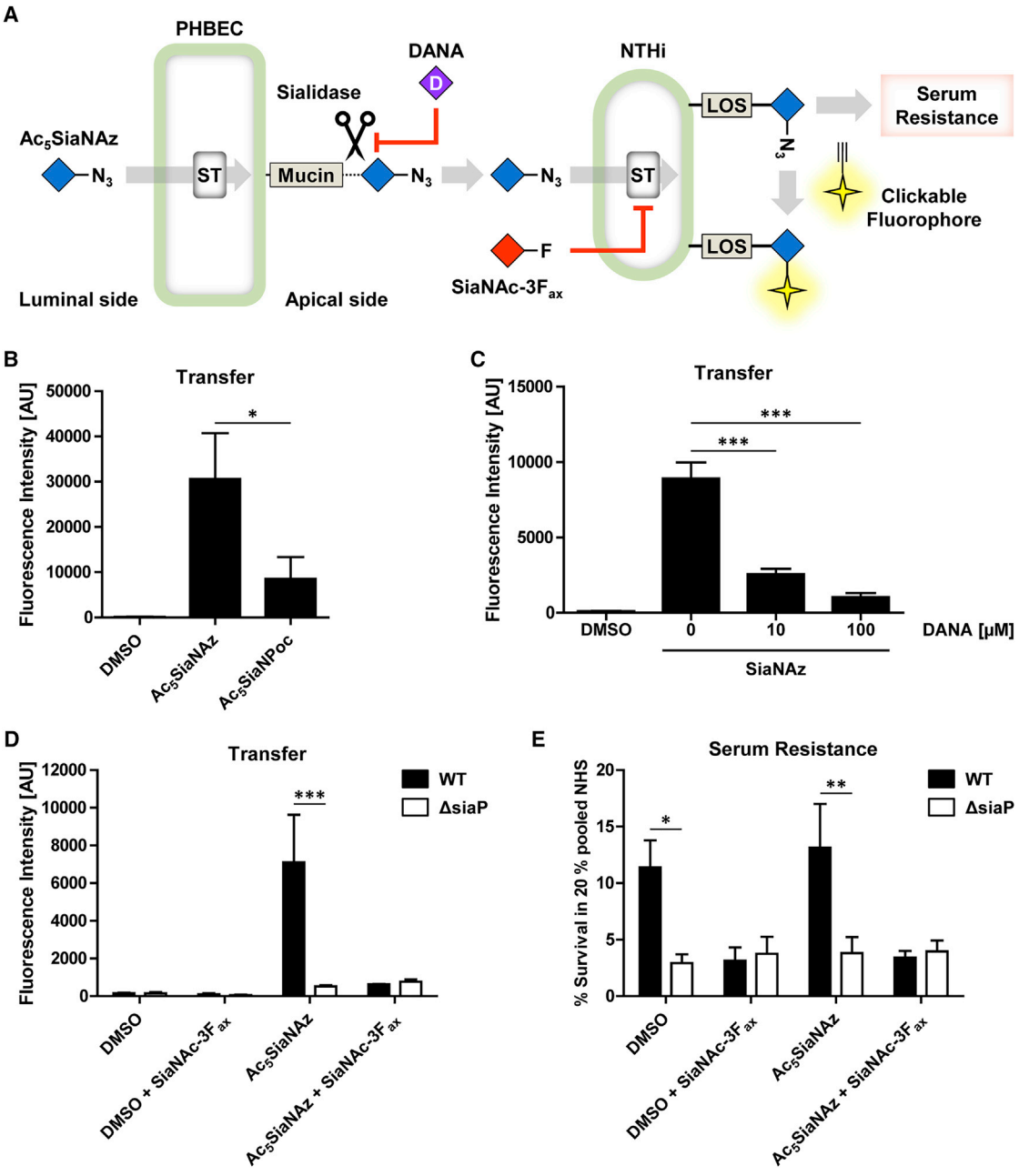


Figure 4. Visualization and Inhibition of Sialic Acid Transfer from PHBECs to NTHi

(A) Schematic presentation of sialic acid transfer from PHBECs to NTHi in the presence of the inhibitors DANA and SiaNAc-3F_{ax}.

(B) PHBECs were grown with 100 μM Ac_5SiaNAz or Ac_5SiaNPoc in basolateral medium for 3 days. NTHi 86-028NP bacteria were grown in Dulbecco's PBS on the apical side for 24 hr, and SiaNAz or SiaNPoc transfer was determined by CuAAC and flow cytometry (n = 4 ± SEM).

(C) PHBECs were grown with 100 μM Ac_5SiaNAz in basolateral medium for 3 days with sialidase inhibitor DANA at the apical and basolateral side. NTHi 86-028NP bacteria were grown in Dulbecco's PBS on the apical side for 24 hr, and SiaNAz transfer was determined by CuAAC followed by flow cytometric analysis (n = 5 ± SEM).

(D and E) PHBECs were grown with 100 μM Ac_5SiaNAz and/or 1 μM SiaNAc-3F_{ax} for 3 days. NTHi 86-028NP WT or Δ*siaP* mutant bacteria were grown in Dulbecco's PBS on the apical side for 24 hr. SiaNAz transfer was determined by CuAAC followed by flow cytometric analysis (D) and serum survival was determined for 1 hr with 20% pooled NHS (E) (n = 4 ± SEM). An unpaired t test (B), one-way ANOVA with Dunnett's multiple comparison test (C) or two-way ANOVA with Bonferroni post-tests (D and E) was used for statistical analysis.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

resistance after transfer. Importantly, bacterial growth was similar in all conditions, and PHBEC viability was not significantly affected by NTHi growth (Figures S4B and S4C). In accordance with our data obtained with defined culture medium, addition of SiaNAc-3F_{ax} abrogated sialic acid-mediated serum resistance of NTHi (Figure 4E).

In conclusion, we have shown that sialic acid mimetics are useful tools to visualize or inhibit sialic acid utilization in NTHi. In a model for the human respiratory tract we demonstrated that sialic acids were release by sialidases and subsequently taken up by NTHi. Moreover, we showed that pharmacological inhibition of NTHi LOS sialylation abolished the resistance to serum-mediated killing. Given the crucial role of sialylation in NTHi infections, these sialic acid mimetics might therefore enable the development of NTHi-specific therapies.

SIGNIFICANCE

The potent sialic acid-based glycotools developed herein can be used to visualize or inhibit sialic acid utilization in NTHi. Exploiting the fact that the sialic acid uptake mechanism differs in host and NTHi makes it possible to orthogonally label and inhibit host or NTHi sialylation. This is an important feature that enables the study of sialic acid exchange between host and NTHi. In addition, the inhibition of sialylation could be achieved in a selective manner thereby reducing the potential for side effects. Given the crucial role of sialylation in NTHi infections, these tools might therefore enable the development of NTHi-specific therapies. Since NTHi sialic acid utilization is phase variable, it may be a non-invasive way to clear the opportunistic subpopulation of NTHi with minimal consequences to rest of the host microbiome.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Bacterial Strains
  - Cell Lines
- METHOD DETAILS
  - Generation of ΔsiaP Gene Deletion Mutants
  - Treatment with Sialic Acid Mimetics and NTHi Co-Culture
  - CuAAC and Flow Cytometry Analysis
  - Lectin Staining
  - Lipooligosaccharide Isolation and SDS-PAGE
  - Silver Staining
  - Western Blot Analysis
  - Serum Survival Assays
  - MTS Cell Proliferation Assay
  - General Synthetic Procedures
  - List of Abbreviations
  - Synthetic Experimental
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and one data file and can be found with this article online at https://doi.org/10.1016/j.chembiol.2018.05.018.

ACKNOWLEDGMENTS

This work was supported by a Radboud University Medical Center PhD grant awarded to C.B. and a KWF grant (KUN2015-7604) awarded to G.J.A., T.J.B., and C.B., and funding from the NWO Netherlands Research School for Chemical Biology (NRSCB).

AUTHOR CONTRIBUTIONS

Conceptualization, T.H., J.D.L., M.I.d.J., G.J.A., C.B., and T.J.B.; Methodology, T.H., J.D.L., C.B., and T.J.B.; Validation, J.D.L. and C.B.; Formal Analysis J.D.L., C.B., and T.J.B.; Investigation, T.H., J.D.L., E.R., and C.B.; Resources, T.H., J.D.L., E.R., C.B., and T.J.B.; Writing – Original Draft, T.H., J.D.L., G.J.A., C.B., and T.J.B.; Writing – Review & Editing, J.D.L., C.B., and T.J.B.; Visualization, T.H., J.D.L., C.B., and T.J.B.; Supervision, J.D.L., M.I.d.J., G.J.A., and T.J.B.; Project Administration, J.D.L. and T.J.B.; Funding Acquisition, J.D.L., M.I.d.J., G.J.A., C.B., and T.J.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: October 13, 2017
Revised: February 26, 2018
Accepted: May 25, 2018
Published: July 5, 2018

REFERENCES

Apicella, M.A. (2012). Nontypeable *Haemophilus influenzae*: the role of N-acetyl-5-neuraminic acid in biology. Front. Cell. Infect. Microbiol. *2*, 19.

Büll, C., Boltje, T.J., Wassink, M., de Graaf, A.M., van Delft, F.L., Den Brok, M.H., and Adema, G.J. (2013). Targeting aberrant sialylation in cancer cells using a fluorinated sialic acid analog impairs adhesion, migration, and in vivo tumor growth. Mol. Cancer Ther. *12*, 1935–1946.

Büll, C., Heise, T., Beurskens, D.M.H., Riemersma, M., Ashikov, A., Rutjes, F.P.J.T., Van Kuppevelt, T.H., Lefeber, D.J., Den Brok, M.H., et al. (2015). Sialic acid glycoengineering using an unnatural sialic acid for the detection of sialoglycan biosynthesis defects and on-cell synthesis of siglec ligands. ACS Chem. Biol. *10*, 2353–2363.

Büll, C., Heise, T., Van Hilten, N., Pijnenborg, J.F., Bloemendal, V.R., Gerrits, L., Kers-Rebel, E.D., Ritschel, T., Den Brok, M.H., et al. (2017). Steering siglec-sialic acid interactions on living cells using bioorthogonal chemistry. Angew. Chem. Int. Ed. *56*, 3309–3313.

Burghout, P., Bootsma, H.J., Kloosterman, T.G., Bijlsma, J.J., de Jongh, C.E., Kuipers, O.P., and Hermans, P.W. (2007). Search for genes essential for pneumococcal transformation: the RADA DNA repair protein plays a role in genomic recombination of donor DNA. J. Bacteriol. *189*, 6540–6550.

Burkart, M.D., Vincent, S.P., and Wong, C.H. (1999). An efficient synthesis of CMP-3-fluoroneuraminic acid. Chem. Commun. 1525–1526.

Cheng, B., Xie, R., Dong, L., and Chen, X. (2016). Metabolic remodeling of cell-surface sialic acids: principles, applications, and recent advances. Chembiochem *17*, 11–27.

Coleman, H.N., Daines, D.A., Jarisch, J., and Smith, A.L. (2003). Chemically defined media for growth of *Haemophilus influenzae* strains. J. Clin. Microbiol. *41*, 4408–4410.

Cross, A.S., Sakarya, S., Rifat, S., Held, T.K., Drysdale, B.E., Grange, P.A., Cassels, F.J., Wang, L.X., Stamatos, N., Farese, A., et al. (2003). Recruitment of murine neutrophils in vivo through endogenous sialidase activity. J. Biol. Chem. *278*, 4112–4120.

de Vries, S.P., Burghout, P., Langereis, J.D., Zomer, A., Hermans, P.W., and Bootsma, H.J. (2013). Genetic requirements for *Moraxella catarrhalis* growth under iron-limiting conditions. Mol. Microbiol. *87*, 14–29.

Figueira, M.A., Ram, S., Goldstein, R., Hood, D.W., Moxon, E.R., and Pelton, S.I. (2007). Role of complement in defense of the middle ear revealed by restoring the virulence of nontypeable *Haemophilus influenzae* siaB mutants. Infect. Immun. *75*, 325–333.

Gantt, R., Millner, S., and Binkley, S.B. (1964). Inhibition of N-acetylneuraminic acid aldolase by 3-fluorosialic acid. Biochemistry *3*, 1952–1960.

Hallström, T., and Riesbeck, K. (2010). *Haemophilus influenzae* and the complement system. Trends Microbiol. *18*, 258–265.

Heise, T., Büll, C., Beurskens, D.M., Rossing, E., de Jonge, M.I., Adema, G.J., Boltje, T.J., and Langereis, J.D. (2017). Metabolic oligosaccharide engineering with alkyne sialic acids confers neuraminidase resistance and inhibits influenza reproduction. Bioconjug. Chem. *28*, 1811–1815.

Herriott, R.M., Meyer, E.M., and Vogt, M. (1970). Defined nongrowth media for stage II development of competence in *Haemophilus influenzae*. J. Bacteriol. *101*, 517–524.

Jenkins, G.A., Figueira, M., Kumar, G.A., Sweetman, W.A., Makepeace, K., Pelton, S.I., Moxon, R., and Hood, D.W. (2010). Sialic acid mediated transcriptional modulation of a highly conserved sialometabolism gene cluster in *Haemophilus influenzae* and its effect on virulence. BMC Microbiol. *10*, 48.

Jones, P.A., Samuels, N.M., Phillips, N.J., Munson, R.S., Jr., Bozue, J.A., Arseneau, J.A., Nichols, W.A., Zaleski, A., Gibson, B.W., and Apicella, M.A. (2002). *Haemophilus influenzae* type b strain A2 has multiple sialyltransferases involved in lipooligosaccharide sialylation. J. Biol. Chem. *277*, 14598–14611.

Langereis, J.D., and de Jonge, M.I. (2015). Invasive disease caused by nontypeable *Haemophilus influenzae*. Emerg. Infect. Dis. *21*, 1711–1718.

Langereis, J.D., de Jonge, M.I., and Weiser, J.N. (2014). Binding of human factor H to outer membrane protein P5 of non-typeable *Haemophilus influenzae* contributes to complement resistance. Mol. Microbiol. *94*, 89–106.

Langereis, J.D., and Hermans, P.W. (2013). Novel concepts in nontypeable *Haemophilus influenzae* biofilm formation. FEMS Microbiol. Lett. *346*, 81–89.

Langereis, J.D., Zomer, A., Stunnenberg, H.G., Burghout, P., and Hermans, P.W. (2013). Nontypeable *Haemophilus influenzae* carbonic anhydrase is important for environmental and intracellular survival. J. Bacteriol. *195*, 2737–2746.

Lantos, A.B., Carlevaro, G., Araoz, B., Ruiz Diaz, P., Camara Mde, L., Buscaglia, C.A., Bossi, M., Yu, H., Chen, X., Bertozzi, C.R., et al. (2016). Sialic acid glycobiology unveils trypanosoma cruzi trypomastigote membrane physiology. PLoS Pathog. *12*, E1005559.

Rillahan, C.D., Antonopoulos, A., Lefort, C.T., Sonon, R., Azadi, P., Ley, K., Dell, A., Haslam, S.M., and Paulson, J.C. (2012). Global metabolic inhibitors of sialyl- and fucosyltransferases. Nat. Chem. Biol. *8*, 661–668.

Severi, E., Hood, D.W., and Thomas, G.H. (2007). Sialic acid utilization by bacterial pathogens. Microbiology *153*, 2817–2822.

Sletten, E.M., and Bertozzi, C.R. (2009). Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. Angew. Chem. Int. Ed. *48*, 6974–6998.

Suzuki, K., and Bakaletz, L.O. (1994). Synergistic effect of adenovirus type 1 and nontypeable *Haemophilus influenzae* in a chinchilla model of experimental otitis media. Infect. Immun. *62*, 1710–1718.

Tra, V.N., and Dube, D.H. (2014). Glycans in pathogenic bacteria - potential for targeted covalent therapeutics and imaging agents. Chem. Commun. *50*, 4659–4673.

Van Eldere, J., Slack, M.P., Ladhani, S., and Cripps, A.W. (2014). Non-typeable *Haemophilus influenzae*, an under-recognised pathogen. Lancet Infect. Dis. *14*, 1281–1292.

Vimr, E.R., Kalivoda, K.A., Deszo, E.L., and Steenbergen, S.M. (2004). Diversity of microbial sialic acid metabolism. Microbiol. Mol. Biol. Rev. *68*, 132–153.

Williams, B.J., Morlin, G., Valentine, N., and Smith, A.L. (2001). Serum resistance in an invasive, nontypeable *Haemophilus influenzae* strain. Infect. Immun. *69*, 695–705.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Sheep polyclonal anti-Factor H	Abcam	Cat# ab8842; RRID: AB_2291797
Donkey polyclonal anti-mouse IgG Alexa Fluor 488	Jackson immunoresearch	Cat# 713-095-003; RRID: AB_2340718
Bacterial and Virus Strains		
R2866	(Williams et al., 2001)	N/A
R2866ΔsiaP	This study	N/A
86-028NP	(Suzuki and Bakaletz, 1994)	N/A
86-028NPΔsiaP	This study	N/A
Biological Samples		
Normal human serum	Immucor	Cat# PHS-N100
Chemicals, Peptides, and Recombinant Proteins		
SiaNAz	This study	N/A
Ac_5SiaNAz	(Büll et al., 2015)	N/A
SiaNPoc	This study	N/A
Ac_5SiaNPoc	(Büll et al., 2015)	N/A
SiaNAc-$3F_{ax}$	This study	N/A
Ac_5SiaNAc-$3F_{ax}$	(Rillahan et al., 2012)	N/A
SiaNAc, N-Acetylneuraminic acid	Carbosynth	Cat# MA00746; CAS: 131-48-6
N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (DANA)	Sigma-Aldrich	Cat# D9050; CAS: 24967-27-9
Copper(II) sulfate pentahydrate	Sigma-Aldrich	Cat# C8027; CAS: 758-99-8
L-histidine	Sigma-Aldrich	Cat# H8000; CAS: 71-00-1
Sodium ascorbate	Sigma-Aldrich	Cat# A7631; CAS:134-03-2
Alkyne-PEG4-biotin	Sigma-Aldrich	Cat# 764213
Azide-PEG3-biotin	Sigma-Aldrich	Cat# 762024
Streptavidin-PE	Invitrogen	Cat# S866
Biotinylated MAIII	Vector Laboratories	Cat# B-1265
Biotinylated SNA-I	Vector Laboratories	Cat# B-1305
Streptavidin-HRP	Thermo Scientific	Cat# 21130
Critical Commercial Assays		
CellTiter AQ_{ueous} One Solution Cell Proliferation Assay (MTS)	Promega	Cat# G3582
DNeasy Blood and Tissue Kit	Qiagen	Cat# 69504
Experimental Models: Cell Lines		
THP-1	ATCC	TIB-202
PHBEC	Lonza	Cat# CC-2540; lot#0000205863
Oligonucleotides		
R2866_0441_L1: TCAACAGAATTGACCGCACT	This study	N/A
R2866_0441_L2: CCACTAGTTCTAGAGCGGCCGCTGCTAAACCAGCAACTA	This study	N/A
R2866_0441_R1: CTATGGCTTGGCAGGCTTAC	This study	N/A
R2866_0441_R2: GCGTCAATTCGAGGGGTATCTTATCGCTTGTCTTGCTCCA	This study	N/A
R2866_0441_C: GGAAAGATCCTTGACCAGCTT	This study	N/A
PBpR412_L: GCCGCTCTAGAACTAGTGG	(Burghout et al., 2007)	N/A
PBpR412_R: GATACCCCTCGAATTGACGC	(Burghout et al., 2007)	N/A
PBMrTn9: CAATGGTTCAGATACGACGAC	(de Vries et al., 2013)	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
FlowJo10.2	Tree Star Inc.	https://www.flowjo.com/
GraphPad Prism version 5.03	GraphPad Software	https://www.graphpad.com/scientific-software/prism/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Thomas J. Boltje (t.boltje@ru.nl).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacterial Strains

NTHi strains used in this study are listed in the Key Resources Table. Growth on plates was performed on brain heart infusion (BHI) broth (Becton Dickinson) agar plates supplemented with 10 μg/mL hemin (Sigma-Aldrich) and 2 μg/mL β-nicotinamide adenine dinucleotide (Merck) (sBHI) at 37°C + 5 % CO_2. For gene deletion mutants, sBHI plates contained 150 μg/mL of spectinomycin (Calbiochem). NTHi was grown shaking with 250 rpm at 37°C in supplemented RPMI-1640 medium as described previously (Coleman et al., 2003).

Cell Lines

THP-1 cells (human acute monocytic leukemia, male) were obtained from and authenticated by ATCC (TIB-202). The cells were cultured in RRMI-1640 medium (Life Technologies) supplemented with 10 % FBS (Greiner Bio-one), 2 mM glutamine (Lonza) and antibiotic-antimycotic solution (Life Technologies) in a humidified CO2 incubator at 37°C. PHBECs (primary human bronchial epithelial cells, male) obtained from (Lonza, CC-2540) were cultured in Bronchial Epithelial Cell Growth (BEGM) medium (Lonza) containing 50 μg/mL gentamycin (Sigma-Aldrich) and 50 ng/mL fungizone (Life Technologies) to 70 % confluence in a T75 tissue culture flask in a humidified CO_2 incubator at 37°C.

METHOD DETAILS

Generation of ΔsiaP Gene Deletion Mutants

Genomic DNA was isolated from mid-log phase cultures with a DNeasy Blood and Tissue Kit (Qiagen). Directed R2866ΔsiaP gene deletion mutant was generated by allelic exchange of the target gene with a spectinomycin resistance cassette, as described previously (Langereis et al., 2013). Overlap extension PCR was performed, which inserted the spectinomycin resistance cassette amplified from the pGSF8 plasmid with primers PBpR412_L and PBpR412_R between the two ~1000-bp flanking sequences surrounding the target gene R2866_0441. R2866ΔsiaP gene deletion mutants were obtained by transformation of the final PCR product by the method of Herriott et al. (Herriott et al., 1970) and selected by plating on sBHI agar plates containing 150 μg/mL of spectinomycin. Directed 86-028NPΔsiaP gene deletion was generated by transformation of a PCR product obtained with primers R2866_0441_L1 + R2866_0441_R1 covering the spectinomycin cassette and ~1000-bp flanking sequences from the R2866ΔsiaP mutant. Gene deletion mutants were validated by PCR with primer sets R2866_0441_L1 + R2866_0441_C and R2866_0441_L1 + PBMrTn9 that control for the presence of the gene or spectinomycin cassette, respectively. Gene deletions were crossed back to the WT strain by using chromosomal DNA of the mutant strains as donor during transformation.

Treatment with Sialic Acid Mimetics and NTHi Co-Culture

NTHi medium was supplemented with SiaNAz, SiaNPoc, SiaNAc-3F_{ax}, Ac_5SiaNAz, Ac_5SiaNPoc, Ac_5SiaNAc-3F_{ax} or N-Acetylneuraminic acid (SiaNAc) for ~8 generations before use in experiments. Viable bacterial counts were determined by plating serial dilutions in phosphate-buffered saline (PBS) on sBHI agar plates. THP-1 cells were cultured 3 days with 100 μM of the sialic acid analogues SiaNAz, SiaNPoc, SiaNAc-3F_{ax}, Ac_5SiaNAz, Ac_5SiaNPoc, Ac_5SiaNAc-3F_{ax} or solvent control. PHBECs were seeded on 24-wells transwells (Greiner) and grown to confluence in PHBEC:DMEM containing 50 μg/mL gentamycin (Sigma-Aldrich) and 50 ng/mL fungizone. PHBECs were differentiated on air-liquid interface with BEGM:DMEM medium containing 50 μg/mL gentamycin (Sigma-Aldrich) and 50 ng/mL fungizone at the basolateral side. Basolateral medium was refreshed and apical side was washed with 200 μL Dulbecco's PBS (Lonza) every 2-3 days for 3 weeks. One week before NTHi growth, antibiotics were omitted. PHBECs were grown with 100 μM Ac_5SiaNAz or Ac_5SiaNPoc for 2-3 days. Sialidase inhibitor N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid

(DANA) was added 2 days prior to NTHi growth. 1 x 10^4 NTHi bacteria in 50 μL in Dulbecco's PBS were added to the apical side of PHBEC with or without DANA or SiaNAc-$3F_{ax}$ inhibitor and grown for 24 hours. After 24 hours, 200 μL Dulbecco's PBS was added to the apical side to collect the NTHi bacteria for CFU counts, flow cytometry or serum resistance assay. PHBEC integrity was measured by transendothelial electrical resistance (TEER) before and after NTHi co-culture using a electrical resistance system (Millipore).

CuAAC and Flow Cytometry Analysis

To detect azide and propargyloxycarbonyl groups at the bacterial and cell surface, the cells were reacted for 20 minutes at 37°C with click buffer (1 x PBS, 250 μM $CuSO_4$, 200 μM L-histidine, 500 μM sodium ascorbate) containing 50 μM alkyne-PEG4-biotin conjugate or azide-PEG3-biotin conjugate, respectively, and stained with streptavidin-PE for 10 minutes at room temperature. Bacteria or cells were resuspended in 1 x PBS containing 1 % BSA for analysis. Surface binding of factor H (fH) was performed by incubating bacteria with heat-inactivated (20 min 56°C) pooled normal human serum (NHS) in Hank's buffered salt solution (HBSS) without phenol red containing Ca^{2+}/Mg^{2+} for 1 hour at 37°C. Bacteria were fixed in 2 % paraformaldehyde, and incubated with 1:100 diluted sheep anti-human fH in HBSS without phenol red containing Ca^{2+}/Mg^{2+} + 5 % FCS for 30 min at 4°C, followed by an incubation with 1:200 diluted donkey anti-goat-Alexa488 conjugated antibodies in HBSS without phenol red containing Ca^{2+}/Mg^{2+} + 5 % FCS 30 minutes at 4°C. Bacteria were washed and taken up in PBS for flow cytometry analysis. Fluorescence was assessed using a LSR II (BD Biosciences) or CyAn ADP flow cytometer (Beckman-Coulter) followed by analysis with FlowJo software.

Lectin Staining

THP-1 cells were stained for 1 hour with biotinylated MALII (5 μg/ml) (Vector Laboratories, Inc.) or SNA-I (1 μg/ml) (Vector Laboratories, Inc.), recognizing α2,3-linked or α2,6-linked sialic acids sialic acids, respectively, in carbo-free blocking solution containing 1 mM $CaCl^{2+}$ and 1 mM $MgCl^{2+}$ at 4°C. The cells were washed in 1 x PBS containing 1 % BSA and cell-bound biotinylated lectins were conjugated for 10 minutes at 4°C with 2 μg/ml streptavidin-PE and analyzed by flow cytometry. Untreated cells stained only with streptavidin-PE were used as background control and the percentage lectin binding was calculated by normalizing the mean fluorescence intensity values from the bound lectins to the respective control.

Lipooligosaccharide Isolation and SDS-PAGE

LOS was isolated as described previously (Jones et al., 2002). Bacteria were grown in sBHI to OD_{600} ~ 0.9 and washed twice with PBS and suspended in 150 uL lysis buffer (60 mM Tris, 10 mM EDTA, 2% SDS, pH 6.8) and boiled 5-10 min. 30 μL proteinase K (2.5 mg/mL) was added and incubated 16-24h at 37°C. The LOS was precipitated with 20 μL 3M sodium acetate and 400 μL 100% EtOH and incubated 1h at -20°C and centrifuged 5 minutes at 15.000g. The pellets were washed twice with 70% EtOH and suspended in 180 μL H_2O. LOS samples were separated on a Tris-Tricine SDS-PAGE gel with a Protean II xi cell electrophoresis system (Bio-Rad) and visualized by silver staining or transferred to PVDF membrane for Western blotting.

Silver Staining

The Tris-Tricine SDS-PAGE gel was fixed for 1 h (45 % methanol, 10 % acetic acid), washed 3 times 20 min. with 50 % methanol and incubated 1 min. in sensitizing solution (0.02 % sodium thiosulfate). The gel was washed 3 times with distilled water and incubated for 20 min. in impregnation solution (0.2 % silver nitrate, 0.075 % formaldehyde). After washing the gel twice with distilled water, it was treated with develop solution (6 % sodium carbonate, 0.02 % sodium thiosulfate, 0.05 % formaldehyde) until the bands were clearly visible. Developing was stopped by incubation in 0.1% acetic acid followed by 30 min. wash with distilled water.

Western Blot Analysis

Membranes were blocked with 5 % BSA in PBS for 1 hour, washed 5 times with PBS. To detect SiaNPoc, membrane was reacted for 20 minutes at 37°C with click buffer (1 x PBS, 250 μM $CuSO_4$, 500 μM sodium ascorbate) containing 50 μM azide-PEG3-biotin conjugate, followed by 3 washes with PBS. Biotin was stained with streptavidin-HRP for 30 minutes at room temperature, followed by 3 washes with PBS. Chemoluminescent signal was detected by ECL plus reagent (GE healthcare).

Serum Survival Assays

NTHi were washed with PBS and diluted to an OD_{620} of 0.1 in HBSS without phenol red containing Ca^{2+}/Mg^{2+} + 0.1 % gelatin (HBSS3+). Samples were finally diluted 10,000-fold in HBSS3+ to obtain a concentration of ~20,000 CFU/mL. 50 μL of the bacteria was mixed with 50 μL 40 % pooled NHS or heat-inactivated (HI)-NHS, diluted in HBSS3+ and incubated 30 min. or 1 h at 37°C. Serial dilutions were plated on sBHI plates and incubated overnight at 37°C in 5 % CO2. Survival was determined by dividing the CFU counts in 20 % NHS with the CFU count in 20 % HI-NHS after incubation.

MTS Cell Proliferation Assay

Five thousand human monocytic THP-1 were cultured in a 96-wells plate with sialic acid analogues SiaNAz, SiaNPoc, SiaNAc-$3F_{ax}$, Ac_5SiaNAz, Ac_5SiaNPoc, Ac_5SiaNAc-$3F_{ax}$ or solvent control in a total volume of 100 μL for 24h or 48h in a humidified CO_2 incubator at 37°C. After 24h or 48h, 20 μL of CellTiter 96®AQ_{ueous} was added to each well and incubated for 4 hours in a humidified CO_2 incubator at 37°C. Absorbance was recorded at 492 nm using a and medium control was subtracted.

General Synthetic Procedures

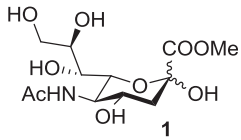
^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz or Bruker Avance III 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz). All NMR signals were assigned on the basis of ^{1}H, ^{13}C, ^{19}F NMR, COSY and HSQC experiments. Mass spectra were recorded on a JEOL JMS-T100CS AccuTOF mass spectrometer. Automatic column chromatography was performed on Biotage Isolera Spektra One, using SNAP cartridges 10-50g filled with normal silica (Biotage, 30-100 μm, 60 Å) or water resistant iatro beads. Microwave reactions were perfomed on a Biotage Initiator 4.1.3. TLC analysis was conducted on TLC Silicagel, 60, F254, Merck, with detection by UV absorption (254 nm) where applicable, and by spraying with 20% sulfuric acid in methanol followed by charring at ~150°C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 gl-1) in 10% sulfuric acid in methanol followed by charring at ~300°C. DCM, ACN and Tol were freshly distilled. All reactions were carried out under an argon atmosphere.

List of Abbreviations

Ac_2O = Acetic anhydride, Acet = Acetone, ACN = Acetonitrile, AzOSu = Azidoaceticacid-Hydroxysuccinimide ester, $BF_3{\cdot}Et_2O$ = Boron trifluoride etherate, CD_3OD = Deuterated methanol, $CDCl_3$ = Deuterated chloroform, D_2O = Deuterium oxide, DMF = *N*,*N*-Dimethylformamide, DCM = Dichloromethane, EtOAc = Ethyl acetate, LiOH = Lithium hydroxide, MeOH = Methanol, NaOH = Sodium hydroxide, PocOSu =Propargylcarboxycarbonyl hydroxysuccinimide carbonate, Pyr = Pyridine, TEA = Triethylamine, TFA = Trifluoroacetic acid, TFAA = Trifluoroacetic acid anhydride, TMSOTf = Trimethylsilyl trifluoromethanesulfonate, Tol = Toluene, I_2 = Iodine, SAda = Adamantyl-thiol.

Synthetic Experimental

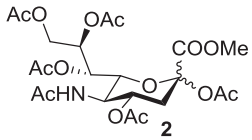
Methyl (5-acetamido-3,5-dideoxy-5-D-glycero-D-galacto)onate (1)



1

The Dowex H^+ resin was washed with MeOH three times before adding 0.2 g resin to a solution of N-acetylneuraminic acid (1 g; 3.24 mmol) in MeOH (20 mL; 0.16 M). After stirring at 80°C for 1 h under microwave radiation, the colorless mixture was filtered. The filtrate was concentrated *in vacuo* affording **1** (1.05 g; 3.24 mmol; quant.) as a white foam. **TLC**: (H_2O:ACN, 20:80 v/v) R_f = 0.39. **^{1}H NMR** (500 MHz, CD_3OD) δ 4.07 – 3.98 (m, 2H, H-4; H-6), 3.85 – 3.79 (m, 2H, H-5; H-9_a), 3.78 (s, 3H, OMe), 3.70 (ddd, *J* = 8.8, 5.7, 2.8 Hz, 1H, H-8), 3.62 (dd, *J* = 11.3, 5.7 Hz, 1H, H-9_b), 3.48 (dd, *J* = 9.2, 1.1 Hz, 1H, H-7), 2.22 (dd, *J* = 12.9, 4.9 Hz, 1H, H-3_{eq}), 2.02 (s, 3H, Me, Ac), 1.89 (dd, *J* = 12.8, 11.5 Hz, 1H, H-3_{ax}). **^{13}C NMR** (126 MHz, CD_3OD) δ 175.10 (C-1), 171.78 (CO, Ac), 96.65 (C-2), 72.06 (C-6), 71.62 (C-8), 70.16 (C-7), 67.83 (C-4), 64.82 (C-9), 54.29 (C-5), 53.71 (OMe), 40.68 (C-3), 22.68 (Me, Ac). **HR-ESI-TOF/MS** (*m/z*): $[M+Na]^+$ calcd. for $C_{12}H_{21}NNaO_9$, 346.11140; found, 346.11286.

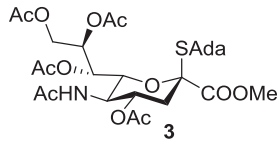
Methyl (5-acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-5-D-glycero-D-galacto)onate (2)



2

To a solution of sialic acid methyl ester **1** (20.9 g; 64.6 mmol) in Pyr (250 mL; 3.09 mol; 47.8 eq.), Ac_2O (125 mL; 1.59 mol; 24.6 eq.) was slowly added. After stirring at r.t. for 24 h, the mixture was concentrated *in vacuo* with Tol co-evaporation. The residue was dissolved in EtOAc and washed successively with HCl (0.1 M) and sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* affording **2** (34.5 g; 64.6 mmol; quant.) as white foam. **TLC**: (Acet:DCM, 40:60 v/v) R_f = 0.51. **^{1}H NMR** (500 MHz, $CDCl_3$, major anomer) δ 5.38 – 5.37 (m, 1H, H-7), 5.26 (ddt, *J* = 10.1, 7.5, 4.9 Hz, 1H, H-4), 5.07 (ddd, *J* = 6.8, 5.1, 2.5 Hz, 1H, H-8), 4.50 (dd, *J* = 12.5, 2.6 Hz, 1H, H-9_a), 4.15 – 4.10 (m, 3H, H-9_b; H-6; H-5), 3.80 (s, 3H, OMe), 2.55 (dd, *J* = 13.5, 5.0 Hz, 1H, H-3_{eq}), 2.15 (s, 3H, Me, OAc), 2.14 (s, 3H, Me, OAc), 2.10 – 2.09 (m, 1H, H-3_{ax}), 2.07 (s, 3H, Me, OAc), 2.05 – 2.03 (m, 6H, 2xMe, OAc), 1.90 (s, 3H, Me, NHAc). **^{13}C NMR** (126 MHz, $CDCl_3$, major anomer) δ 171.15 (CO, Ac), 170.75 (CO, Ac), 170.41 (2xCO, Ac), 170.38 (CO, Ac), 168.37 (C-1), 166.46 (CO, Ac), 97.65 (C-2), 73.00 (C-6), 71.52 (C-8), 68.44 (C-4), 67.98 (C-7), 62.27 (C-9), 53.35 (OMe), 49.49 (C-5), 36.05 (C-3), 23.33 (Me, Ac), 21.05 (Me, Ac), 20.99 (Me, Ac), 20.93 (2xMe, Ac) 20.91 (Me, Ac). **HR-ESI-TOF/MS** (*m/z*): $[M+Na]^+$ calcd. for $C_{22}H_{31}NNaO_{14}$, 556.16422; found, 556.16487.

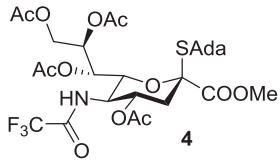
Methyl 5-acetamido-4,7,8,9-penta-O-acetyl-2,3,5-tri-deoxy-2-thioadamantyl-D-glycero-α-galacto-non-2-ulopyranosonate (3)



3

Peracetylated sialic acid **2** (5 g; 9.37 mmol) was solved in DCM (60 ml; 0.16 M) and HSAda (1.735 g; 10.31 mmol; 1.1 eq) was added. $BF_3 \cdot Et_2O$ (2.375 ml; 18.74 mmol; 2 eq) was added slowly and the reaction was stirred at room temperature for 16 h. Diluted with DCM and ice-cold sat. aq. $NaHCO_3$. The organic layer was separated, dried with $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo*. Silicagel flash column chromatography (0% → 25% Acet in DCM) afforded **3** (4.95 g; 9.37 mmol; 82%) as a white foam. **TLC**: (Acet:DCM, 25:75 v/v) $R_f = 0.46$. **^{1}H NMR** (500 MHz, $CDCl_3$, major anomer) δ 5.88 (d, *J* = 10.2 Hz, 1H, NH), 5.43 (dd, *J* = 2.8, 1.4 Hz, 1H, H-7), 5.29 – 5.17 (m, 1H, H-4), 5.10 (dt, *J* = 8.9, 1.7 Hz, 1H, H-8), 4.96 (dd, *J* = 12.4, 1.8 Hz, 1H, H-9_a), 4.52 (dd, *J* = 10.5, 2.7 Hz, 1H, H-6), 4.16 (ddd, *J* = 11.7, 9.0, 2.4 Hz, 1H, H-9_b), 4.01 (d, *J* = 10.4 Hz, 1H, H-5), 3.77 (s, 3H, OMe), 2.48 (dd, *J* = 13.5, 4.6 Hz, 1H, H-3_{eq}), 2.07 (s, 3H, Me, Ac), 2.03 (s, 3H, Me, Ac), 2.01 – 1.78 (m, 19H, 3xMe, Ac; H-3_{ax}; $(CH_2)_3$CS, SAda; 3x(CH_2CH), SAda), 1.66 – 1.54 (m, 6H, 3x(CH_2CH), SAda). **^{13}C NMR** (126 MHz, $CDCl_3$, major anomer) δ 171.33 (CO, Ac), 170.74 (CO, Ac), 170.29 (CO, Ac), 170.26 (CO, Ac), 170.09 (CO, Ac), 169.80 (C-1), 86.04 (C-2), 74.00 (C-8), 72.57 (C-6), 69.20 (C-7), 68.91 (C-4), 63.24 (C-9), 52.69 (OMe), 50.36 ((CH_2)$_3$CS, SAda), 49.31 (C-5), 43.38 ((CH_2)$_3$CH, SAda), 39.84 (C-3), 35.84 (3x(CH_2CH), SAda), 29.64 (3x(CH_2CH), SAda), 22.99 (Me, Ac), 20.98 (Me, Ac), 20.75 (Me, Ac), 20.62 (Me, Ac), 20.52 (Me, Ac). **HR-ESI-TOF/MS** (*m/z*): $[M+Na]^+$ calcd. for $C_{30}H_{43}NNaO_{12}S$, 664.24036; found, 664.23780.

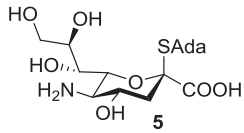
Methyl 5-trifluoroacetamido-4,7,8,9-penta-O-acetyl-2,3,5-tri-deoxy-2-thioadamantyl-D-glycero-α-galacto-non-2-ulopyranosonate (4)



4

Thioadamantyl sialic acid **3** (100 mg; 0.156 mmol) was solved in ACN (4 ml; 0.04 M) and TFAA (88 μL; 0.623 mmol; 4 eq.) was added. The reaction vessel was sealed and stirred at 135°C and 6 bar for 10 min under microwave radiation. After cooling to room temperature, the reaction was quenched by an excess of TEA and MeOH, diluted in EtOAc and washed three times with sat. aq. $NaHCO_3$. The organic layer was separated, dried with $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo*. Silicagel flash column chromatography (0% → 10% Acet in DCM) afforded **4** (80 mg; 0.12 mmol; 74%) as a white foam. **TLC**: (Acet:DCM, 10:90 v/v) $R_f = 0.75$. **^{1}H NMR** (500 MHz, $CDCl_3$, major anomer) δ 5.48 (t, *J* = 2.1 Hz, 1H, H-7), 5.43 (ddd, *J* = 11.8, 10.4, 4.7 Hz, 1H, H-4), 5.18 (dt, *J* = 8.7, 1.9 Hz, 1H, H-8), 4.98 (dd, *J* = 12.3, 1.8 Hz, 1H, H-9_a), 4.75 (dd, *J* = 10.4, 2.7 Hz, 1H, H-6), 4.25 (dd, *J* = 12.4, 8.7 Hz, 1H, H-9_b), 4.08 (q, *J* = 10.1 Hz, 1H, H-5), 3.84 (s, 3H, OMe), 2.59 (dd, *J* = 13.6, 4.7 Hz, 1H, H-3_{eq}), 2.14 (s, 3H, Me, OAc), 2.10 (s, 3H, Me, OAc), 2.04 – 1.83 (m, 16H, 2xMe, OAc; H-3_{ax}; $(CH_2)_3$CS Sada; 3x(CH_2CH), SAda), 1.71 – 1.62 (m, 6H, 3x(CH_2CH), SAda). **^{13}C NMR** (126 MHz, $CDCl_3$, major anomer) δ 171.39 (CO, Ac), 170.84 (CO, Ac), 170.37 (CO, Ac), 169.80 (CO, Ac), 169.67 (C-1), 157.72 (q, *J* = 37.8 Hz, $COCF_3$), 115.47 (q, *J* = 288.3 Hz, $COCF_3$), 86.03 (C-2), 73.61 (C-8), 71.56 (C-6), 69.11 (C-7), 68.43 (C-4), 63.15 (C-9), 52.81 (OMe), 50.61 ((CH_2)$_3$CH, SAda), 50.34 (C-5), 43.38 ((CH_2)$_3$CS, SAda), 39.86 (C-3), 35.84 (3x(CH_2CH), SAda), 29.75 (3x(CH_2CH), SAda), 21.04 (Me, Ac), 20.59 (Me, Ac), 20.56 (Me, Ac), 20.53 (Me, Ac). **HR-ESI-TOF/MS** (*m/z*): $[M+Na]^+$ calcd. for $C_{30}H_{40}F_3NNaO_{12}S$, 718.21210; found, 718.21045.

5-amido-2,3,5-tri-deoxy-2-thioadamantyl-D-glycero-α-galacto-non-2-ulopyranosonate (5)

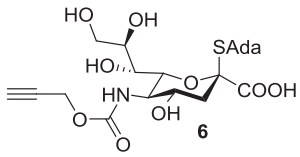


5

TFA protected sialic acid **4** (1g; 1.437 mmol) was solved in MeOH (10 ml; 0.144 M) and LiOH (482 mg; 20.12 mmol; 14 eq.) and H_2O (5 ml) were added. The reaction vessel was sealed and stirred at 120°C and 2 bar for 30 min under microwave radiation. The reaction was filtered and silica was added to the filtrate. After concentration *in vacuo*, the crude product on silica was loaded on water-resistant iatro-beads crosslinked-silicagel flash column chromatography (0% → 20% H_2O (+1% TFA) in ACN) afforded **5** (591 mg; 1.1416 mmol; 98%) as a white foam. **TLC**: (H_2O:ACN, 20:80 v/v) R_f = 0.19. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.17 (dd, *J* = 10.0, 1.0 Hz, 1H, H-5), 3.93 – 3.81 (m, 5H, H-7; H-8; H-5; H-9_a; H-4), 3.80 – 3.72 (m, 1H, H-9_b), 2.68 (t, *J* = 9.8 Hz, 1H, H-6), 2.42 (dd, *J* = 13.5, 4.6 Hz, 1H, H-3_{eq}), 2.03 – 1.95 (m, 9H, $(CH_2)_3$CS, SAda; 3x(CH_2CH), SAda), 1.74 – 1.60

(m, 7H, H-3_{ax}; 3x(C***H*****$_2$**CH), SAda). **^{13}C NMR** (126 MHz, D_2O, major anomer) δ 177.24 (C-1), 88.40 (C-2), 73.36 (C-5), 70.14 (C-7), 69.58 (C-8), 68.94 (C-4), 63.32 (C-9), 53.44 (C-6), 49.44 ((CH$_2$)$_3$CS, SAda), 43.63 (C-3), 43.32 ((CH$_2$)$_3$CS, SAda), 35.66 (3x*C*H$_2$CH, SAda), 29.68 (3xCH$_2$*C*H, SAda). **HR-ESI-TOF/MS** (*m/z*): $[M+H]^+$ calcd. for $C_{19}H_{32}NO_7S$, 418.18995; found, 418.19152.

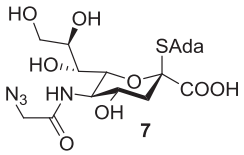
5-propargyloxycarbamado-2,3,5-tri-deoxy-2-thioadamantyl-D-glycero-α-galacto-non-2-ulopyranosonate (6)



6

Sialic acid amine **5** (50 mg; 0.12 mmol) was solved in H_2O (0.5 ml). PocOSu (28 mg; 0.144 mmol; 1.2 eq) was solved in ACN (1 ml) and added to the sialic acid solution. The reaction was stirred at r.t. for 16 h, diluted with an excess of MeOH and concentrated on silica. The product was purified on water-resistant iatro-beads crosslinked-silicagel flash column chromatography (0% → 20% H_2O (+1% TFA) in ACN) affording **7** (50 mg; 0.12 mmol; 84%) as a white foam. **TLC**: (H_2O:ACN, 25:75 v/v) R_f = 0.57. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.71 (dd, *J* = 4.8, 2.4 Hz, 2H, CH_2, Poc), 4.30 (d, *J* = 10.5 Hz, 1H, H-6), 4.04 (ddd, *J* = 11.9, 10.0, 4.8 Hz, 1H, H-4), 3.91 – 3.83 (m, 2H, H-8; H-9a), 3.73 (dd, *J* = 11.8, 5.1 Hz, 1H, H-9_b), 3.61 (d, *J* = 9.2 Hz, 1H, H-7), 3.55 (t, *J* = 10.3 Hz, 1H, H-5), 2.92 (t, *J* = 2.4 Hz, 1H, C≡CH, Poc), 2.50 (dd, *J* = 13.5, 4.9 Hz, 1H, H-3_{ax}), 2.04 – 1.95 (m, 9H, (CH$_2$)$_3$CS, SAda; 3xCH$_2$C*H*, SAda), 1.74 (dd, *J* = 13.6, 12.0 Hz, 1H, H-3_{ax}), 1.68 (d, *J* = 2.9 Hz, 6H, 3xC***H*****$_2$**CH, SAda). **^{13}C NMR** (126 MHz, D_2O) δ 176.82 (C-1), 157.52 (CO, Poc), 88.10 (*C*≡CH, Poc), 78.52 (C-2), 75.67 (C≡*C*H, Poc), 70.99 (C-6), 69.87 (C-8), 68.93 (C-7), 67.39 (C-4), 63.28 (C-9), 54.31 (C-5), 52.88 (CH_2, Poc), 49.46 ((CH$_2$)$_3$CS, SAda), 43.56 (C-3), 43.20 ((CH$_2$)$_3$CH, SAda), 35.59 (3x*C*H$_2$CH, SAda), 29.64 (3xCH$_2$*C*H, SAda). **HR-ESI-TOF/MS** (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{33}NNaO_9S$, 522.17737; found, 522.17847.

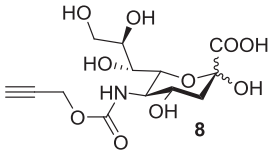
5-azidoacetamido-2,3,5-tri-deoxy-2-thioadamantyl-D-glycero-α-galacto-non-2-ulopyranosonate (7)



7

Sialic acid amine **5** (50 mg; 0.12 mmol) was solved in H_2O (0.5 ml). AzOSu (31 mg; 0.156 mmol; 1.3 eq) was solved in ACN (1 ml) and added to the sialic acid solution. The reaction was stirred at r.t. for 16 h, diluted with an excess of MeOH and concentrated on silica. The product was purified on water-resistant iatro-beads crosslinked-silicagel flash column chromatography (0% → 20% water (+1% TFA) in ACN) affording **7** (45 mg; 0.09 mmol; 75%) as a white foam. Note: Surprisingly, the conversion was not complete - 5 mg amine starting material was retrieved. **TLC**: (H_2O:ACN, 25:75 v/v) R_f = 0.50. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.39 (d, *J* = 10.6 Hz, 1H, H-6), 4.17 (ddd, *J* = 11.8, 10.0, 4.7 Hz, 1H, H-4), 4.11 (s, 2H, CH_2, Az), 3.93 (t, *J* = 10.2 Hz, 1H, H-5), 3.89 – 3.81 (m, 2H, H-8; H-9_a), 3.72 (dd, *J* = 12.1, 5.2 Hz, 1H, H-9_b), 3.54 (d, *J* = 9.4 Hz, 1H, H-7), 2.52 (dd, *J* = 13.6, 4.7 Hz, 1H, H-3_{eq}), 2.06-1.98 (m, 9H, (CH$_2$)$_3$CS, SAda; 3xCH$_2$C*H*, SAda), 1.91 (dd, *J* = 13.6, 11.8 Hz, 1H, H-3_{ax}), 1.68 (d, *J* = 2.9 Hz, 6H, 3xC***H*****$_2$**CH, SAda). **^{13}C NMR** (126 MHz, D_2O, major anomer) δ 176.47 (C-1), 175.04 (CO, Az), 86.50 (C-2), 70.78 (C-6), 69.67 (C-8), 68.65 (C-7), 66.56 (C-4), 63.09 (C-9), 52.52 (C-5), 51.93 (CH_2, Az), 50.44 ((CH$_2$)$_3$CS, SAda), 43.10 ((CH$_2$)$_3$CS, SAda), 42.53 (C-3), 35.46 (3x*C*H$_2$CH, SAda), 29.65 (3xCH$_2$*C*H, SAda). **HRMS** (*m/z*): [M+Na]+ calcd for $C_{21}H_{32}N_4NaO_8S$, 523.18385; found, 523.18332.

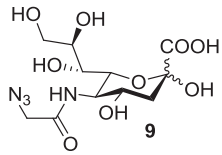
5-propargyloxycarbamado-3,5-dideoxy-D-glycero-α-galacto-non-2-ulopyranosonate (8)



8

Sialic acid **6** (55 mg; 0.11 mmol) was solved in H_2O (1 ml; 0.11 M) and I_2 (31 mg; 0.121 mmol; 1.1 eq.) was added. The reaction was stirred vigourisly for 30 min and diluted with an excess of EtOAc. The reaction was left stirring for 1 min and then washed with water. The water layer was lyophilized to yield compound **8** (33 mg; 0.110 mmol; 86%) as a white foam. **TLC**: (H_2O:ACN, 20:80 v/v) R_f = 0.11. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.63 (dd, *J* = 4.1, 2.4 Hz, 2H, CH_2, Poc), 3.98 – 3.88 (m, 2H, H-6; H-4), 3.78 (dd, *J* = 11.9, 2.7 Hz, 1H, H-9_a), 3.69 (ddd, *J* = 9.2, 6.6, 2.7 Hz, 1H, H-8), 3.58 (t, *J* = 10.2 Hz, 1H, H-5), 3.56 – 3.51 (m, 2H, H-7; H-9_b), 2.84 (t, *J* = 2.4 Hz, 1H, C≡CH, Poc), 2.13 (dd, *J* = 12.9, 4.9 Hz, 1H, H-3_{eq}), 1.75 (t, *J* = 12.3 Hz, 1H, H-3_{ax}). **^{13}C NMR** (126 MHz, D_2O, major anomer) δ 176.71 (C-1), 157.47 (CO, Poc), 92.41 (C-2), 78.52 (*C*≡CH, Poc), 75.66 (C≡*C*H, Poc), 70.46 (C-8), 70.27 (C-6) , 68.50 (C-7), 67.37 (C-4), 63.31 (C-9), 53.79 (C-5), 52.87 (CH_2, Poc), 39.35 (C-3). **HRMS** (*m/z*): [M+Na]+ calcd for $C_{13}H_{19}NO_{10}$, 372.09066; found, 372.09041.

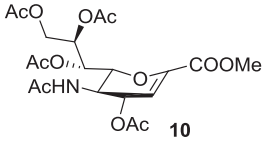
5-azidoacetamido-3,5-dideoxy-D-glycero-α-galacto-non-2-ulopyranosonate (9)



9

Sialic acid **7** (6 mg; 0.012 mmol) was solved in H_2O (1 ml; 0.012 M) and I_2 (9 mg; 0.036 mmol; 3 eq.) was added. The reaction was stirred vigourisly for 10 min and diluted with an excess of EtOAc. The reaction was left stirring for 1 min and then washed with water. The water layer was lyophilized to yield compound **9** (4 mg; 0.011 mmol; 95%) as a white foam. **TLC**: (H_2O:ACN, 20:80 v/v) R_f = 0.09. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.20 – 4.11 (m, 2H, H-4; H-6), 4.11 (s, 2H, CH_2, Az), 4.03 (d, *J* = 10.3 Hz, 1H, H-5), 3.85 (dd, *J* = 12.1, 2.7 Hz, 1H, H-9_a), 3.81 – 3.73 (m, 1H, H-8), 3.63 (dd, *J* = 11.9, 6.4 Hz, 1H, H-9_b), 3.57 (d, *J* = 9.1 Hz, 1H, H-7), 2.32 (dd, *J* = 13.0, 5.0 Hz, 1H, H-3_{eq}), 1.90 (t, *J* = 12.2 Hz, 1H, H-3_{ax}). **^{13}C NMR** (126 MHz, D_2O, major anomer) δ 176.47 (C-1), 173.82 (CO, Az), 95.52 (C-2 *hardly visible*) 70.20 (C-8), 68.20 (C-6), 52.17 (C-5), 51.92 (CH_2, Az), 38.93 (C-3). **HRMS** *(m/z)*: [M+Na]+ calcd for $C_{11}H_{18}N_4NaO_9$, 373.09715; found, 373.09913.

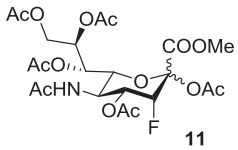
Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3-dehydro-2,3,5-trideoxy-5-β-D-glycero-D-galacto)onate (10)



10

Peracetylated sialic acid **2** (1.02 g, 1.912 mmol) was dissolved in ACN (4 ml; 0.478 M) and cooled to 0°C. TMSOTf (1.03 ml; 5.736 mmol; 3 eq.) was added dropwise to the solution which turned slightly yellow. After 4 hours, the reaction was diluted with an excess EtOAc and washed three times with sat. aqueous $NaHCO_3$ (30 mL). The organic layer was concentrated *in vacuo* and the product was purified on silicagel flash column chromatography (0 → 30% Acet in DCM), and a white solid was obtained (0.552 g, 1.166 mmol, 61% yield as a white foam). **TLC:** (Acet:DCM, 30:70 v/v) R_f = 0.50. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.05 (d, *J* = 8.9 Hz, 1H, NH), 5.99 (d, *J* = 3.1 Hz, 1H, H-3), 5.54 – 5.48 (m, 2H, H4; H7), 5.35 (ddd, *J* = 7.5, 4.4, 3.1 Hz, 1H, H-8), 4.65 (dd, *J* = 12.3, 3.1 Hz, 1H, H-9_a), 4.40 (dd, *J* = 7.8, 5.1 Hz, 2H, H-6; H-5), 4.20 (dd, *J* = 12.3, 7.3 Hz, 1H, H-9_b), 3.81 (s, 3H, OMe), 2.13 (s, 3H, Me, Ac), 2.08 (s, 3H, Me, Ac), 2.07 (s, 3H, Me, Ac), 2.06 (s, 3H, Me, Ac), 1.93 (s, 3H, Me, Ac). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.82 (CO, Ac), 170.61 (CO, Ac), 170.19 (CO, Ac), 170.17 (CO, Ac), 161.63 (C-1), 145.06 (C-2), 107.98 (C-3), 76.64 (C-6), 70.85 (C-8), 68.03 (C-7), 67.64 (C-4), 61.97 (C-9), 52.57 (OMe), 46.46 (C-5), 23.09 (Me, Ac), 20.85 (Me, Ac), 20.76 (Me, Ac), 20.72 (Me, Ac), 20.70 (Me, Ac). **HRMS** *(m/z)*: [M+Na]+ calcd for $C_{20}H_{27}NO_{12}$, 496.1431; found, 496.1430.

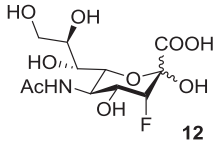
Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (11)



11

Sialic acid glycal **10** (1.5 g, 3.17 mmol) was solved in DMF (23.8 ml) and H_2O (7.9 ml; 0.1 M). Selectfluor (3.37 g, 9.51 mmol; 3 eq.) was added and the reaction was heated to 60°C for 16 hours, then concentrated *in vacuo*. The compound was redissolved in EtOAc washed with sat. aqueous $NaHCO_3$, dried with $MgSO_4$, filtered, concentrated *in vacuo*. **TLC:** (Acet:DCM, 30:70 v/v) R_f = 0.40. **HRMS** *(m/z)*: [M+Na]+ calcd for $C_{20}H_{28}FNO_{13}$, 532.1443; found, 532.1442. The crude fluorinated alcohol was dissolved in PYR (14.5 ml) and Ac_2O (7.3 ml), stirred for 16 hours at r.t. and was evaporated *in vacuo*. The resulting solid dissolved in EtOAc and sat. aqueous $NaHCO_3$. The organic phase was separated and the solvent evaporated *in vacuo* and purified on silica flash-column chromatography (0 → 30% Acet in DCM) affording **11** (1.46 g, 2.54 mmol, 80% yield over two steps) as a slightly yellow foam. **TLC:** (Acet:DCM, 30:70 v/v) R_f = 0.55. **^{1}H-NMR** (500 MHz, CD_3OD, major anomer) δ 5.58 (d, *J* = 8.9 Hz, 1H, NH), 5.46 (dd, J = 27.9, 10.7 Hz, 1H, H-4), 5.29 (dd, J = 5.0, 1.8 Hz, 1H, H-7), 5.05 (ddd, J = 6.7, 5.2, 2.5 Hz, 1H, H-8), 4.87 (dd, J = 49.1, 2.5 Hz, 1H, H-3), 4.51 (dd, J = 12.5, 2.5 Hz, 1H, H-9_a), 4.21 – 4.10 (m, 3H, H-9_b; H-5; H-6), 3.77 (s, 3H, OMe), 2.12 – 2.09 (m, 6H, 2xMe, OAc), 2.04 (s, 3H, Me, OAc), 1.98 (s, 3H, Me, OAc), 1.97 (s, 3H, Me, OAc), 1.85 (s, 3H, Me, NHAc).; **^{13}C-NMR** (126 MHz, CD_3OD) δ 170.58 (CO, Ac), 170.56 (CO, Ac), 170.50 (CO, Ac), 170.34 (CO, Ac), 167.11 (C-1), 95.16 (d, *J* = 28.8 Hz, C-2), 86.95 (d, *J* = 185.3 Hz, C-3), 71.91 (C-6), 71.37 (C-8), 68.40 (d, *J* = 17.2 Hz, C-4), 67.93 (C-7), 62.09 (C-9), 53.49 (OMe), 45.53 (C-5), 29.27 (Me, Ac), 20.88 (Me, Ac), 20.79 (Me, Ac) , 20.74 (Me, Ac), 20.65 (Me, Ac), 20.51 (Me, Ac); **HRMS** *(m/z)*: [M+Na]+ calcd for $C_{22}H_{30}FNO_{14}$, 574.1548; found, 574.1548.

(5-acetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (12)



12

Fluorinated sialic acid **11** (150 mg; 0.294 mmol) was solved in a 1 molar solution of NaOH (0.8 g; 0,02 mol) in MeOH (19 ml) and H_2O (1 ml) and stirred at r.t. for 16 h. Silica powder was added to the solution before concentrating *in vacuo* and purification on water-resistant iatro-beads crosslinked-silicagel flash column chromatography (0% → 20% H_2O (+1% TFA) in ACN) to afford **12** (52 mg; 0.15 mmol; 51%) as a white foam. **TLC**: (H_2O:ACN, 25:75 v/v) R_f = 0.27. **^{1}H NMR** (500 MHz, D_2O, major anomer) δ 4.78 (dd, *J* = 49.3, 2.5 Hz, 1H, H-3), 4.17 (t, *J* = 10.6 Hz, 1H, H-5), 4.04 (ddd, *J* = 30.0, 10.8, 2.5 Hz, 1H, H-4), 3.97 (d, *J* = 10.6 Hz, 1H, H-6), 3.86 – 3.75 (m, 3H, H-8; H-9_a), 3.57 (dd, *J* = 12.5, 7.0 Hz, 1H, H-9_b), 3.47 (dd, *J* = 8.8, 1.1 Hz, 1H, H-7), 1.98 (s, 3H, Me, NHAc). **^{13}C NMR** (126 MHz, D_2O,) δ 174.89 (CO, NHAc), 171.43 (C-1), 94.49 (d, *J* = 26.5 Hz, C-2), 89.59 (d, *J* = 177.4 Hz, C-3), 70.35 (C-8), 70.10 (C-6), 68.11 (C-7), 67.69 (d, *J* = 18.3 Hz, C-4)., 63.10 (C-9), 47.26 (C-5), 22.03 (Me, NHAc). **^{19}F NMR** (470 MHz, D_2O) δ -208.11 (dd, *J* = 49.3, 30.0 Hz, major anomer), -217.73 (dd, *J* = 51.3, 30.0 Hz, minor anomer). **HRMS** *(m/z)*: [M+Na]+ calcd for $C_{11}H_{18}FNNaO_9$, 350.08633; found, 350.08670.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are presented as mean values ± standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism version 5.03 for Windows (GraphPad Software, Inc.). A student T-test was used to compare two variables tested in a single condition. A One-way ANOVA with Tukey's Multiple Comparison Test was used to compare more than two variables with each other. An one-way ANOVA with Dunnett's Multiple Comparison Test was used to compare more than two variables with a control condition. A Two-way ANOVA with Bonferroni was used to compare two variables under multiple conditions. Differences were considered significant at $P < 0.05$. NS, not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.