A Rigid Bicyclic Platform for the Generation of Conformationally Locked Neuraminidase Inhibitors

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Organic Letters

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**General Experimental Procedures.** All reactions were performed in single-neck, flame-dried, round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Liquid reagents were transferred via glass microsyringe. Solvents were transferred via syringe with a stainless steel needle. Organic solutions were concentrated at 35 °C by rotary evaporation under vacuum. Analytical thin-layer chromatography (TLC) was performed using aluminum plates pre-coated with silica gel (0.20 mm, 60 Å pore-size, 230-400 mesh, Macherey-Nagel) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light. Flash-column chromatography was employed over silica gel (60 Å, 63-200 µM, Caledon).

**Materials.** Commercial solvents and reagents were used as received with the following exceptions. Tetrahydrofuran was dried by distillation over sodium and benzophenone. Dichloromethane was dried by passage through alumina in a commercial solvent purification system (SPS).

**Instrumentation.** Proton nuclear magnetic resonance spectra (\(^1\)H NMR) were recorded at 300 MHz or 500 MHz at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane, and are referenced to residual protium in the NMR solvent (CD\(_3\)C(O)CD\(_3\), δ 2.05; CDCl\(_3\), δ 7.26; CD\(_3\)OD, 3.31; CD\(_3\)S(O)CD\(_3\), 2.50; D\(_2\)O, 4.79). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, pent = pentet, sext = sextet, m = multiplet and/or multiple resonances, br = broad, app = apparent), coupling constant in Hertz, and integration. Carbon nuclear magnetic resonance spectra (\(^{13}\)C NMR) were recorded at 75 MHz or 125 MHz at 23 °C. Carbon chemical shifts are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent (CD\(_3\)C(O)CD\(_3\), δ 29.85; CDCl\(_3\), δ 77.22; CD\(_3\)OD, 49.00; CD\(_3\)S(O)CD\(_3\), 39.52). Infrared (IR) spectra were obtained using an FT-IR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm\(^{-1}\)), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Accurate masses were obtained using an orbitrap MS.
Synthetic Procedures.

Butadiene sulfone (1) (4.15 g, 35.2 mmol) and ketone 2b (10.3 g, 35.2 mmol) were dissolved in tetrahydrofuran (200 mL), and the solution was cooled to −78 °C. LiHMDS (42.3 mL, 1 M in tetrahydrofuran, 42.3 mmol) was added in one portion. The reaction mixture was stirred for 1 h at −78 °C, then removed from the cooling bath and stirred 1 h at room temperature. The reaction was quenched by the addition of 10% aqueous HCl (50 mL), and the mixture was partially concentrated in vacuo at 30 °C. The resulting orange solution was partitioned between 10% aqueous HCl and dichloromethane. The organic fraction was washed with brine and dried with Na₂SO₄ then concentrated in vacuo at 30 °C to provide 14.5 g of crude keto sulfone 4b as a light orange foam. The crude product was carried to the next step with no further purification. IR (film) 1683, 1305, 1173 cm⁻¹; ¹H NMR (CDCl₃ 300 MHz) δ 7.51 (d, J = 16 Hz, 1 H), 7.47 (d, J = 8.7 Hz, 2 H), 7.27 (d, J = 8.7 Hz, 2 H), 6.90 (d, J = 8.7 Hz, 2 H), 6.83 (d, J = 8.7 Hz, 2 H), 6.56 (d, J = 16 Hz, 1 H), 6.20-6.06 (m, 2 H), 4.11-4.05 (m, 1 H), 4.00-3.91 (m, 1 H), 3.83 (s, 3 H), 3.76 (s, 3 H), 3.72-3.64 (m, 1 H), 3.52 (ddd, J = 16, 4.5, 2.3 Hz, 1 H), 3.33 (dd, J = 16, 5.2 Hz, 1 H), 3.14 (dd, J = 16, 9.2 Hz, 1 H); ¹³C NMR (CDCl₃ 75 MHz) δ 197.9 (C), 161.8 (C), 158.8 (C), 143.2 (CH), 131.3 (C), 130.2 (CH), 129.5 (CH), 128.6 (CH), 127.0 (C), 124.6 (CH), 123.6 (CH), 114.5 (CH), 114.0 (CH), 69.1 (CH), 56.0 (CH₂), 55.4 (CH₃), 55.2 (CH₃), 42.4 (CH₂), 40.0 (CH); MS (ES+) m/z 435 (100); HRMS calcd for C₂₃H₂₄O₅S (M+H): 413.1423. Found: 413.1423.
Compound 4b (crude, 14.5 g, 35.2 mmol) was dissolved in tetrahydrofuran (400 mL), and the solution was cooled to –78 °C. LiHMDS (42.3 mL, 1 M in tetrahydrofuran, 42.3 mmol) was added in one portion. The reaction mixture was stirred for 30 min at –78 °C then removed from the cooling bath and stirred 2.5 h at room temperature. The reaction was quenched by the addition of saturated aqueous NH₄Cl and the mixture was partially concentrated in vacuo. The resulting red solution was partitioned between saturated aqueous NH₄Cl and dichloromethane. The organic fraction was washed with brine, dried with Na₂SO₄ and concentrated in vacuo at 30 °C to provide 14.5 g of crude vinyl sulfone 5b as a brick red solid. The crude product was carried to the next step with no further purification. IR (film) 3479 (br), 1250, 1132 cm⁻¹; ¹H NMR (CDCl₃ 300 MHz) δ 7.35 (d, J = 8.6 Hz, 2 H), 7.28 (d, J = 8.6 Hz, 2 H), 6.89 (d, J = 8.8 Hz, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 6.74 (d, J = 16 Hz, 1 H), 6.66 (dd, J = 6.8, 1.5 Hz, 1 H), 6.59 (dd, J = 6.8, 3.1 Hz, 1 H), 6.23 (d, J = 16 Hz, 1 H), 4.25-4.13 (m, 1 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.77 (dd, J = 9.7, 3.1, 2.5 Hz, 1 H), 3.72 (dd, J = 9.7, 7.4 Hz, 1 H), 2.42 (d, J = 9.8 Hz, 2 H); ¹³C NMR (CDCl₃ 75 MHz) δ 159.8 (C), 158.8 (C), 136.3 (CH), 133.3 (CH), 132.7 (C), 129.6 (CH), 128.8 (CH), 128.6 (C), 128.4 (CH), 128.0 (CH), 114.4 (CH), 114.3 (CH), 80.7 (C), 67.9 (CH), 58.0 (CH), 55.5 (CH₃), 55.5 (CH₃), 50.6 (CH₂), 42.6 (CH); MS (ES+) m/z 435 (100); HRMS calcd for C₂₃H₂₄O₅S (M+Na): 435.1242. Found: 435.1239.
Compound 5b (crude, 5.01 g, 12.5 mmol) was dissolved in tetrahydrofuran (200 mL). The solution was cooled to 0°C and Red-Al (5.7 mL, 65% in toluene, 18.5 mmol) was added in one portion. The solution was stirred at 0°C for 45 min. An aqueous 10% solution of Rochelle’s salt was added in small portions over 5 min until gas evolution desisted. The reaction mixture was partitioned between ethyl acetate and water. The organic fraction was dried with Na$_2$SO$_4$ and concentrated in vacuo. Flash-column chromatography (dichloromethane:ethyl acetate 25:1) afforded 1.33 g (26% over 3 steps from 1) of sulfone 6. IR (film) 3477 (br), 1250, 1108 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.22 (d, $J = 8.8$ Hz, 2 H), 7.14 (d, $J = 8.8$ Hz, 2 H), 6.76 (d, $J = 8.8$ Hz, 2 H), 6.74 (d, $J = 8.8$ Hz, 2 H), 6.56 (d, $J = 16$ Hz, 1 H), 6.01 (d, $J = 16$ Hz, 1 H), 4.00-3.89 (m, 1 H), 3.70 (s, 3 H), 3.66 (s, 3 H), 3.44-3.30 (m, 2 H), 2.98-2.80 (m, 2 H), 2.20-1.87 (m, 4 H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 159.5 (C), 158.6 (C), 133.4 (C), 129.6 (CH), 129.0 (CH), 128.9 (C), 128.5 (CH), 127.8 (CH), 114.2 (CH), 114.2(CH), 82.4 (C), 70.0 (CH), 55.4 (CH$_3$), 55.4 (CH$_3$), 51.3 (CH$_2$), 51.0 (CH), 49.0 (CH$_2$), 44.8 (CH), 19.3 (CH$_2$); MS (ES+) $m/z$ 437 (100); HRMS calcd for C$_{23}$H$_{26}$O$_5$S (M+Na): 437.1399. Found: 437.1393.
Compound 6 (1.33 g, 3.21 mmol) was dissolved in dichloromethane (80 mL). The solution was cooled to −78 ⁰C, then ozone was bubbled through until a light blue color persisted. The solution was purged with argon for 10 min, then dimethylsulfide (3.21 mL) was added at −78 ⁰C. The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was concentrated in vacuo, and the crude product was carried on to the next step with no further purification. The crude aldehyde was dissolved in 爷-butanol (16 mL), tetrahydrofuran (8 mL) and water (8 mL). KH₂PO₄ (2.2 g, 16 mmol) was added, followed by 2-methyl-2-butene (1.7 mL, 16 mmol). The reaction mixture was stirred for 5 min, then sodium chlorite (725 mg, 8.03 mmol) in water (2 mL) was added drop-wise. The reaction mixture was stirred for a further 60 min at room temperature then partitioned between ethyl acetate and 10% aqueous HCl. The aqueous layer was washed with ethyl acetate, and the combined organic fractions were dried with Na₂SO₄ and concentrated in vacuo. Flash column chromatography (dichloromethane:methanol:acetic acid 100:1:1 to dichloromethane:methanol:acetic acid 100:5:2) afforded 752 mg (72% over 2 steps) of sulfone 7 as a white solid. IR (film) 3460 (br), 1732, 1251, 1113 cm⁻¹; ¹H NMR (CD₃C(O)CD₃ 300 MHz)  δ 7.35 (d, J = 8.6 Hz, 2 H), 6.90 (d, J = 8.6 Hz, 2 H), 4.02-3.89 (m, 1 H), 3.78 (s, 3 H), 3.71 (dd, J = 10, 9.2 Hz, 1 H), 3.55 (dd, J = 10, 9.2 Hz, 1 H), 3.35 (td, J = 13, 7.6 Hz, 1 H), 3.00-2.91 (m, 1 H), 2.45 (t, J = 13 Hz, 1 H), 2.34-2.22 (m, 2 H), 2.19-2.08 (m, 1 H); ¹³C NMR (CD₃C(O)CD₃ 75 MHz)  δ 175.4 (C), 159.5 (C), 134.0 (C), 129.6 (CH), 114.8 (CH), 82.9 (C), 69.9 (CH), 55.4 (CH₃), 51.6 (CH₂), 47.4 (CH₂), 46.6 (CH), 20.9 (CH₂); MS (ES+) m/z: 349 (100); HRMS calcd for C₁₅H₁₈O₆S (M+Na): 349.0722. Found: 349.0717.
Compound 7 (752 mg, 0.576 mmol) was dissolved in benzene (60 mL) and ethanol (5 mL). p-Tolylsulfonic acid (4 mg, 0.023 mmol) was added and the solution was heated at 80 °C using a Dean-Stark apparatus for 16 h. The reaction mixture was cooled to room temperature, then partitioned between water and ethyl acetate. The organic fraction was dried with Na₂SO₄ and concentrated in vacuo to provide 773 mg of crude ethyl ester 7a as a white solid. The crude product was carried to the next step with no further purification. IR (film) 3465 (br), 1732, 1252, 1113 cm⁻¹; ¹H NMR (CDCl₃ 300 MHz) δ 7.28 (d, J = 8.6 Hz, 2 H), 6.88 (d, J = 8.6 Hz, 2 H), 4.32 (qd, J = 7.2, 1.4 Hz, 2 H), 3.99 (ddd, J = 13, 10, 7.3 Hz, 1 H), 3.80 (s, 3 H), 3.67-3.51 (m, 2 H), 3.50-3.34 (m, 1 H), 3.02-2.92 (m, 1 H), 2.36 (t, J = 13 Hz, 1 H), 2.24 (dd, J = 13, 7.2 Hz, 1 H), 2.19-2.06 (m, 2 H), 1.34 (t, J = 7.2 Hz, 3 H); ¹³C NMR (CDCl₃ 75 MHz) δ 174.3 (C), 158.8 (C), 132.3 (C), 128.6 (CH), 114.3 (CH), 82.4 (C), 69.1 (CH), 63.1 (CH₂), 55.4 (CH₃), 50.6 (CH₂), 49.6 (CH), 46.8 (CH₂), 46.0 (CH), 20.5 (CH₂), 14.3 (CH₃); MS (ES+) m/z 377 (100); HRMS calcd for C₁₇H₂₂O₆S (M+Na): 377.1035. Found: 377.1032.

Compound 7a (crude, 640 mg, 1.81 mmol) was dissolved in CCl₄ (5 mL), acetonitrile (5 mL) and water (15 mL). NaHCO₃ (152 mg, 1.81 mmol) was added, followed by NaIO₄ (5.81 g, 27.2 mmol). RuCl₃ (18 mg, 0.091 mmol) was added and the reaction mixture was stirred 16 h. Due to the volatility of RuO₄ additional aliquots of RuCl₃ (18 mg, 0.019 mmol) were added as necessary. The reaction mixture was partitioned between 10% aqueous HCl and ethyl acetate.
The organic fraction was dried with Na$_2$SO$_4$ then concentrated in vacuo at 30 °C. The crude solid was dissolved in chloroform, filtered through cotton, then concentrated in vacuo to provide 373 mg of crude acid 7b as a white solid. The crude product was carried to the next step with no further purification. IR (film) 3470 (br), 1734, 1265, 1114 cm$^{-1}$; $^1$H NMR (CDCl$_3$ 300 MHz) δ 4.29 (qd, $J$ = 7.3, 2.0 Hz, 2 H), 3.99-3.90 (m, 1 H) 3.73 (td, $J$ = 10, 8.4 Hz, 1 H), 3.50-3.41 (m, 1 H), 3.41-3.38 (m, 1 H), 3.07-2.96 (m, 1 H), 2.42-2.32 (m, 2 H), 2.25-2.06 (m, 2 H), 1.32 (t, $J$ = 7.3 Hz, 3 H); $^{13}$C NMR (CDCl$_3$ 75 MHz) δ 177.1 (C), 173.6 (C), 82.4 (C), 63.9 (CH), 63.3 (CH$_2$), 50.8 (CH$_2$), 49.1 (CH), 45.6 (CH), 42.6 (CH$_2$), 20.5 (CH$_2$), 14.3 (CH$_3$); MS (ES+) m/z 315 (100); HRMS calcd for C$_{11}$H$_{16}$O$_7$S (M+Na): 315.0515. Found: 315.0516.

Compound 7b (crude, 373 mg, 1.28 mmol) was dissolved in benzene (30 mL). Triethylamine (271 µL, 1.92 mmol) was added, followed by diphenylphosphoryl azide (277 µL, 1.28 mmol). Benzyl alcohol (200 µL, 1.92 mmol) was added and the reaction mixture was stirred at 80 °C for 16 h. The reaction mixture was quenched with saturated aqueous NH$_4$Cl then partitioned between saturated aqueous NH$_4$Cl and ethyl acetate. The organic fraction was dried with Na$_2$SO$_4$ and then concentrated in vacuo. Flash column chromatography (dichloromethane:ethyl acetate 20:1 to dichloromethane:ethyl acetate 2:1) afforded 171 mg (23% over 3 steps from 7) of 8 as a light brown solid. IR (film) 3349 (br), 1722, 1265, 1113 cm$^{-1}$; $^1$H NMR (CD$_3$C(O)CD$_3$ 300 MHz) δ 7.43-7.23 (m, 5 H), 6.83 (s, 1 H), 5.08 (s, 2 H), 4.76-4.63 (m, 1 H), 4.21 (q, $J$ = 7.3 Hz, 2 H), 3.69-3.48 (m, 2 H) 3.26 (td, $J$ = 10, 8.4 Hz, 1 H), 2.98-2.88 (m, 1 H), 2.80 (d, $J$ = 9.2 Hz, 2 H), 2.45-2.13 (m, 3 H), 1.26 (t, $J$ = 13 Hz, 3 H); $^{13}$C NMR (CD$_3$OD 75 MHz) δ 174.4 (C), 157.8 (C), 138.1 (C), 129.5 (CH), 129.2 (CH), 129.0 (CH), 82.3 (C), 67.6 (CH), 67.5 (CH$_2$), 67.3 (CH), 62.9 (CH$_2$), 55.0 (CH), 52.0 (CH$_2$), 45.3 (CH$_2$), 21.3 (CH$_2$), 14.4 (CH$_3$); MS (ES+) m/z 420 (100); HRMS calcd for C$_{18}$H$_{23}$NO$_7$S (M+Na): 420.1093. Found: 420.1092.
Compound 8 (151 mg, 0.380 mmol) was dissolved in methanol (8 mL). Acetic acid (~ 100 µL) was added, followed by Pd/C 10% (~ 10 mg). The reaction mixture was pressurized inside a Parr reactor to 300 PSI of H₂ and was stirred for 16 h. The reaction mixture was filtered through cotton, diluted with cyclohexane (10 mL) then concentrated in vacuo to provide 111 mg of the acetic acid salt of amine 8a as a white solid. The crude product was carried to the next step with no further purification. IR (film) 3261 (br), 1739, 1262, 1114 cm⁻¹; ¹H NMR (CD₃C(O)CD₃ 300 MHz) δ 4.63 (dt, J = 11, 6.8 Hz, 1 H), 4.20 (qd, J = 7.4, 1.1 Hz, 2 H), 3.66-3.47 (m, 2 H), 3.31-3.17 (m, 1 H), 2.97-2.85 (m, 1 H), 2.33-2.07 (m, 4 H), 1.26 (t, J = 7.4 Hz, 3 H); ¹³C NMR (CD₃C(O)CD₃ 75 MHz) δ 173.7 (C), 83.9 (C), 71.0 (CH), 64.1 (CH), 63.4 (CH₂), 52.7 (CH₂), 50.6 (CH), 46.9 (CH₂), 22.3 (CH₂), 15.5 (CH₃); MS (ES+) m/z 264 (100); HRMS calcd for C₁₀H₁₇NO₅S (M+H): 264.0906. Found: 264.0901.

Compound 8a (crude, 54 mg, 0.167 mmol) was dissolved in DMF (5 mL). Triethylamine (120 µL, 0.836 mmol) was added, followed by HgCl₂ (45 mg, 0.167 mmol). 1,3-bis(benzoyloxy carbonyl)-2-methylisothiourea (9, 60 mg, 0.167 mmol) was added and the reaction mixture was stirred 16 h. The reaction mixture was diluted ethyl acetate, filtered through celite, then concentrated in vacuo. Flash column chromatography (dichloromethane:ethyl acetate 25:1 to dichloromethane:ethyl acetate 10:1) afforded 55 mg (53% over 2 steps) of...
8b as a white solid. IR (film) 3477 (br), 3288 (br), 1771, 1732, 1622, 1271, 1114 cm\(^{-1}\); \(^1\)H NMR (CD\(_3\)C(O)CD\(_3\) 300 MHz) \(\delta\) 11.89 (s, 1 H), 8.70 (d, \(J = 8.0\) Hz, 1 H), 7.49-7.29 (m, 10 H), 5.26 (s, 2 H), 5.12 (s, 2 H), 4.21 (q, \(J = 7.1\) Hz, 2 H), 3.88 (dd, \(J = 10, 8.9\) Hz, 1 H), 3.66-3.57 (m, 1 H), 3.35-3.22 (m, 1 H), 3.01-2.90 (m, 1 H), 2.45-2.37 (m, 2 H), 2.28-2.06 (m, 3 H), 1.24 (t, \(J = 7.1\) Hz, 3 H); \(^{13}\)C NMR (CD\(_3\)C(O)CD\(_3\) 75 MHz) \(\delta\) 173.7 (C), 164.5 (C), 156.5 (C), 154.2 (C), 138.1 (C), 136.3 (C), 129.5 (CH), 129.4 (CH), 129.4 (CH), 129.2 (CH), 129.1 (CH), 128.7 (CH), 82.3 (C), 68.9 (CH\(_2\)), 67.8 (CH\(_2\)), 67.3 (CH), 62.4 (CH\(_2\)), 54.5 (CH), 51.7 (CH\(_2\)), 49.1 (CH), 44.9 (CH\(_2\)), 21.2 (CH\(_2\)), 14.4 (CH\(_3\)); MS (ES+) \(m/z\) 596 (100); HRMS calcd for C\(_{27}\)H\(_{31}\)N\(_3\)O\(_9\)S (M+Na): 596.1678. Found: 596.1674.

Compound 8b (54 mg, 0.094 mmol) was dissolved in methanol (5 mL). Acetic acid (~ 100 µL) and 10% aqueous HCl (~ 50 µL) was added, followed by Pd/C 10% (~10 mg). The reaction mixture was stirred inside a Parr reactor pressurized to 300 PSI H\(_2\) and was stirred for 16 h. The reaction mixture was filtered through cotton, diluted with cyclohexane (10 mL) then concentrated in vacuo to provide 29 mg (90%) of the hydrochloride salt of 10, ethyl ester. Prior to biological testing the compound was purified through recrystallization from methanol and dichloromethane. Mp: decomposed at 218°C; IR (film) 3350 (br), 1732, 1667, 1260, 1108 cm\(^{-1}\); \(^1\)H NMR (CD\(_3\)OD 300 MHz) \(\delta\) 4.61-4.51 (m, 1 H), 4.25 (qd, \(J = 7.1, 1.2\) Hz, 2 H), 3.59-3.51 (m, 2 H), 3.38 (td, \(J = 13, 7.6\) Hz, 1 H), 3.13-3.02 (m, 1 H), 2.26-2.07 (m, 4 H), 1.30 (t, \(J = 7.1\) Hz, 3 H); \(^{13}\)C NMR (CD\(_3\)OD 75 MHz) \(\delta\) 173.7 (C), 158.3 (C), 82.0 (C), 69.3 (CH), 63.2 (CH\(_2\)), 56.0 (CH), 52.1 (CH\(_2\)), 50.1 (CH), 45.3 (CH\(_2\)), 21.7 (CH\(_2\)), 14.4 (CH\(_3\)); MS (ES+) \(m/z\) 306(100); HRMS calcd for C\(_{11}\)H\(_{19}\)N\(_3\)O\(_5\)S (M+H): 306.1124. Found: 306.1117.
The ethyl ester of 10 (HCl salt, 22.6 mg, 0.0661 mmol) was dissolved in 750 µL of 10% NaOH in H₂O and 100 µL DMSO. The reaction mixture was stirred for 16 h, then neutralized with 12 M HCl to provide a standard solution (61.9 mM) of the free acid. Hydrolysis of the ester function was confirmed through MS (ES+) m/z 276 (100).

Compound 5b (crude, 10.3 g, 25.0 mmol) was dissolved in 50 mL acetonitrile and 50 mL of aqueous ammonia (28% w/w). The reaction mixture was heated at 60°C for 3 days in a sealed vessel. The reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was washed with ethyl acetate, and the combined organic extracts were dried with Na₂SO₄ and concentrated in vacuo to provide 10.5 g of compound 5c as yellow solid. The crude product was carried to the next step with no further purification. IR (film) 3470 (br), 3348 (br) 1513, 1250, 1112 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.34 (d, J = 8.6 Hz, 2 H), 7.25 (d, J = 8.6 Hz, 2 H), 6.87 (d, J = 8.6 Hz, 2 H), 6.87 (d, J = 8.7 Hz, 2 H), 6.70 (d, J = 16 Hz, 1 H), 6.21 (d, J = 16 Hz), 4.15-4.03 (m, 1 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.80-3.68 (m, 3 H), 3.04-2.95 (m, 2 H), 2.25-2.17 (m, 2 H); ¹³C NMR (CDCl₃, 75 MHz) δ 159.7 (C), 158.7 (C), 133.2 (C), 129.0 (C), 129.6 (CH), 129.2 (CH), 128.8 (C), 128.5 (C), 128.0 (CH), 114.4 (CH), 114.3 (CH), 81.4 (C), 68.8
Compound 5c (crude, 10.5 g, 24.5 mmol) was dissolved in 200 mL dichloromethane. DMAP (599 mg, 4.9 mmol) followed by acetic anhydride (6.95 mL, 73.5 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between 10% aqueous NaHCO₃ and dichloromethane. The aqueous layer was washed with dichloromethane, and the combined organic extracts were dried with Na₂SO₄ and concentrated in vacuo. Flash column chromatography (dichloromethane:ethyl acetate 4:1 to dichloromethane:ethyl acetate 1:1) afforded 4.6 g (40% over 4 steps from 1) of 11 as a yellow solid. IR (film) 3360 (br), 1658, 1250, 1113 cm⁻¹; ¹H NMR (CDCl₃ 300 MHz) δ 7.36 (d, J = 8.6 Hz, 2 H), 7.26 (d, J = 8.6 Hz, 2 H), 6.88 (d, J = 8.6 Hz, 2 H), 6.86 (d, J = 8.6 Hz, 2 H), 6.73 (d, J = 16 Hz, 1 H), 6.44 (d, J = 8.7 Hz, 1 H), 6.36 (d, J = 16 Hz, 1 H), 4.91 (dd, J = 8.5, 7.0 Hz, 1 H), 4.10-3.99 (m, 1 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79-3.74 (m, 1 H), 3.67-3.58 (m, 1 H), 3.13-3.06 (m, 1 H), 2.97-2.89 (m, 1 H), 2.30-2.15 (m, 2 H) 1.95 (s, 3 H); ¹³C NMR (CDCl₃ 75 MHz) δ 169.9 (C), 159.4 (C), 158.5 (C), 132.7 (C), 129.0 (C), 129.0 (CH), 128.9 (CH), 128.3 (CH), 127.8 (CH), 114.1 (CH), 114.1 (CH), 80.6 (C), 69.5 (CH), 58.9 (CH), 56.1 (CH₂), 55.2 (CH₃), 55.2 (CH₃), 48.1 (CH₂), 45.5 (CH), 44.0 (CH), 23.0 (CH₃); MS (ES+) m/z 494 (100); HRMS calcd for C₂₅H₂₉NO₆S (M+Na): 494.1608. Found: 494.1598.
Compound 11 (5.90 g, 12.5 mmol) was dissolved in 200 mL acetone. N-Methylmorpholine-N-oxide (2.92 g, 25 mmol) was added, followed by OsO$_4$ (4.0 mL, 4% in water, 0.625 mmol). The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature and stirred for 1 h. The reaction mixture was partitioned between 0.5 M aqueous sodium thiosulfate and ethyl acetate. The aqueous layer was washed with ethyl acetate, and the combined organic extracts were dried with Na$_2$SO$_4$ and concentrated in vacuo. The crude triol was dissolved in 160 mL acetone and 40 mL water. NaIO$_4$ (10.7 g, 50 mmol) was added, and the reaction mixture was stirred for 16 h. The reaction mixture was partitioned between 0.5 M aqueous sodium thiosulfate and ethyl acetate. The aqueous layer was washed with ethyl acetate, and the combined organic extracts were dried with Na$_2$SO$_4$ and concentrated in vacuo. Flash column chromatography (dichloromethane:tetrahydrofuran 20:1 to dichloromethane: tetrahydrofuran 5:1) afforded 2.2 g (52% over 2 steps from 11) of 12 as a white solid. IR (film) 3335 (br), 1751, 1652, 1255, 1114 cm$^{-1}$; $^1$H NMR (CD$_3$S(O)CD$_3$ 300 MHz) $\delta$ 8.39 (d, $J = 7.2$ Hz, 1 H), 7.32 (d, $J = 8.6$ Hz, 2 H), 6.93 (d, $J = 8.6$ Hz, 2 H), 4.46 (pentet, $J = 6.3$ Hz, 1 H), 4.10 (dd, $J = 9.8$, 8.1 Hz, 1 H), 3.93-3.81 (m, 1 H), 3.74 (s, 3 H), 3.60 (dd, $J = 13$, 6.9 Hz, 1 H), 3.51 (dd, $J = 10$, 6.0 Hz, 1 H), 3.13 (dd, $J = 13$, 7.1 Hz, 1 H), 2.79 (dd, $J = 18$, 8.6 Hz, 1 H), 2.68 (dd, $J = 18$, 10 Hz, 1 H), 1.95 (s, 3 H); $^{13}$C NMR (CD$_3$S(O)CD$_3$ 75 MHz) $\delta$ 211.3 (C), 169.2 (C), 158.3 (C), 133.6 (C), 128.4 (CH), 114.1 (CH), 67.3 (CH), 55.1 (CH$_2$), 55.1 (CH$_3$), 54.2 (CH), 46.0 (CH$_2$), 45.1 (CH), 33.8 (CH), 22.5 (CH$_3$); MS (ES+) $m/z$ 360 (100); HRMS calcd for C$_{16}$H$_{19}$NO$_5$S (M+Na): 360.0876. Found: 360.0870.
Compound 12 (2.20 g, 6.53 mmol) was dissolved in 80 mL tetrahydrofuran. The solution was cooled to –78°C, then L-Selectride (9.80 mL, 1 M in tetrahydrofuran, 9.80 mmol) was added. The solution was stirred at –78°C for 1 h. The reaction mixture was quenched with methanol at –78°C, then warmed to room temperature and partitioned between ethyl acetate and 10% NaHCO₃. The aqueous layer was washed with ethyl acetate, and the combined organic extracts were dried with Na₂SO₄ and concentrated in vacuo to provide 2.21 g of a 12a as a white solid. The crude product was carried to the next step with no further purification.

Compound 12a (2.21 g, 6.53 mmol) was dissolved in 40 mL pyridine. The solution was cooled to 0°C, then methanesulfonyl chloride (1.02 mL, 13.06 mmol) was added. The solution was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo, then partitioned between dichloromethane and water. The aqueous layer was washed with ethyl acetate, and the combined organic extracts were dried with Na₂SO₄ and concentrated in vacuo. The crude mesylate (2.72 g, 6.53 mmol) was dissolved in 25 mL dimethylformamide. NaN₃ (1.27 g, 19.6 mmol) was added. The reaction mixture was heated at 60°C for 16 h. The reaction mixture was partitioned between dichloromethane and water. The aqueous layer was washed with dichloromethane, and the combined organic extracts were dried with Na₂SO₄ and concentrated in vacuo to provide 2.35 g of crude azide 12b. The crude product was typically carried to the next step with no further purification. Flash column chromatography of a 776 mg portion of crude azide-12b (dichloromethane:tetrahydrofuran 10:1 to dichloromethane:
tetrahydrofuran 1:1) afforded 383 mg (48% over 3 steps from 12) of 12b as a yellow solid. IR (film) 3354 (br), 2103, 1661, 1252, 1119 cm\(^{-1}\); \(^1\)H NMR (CD\(_3\)OD 300 MHz) \(\delta\) 7.27 (d, \(J = 8.7\) Hz, 2 H), 6.89 (d, \(J = 8.7\) Hz, 2 H), 4.53 (dt, \(J = 8.0, 6.3\) Hz, 1 H), 4.17-4.02 (m, 1 H), 3.89 (dd, \(J = 9.8, 8.1\) Hz, 1 H), 3.78 (s, 3 H), 3.79-3.67 (m, 2 H), 3.55 (dd, \(J = 13, 6.0\) Hz, 1 H), 3.24 (dd, \(J = 13, 8.1\) Hz, 1 H), 3.04-2.94 (m, 1 H), 2.59 (dt, \(J = 12, 6.3\) Hz, 1 H), 2.00 (s, 3 H); \(^{13}\)C NMR (CD\(_3\)OD 75 MHz) \(\delta\) 173.2 (C), 160.3 (C), 133.8 (C), 129.4 (CH), 115.2 (CH), 70.5 (CH), 66.3 (CH), 56.5 (CH\(_2\)), 56.1 (CH), 55.7 (CH\(_3\)), 49.6 (CH), 44.0 (CH), 42.4 (CH\(_2\)), 22.5 (CH\(_3\)); MS (ES+) \(m/z\) 387 (100); HRMS calcd for C\(_{16}\)H\(_{20}\)N\(_4\)O\(_4\)S (M+Na): 387.1098. Found: 387.1098.

Compound 12b (crude, 1.02 g, 2.80 mmol) was dissolved in CCl\(_4\) (5 mL), acetonitrile (5 mL) and water (15 mL). NaIO\(_4\) (7.80 g, 36.4 mmol) was added, followed by RuCl\(_3\) (29 mg, 0.140 mmol). The reaction mixture was stirred 16 h. Due to the volatility of RuO\(_4\) additional aliquots of RuCl\(_3\) (29 mg, 0.140 mmol) were added as necessary. The reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was washed with ethyl acetate, and the combined organic fractions were dried with Na\(_2\)SO\(_4\) then concentrated in vacuo at 30 °C. The crude solid was dissolved in ethyl acetate, filtered through cotton, then concentrated in vacuo to provide 610 mg of crude acid 13 as a white solid. Flash column chromatography (dichloromethane:methanol:acetic acid 100:2:1 to dichloromethane:methanol:acetic acid 100:5:1) afforded 312 mg (35% over 4 steps from 12) of 13 as a white solid. IR (film) 3352 (br), 2105, 1731, 1654, 1253, 1121 cm\(^{-1}\); \(^1\)H NMR (CD\(_3\)S(O)CD\(_3\)) 300 MHz) \(\delta\) 8.34 (d, \(J = 7.3\) Hz, 1 H), 4.20-4.05 (m, 2 H), 3.60-3.47 (m, 1 H), 3.41-3.25 (m, 2 H), 3.15 (dd, \(J = 13, 9.3\) Hz, 1 H), 2.91-2.80 (m, 1 H), 2.39-2.26 (m, 1 H), 2.16-2.03 (m, 1 H), 1.85 (s, 3 H); \(^{13}\)C NMR (CD\(_3\)S(O)CD\(_3\)) 75 MHz) \(\delta\) 173.0 (C), 170.4 (C), 64.0 (CH), 63.4 (CH), 54.5 (CH\(_2\)), 53.9 (CH), 47.0 (CH), 42.5 (CH), 34.6 (CH\(_2\)), 22.6 (CH\(_3\)); MS (ES+) \(m/z\) 325 (100); HRMS calcd for C\(_{10}\)H\(_{14}\)N\(_4\)O\(_3\)S (M+Na): 325.0577. Found: 325.0579.
Compound 13 (236 mg, 0.779 mmol) was dissolved in methanol (5 mL). Pd(OH)$_2$/C 10% (~ 50 mg) and 10% aqueous HCl (~ 50 µL) were added, then H$_2$ was bubbled through the solution for 5 min. The reaction mixture was stirred under 1 atm H$_2$ for 16 h. The reaction mixture was filtered through cotton, then concentrated in vacuo to provide 225 mg of crude 14. Prior to biological testing, flash column chromatography of a 65 mg portion of crude 14 (dichloromethane:methanol:acetic acid 100:2:1 to dichloromethane:methanol:acetic acid 60:40:20) afforded 23.0 mg (35%) of the hydrochloride salt of amine 14 as a white solid. Compound 14 (HCl salt, 23.0 mg, 0.736 mmol) was dissolved in 500 µL of DMSO to provide a standard solution (147 mM) for testing. IR (film) 3351 (br), 3248 (br), 1727, 1653, 1299, 1122 cm$^{-1}$; $^1$H NMR (D$_2$O 300 MHz) δ 4.46 (ddd, $J = 7.3$, 6.5, 6.5 Hz, 1 H), 4.30 (dd, $J = 10$, 6.6 Hz, 1 H), 3.83 (ddd, $J = 7$, 6.7, 6.7 Hz, 1 H), 3.69 (dd, $J = 14$, 6.5 Hz, 1 H), 3.54-3.45 (m, 2 H), 3.25 (ddd, $J = 10$, 6, 6 Hz, 1 H), 2.64 (ddd, $J = 13$, 7.5, 7.5 Hz, 1 H), 2.15 (ddd, $J = 13$, 8.1, 8.1 Hz, 1 H), 2.04 (s, 3 H); $^{13}$C NMR (D$_2$O 75 MHz) δ 175.0 (C), 172.2 (C), 63.0 (CH), 52.6 (CH), 51.1 (CH$_2$), 50.6 (CH), 45.6 (CH), 42.6 (CH), 32.2 (CH$_2$), 19.6 (CH$_3$); MS (ES+) $m/z$ 299 (100); HRMS calcd for C$_{10}$H$_{16}$N$_2$O$_5$S (M+Na): 299.0672. Found: 299.0678.
Compound 14 (crude, 130 mg, 0.429 mmol) was dissolved in dimethylformamide (6 mL). Triethylamine (300 µL, 2.14 mmol) was added, followed by HgCl₂ (116 mg, 0.429 mmol). 1,3-Bis(benzyloxy carbonyl)-2-methyl isothiourea (9, 154 mg, 0.429 mmol) was added and the reaction mixture was stirred 16 h. The reaction mixture was concentrated in vacuo. Flash column chromatography (100:1 dichloromethane:methanol to 10:1 dichloromethane:methanol) afforded 50 mg (20% over 2 steps from 13) of 14a as a white solid. IR (film) 3313 (br), 1771, 1714, 1651, 1302, 1116 cm⁻¹; ¹H NMR (CD₃OD 500 MHz) δ 7.43-7.29 (m, 10 H), 5.20 (s, 2 H), 5.17 (s, 1 H), 4.42 (dd, J = 11, 6.0 Hz, 1 H), 4.31 (dd, J = 10, 6.9 Hz, 1 H), 4.23-4.17 (m, 1 H), 3.73-3.65 (m, 1 H), 3.51 (dd, J = 13, 6.5 Hz, 1 H), 3.22 (dd, J = 13, 6.0 Hz, 1 H), 3.04-2.97 (m, 1 H), 2.63-2.54 (m, 1 H), 1.94 (s, 3 H), 1.93-1.89 (m, 1 H); ¹³C NMR (CD₃OD 125 MHz) δ 173.7 (C), 173.3 (C), 155.9 (C), 155.1 (C), 153.1 (C), 137.0 (C), 136.9 (C), 129.6 (CH), 129.6 (CH), 129.5 (CH), 129.4 (CH), 129.4 (CH), 129.3 (CH), 68.6 (CH₂), 68.5 (CH₂), 64.4 (CH), 55.9 (CH), 55.7 (CH), 55.6 (CH₂), 54.8 (CH), 46.5 (CH), 38.0 (CH₂), 22.5 (CH₃); MS (ES⁺) m/z 609 (100); HRMS calcd for C₂₇H₃₀N₄O₉S (M+Na): 609.1626. Found: 609.1614.
Compound 14a (50 mg, 0.085 mmol) was dissolved in methanol (8 mL). Acetic acid (~ 100 µL) and 10% aqueous HCl (~ 50 µL was added, followed by Pd/C 10% (~ 10 mg). The reaction mixture was stirred under 1 atm H₂ for 16 h. The reaction mixture was filtered through cotton, diluted with cyclohexane (10 mL) then concentrated in vacuo. Prior to biological testing, flash column chromatography (dichloromethane:methanol:acetic acid 100:2:1 to dichloromethane: methanol:acetic acid 60:40:20) afforded 14.3 mg (43%) of the acetic acid salt of guanidine 15 as a white solid. Compound 15 (AcOH salt, 14.3 mg, 0.0378 mmol) was dissolved in 500 µL of DMSO to provide a standard solution (75.6 mM) for testing. IR (film) 3348 (br), 1667, 1557, 1650, 1317, 1120 cm⁻¹; ¹H NMR (D₂O 500 MHz) δ 4.47 (ddd, J = 5.6, 5.6 , 5.1 Hz, 1 H), 4.14 (dd, J = 11, 8.2 Hz, 1 H), 4.08-4.00 (m, 1 H), 3.68 (dd, J = 14, 6.5 Hz, 1 H), 3.67-3.44 (m, 2 H), 2.97 (ddd, J = 11, 7.4, 4.6 Hz, 1 H) 2.49 (ddd, J = 12, 6.6, 6.6 Hz, 1 H), 2.00 (s, 3 H), 1.85 (ddd, J = 12, 11, 11 Hz, 1 H); ¹³C NMR (D₂O 125 MHz) δ 176.7 (C), 173.9 (C), 160.7 (C), 63.6 (CH), 54.8 (CH), 54.0 (CH₂), 53.8 (CH), 47.8 (CH), 43.8 (CH), 37.0 (CH₂) 21.9 (CH₃); MS (ES+) m/z 341 (100); HRMS calcd for C₁₁H₁₈N₄O₅S (M+Na): 341.0890. Found: 341.0890.
Enzyme Assay conditions.

All enzyme assays were conducted on a SpectraMax M5 platereader using standard sensitivity settings. The following solutions were prepared for the enzyme assays:

1. **Assay Buffer**: 50 mM Tris, 5 mM CaCl₂, 200 mM NaCl, pH 7.5;
2. **Protein Stock Solution**: inactivated virus suspension was diluted in Assay Buffer at 4 °C to obtain a concentration which, when used in the assays described below, gave a slope of approximately 2 fluorescence units per minute in the absence of inhibitors.
3. **Substrate Stock Solution**: 2’-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (Aldrich) was dissolved in DMSO to a concentration of 10 mM;
4. **Substrate Working Solution**: 20 μL of the substrate stock solution was diluted to 1000 μL with assay buffer, for a final concentration of 200 μM (2% DMSO);
5. **Inhibitor Solutions**: Inhibitors were diluted in assay buffer to provide a range of working concentrations.

Sample wells of a black 96-well plate (Nunc, optical bottom) were charged with 40 μL of protein stock solution, followed by 10 μL of inhibitor solution. The samples were incubated at room temperature for 2 h, after which 50 μL of substrate working solution was added (i.e. final substrate concentration = 100 μM). The samples were mixed briefly by pipetting, and fluorescence was monitored over 5 min (λ exc = 365 nm; λ em = 445 nm).

For kinetic data, the working solution was subjected to serial dilution to obtain a range of substrate concentrations. Progress of the reaction was measured over 10 min at various concentrations of substrate and inhibitor, as indicated in Figure 5. Control experiments (substrate buffer only) showed no significant background reaction.

IC₅₀ values were obtained by plotting percent inhibition against inhibitor concentration using XLfit (IDBS software) and identifying the concentration required to achieve 50% inhibition of enzymatic activity. Kₘ values at a range of inhibitor concentrations were obtained by fitting the kinetic data to Michaelis-Menten curves in XLfit, and Kᵢ values were obtained by plotting Kₘ against inhibitor concentration. Kᵢ was reported as the negative of the x-intercept. Estimates of error for IC₅₀ values were obtained by plotting the data from 3 separate experiments (each of which was done in triplicate) and determining the goodness of fit. Error values correspond to the measured standard error. Error values reported for Kᵢ results correspond to the standard deviation for two separate determinations of Kᵢ, each of which was run in duplicate.
Spectral Data.

1. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 6 in CDCl$_3$:
2. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 7 in CD$_3$C(O)CD$_3$:
3. $^1$H (in CD$_3$C(O)CD$_3$), $^{13}$C and DEPT-135 NMR Spectra for Compound 8 in CD$_3$OD:
4. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 10 (as the ethyl ester, HCl salt) in CD$_3$OD:
5. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 11 in CDCl$_3$: 

![NMR Spectra](image)
6. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 12 in CD$_3$S(O)CD$_3$: 
7. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 12b in CD$_3$OD:
8. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 13 in CD$_3$S(O)CD$_3$: 
9. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 14 (HCl salt) in D$_2$O:
10. $^1$H, $^{13}$C and DEPT-135 NMR Spectra for Compound 15 (AcOH salt) in D$_2$O:
ORTEP Diagram for 10 (ethyl ester, hydrochloride).

Supplementary crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. Data for compound 10 (ethyl ester, hydrochloride) are available by quoting deposition number 900422. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK or via www.ccdc.cam.ac.uk/conts/retrieving.html.