Supporting information

Structure-activity relationship of fluorinated sialic acid inhibitors for bacterial sialylation

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Bacterial growth conditions
NTHi strain 86-028NP was cultured overnight at 37°C + 5% CO₂ on brain heart infusion (BHI) agar plates supplemented with 1 μg/mL hemin (Sigma-Aldrich) and 2 μg/mL β-nicotinamide adenine dinucleotide (Merck). Bacteria were grown static in 200 μL supplemented RPMI medium with addition of 100 μM N-Azidoacetylneuraminic acid (SiaNAz) with or without 10-fold dilutions of 1000 – 0.1 μM inhibitors at 37°C + 5% CO₂ in a 96-wells plate for 8 hours in a Tecan Spark 10M. Fifty μL 80% glycerol was added to the wells (end concentration 16%) and the plate was stored at -20°C for further experiments.

Copper-catalyzed-alkyne-azide-cycloaddition (CuAAC) and Flow Cytometry Analysis
To detect SiaNAz at the bacterial surface, bacteria were reacted for 20 minutes at 37°C with click buffer (250 μM CuSO₄, 200 μM L-histidine, 500 μM sodium ascorbate in PBS) containing 50 μM alkyne-PEG4-biotin conjugate (Sigma-Aldrich), and stained with streptavidin-PE for 10 minutes at room temperature. Bacteria without CuAAC reaction were used as antibody control. Bacteria were fixed in 2 % paraformaldehyde and taken up in PBS for flow cytometry analysis. Fluorescence was determined by flow cytometry using a FACS LSR II instrument (BD Biosciences) and expressed in mean fluorescence intensity (MFI) in arbitrary units (AU). Control condition was growth with 100 μM SiaNAz without inhibitor. Data was presented as % of control condition. Data were analyzed by using FlowJo version 10.4.1.

Metabolite extraction
NTHi strain 86-028NP was cultured n=2 with either 100 μM SiaFNAc or 1/1000 v/v DMSO in 7 mL Brain-Heart Infusion (BHI) medium (BD biosciences) supplemented with 1 μg/mL hemin and 2 μg/mL β-nicotinamide adenine dinucleotide (Merck) to an optical density at 620 nm (OD620) of 0.5 in a shaking incubator at 37°C. Bacteria were pelleted at 4°C, 3,220 g for 10 minutes. Supernatant was removed and pellets were resuspended in 2 mL 75 mM ammonium carbonate, pH 7.4 and bacteria were pelleted at 4°C, 3,220 g for 10 minutes. Pellets were resuspended in 1 mL cold acetonitrile:methanol:miliQ water 40:40:20 v:v, transferred to 1.5 mL Eppendorf tubes and incubated for 5 minutes at -20°C. samples were centrifuged for 3 minutes,16,100 g at 4°C. Supernatant was collected and concentrated at rt in vacuo (SpeedVac, Thermo Fisher Scientific) and stored at -80°C until further analysis.

Liquid chromatography – mass spectrometry analysis of nucleotide sugars
UHPLC and QQQ Mass-spectrometry were performed as previously described. All samples were measured on a QQQ LC/MS system with a highflow iFunnel ionization source (Agilent) and analysis was done using MassHunter Workstation software (Agilent) using default settings. For data interpretation, peak areas for all nucleotide sugars were normalized to the sum of all areas to obtain the relative abundance. Data was processed using Microsoft Excel 365.
Figure S1: Pie charts containing relative abundances (% of total response) for n=2 samples from nthi, grown in medium containing either 1/1000 v/v DMSO (left) or 100 μM SiaF. Miscellaneous fraction is comprised of the sum of abundance of peaks for nucleotide sugars for which no standards were measured.
Experimental methods

NMR spectra were recorded on a Bruker Avance III 400 MHz or a Bruker 500 MHz spectrometer and the compounds were assigned using $^1$H NMR, $^{13}$C NMR, $^{19}$F NMR, COSY, HSQCED and HMBC spectra. Chemical shifts were reported in parts per million (ppm.) relative to reference (CDCl$_3$: $^1$H: 7.26 ppm. and $^{13}$C 77.16 ppm.; CD$_3$OD: $^1$H: 3.31 ppm. and $^{13}$C 49.00 ppm.; D$_2$O: $^1$H 4.79 ppm.) NMR data are presented in the following way: chemical shift, multiplicity ($s$ = singlet, $bs$ = broad singlet, $d$ = doublet, $t$ = triplet, $dd$ = doublet of doublets, $ddd$ = doublet of doublet of doublets, $dt$ = doublet of triplet of doublets $h$ = heptet, $m$ = multiplet and/or multiple resonances) and coupling constants $J$ in Hz. Reactions were monitored using TLC F$_254$ (Merck KGaA) using UV absorption detection (254 nm) and by spraying them with dinitrophenylhydrazine (DNP) or 5% conc. H$_2$SO$_4$ in MeOH or cerium ammonium molybdate stain (Hannesian’s stain) followed by charring at $\pm$300 °C. Mass spectra were recorded on a JEOL AccuTOF CS JMS-T100CS (ESI) mass spectrometer. Purification by flash column chromatography was executed using silica gel 60 (Merck, 0.040‐0.063mm) or using automatic flash column chromatography on a Biotage Isolera Spektra One using SNAP or Silicycle cartridges (Biotage, 30‐100 μm, 60 Å) 4‐50 g. Reactions under protective atmosphere were performed under positive Ar./N2 flow in flame-dried flasks.

Compounds 1a, 1d, 1f, 1g, 1h, 1i, 1j, 2e and 10 have been described previously.

| Compound | 95% Confidence interval | EC50 calculated | lower 95% confidence limit | higher 95% confidence limit |
|----------|-------------------------|-----------------|---------------------------|---------------------------|
| SiaFNAc  | 0.08492                 | 0.0693          | 0.1041                    |                           |
| SiaFNFMFA| 0.1185                  | 0.09304         | 0.1509                    |                           |
| SiaFNDFA | 0.6279                  | 0.3945          | 0.9993                    |                           |
| SiaFNTFA | 62.92                   | 34.11           | 116.1                     |                           |
| SiaFNPra | 0.2286                  | 0.1139          | 0.4585                    |                           |
| SiaFNAz  | 21.95                   | 11.78           | 40.91                     |                           |
| SiaFNAlloc| 76.63                   | 48.01           | 122.3                     |                           |
| SiaFNEtoc| >1000                   |                 |                           |                           |
| SiaFNMee | 0.7077                  | 0.4596          | 1.067                     |                           |
| SiaFNPoc | >1000                   |                 |                           |                           |
| SiaFHept | >1000                   |                 |                           |                           |
| SiaFOct  | 11.32                   | 6.467           | 19.8                      |                           |
| SiaF9Az  | 229.4                   | 86.42           | 609                       |                           |
| SiaFForm  | 315.9                   | 221.1           | 451.2                     |                           |
| SiaFNMee | 33.64                   | 18.43           | 61.4                      |                           |
| SiaF4Az  | >1000                   |                 |                           |                           |

**General method A: Synthesis of sialic acid derivatives 2a-2k**

Mannosamine derivative 1a-1k (5 eq) and sodium fluoropyruvate (1 eq.) were dissolved in H$_2$O. Neu$_5$Ac aldolase (8 U mg$^{-1}$) was added and the mixture was heated to 37°C and stirred overnight. The solvents were evaporated and the crude product was purified by column chromatography (0 – 20% 0.04M HCl in MeCN, v/v), yielding the product.

2-Formamido-2-deoxy-o-mannopyranose (1k)

To a solution of mannosamine hydrochloride (299 mg, 1.39 mmol) in anhydrous MeOH (2.10 mL), a 5.4M solution of NaOMe in MeOH (385 μL, 1.5 eq.) was added, followed by triethylamine (193 μL, 1.39 mmol, 1 eq.) and methyl formate (600 μL, 9.71 mmol, 7 eq.). The solution was stirred at rt overnight. The mixture was concentrated and purified using column chromatography (0 – 20% H$_2$O in MeCN, v/v), yielding the product (195mg, 68%) as a mixture of anomers and rotamers.
1H NMR (400 MHz, D2O, major anomer, rotamer) δ 8.11 (s, 1H), 5.09 (d, J = 1.6 Hz, 1H), 4.36 – 4.28 (m, 1H), 4.02 (dd, J = 9.9, 4.5 Hz, 1H), 3.89 – 3.68 (m, 3H), 3.51 (t, J = 9.7 Hz, 1H). 13C NMR (101 MHz, D2O) δ 164.64, 92.67, 72.13, 68.57, 67.02, 60.65, 51.90. HRMS (ESI+) m/z calculated for C11H13NO2Na [M+Na]+ 230.0646, found 230.06284. TLC (H2O/MeCN 20/80, v/v) Rf = 0.4

(5-N-methoxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2a)
Using general method A starting from 1a afforded 2a (27.5 mg, 38%) 1H NMR (500 MHz, D2O) δ 4.92 – 4.81 (m, 1H), 4.12 (m, 2H), 3.98 (t, J = 10.6 Hz, 1H), 3.89 (dd, J = 14.5, 10.3, 2.8 Hz, 2H), 3.70 (s, 3H). 13C NMR (126 MHz, D2O) δ 174.4, 159.1, 159.1, 119.1, 91.8, 97.4, 70.4, 70.2, 68.5, 68.4, 63.3, 52.6, 48.9. HRMS (ESI+) m/z calculated for C13H15FNO26Na [M+Na]+ 366.08124, found 366.08159. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.1

(5-N-ethoxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2b)
Using general method A starting from 1b afforded 2b (22.5 mg, 87%) 1H NMR (500 MHz, D2O) δ 4.95 (dd, J = 49.3, 2.5 Hz, 1H), 4.22 – 4.11 (m, 4H), 4.00 (t, J = 10.7 Hz, 1H), 3.89 (dd, J = 11.7, 2.8 Hz, 1H). 13C NMR (126 MHz, D2O) δ 172.6, 158.7, 94.7, 90.6, 89.2, 70.5, 70.3, 68.3, 68.0, 63.2, 62.0, 48.7, 13.8. HRMS (ESI+) m/z calculated for C13H15FNO26Na [M+Na]+ 380.09689, found 380.09839. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.2

(5-N-allyloxy carbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2c)
Using general method A starting from 1c afforded 2c (26.5 mg, 99%) 1H NMR (500 MHz, D2O) δ 6.00 (ddt, J = 17.4, 10.5, 5.2 Hz, 1H), 5.36 (dd, J = 17.2, 1.9 Hz, 1H), 5.29 (dd, J = 10.6, 1.5 Hz, 1H), 4.94 (dd, J = 49.3, 2.4 Hz, 1H). 13C NMR (126 MHz, D2O) δ 172.3, 158.3, 132.7, 117.3, 94.8, 90.7, 89.3, 70.5, 70.4, 68.2, 68.0, 63.2, 48.8. HRMS (ESI+) m/z calculated for C13H20FNO26Na [M+Na]+ 392.09689, found 392.09791. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.2

(5-N-proparglyoxy carbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2d)
Using general method A starting from 1k afforded 2d (17.3 mg, 65%) 1H NMR (500 MHz, D2O) δ 4.95 (dd, J = 49.3, 2.6 Hz, 1H), 4.73 (d, J = 2.9 Hz, 2H), 4.23 – 4.12 (m, 2H), 4.02 (t, J = 10.6 Hz, 1H), 3.93 – 3.83 (m, 2H). 13C NMR (126 MHz, D2O) δ 172.1, 157.4, 94.8, 90.6, 89.2, 75.8, 70.4, 70.3, 68.2, 67.9, 63.2, 53.0, 48.9. HRMS (ESI+) m/z calculated for C13H23FNO26Na [M+Na]+ 390.08143, found 390.08143. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.2

(5-propionamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2f)
Using general method A starting from 1f afforded 2f (10.8 mg, 22%) 1H NMR (500 MHz, D2O) δ 4.78 (dd, J = 49.3, 2.5 Hz, 1H), 4.19 (t, J = 10.6 Hz, 1H). 13C NMR (126 MHz, D2O) δ 178.79, 174.46, 95.37, 95.15, 91.08, 89.69, 70.28, 70.09, 68.58, 68.23, 68.09, 63.28, 47.29, 47.27, 29.36, 9.57. HRMS (ESI+) m/z calculated for C13H18FNO26Na [M+Na]+ 364.10198, found 364.10293. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.2

(5-azidoacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2g)
Using general method A starting from 1g afforded 2g (38.7 mg, 51%) 1H NMR (500 MHz, D2O) δ 3.49 (d, J = 14.5, 10.3 Hz, 1H), 4.17 – 4.05 (m, 2H). 13C NMR (126 MHz, D2O) δ 174.42, 171.03, 91.03, 89.63, 70.31, 69.78, 68.48, 68.04, 67.89, 63.23, 51.96, 47.59. HRMS (ESI+) m/z calculated for C13H17F2N4O2Na [M+Na]+ 391.08773, found 391.08821. TLC (0.04M HCl/MeCN 20/80, v/v) Rf = 0.2

(5-fluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2h)
Using general method A starting from 1h afforded 2h (30.3 mg, 72%) 1H NMR (500 MHz, D2O) δ 4.88 (d, J = 46.2 Hz, 2H), 4.80 (dd, J = 49.2, 2.4 Hz, 1H), 4.31 (t, J = 10.6 Hz, 1H), 4.21 – 4.08 (m, 2H), 3.84 – 3.75 (m, 2H), 3.62 – 3.54 (m, 1H). 13C NMR (126 MHz, D2O) δ 174.43, 171.43, 171.29, 95.38, 95.16, 91.04, 89.64, 80.58, 79.13, 70.30, 69.73, 68.46, 68.03, 67.89, 63.25, 47.11,
HRMS (ESI+) m/z calculated for C$_{12}$H$_{12}$F$_{5}$NO$_{9}$Na [M+Na]$^+$ 368.07691, found 368.07903. **TLC** (0.04M HCl/MeCN 20/80, v/v) **R$_f$** = 0.3

(S-difluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2i)

Using general method A starting from 1i afforded 2i (38.7 mg, 68%)

**1H NMR** (500 MHz, D$_2$O) δ 6.14 (t, $J = 5.36$ Hz, 1H), 4.81 (dd, $J = 49.4$, 2.4 Hz, 1H), 4.30 (t, $J = 10.6$ Hz, 1H), 4.22 – 4.10 (m, 2H), 3.81 (dt, $J = 10.3$, 2.5 Hz, 2H), 3.58 (dd, $J = 12.5$, 7.0 Hz, 1H), 3.45 (dd, $J = 9.0$, 1.1 Hz, 1H). **13C NMR** (126 MHz, D$_2$O) δ 174.34, 165.79, 165.58, 165.38, 110.29, 108.31, 106.34, 95.40, 95.18, 91.01, 89.60, 70.32, 69.54, 68.45, 68.72, 67.67, 63.23, 47.61, 47.59. **HRMS** (ESI+) m/z calculated for C$_{12}$H$_{12}$F$_{5}$NO$_{9}$Na [M+Na]$^+$ 386.06748, found 386.06977. **TLC** (0.04M HCl/MeCN 20/80, v/v) **R$_f$** = 0.4

(S-trifluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2j)

Using general method A starting from 1j afforded 2j (13.7 mg, 97%)

**1H NMR** (500 MHz, D$_2$O) δ 4.82 (dd, $J = 49.3$, 2.1 Hz, 1H), 4.32 (t, $J = 10.6$ Hz, 1H), 4.24 – 4.08 (m, 2H), 3.87 – 3.79 (m, 2H), 3.61 – 3.56 (m, 1H), 3.43 (dt, $J = 9.0$, 1.1 Hz, 1H). **13C NMR** (126 MHz, D$_2$O) δ 174.30, 159.61, 159.31, 119.19, 116.91, 114.63, 95.36, 95.14, 90.95, 85.94, 70.22, 69.32, 68.58, 67.69, 67.55, 63.25, 48.15, 48.13. **HRMS** (ESI+) m/z calculated for C$_{12}$H$_{12}$F$_{5}$NO$_{9}$Na [M+Na]$^+$ 404.05806, found 404.05936. **TLC** (0.04M HCl/MeCN 20/80, v/v) **R$_f$** = 0.6

(S-formamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2k)

Using general method A starting from 1k afforded 2k (14.4 mg, 59%)

**1H NMR** (400 MHz, D$_2$O) δ 8.16 (s, 1H), 4.86 – 4.73 (m, 1H), 4.27 (t, $J = 10.6$ Hz, 1H), 4.17 – 3.99 (m, 3H), 3.86 – 3.75 (m, 3H), 3.58 (dd, $J = 12.5$, 6.9 Hz, 2H), 3.49 (d, $J = 9.0$, 1.1 Hz). **13C NMR** (101 MHz, D$_2$O) δ 164.86, 91.12, 89.37, 70.29, 69.84, 68.40, 68.09, 67.91, 63.26, 46.25. **HRMS** (ESI+) m/z calculated for C$_{12}$H$_{12}$F$_{5}$NO$_{9}$Na [M+Na]$^+$ 336.07068, found 336.07259. **TLC** (0.04M HCl/MeCN 20/80, v/v) **R$_f$** = 0.2

(S-N-methanesulfonamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-o-glycero-o-galacto)onate (2l)

To a solution of 4 (260 mg, 0.76 mmol) in DMF (5.40 mL) and H$_2$O (1.80 mL), selectfluor (1.08 mg, 3.05 mmol, 4 eq.) was added. The solution was stirred for 24h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (0 – 20% H$_2$O in MeCN) to obtain the product, which was dissolved in H$_2$O (3 mL) after which NaOH (1.0M, 0.38 mL, 1 eq.) was added. The mixture was stirred for 2h at rt. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (0 – 20% H$_2$O in MeCN) to obtain the desired product 2l (39 mg, 28% over two steps)

**1H NMR** (400 MHz, D$_2$O) δ 4.86 – 4.74 (m, 1H), 4.07 – 3.89 (m, 2H), 3.86 – 3.78 (m, 3H), 3.72 – 3.57 (m, 2H), 3.12 (m, 3H). **13C NMR** (101 MHz, D$_2$O) δ 174.29, 95.18, 91.70, 89.95, 70.83, 70.49, 68.71, 68.53, 68.10, 63.33, 51.55, 51.53, 41.35. **HRMS** (ESI+) m/z calculated for C$_{12}$H$_{12}$F$_{5}$NO$_{9}$Na [M+Na]$^+$ 386.05331, found 386.05517. **TLC** (0.04M HCl/MeCN 20/80, v/v) **R$_f$** = 0.2

Methyl 5-methanesulfonamido-2,6-anhydro-2,3,5-trideoxy-5-L-arabinono-2-enonate (4)

To a solution of amine 3 (1.41g, 2.98 mmol) in DCM (0.1M) was added DIPEA (1.6 mL, 3 eq.). After cooling to 0°C, MeCl$_2$ (0.70 mL, 3 eq.) was added and the solution was warmed up to rt and stirred overnight. The mixture was concentrated, dissolved in EtOAc and washed with 2x 0.1M HCl and brine. The solution was dried using Na$_2$SO$_4$ and concentrated. The crude mixture was purified by column chromatography (0 – 100% EtOAc in heptane, v/v), yielding the product which was immediately dissolved in MeOH (29.8 mL, 0.1M), followed by addition of NaOMe (110 μL, 5.4M in MeOH, 0.1 eq.). The solution was stirred at rt until TLC showed completion of the reaction. The mixture was concentrated and purified by column chromatography (0 – 10% MeOH in EtOAc, v/v), yielding the product (260 mg, 26% over two steps)

**1H NMR** (500 MHz, MeOD) δ 5.95 (d, $J = 2.5$ Hz, 1H), 4.48 (dd, $J = 8.7$, 2.6 Hz, 1H), 4.17 (dd, $J = 10.8$, 1.2 Hz, 1H), 4.02 (dd, $J = 9.2$, 1.2 Hz, 1H), 3.94 – 3.85 (m, 2H), 3.80 (s, 3H), 3.71 (dd, $J = 11.2$, 5.4 Hz, 1H), 3.58 (dd, $J = 10.8$, 8.7 Hz, 1H), 3.15 (s, 3H). **13C NMR** (126 MHz, MeOD) δ 162.95, 143.55, 112.38, 77.38, 69.98, 68.14, 67.86, 63.58, 53.75, 53.42, 40.67. **HRMS** (ESI+) m/z calculated for C$_{10}$H$_{12}$F$_{4}$NSO$_{4}$ [M+Na]$^+$ 364.06728, found 364.06810. **TLC** (MeOH/EtOAc 15/85, v/v) **R$_f$** = 0.6

Methyl (5-acetemido-3-dehydro-2,3,5-trideoxy-5-L-arabinono)onate (6)

To a solution of sialic acid glucal 5 (51.2 mg, 0.168 mmol) in anhydrous MeOH (4 mL), NaIO$_4$ (94.2 mg, 440 μmol, 2.6 eq.) was added. The reaction mixture was stirred for 17 h at rt under inert atmosphere, before it was filtered
through a celite pad. The pad was washed with methanol and the filtrated and washings were evaporated under reduced pressure. The residue was dissolved in anhydrous MeOH (6 mL), the reaction mixture was cooled to 0°C and then NaBH₄ (35.2 mg, 0.93 mmol, 5.5 eq.) was added. The reaction mixture was stirred for 1 h under inert atmosphere, before it was filtered through a celite pad. The filtrate was evaporated under reduced pressure and the product was purified using column chromatography on silica gel (0 - 20% MeOH in DCM, v/v), giving the desired product as a white solid (40 mg, 97%).

**1H NMR** (400 MHz, D2O) δ 6.10 (d, J = 2.7 Hz, 1H), 4.49 (dd, J = 8.3, 2.7 Hz, 1H), 4.14 (dd, J = 10.2, 5.2, 2.5 Hz, 1H), 4.01 (dd, J = 10.2, 8.3 Hz, 1H), 3.86 (d, J = 0.8 Hz, 4H), 3.79 (dd, J = 12.9, 5.1 Hz, 1H), 2.08 (s, 3H). **13C NMR** (400 MHz, D2O) δ 111.15, 78.67, 66.36, 60.14, 52.89, 49.67, 22.05

HRMS (ESI+) m/z calculated for C₇H₁₃NO₄Na [M+Na]+ 268.07971, found 268.0781.

**TLC** (DCM/MeOH, 9/1, v/v) Rf = 0.6

Methyl (5-acetamido-3-dehydro-2,3,5-trideoxy-5-D-galacto)onate (7)

To a solution of sialic acid glucal (94 mg, 0.29 mmol) in anhydrous MeOH (30 mL), NaIO₄ (557.2 mg, 2.05 mmol, 1.0 eq.) was added. The reaction mixture was stirred for 17 h at rt under inert atmosphere, before it was filtered through a celite pad. The pad was washed with methanol and the filtrated and washings were evaporated under reduced pressure. The residue was dissolved in anhydrous MeOH (30 mL), the reaction mixture was cooled to 0°C and then NaBH₄ (236.7 mg, 6.257 mmol, 2.4 eq.) was added. The reaction mixture was stirred for 1 h under inert atmosphere, before it was filtered through a celite pad the filtrate was evaporated under reduced pressure and the product was purified using column chromatography on silica gel (0 - 20% MeOH in DCM). Giving the desired product as a white solid (131 mg, 51.6%).

**1H NMR** (500 MHz, D2O) δ 4.91 (dd, J = 29.2, 10.7, 2.4 Hz, 1H), 4.17 – 4.08 (m, 1H), 3.90 – 3.84 (m, 1H), 3.78 – 3.65 (m, 3H), 2.08 (d, J = 1.7 Hz, 3H). **13C NMR** (126 MHz, D2O) δ 174.83, 164.26, 143.31, 112.42, 76.87, 68.57, 66.66, 61.98, 52.95, 49.66, 22.11

HRMS (ESI+) m/z calculated for C₁₀H₁₅NO₆Na [M+Na]+ 298.09027, found 298.09214.

**TLC** (DCM/MeOH, 9/1, v/v) Rf = 0.2

5-acetamido-3-fluoro-3,5-dideoxy-D-galacto-oct-2-ulosonic acid (8)

To a solution of 6 (68 mg, 0.25 mmol) in DMF (0.37 mL) and H₂O (0.58 mL), selectfluor (358 mg, 1.01 mmol, 4.1 eq.) was added. The solution was stirred at 50°C for 28h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (0 – 20% H₂O in MeCN) to obtain the product, which was dissolved in MeOH (3 mL), after which NaOH (1M, 0.3 mL, 1 eq.) was added. The mixture was stirred for 42h at rt. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (0 – 20% H₂O in MeCN) to obtain the desired product (21 mg, 26% over two steps)

**1H NMR** (500 MHz, D₂O) δ 4.95 – 4.51 (m, 1H), 4.19 – 4.08 (m, 2H), 3.92 – 3.88 (m, 1H), 3.86 (d, J = 1.6 Hz, 3H), 3.79 (td, J = 6.3, 5.7, 1.5 Hz, 2H), 2.11 (s, 3H). **13C NMR** (126 MHz, D₂O) δ 174.83, 164.26, 143.31, 112.42, 76.87, 68.57, 66.66, 61.98, 52.95, 49.66, 22.11

HRMS (ESI+) m/z calculated for C₁₀H₁₅NO₆Na [M+Na]+ 298.09027, found 298.09214.

**TLC** (DCM/MeOH, 9/1, v/v) Rf = 0.2

5-acetamido-3-fluoro-3,5-dideoxy-L-arabinino-hept-2-ulosonic acid (9)

To a solution of 7 (105.4 mg, 429.8 µmol) in DMF (0.64 mL) and H₂O (1.01 mL), selectfluor (611.1 mg, 1.725 mmol, 4.1 eq.) was added. The solution was stirred at 50°C for 48h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (0 - 20% H₂O in MeCN) to obtain the product, which was dissolved in 0.04M HCl (7 mL) and stirred for 23h at rt. The solvent was lyophilized and the crude product was purified by column chromatography (0 - 20% H₂O in MeCN) to obtain the desired product (12.5 mg, 17% over two steps)

**1H NMR** (500 MHz, D₂O) δ 4.91 (dd, J = 49.2, 2.4 Hz, 1H), 4.11 (dd, J = 29.2, 10.7, 2.4 Hz, 1H), 4.04 (td, J = 10.5, 1.5 Hz, 1H), 3.91 – 3.86 (m, 1H), 3.77 – 3.66 (m, 3H), 2.07 (s, 3H).

**13C NMR** (126 MHz, D₂O) δ 174.79, 174.46, 95.37, 95.15, 91.08, 89.69, 70.28, 70.09, 68.58, 68.23, 68.09, 63.28, 47.29, 47.27, 29.36, 9.57.

HRMS (ESI+) m/z calculated for C₁₀H₁₅FNO₆Na [M+Na]+ 320.07576, found 320.07565. **TLC** (MeCN/H₂O, 8/2, v/v) Rf = 0.2

5-azido-5-acetamido-3-dehydro-3,5,9-trideoxy-3-fluoro-5-β-D-glycero-o-galactonoate (11)

To a solution of 10⁸ (62 mg, 0.19 mmol) in DMF (0.28 mL) and H₂O (0.44 mL) was added selectfluor (130.1 mg, 0.37 mmol, 2 eq.). The solution was stirred at 50°C for 24h. An extra 2 eq. of selectfluor was added and the solution was stirred at 50°C for another 24h. The mixture was concentrated and the crude product was purified using column chromatography (0 – 20% H₂O in MeCN, v/v)
$^1$H NMR (500 MHz, D$_2$O) $\delta$ 4.84 (dd, $J$ = 49.2, 2.3 Hz, 1H), 4.19 (t, $J$ = 10.6 Hz, 1H), 4.12 – 4.04 (m, 1H), 4.04 – 4.00 (m, 1H), 3.96 – 3.91 (m, 1H), 3.63 – 3.51 (m, 2H), 3.51 – 3.46 (m, 1H), 1.99 (s, 3H).

$^{13}$C NMR (126 MHz, D$_2$O) $\delta$ 174.79, 172.90, 92.19, 89.97, 70.01, 68.99, 68.77, 67.97, 53.90, 47.36, 22.08.

HRMS (ESI+) $m/z$ calculated for C$_{11}$H$_{17}$FN$_4$O$_8$Na $[M+Na]^+$ 375.09281, found 375.09465. TLC (H$_2$O/MeCN 20/80, v/v) $R_f$ = 0.3
2-Formamido-2-deoxy-d-mannopyranose (1k)
(S-N-methoxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (2a)
(S-N-ethoxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (2b)
(S-N-allyloxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-β-D-galacto)onate (2c)
(5-N-propargyloxycarbamado-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-β-D-galacto)onate (2d)
(5-propionamido-3-dehydro-3,5-dideoxy-3-fluoro-5-\(\beta\)-D-glycero-\(\alpha\)-galacto)onate (2f)
(S-azidoacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-β-D-galacto)onate (2g)
(5-fluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (2h)
(5-difluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-β-D-galacto)onate (2i)
(5-trifluoroacetamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (2j)
(5-formamido-3-dehydro-3,5-dideoxy-3-fluoro-5-β-D-glycero-D-galacto)onate (2k)
(S-\(N\)-methanesulfonamido-3-dehydro-3,5-dideoxy-3-fluoro-5-\(\beta\)-\(D\)-glycero-\(D\)-galacto)onate (2l)
Methyl 5-methanesulfonamido-2,6-anhydro-2,3,5-trideoxy-α-glycero-α-galacto-non-2-enonate (4)
Methyl (5-acetemido-3-dehydro-2,3,5-trideoxy-5-L-arabino)onate (6)
5-acetamido-3-fluoro-3,5-dideoxy-D-galacto-oct-2-ulosonic acid (8)
5-acetamido-3-fluoro-3,5-dideoxy-L-arabino-hept-2-ulosonic acid (9)
(9-azido-5-acetamido-3-dehydro-3,5,9-trideoxy-3-fluoro-5-β-D-glycero-α-galacto)onate (11)
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