Inhibitory Efficiencies for Mechanism-Based Inactivators of Sialidases

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Supporting Information
5-Acetamido-3,5-dideoxy-3-fluoro-D-erythro-1-manno-non-2-ulopyranosonic acid (S1-1)

Neu5Ac aldolase (60 mg, 30 units/mg) was added to solution of N-acetylmannosamine monohydrate (6.0 g, 27.5 mmol) and sodium 3-fluoropyruvate (900 mg, 7.04 mmol) in water (34 mL). The pH was adjusted to 8.0–8.5 using NaOH (1 M). The resultant mixture was left at room temperature for 3 days. The progress of the reaction was monitored by $^{19}$F NMR spectroscopy by following the disappearance of the 3-fluoropyruvate resonance ($\delta = -233$ ppm) and the appearance of the signal for 3-fluorosialic acid ($\delta = -208.2$ ppm). The protein was denatured by heating to 50 ºC for 10 min and then removed by centrifugation. The resulting supernatant was filtered over an Amicon filter (0.2 µm). The mixture was purified by ion exchange column chromatography (Dowex 1×2 200 formate form) by eluting using a formic acid gradient (0 to 2 M). Fractions containing the desired diastereomers were combined and lyophilised to afford 3-fluorosialic acid S1-1 as a white solid (1.86 g, 81%); mp = 157–159 ºC (lit. 160 ºC).$^{1}$H NMR (400 MHz, D$_2$O) 4.92 (m, 1H, $J_{3,4} = 2.6$, H-3), 4.25 (t, 1H, $J_{5,4} = 10.8, J_{5,6} = 10.8$, H-5), 4.16 (dd, 1H, $J_{4,5} = 10.8, J_{4,F3} = 30.0$, H-4), 3.85 (d, 1H, $J_{6,5} = 10.8, J_{6,5} = 1.1$, H-6), 3.70–3.66 (m, 2H), 3.58 (dd, 1H, $J_{7,6} = 5.4, J_{7,8} = 10.8$, H-7), 3.33 (d, 1H, $J = 9.8$), 2.05 (s, 3H, CH$_3$, NCOMe).$^{19}$F (376 MHz, D$_2$O) –208.13 (dd, $J_{F3,4} = 29.9, J_{F3,3} = 49.3$, F-3). The above NMR spectral data match those reported in the literature.$^{1}$

Methyl 5-acetamido-3,5-dideoxy-3-fluoro-D-erythro-1-manno-non-2-ulopyranosonate (S1-2).

To a solution of 3-fluorosialic acid S1-1 (1.54 g, 4.71 mmol) in dry methanol (29 ml) was added Amberlite IR-120 resin (H$^+$ form) (1.54 g). This mixture was left to stir at room temperature overnight. Progress of the reaction was monitored by thin layer chromatography (TLC) using EtOAc/MeOH/H$_2$O/HOAc (4/2/1/0.1 v/v/v/v) as solvent. After the reaction was complete the resin was removed by filtration and it was washed with dry methanol (10 ml). The combined
filtrates were evaporated to obtain 3-fluorosialic acid methyl ester \( \text{S1-2} \) as a colourless solid (1.50 g, 94%). \( \text{mp} = 129–132 \degree \text{C} \) (lit. 131–133 \degree \text{C}),\(^2\) \( ^1\text{H} \) NMR (400 MHz, D\(_2\)O) 4.98 (dd, 1H, \( J_{3,4} = 2.4, J_{3,F} = 49.2, \text{H-3} \)), 4.27 (t, 1H, \( J = 10.0, \text{H-5} \)), 4.17 (dd, 1H, \( J_{4,5} = 10.6, J_{4,3} = 2.4, J_{4,F3} = 29.5, \text{H-4} \)), 4.13 (dd, 1H, \( J_{6,5} = 10.4, J_{6,7} = 1.0, \text{H-6} \)), 3.81–3.87 (m, 2H, H-8, H-9'), 3.89 (s, 3H, OMe), 3.66 (dd, 1H, \( J_{9,9'} = 11.6, J_{9,8} = 5.9, \text{H-9} \)), 3.59 (dd, 1H, \( J_{7,8} = 8.2, J_{7,6} = 1.0, \text{H-7} \)), 2.06 (s, 3H, NHCOMe); \( ^{19}\text{F} \) (376 MHz, D\(_2\)O) \( -208.13 \) (dd, \( J_{F3,4} = 29.5, J_{F3,3} = 49.1, \text{F-3} \)). The above NMR spectral data match those reported in the literature.\(^1\)

**Methyl 5-acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-3-fluoro-\( \text{d-erythro-a-1-manno-} \)

**non-2-ulopyranosonate \( \text{S1-3} \)**

To a cooled solution of methyl ester \( \text{S1-2} \) (1.51 g, 4.4 mmol) in dry pyridine (19.2 mL) at 0 \degree \text{C} was added acetic anhydride (22.3 mL, 240 mmol) and 4-(\( \text{N}, \text{N}\)-dimethylamino)pyridine (0.16 g, 1.3 mmol). The reaction mixture was stirred at 0 \degree \text{C} for 4 h and then left at rt for 48 h. The resultant mixture was extracted with dichloromethane (4 \times 45 mL) and then the combined organic layers were washed with saturated sodium bicarbonate solution (100 mL), dilute sulfuric acid (10% v/v, 100 mL), water (100 mL) and brine (100 mL). The organic layer was then dried over the sodium sulfate and the volatiles were removed under reduced pressure. Flash chromatography (EtOAc/Hex, 9:1 v/v) afforded fully protected 3-fluorosialic acid \( \text{S1-3} \) as a colorless oil (1.97 g, 81%). \( ^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) 5.58 (ddd, 1H, \( J_{4,F3} = 27.8, J_{4,5} = 10.8, J_{4,3} = 2.5, \text{H-4} \)), 5.36 (dd, 1H, \( J_{7,8} = 5.2, J_{7,6} = 2, \text{H-7} \)), 5.15 (ddd, 1H, \( J_{8,9} = 6.4, J_{8,9'} = 2.5, \text{H-8} \)), 4.95 (br dd, 1H, \( J_{3,4} = 49.1, J_{3,F3} = 2.5, \text{H-3} \)), 4.57 (dd, 1H, \( J_{9,9'} = 12.5, J_{9,8} = 2.5, \text{H-9} \)), 4.26 (d, 1H, \( J_{6,5} = 10.6, 5\text{H-9} \)), 4.22 (dd, 1H, \( J_{9,9'} = 12.5, J_{9,8} = 6.4, \text{H-9} \)), 4.20–4.14 (m, 1H, H-5), 3.86 (s, 3H, OCH\(_3\)), 2.20, 2.18, 2.13, 2.06, 2.05 (5 s, 15H, OAc). \( ^{19}\text{F} \) (376 MHz, CDCl\(_3\)) \(-208.87 \) (dd,
$J_{F_3,H_4} = 27.9, J_{F_3,H_4} = 49.1, F-3)$. The above NMR spectral data match those reported in the literature.¹

**Methyl 5-acetamido-4,7,8,9-tetra-$O$-acetyl-3,5-dideoxy-3-fluoro-$d$-erythro-$\alpha$-$L$-manno-non-2-ulopyranosonate S1-4**

Hydrazinium acetate (615 mg, 6.7 mmol) was dissolved in dry methanol (12 mL) and this solution was added to a solution of S1-3 (1.23 g, 2.2 mmol) in dry dichloromethane (12 mL). This reaction mixture was stirred at 0 °C for 6 h. Reaction progress was monitored by TLC using CH2Cl2/MeOH (9.5:0.5; v/v) as solvent. After completion the reaction mixture was concentrated *in vacuo* and the residue was extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with water (100 mL) and then dried over anhydrous sodium sulphate (Na2SO4). Removal of solvent under vacuum followed by purification using flash chromatography CH2Cl2/MeOH (9.5:0.5 v/v) afforded hemiketal S1-4 as a colorless syrup (1.03 g, 91%). ¹H NMR (400 MHz, CDCl3) 5.58 (d, 1H, $J_{NHAc,5} = 9.9$, NH), 5.36 (br dd, 1H, $J_{4,F_3} = 27.9$, $J_{4,5} = 10.8$, H-4), 5.23–5.35 (m, 2H), 4.85 (br dd, 1H, $J_{3,F_3} = 49.8$, $J_{3,4} = 1.9$, H-3), 4.32–4.48 (m, 1H), 4.24 (d, 1H), 4.02–4.14 (m, 2H), 3.85 (s, 3H, OCH3), 2.14, 2.08, 2.02, (3 s, 12H, OAc), 1.91 (s, 3H, NHCOCH3). ¹⁹F (376 MHz, CDCl3) –204.83 (dd, $J_{F_3,H_4} = 27.8$, $J_{F_3,H_4} = 49.7$, F-3).

**3-Deoxy-3-fluoro-$d$-erythro-$\beta$-$L$-manno-non-2-ulopyranosonic acid S2-1**

Neu5Ac aldolase (30 mg, 30 u/mg) was added to a solution of d-mannose (3.0 g, 16.7 mmol) and 3-fluoropyruvic acid sodium salt (450 mg, 3.49 mmol) in water (17 mL). The pH of the solution was adjusted to 8.0–8.5 using NaOH (1 M). The resultant mixture was left at rt for 4 days, during which time the reaction progress was monitored by ¹⁹F NMR spectroscopy. The protein was denatured by heating to 50 °C for 10 min and then removed by centrifugation. The resulting supernatant was filtered over an Amicon filter (0.2 µm) and the mixture was purified by ion
exchange column chromatography (Dowex 1×2 200 formate form) by eluting using a formic acid gradient (0 to 2 M). Fractions containing the product were combined and lyophilised to afford 3-fluoro-Kdn (S2-1) as a white powder (828 mg, 83%). mp = 150–152 °C, ¹H NMR (400 MHz, D₂O) 4.90 (dd, 1H, J₃,₄ = 2.1, J₃,F₃ = 49.2, H-3), 4.04 (dd, 1H, J₆,₇ = 2.0, J₆,₅ = 10.3, H-6), 3.82–3.78 (m, 2H), 3.74–3.67 (m, 2H), 3.55–3.49 (m, 2H). ¹⁹F (376 MHz, D₂O) –208.13 (dd, J₃,F₄ = 32.2, J₃,F₃ = 49.2, F-3).

**Methyl 3-deoxy-3-fluoro-D-erythro-β-L-manno-non-2-ulosonate S2-2**

To a solution of S2-1 (541 mg, 1.9 mmol) in dry methanol (30 mL) was added Amberlite IR-120 resin (H⁺ form) (600 mg). This mixture was stirred at room temperature overnight. Progress of the reaction was monitored by TLC using EtOAc/MeOH/H₂O/HOAc (4/2/1/0.1, v/v) as solvent. When the reaction was completed, it was filtered to remove the resin, which was rinsed with dry methanol (10 ml). The resultant filtrates were dried in vacuo to obtain a white solid of pure S2-2 (482 mg, 85%). ¹H NMR (400 MHz, D₂O) 4.93 (dd, 1H, J₃,₄ = 2.5, J₃,F₃ = 49.2, H-3), 4.05 (d, 1H, J₆,₅ = 10.4, H-6), 4.01 (ddd, 1H, J₄,₅ = 9.61, J₄,₃ = 2.4, J₄,F₃ = 30.2, H-4), 3.75–3.95 (m, 4H, H-5, H-7, H-8, H-9'), 3.85 (s, 3H, OMe), 3.68 (dd, 1H, J₉,₉' = 11.9, J₉,₈ = 5.8, H-9); ¹⁹F (376 MHz, D₂O) –207.25 (dd, J₃,F₄ = 30.2, J₃,F₃ = 49.2, F-3).

**Methyl 2,4,5,7,8,9-hexa-O-acetyl-3-deoxy-3-fluoro-D-erythro-α-L-manno-non-2-ulosonate S2-3**

To a cooled solution of methyl ester S2-2 (482 mg, 1.61 mmol) in dry pyridine (3.4 mL) at 0 °C was added acetic anhydride (3.9 mL, 42 mmol) and 4-(N,N-dimethylamino)pyridine (28.1 mg, 0.23 mmol). The reaction mixture was stirred at 0 °C for 4 h and then left at room temperature for 48 h. The resultant mixture was extracted with dichloromethane (5 × 30 mL) and then the combined organic layers were washed with saturated sodium bicarbonate solution (75 mL), dilute
sulfuric acid (10% v/v, 75 mL), water (75 mL) and brine (75 mL). The organic layer was then
dried over the sodium sulfate and the volatiles were removed under reduced pressure. Flash
chromatography (EtOAc:Hex, 1/1 v/v) was used to purify the residue to afford per-acetylated 3-
fluoro-Kdn S2-3 as a colorless syrup (776.1 mg, 87.5%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) 5.38 (dd,
1H, \(J_{7,8} = 5.5, J_{7,6} = 2.3, H-7\)), 5.25–5.35 (m, 2H, H-4, H-5), 5.17 (td, 1H, \(J_{8,7} = 5.85, J_{8,9'} = 5.95,
J_{8,9} = 2.4, H-8\)), 4.97 (br dd, 1H, \(J_{3,F3} = 48.7, J_{3,4} = 2.2, H-3\)), 4.55 (dd, 1H, \(J_{9,9'} = 12.4, J_{9,8} = 2.4,
H-9\)), 4.18 (dd, 1H, \(J_{9,9'} = 12.5, J_{9',8} = 6.2, H-9'\)), 4.10 (d, 1H, \(J_{6,5} = 9.2, H-6\)), 3.84 (s, 3H, OCH\(_3\)),
2.19, 2.13, 2.09, 2.05, 2.04, 2.03 (6 s, 18H, OAc). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)): 20.08, 20.12,
20.15, 20.26, 20.39, (6 s OAc), 53.09 (OMe), 61.40 (C-9), 63.13 (C-5), 66.49 (C-7), 69.27 (d,
\(J_{4,F3} = 16.8, C-4\)), 70.31 (C-8), 71.13 (C-6), 86.98 (br d, \(J_{3,F3} = 185.6\) Hz, C-3), 94.67 (d, \(J_{2,F3} =
29.1, C-2\)), 164.28, 166.46, 168.74, 169.45, 169.59, 170.08 (7C, C-1, C=O). \(^{19}\)F (376 MHz,
CDCl\(_3\)) –207.80 (dd, \(J_{F3,H3} = 48.3, J_{F3,H4} = 30.9, F-3\)). HRMS-FAB (m/z): [M + NH\(_4^+\)] calcd for
C\(_{22}\)H\(_{29}\)FNO\(_{15}\), 570.1834; Found, 570.1850. The above NMR spectral data match those reported
in the literature.\(^3\)

**Methyl 4,5,7,8,9-penta-O-acetyl-3-deoxy-3-fluoro-D-erythro-\(\alpha\)-L-manno-non-2-ulosonate S2-4**

A solution of hydrazinium acetate (125 mg, 1.36 mmol) in dry methanol (15 mL) was added to a
solution of S2-3 (705 mg, 1.28 mmol) in dry dichloromethane (15 mL). This reaction mixture
was stirred at 0 °C for 6 h. Following completion of the reaction, which was monitored by TLC
using EtOAc/MeOH (1:1 v/v) as the solvent, the volatiles were removed in vacuo and the residue
was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with
water (100 mL) and then dried over sodium sulphate (Na\(_2\)SO\(_4\)). The solvent was removed under
vacuum and the resulting residue was purified by flash chromatography EtOAc/Hexanes (1:1,
v/v) to afford the hemiketal S2-4 as a colorless syrup (482 mg, 74%). $^1$H NMR (400 MHz, CDCl$_3$) 5.38–5.18 (m, 4H, H-4, H-5, H-7, H-8), 4.93 (br dd, 1H, $J_{3,F3} = 49.0$, $J_{3,4} = 2.3$, H-3), 4.55 (dd, 1H, $J_{9,9'} = 12.4$, $J_{9,8} = 2.4$, H-9) 4.18 (dd, 1H, $J_{9,9'} = 12.5$, $J_{9',8} = 6.2$, H-9'), 4.10 (d, 1H, $J_{6,5} = 9.2$, H-6), 3.85 (s, 3H, OCH$_3$), 2.11, 2.08, 2.06, 2.03, 2.01 (5 s, 15H, OAc). $^{13}$C NMR (151 MHz, CDCl$_3$): 20.78, 20.83, 20.85, 20.97, 21.09 (OAc), 53.94 (OMe), 62.10 (C-9), 63.84 (C-5), 67.19 (C-7), 69.98 (d, $J_{4,F3} = 16.8$, C-4), 71.02 (C-8), 71.84 (d, C-6), 87.69 (d, $J_{3,F3} = 185.5$ Hz, C-3), 95.37 (d, $J_{2,F3} = 29.1$, C-2), 164.98 (C-1), 167.15, 169.44, 170.43, 170.14, 170.28, 170.77 (s, C=O). $^{19}$F (376 MHz, CDCl$_3$) –204.57 (dd, $J_{F3,H3} = 49.3$, $J_{F3,H4} = 28.1$, F-3). The above NMR spectral data match those reported in the literature.$^3$

Methyl 4,5,7,8,9-penta-$O$-acetyl-3-deoxy-3-fluoro-$D$-erythro-$\beta$-L-manno-non-2-ulopyranosyl fluoride S2-5

XtalFluor-E (390 mg, 1.7 mmol) and hemiacetal S2-4 (290 mg, 0.6 mmol) were added to a mixture of Et$_3$N.3HF (0.38 mL, 2.3 mmol) in anhydrous dichloromethane (3 mL). The resultant mixture was left to stir at rt for 4 h. The reaction progress was monitored by using TLC with EtOAc/hexanes (1:1, v/v) as solvent. After completion the reaction mixture was neutralized by the addition of an aqueous solution of sodium bicarbonate (5%) and extracted with dichloromethane (3 × 40 mL). The combined organic layers were washed with brine (100 mL) and dried over sodium sulphate (Na$_2$SO$_4$). The solvent was removed under vacuum and the resulting residue was purified by flash chromatography EtOAc/hexanes (1:1, v/v) to afford pure S2-5 as a colorless oil (159.6 mg, 55%). $^1$H NMR (400 MHz, CDCl$_3$) 5.40–5.33 (m, 2H, H-8, H-7), 5.29 (dd, 1H, $J_{5,6} = 9.6$, $J_{5,4} = 9.6$, H-5), 5.19 (dd, 1H, $J_{4,5} = 9.6$, $J_{4,F3} = 24.9$, H-4), 5.12 (br dd, 1H, $J_{3,F3} = 49.9$, $J_{3,4} = 2.35$, H-3), 4.33 (dd, 1H, $J_{9,9'} = 12.6$, $J_{9',8} = 2.1$, H-9), 4.22–4.14 (m, 2H, H-9), 3.89 (s, 3H, OCH$_3$), 2.12, 2.10, 2.07, 2.05, 2.04 (5s, 18H, OAc). $^{13}$C NMR (151 MHz,
CDCl₃): 20.04, 20.10, 20.24, 20.45 (OAc), 53.39 (OMe), 61.21 (C-9), 63.03, 65.55, 68.07, (C-5, 7, 8), 69.44 (dd, J₄,F₃ = 17.1, J₄,F₂ = 5.5, C-4), 71.52 (d, J₆,F₃ = 4.0 Hz, C-6), 85.18 (dd, J₃,F₂ = 19.6, J₃,F₃ = 194.2 Hz, C-3), 104.03 (dd, J₂,F₃ = 17.7, J₂,F₂ = 228.1 Hz, C-2), 163.64 (br d, J₁,F₂ = 29.9 Hz, C-1), 168.65, 169.12, 169.30, 169.45, 170.06 (s, C=O). ¹⁹F (376 MHz, CDCl₃), –215.6 (ddd, J₃,F₃,H₃ = 49.8, J₃,F₃,H₄ = 24.6, J₃,F₂ = 10.9, F-3), –124.5 (d, J₃,F₂,F₃ = 10.3, F-2). HRMS-FAB (m/z): [M + H⁺] calcd for C₂₀H₂₆F₂O₁₃, 513.1420; Found, 513.1398.

Methyl 3-deoxy-3-fluoro-D-erythro-β-L-manno-non-2-ulopyranosyl fluoride S₂-6

To a solution of S₂-5 (110 mg, 0.22 mmol) in dry methanol (5 mL) at 0 °C was added sodium methoxide (12 mg, 0.22 mmol) and this mixture was stirred for 3 h. After which the reaction mixture was neutralized by addition of Amberlite IR-120+ resin (H⁺ form) this solution was then filtered and the resin was washed with dry methanol. The combined filtrates were concentrated to afford the desired product as a colorless oil S₂-6 (61.7 mg, 95%). This product was used in the next step without further purification.

3-Deoxy-3-fluoro-D-erythro-β-L-manno-non-2-ulopyranosyl fluoride 7

Compound S₂-6 (61.7 mg, 0.21 mmol) was dissolved in THF/H₂O (3/1 v/v) (4 mL) followed by the addition of LiOH.H₂O (8.2 mg, 0.21 mmol). The resultant mixture was stirred at 0 °C for 1 h, at which time the reaction was completed as shown by TLC analysis using EtOAc/MeOH/H₂O/HOAc (4:2:1:0.1, v/v) as solvent. The reaction mixture was then neutralized by adding Amberlite IR-120+ resin (H⁺ form), which was subsequently filtered to remove the resin. The resin was washed with dry methanol and then the combined filtrate was concentrated. The remaining aqueous residue was then lyophilized to afford 7 as a colourless solid (54 mg, 91%). ¹H NMR (600 MHz, D₂O).5.21 (br dt, 1H, J₃,F₃ = 51.3, J₃,F₂ = 3.1, J₃,4 = 2.9, H-3), 4.10–4.02 (m, 1H, H-4), 3.98–3.85 (m, 4H, H-5, H-7, H-8, H-9’), 3.74 (d, 1H, J₆,5 = 10.1, H-6), 3.70
(dd, 1H, $J_{9,9'} = 12.4$, $J_{9,8} = 6.5$, H-9). $^{13}$C NMR (151 MHz, D$_2$O) 62.51 (s, C-9); 64.86 (s, C-5), 67.11 (s, C-7), 70.29 (s, C-8), 70.79 (dd, $J_{4,F2} = 5.4$, $J_{4,F3} = 17.4$, C-4), 73.28 (d, 1C, $J_{6,F2} = 3.4$, C-6), 89.24 (dd, $J_{3,F3} = 182.6$, $J_{3,F2} = 18.5$, C-3), 106.30 (br dd, $J_{2,F} = 219.4$, $J_{2,F3} = 14.8$, C-2), 169.24 (d, $J_{1,F2} = 26.6$, C-1). $^{19}$F (376 MHz, D$_2$O) –216.8 (ddd, $J_{F3,H3} = 51.4$, $J_{F3,H4} = 28.5$, $J_{F3,F2} = 11.2$, F-3), –121.3 (d, $J_{F2,F3} = 11.2$, F-2). HRMS-FAB (m/z): [M + H$^+$] calcd for C$_9$H$_{14}$F$_2$O$_8$, 289.0735; Found, 289.0723, $\alpha_D^{20} = -31.5$ (c = 0.008, H$_2$O).

Evaluation of the Kinetic Parameters for Inactivation of M. viridifaciens sialidase (MvS) and Aspergillus fumigatus Sialidase (AfS) by 7.

All kinetic experiments were performed in 1.0 cm path-length quartz cuvettes using either a Cary 3E UV-visible spectrophotometer (MvS; $\lambda = 400$ nM) or a Cary Eclipse Spectrofluorimeter (AfS, $\lambda_{ex} = 365$ nM and $\lambda_{em} > 440$ nM), which were equipped with Peltier temperature controllers. For all experiments, the sialidase was incubated in buffer (NaOAc-HOAc 100 mM, pH 5.25 containing BSA 0.1% w/v) with 7 at 25°C for various times ranging from 0–60 min. After incubation the remaining catalytic activity was measured at pH values of 7.00 (MvS) or 5.25 (AfS). Specifically, the buffered inactivation solution of MvS with various concentrations of 7 (0.25–100 μM) was made up to a final volume of 500 μL. At various time intervals an aliquot (50 μL) of this solution was added to a cuvette containing 450 μL buffer (pH 7.00), KCl (100 mM), BSA (0.01% w/v) and 4-nitrophenyl 5-N-acetyl-α-D-neuraminide (80 μM; PNP-αNeu5Ac). In the case of AfS, the stock solution of sialidase was incubated with various concentrations of 7 (50–400 μM) in the inactivation buffer (total volume = 200 μL). At various time intervals an aliquot (20 μL) of this solution was added to a cuvette containing 180 μL buffer (pH 5.25) containing BSA (0.01% w/v) and 4-methylumbelliferyl α-D-Kdn (1 mM; MU-Kdn). Several repeat measurements were averaged for each concentration and incubation time. Control
experiments were performed in which it was shown that: 1) when water was used instead of 7 no decrease in sialidase activity was observed; and 2) the observed inactivation rate constants ($k_{obs}$) were independent of the concentration of sialidase.
Figure S1. $^1$H NMR spectrum of 5-acetamido-3,5-dideoxy-3-fluoro-D-erythro-$\beta$-L-manno-non-2-ulopyranosyl fluoride (6) in D$_2$O.
Figure S2. $^{19}$F NMR spectrum of 5-acetamido-3,5-dideoxy-3-fluoro-D-erythro-β-L-manno-non-2-ulopyranosonyl fluoride (6) in D$_2$O.
Figure S3. $^{13}$C NMR spectrum of 5-acetamido-3,5-dideoxy-3-fluoro-$d$-erythro-$\beta$-$L$-manno-non-2-ulopyranosonyl fluoride (6) in D$_2$O.
Figure S4. $^1$H NMR spectrum of 3,5-dideoxy-3-fluoro-\(\delta\)-erythro-\(\beta\)-L-manno-non-2-ulpopyranosonyl fluoride (7) in D$_2$O.
Figure S5. $^{19}$F NMR spectrum of 3,5-dideoxy-3-fluoro-D-erythro-$\beta$-L-manno-non-2-ulopyranosonyl fluoride (7) in D$_2$O.
Figure S6. $^{13}$C NMR spectrum of 3,5-dideoxy-3-fluoro-D-erythro-β-L-manno-non-2-ulopyranosonyl fluoride (7) in D$_2$O.
Figure S7. Experimental setup for the measurement of the kinetic parameters for inactivation of MvS by 6.
Figure S8. Structures for compounds listed in the supporting information section.
Table S1. The derived first-order rate constants ($k_{obs}$) for inactivation of $Mv$ and $Af$ as function of the inactivator concentrations.

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<th></th>
<th>$Af$</th>
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<td>$k_{obs}$ (s⁻¹)</td>
<td>[7] (µM)</td>
<td>$10^3 x k_{obs}$ (s⁻¹)</td>
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<td>0.25</td>
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<td>0.45 ± 0.09</td>
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<td>0.5</td>
<td>0.81 ± 0.35</td>
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References

