Supplementary Information

Highly Specific and Rapid Glycan based Amperometric Detection of Influenza Viruses.

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**Chemical synthesis and characterization:**

**General:**

Glycosylation reactions were performed under argon with solvents dried using a solvent purification system Innovative Technology. All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. The acidic ion exchange resin used was Amberlite® IR 120 (H⁺) resin. Column chromatography was performed using silica gel (230-400 mesh). Analytical thin layer chromatography (TLC) was performed on silica gel 230-400 mesh (Sicicycle). Plates were visualized under UV light, and/or by staining with acidic CeH₈Mo₃N₂O₁₂ followed by heating. ¹H and ¹³C NMR spectra were recorded on Bruker 400MHz spectrometer. Chemical shifts are reported in δ (ppm) units using residual ¹³C and ¹H signals from deuterated solvents as references. Spectra were analyzed with MestReNova® (Mestrelab Research). Electrospray ionization mass spectra were recorded on a Micromass Q T 2 (Waters) and data were analyzed with MassLynx® 4.0 (Waters) software. Reported yields refer to spectroscopically and chromatographically pure compounds that were dried under high vacuum (10⁻² mbar) before analytical characterization, unless otherwise specified.

**Abbreviations:** Ethyl acetate, EtOAc; Dichloromethane, DCM; Triflic acid, TfOH; N-Iodosuccinimide, NIS; Sodium methoxide, NaOMe; Methanol, MeOH; Palladium hydroxide, Pd(OH)₂; Ethanol, EtOH; Sodium hydroxide, NaOH; Triethyl amine, NEt₃; Thin layer chromatography, TLC. Sodium thiosulfate, Na₂S₂O₃; Sodium sulfate, Na₂SO₄.
Scheme 1. Reagents and conditions: a) 4-methyl thiophenol, DCM, DIEPA, rt, 12h; b) TfOH, NIS, DCM, -50°C, 2h, 40% for compound 4 and 37% for compound 6, respectively; c) i. NaOMe, MeOH, rt, 1h; ii. Pd(OH)₂/C/H₂, EtOH, rt, 12h; iii. 0.05 N NaOH in H₂O, rt, 2h, 94% for (4,7-di-OMe)Sa₂,6Gal and 88% for (4,7-di-OMe)Sa₂,3Gal over three steps, respectively.

5-acetamido-8,9-di-O-acetyl-4,7-di-O-methyl-3,5-dideoxy-D-glycero-α-D-galacto-2-ulopyranosylonate-(2,6)-1,2,3,4-tetra-O-benzyl-α-D-galactopyranoside(4):

To the stirring solution of acceptor 3 (0.075g, 0.14 mmol) and donor 2 (0.050g, 0.092 mmol) in anhydrous DCM under an argon atmosphere at -50°C, NIS (0.052g, 0.23 mmol) was added followed by the addition of TfOH (0.014g, 0.092 mmol, 10% in anhydrous DCM). The reaction mixture was stirred at -50°C until the donor was consumed as determined by TLC. Et₃N (0.5 mL) was added to quench the reaction and warmed to rt. The reaction mixture was diluted with DCM, washed with saturated Na₂S₂O₃, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was subjected to flash silica gel column chromatography eluting with hexane:EtOAc (10:90) to afford compound 4 as a white solid (0.035g, 0.036 mmol, 40%).

H NMR (400 MHz, CDCl₃): δ 7.39-7.23 (m, 20H), 5.38-5.35 (m, 1H), 5.25-5.22 (m, 1H), 4.90 (d, J= 4.0 Hz, 1H), 4.76 - 4.70 (m, 3H), 4.66-4.63 (m, 1H), 4.63-4.60 (m, 1H), 4.56 - 4.49 (m, 2H), 4.45 - 4.43 (m, 3H), 4.20 (dd, J = 4.0 Hz, 12.0 Hz, 1H), 4.05 - 3.98 (m, 4H), 3.69 (s, 3H), 3.64-3.62 (m, 2H), 3.55 (d, J = 4.0 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 1H), 3.32 (s, 3H), 2.74 (dd, J = 8.0
Hz, 1H), 2.19 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.75-1.69 (m, 1H). 13C NMR (100 MHz, CDCl3): δ 170.5, 170.3, 169.8, 168.4, 138.7, 138.4, 137.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 127.2, 99.0, 97.7, 84.1, 80.4, 79.6, 73.2, 72.5, 72.4, 70.9, 68.2, 63.2, 62.8, 60.9, 55.7, 52.4, 50.3, 36.7, 29.7, 23.7, 22.7, 21.1, 20.8. HRMS (ESI): Calculated for C52H63NO16Na [M+Na] 980.4045; Found 980.4043.

5-acetamido-4,7-di-O-methyl-3,5-dideoxy-D-glycero-α-D-galacto-2-ulopyranosylonate-(2,6)-α, β-D-galactopyranoside ((4,7di-OMe)Sα2,6Gal):

Compound 4 (0.035g, 0.036 mmol) was dissolved in MeOH and treated with 30% NaOMe in MeOH and stirred at rt for 1 h. The solution was neutralized with Amberlite® IR 120 (H+) resin, filtered and concentrated to dry. The dried compound was further treated with Pd(OH)2/C in absolute EtOH and stirred at rt for 12h under H2 at 1 atm. After completion of reaction as monitored by TLC, the reaction mixture was filtered through celite pad, washed with EtOH and the combined solvent was concentrated to dry. The residue was then treated with 0.05 N NaOH in H2O and stirred at rt for 2h. After completion of reaction, as monitored by TLC, the mixture was neutralized using Amberlite® IR 120 (H+) resin, filtered, concentrated and subjected to P-2 column to afford compound (4,7di-OMe)Sα2,6Gal (0.017g, 0.034 mmol, 94% over three steps).1H NMR (400 MHz, D2O): 5.15 (d, J = 4.0 Hz, 0.6H, H1α), 4.47 (d, J = 8.0 Hz, 0.4H, H1β), 4.13-4.08(m, 1H), 3.89 - 3.77 (m, 5H), 3.76 - 3.74 (m, 1H), 3.71-3.52(m, 5H)3.41-3.37 (m, 1H), 3.35 (s, 3H), 3.30 (s, 3H), 2.78-2.72 (dd, 1H, J = 4.0, 12.0 Hz), 1.96 (s, 3H), 1.64-1.57 (m, 1H). 13C NMR (100 MHz, D2O): 174.2, 171.3, 99.3, 96.4, 92.1, 78.0, 77.3, 73.3, 72.8, 72.6, 71.7, 70.8, 68.2, 62.4, 60.3, 56.7, 49.9, 36.0, 22.2. HRMS (ESI): Calculated for C19H33NO14Na [M+Na] 522.1799; Found 522.1786.

5-acetamido-8,9-di-O-acetyl-4,7-di-O-methyl-3,5-dideoxy-D-glycero-α-D-galacto-2-ulopyranosylonate-(2,3)-1,2,4,6-tetra-O-benzyl-α-D-galactopyranoside (6):
Compound 6 was synthesized using the procedure as described for the synthesis of compound 5 (0.033 g, 0.034 mmol, 37%). \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}): 7.37-7.26 (m, 20H), 5.39-5.36 (m, 1H). 5.21 (d, \(J = 8.0\) Hz, 1H), 5.16-5.12 (s, 1H), 4.78 (d, \(J = 12.0\) Hz, 1H), 4.74 (d, \(J = 12.0\) Hz, 1H), 4.65 (d, \(J = 12.0\) Hz, 1H), 4.54-4.40 (m, 4H), 4.35 (d, \(J = 12.0\) Hz, 1H), 4.21 (br, 2H), 4.15-4.01 (m, 4H), 3.98-3.91 (m, 1H), 3.37 (s, 3H), 3.58-3.54 (br, 2H), 3.50 (s, 3H), 3.38 (s, 1H), 3.32 (s, 3H), 2.75 (dd, \(J = 4.0\) Hz, 12.0 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 6H), 1.76-1.70 (m, 1H).

\(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): 171.2, 170.3, 170.2, 168.2, 138.3, 138.0, 137.9, 137.8, 131.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 104.8, 99.1, 88.6, 82.5, 80.1, 75.3, 73.3, 72.8, 72.1, 71.7, 70.5, 68.6, 63.3, 62.9, 61.0, 60.4, 55.8, 52.5, 50.2, 36.7, 29.7, 23.8, 21.1, 21.0, 20.8. HRMS (ESI): Calculated for C\textsubscript{52}H\textsubscript{63}NO\textsubscript{16}Na [M+Na] 980.4045; Found 980.4048.

5-acetamido-4,7-di-O-methyl-3,5-dideoxy-D-glycero-α-D-galacto-2-ulopyranosyl(2,3)-α, β-D-galactopyranoside ((4,7di-OME)Sα2,3Gal):

Compound (4,7di-OME)Sα2,3Gal was synthesized using the procedure as described in the synthesis of compound (4,7di-OME)Sα2,6Gal (0.015 g, 0.030 mmol, 88%) from 6. \(^1\)H NMR (400 MHz, D\textsubscript{2}O): 5.14 (d, \(J = 4.0\) Hz, 0.4H, H1α). 4.47 (d, \(J = 8.0\) Hz, 0.4H, H1β). 3.99-3.96 (m, 1H). 3.89-3.69 (m, 7H), 3.61-3.57 (m, 5H), 3.35 (s, 3H), 3.30 (s, 3H), 2.78-2.72 (m, 1H), 1.96 (s, 3H), 1.67-1.57 (m, 1H). \(^{13}\)C NMR (100 MHz, D\textsubscript{2}O): 174.1, 173.6, 100.1, 96.3, 92.2, 78.4, 77.9, 76.0, 74.9, 72.7, 71.2, 70.2, 67.7, 62.2, 61.0, 60.0, 56.7, 49.8, 36.4, 22.2. HRMS (ESI): Calculated for C\textsubscript{19}H\textsubscript{33}NO\textsubscript{14}Na [M+Na] 522.1799; Found 522.1788.
**Figure S1.** Space filling model of active site of NAs with sialic acid derivatives as stick model. **Left.** X-ray structure of *S. pneumoniae*NanA with zanamivir (PDB: 2YA7). [1]**Right.** X-ray structure of influenza NA with 4-guanidino-Neu5Ac2en (PDB: 1NNC). [2] Note the smaller pocket of the bacterial NA, which can be exploited to develop influenza specific compounds. Structures were downloaded from protein database bank (PDB) and PyMOL (www.pymol.org) was used to create the models.
Figure S2. Standard curve galactose concentration versus current using Acck-Chek Aviva strips.
Figure S3. Detection of N1 using Sα23Gal. 50 U of A/Anhui/1/2005 H5N1 NA was incubated with Sα23Gal at 37°C. The current was measured at 15 min and 60 min.
Figure S4: Specificity towards Influenza B, C, and H. Influenzae in 15 min (Top) and 60 min (Bottom). 50 μL of B/Memphis/20/96, C/Taylor/1233/1947, or H. Influenzae was incubated with Sα23Gal (8.8 μL, 2 mM) or (4,7di-OMe)Sα23Gal (8.8 μL, 2 mM) at 37°C.
**Figure S5.** *Time course studies.* Time curve of different concentration of virus for 24 h (top) and 6 h (bottom). 50 μL of A/victoria/361/2011 ($10^1$−$10^4$ pfu) was incubated with Sa23Gal (8.8 μL, 2 mM) at 37 °C. The current was measured using Accu-Chek Aviva strips at 15 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h.
Figure S6. Limit of detection. **TOP:** The limit of detection of this assay with (4,7di-OMe)Sα23Gal and A/Brisbane/59/2007 was $10^2$ pfu in 15 min. **Bottom:** The limit of detection of this method with (4,7di-OMe)Sα23Gal and A/Victoria/361/2011 in clinical sample B was $10^2$ pfu in 15 min. The current was measured using Accu-Chek Aviva strips.
Figure S7: Limit of detection. The limit of detection of this method with Sa23Gal and S. pneumoniae (serotype 1, ATCC 6305) was $10^2$ cfu in 15 min. The current was measured using Accu-Chek Aviva strips.
References:

Compound 3: $^1$H NMR CDCl$_3$, 400 MHz
Compound 3: COSY, CDCl$_3$, 400 MHz
Compound 3: $^{13}$C NMR CDCl$_3$, 100 MHz
Compound 3: HSQC, CDCl3, 100 MHz
Compound (4,7di-OMe)Sa26Gal: $^1$H NMR D$_2$O, 400 MHz
Compound (4,7di-OME)Sa26Gal: COSY, D₂O, 400 MHz
Compound \((\text{4,7-di-OMe})\text{Sa}_2\text{Gal}\): $^{13}$C NMR D$_2$O, 100 MHz
Compound (4,7di-OMe)Sa26Gal: HSQC, D₂O, 100 MHz

Compound 5: ¹H NMR CDCl₃, 400 MHz
Compound 5: COSY, CDCl₃, 400 MHz
Compound 5: $^{13}$C NMR CDCl$_3$, 100 MHz
Compound 5: HSQC, CDCl₃, 100 MHz
Compound (4,7di-OMe)Sa23Gal: $^1$H NMR D$_2$O, 400 MHz
Compound \((4,7\text{-OMe})\text{Gal}_{23}\): COSY, D\(_2\)O, 400 MHz
Compound (4,7-di-OMe)Sa23Gal: $^{13}$C NMR D$_2$O, 100 MHz
Compound $(4,7\text{di-OMe})\text{Sa}_2\text{Gal}$: HSQC, $\text{D}_2\text{O}$, 100 MHz