Polymeric mannosides prevent DC-SIGN-mediated cell-infection by cytomegalovirus

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Contents

General chemical procedures and analytical data S2-S11
General biological procedures S12-S13
NMR spectra of synthetic products S14-S33
Titration curves and inhibitory concentrations S34-S36
General Methods

All reagents were purchased from Acros Organics, Alfa Aesar, Carbosynth or Aldrich and were used without further purification. Dichloromethane ethyl acetate and petroleum ether were distilled on a Buchi rotavapor R-220-SE. Acetonitril, pyridine, benzene and DMF were freshly distilled from calcium hydride under argon. THF was distilled on sodium, benzophenone and under argon. Reactions requiring anhydrous conditions were performed under argon. Column chromatography was conducted on silica gel Kieselgel Si60 (40–63 μm) from Merck, or on Silica cartridge from Interchim and eluted via a Puriflash 430 with an UV and ELSD detection. Thin layer chromatography (TLC): Merck Silica gel 60 F254 analytical plates, detection either with UV (254 nm) or dipping in a solution of cerium molybdate, potassium permanganate, ninhydrine and subsequent heating. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures. 1H and 13C NMR spectra were recorded on Bruker Avance 300 spectrometer fitted with a 5 mm i.d. BBO probe carefully tuned to the recording frequency of 300.13 MHz (for 1H) and 75.47 MHz (for 13C), the temperature of the probe was set at room temperature (around 293-294 K), on a Bruker Avance 400 spectrometer fitted with a 5 mm i.d. BBFO+ probe carefully tuned to the recording frequency of 400.13 MHz (for 1H) and 100.61 MHz (for 13C), the temperature of the probe was set at 303 K, on a Bruker Avance III 500 spectrometer fitted with a 5mm i.d. 13C/1H cryoprobe carefully tuned to the recording frequency of 500.13 MHz (for 1H) and 125.76 MHz (for 13C), the temperature of the probe was set at 303 K. The spectra are referenced to the solvent in which they were run (7.26 ppm for 1H CDCl3 and 77.16 ppm for 13C CDCl3, 2.50 ppm for 1H DMSO-d6 and 39.52 ppm for 13C DMSO-d6, 3.31 ppm for 1H CD3OD and 49.00 ppm for 13C, 7.16 ppm for 1H C6D6 and 128.06 ppm for 13C C6D6, 5.32ppm for 1H CD2Cl2 and 53.84 ppm for 13C, 4.79 ppm for 1H D2O). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are given in Hz. Multiplicity of signals is indicated as following: s (singulet), d (doublet), t (triplet), q (quadruplet), m (multiplet), brs (broad singulet), dd (doublet of doublet), dt (doublet of triplet)... The numbering used for NMR attribution is different from UPAC numbering, and written on each molecule.

Copper Catalyzed Azide-Alkyne Cycloaddition Method 1.
The azide derivative and the alkyne derivative (1.1 eq./azide function) were dissolved in dioxane (2mL/mmol). A solution of copper sulfate (0.2 eq./azide function) and sodium ascorbate (0.4 eq./azide function) in water (0.5mL/mmol) was added and the mixture was heated at 60°C until completion. The mixture was dissolved in DCM (50mL/mmol), washed with a solution of EDTA (50 mL/mmol) and the aqueous layer was extracted twice with DCM (2 x 50mL/mmol). The organic layer was then dried over MgSO4, filtered, concentrated under reduced pressure and purified.
Deprotection of acetyl groups using sodium methanolate method 2.
The protected carbohydrate (1eq.) was dissolved in MeOH/H₂O (1:1, 1 mL/mmol). Amberlite resin IRN 78 OH- 1.25 meq/mL (150 mg/mmol) was added, and the mixture was stirred overnight at rt. The resin was filtered off and washed with methanol and water. The solvent was evaporated under reduced pressure.

Compound 1

Obtained following the acetate deprotection method 2 to afford 1 (89% yield) as a colorless gum.

1H NMR (400 MHz, MeOD): δ = 8.07 (1H, s, H-9), 4.85 (1H, d, J = 1.9Hz, H-1), 4.80 (1H, d, J = 12.7Hz, H-7a), 4.65 (1H, d, J = 12.7Hz, H-7b), 4.59 (1H, t, J = 5.2Hz, H-10), 3.90 (2H, t, J = 5.2Hz, H-11), 3.85 (1H, dd, J = 1.9Hz, J = 12.4Hz, H-6a), 3.79 (1H, dd, J = 1.9Hz, J = 3.7Hz, H-2), 3.74-3.83 (1H, m, H-3, H-4, H-5, H-6b, 12xCH₂O); 13C NMR (100 MHz, MeOD): δ = 145.1 (C-8), 126.2 (C-9), 100.8 (C-1), 75.0 (C-5), 73.7 (C-3), 72.5 (C-2), 71.5-71.4 (CH₂O), 70.3 (C-10), 68.6 (C-4), 63.0 (C-6), 62.2 (C-7), 51.5 (C-7); HRMS (MALDI) m/z calcd for C₁₇H₃₁N₃O₁₀Na: 460.1907, found 460.1903.

Compound 2

Obtained following the acetate deprotection method 2 to afford 2 (83% yield) as a colorless gum.

1H NMR (400 MHz, MeOD): δ = 7.90 (1H, s, H-10), 4.72 (1H, d, J = 1.6Hz, H-1), 4.67 (2H, s, H-12), 4.40 (2H, t, J = 7.1Hz, H-9), 3.82 (1H, dd, J = 2.4Hz, J = 11.8Hz, H-6a), 3.77 (1H, dd, J = 1.7Hz, J = 3.3Hz, H-2), 3.74-3.65 (3H, m, H-3, H-6b, H-7a), 3.55-3.57 (1H, m, H-4), 3.55-3.48 (1H, m, H-5), 3.44-3.37 (1H, m, H-7b), 1.97-1.85 (2H, m, 2XH-8), 1.65-1.51 (2H, m, 2XH-8), 1.44-1.27 (6H, m, 6XH-8); 13C NMR (100 MHz, MeOD): δ = 149.1(C-11), 124.0 (C-10), 101.5 (C-1), 74.6 (C-5), 72.7 (C-3), 72.3 (C-2), 68.7 (C-4), 68.4 (C-7), 63.0 (C-6), 56.5 (C-12), 51.3 (C-9), 31.2, 30.4, 29.8, 27.4, 27.1(C-8); HRMS (ES⁺) m/z calcd for C₁₆H₃₀N₃O₇: 376.2084, found 373.2073.
Compound 3

Obtained following the acetate deprotection to afford 3 (97% yield) as a colorless gum. $\left[\alpha\right]_{D}^{20} = +78$ (C = 1, MeOH); $^1$H NMR (400 MHz, D$_2$O): $\delta = 8.14$ (2H, s, H-9), 5.03 (2H, d, $J = 1.9$Hz, H-1), 4.88 (2H, d, $J = 12.7$Hz, H-7a), 4.75 (2H, d, $J = 12.7$Hz, H-7b), 4.69 (4H, t, $J = 5.2$Hz, H-10), 4.02 (t, $J = 5.2$Hz, 4H, H-11), 3.98 (dd, $J = 1.9$Hz, $J = 12.4$Hz, 2H, H-6a), 3.91 (dd, $J = 1.9$Hz, $J = 3.7$Hz, 2H, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.70 (4H, m, H-4, H-5), 3.69-3.65 (4H, m, CH$_2$O), 3.62-3.57 (4H, m, CH$_2$O); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 143.5$ (C-8), 126.6 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.7-69.5 (CH$_2$O), 68.7 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for C$_{26}$H$_{44}$N$_{6}$O$_{15}$Na: 703.2762, found 703.2766.

Compound 4

Obtained following the acetate deprotection to afford 4 (73% yield) as a colorless gum. $\left[\alpha\right]_{D}^{20} = +64$ (C = 1, MeOH); $^1$H NMR (400 MHz, D$_2$O): $\delta = 8.19$ (2H, s, H-9), 5.04 (2H, d, $J = 1.9$Hz, H-1), 4.91 (2H, d, $J = 12.7$Hz, H-7a), 4.79 (2H, d, $J = 12.7$Hz, H-7b), 4.71 (4H, t, $J = 5.2$Hz, H-10), 4.05 (4H, t, $J = 5$Hz, H-11), 3.99 (2H, dd, $J = 1.9$Hz, $J = 12.4$Hz, H-6a), 3.92 (2H, dd, $J = 1.9$Hz, $J = 3.7$Hz, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.73 (28H, m, H-4, H-5, CH$_2$O), 3.72-3.66 (16H, m, CH$_2$O); $^{13}$C NMR (100 MHz, D$_2$O): $\delta = 143.6$ (C-8), 125.7 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.7-69.3 (CH$_2$O), 68.8 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-8), 50.1 (C-11); HRMS (ES+) m/z calcd for C$_{42}$H$_{76}$N$_{6}$O$_{23}$Na: 1055.4860, found 1055.4860.

Compound 5

Obtained following the acetate deprotection to afford 5 (71% yield) as a colorless gum. $\left[\alpha\right]_{D}^{20} = +60$ (C = 1, MeOH); $^1$H NMR (400 MHz, D$_2$O): $\delta = 8.19$ (2H, s, H-9), 5.04 (2H, d, $J = 1.9$Hz, H-1), 4.91 (2H, d, $J = 12.7$Hz, H-7a), 4.79 (2H, d, $J = 12.7$Hz, H-7b), 4.70 (4H, t, $J = 5.2$Hz, H-10),
4.04 (4H, t, J = 5.2Hz, H-11), 3.96 (dd, J = 1.9Hz, J = 12.4Hz, H-6a), 3.90 (2H, dd, J = 1.9Hz, 2H, J = 3.7Hz, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.69 (4H, m, H-4, H-5), 3.70-3.63 (m, 76 H, CH₂O); ¹³C NMR (100 MHz, D₂O): δ = 143.6 (C-8), 125.6 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.6 (CH₂O), 68.8 (C-10), 66.7 (C-4), 60.9 (C-6), 59.7 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for C₅₈H₁₀₈N₆O₃Na: 1407.6957, found 1407.6953.

Compound 6

![Structure of Compound 6](image)

Obtained following the acetate deprotection to afford 6 (74% yield) as a colorless gum. [α]₂₀⁰₀⁺ = + 58 (C = 1, MeOH); ¹H NMR (400 MHz, CD₃OD): δ = 8.07 (2H, s, H-9), 4.86 (2H, d, J = 1.9Hz, H-1), 4.79 (2H, d, J = 12.7 Hz, H-7a), 4.65 (2H, d, H-7b), 4.59 (4H, t, J = 5.2Hz, H-10), 3.91 (4H, t, J = 5.2Hz, H-11), 3.96 (2H, dd, J = 1.9Hz, J = 12.4Hz, H-6a), 3.90 (2H, dd, J = 1.9Hz, J = 3.7Hz, H-2), 3.75-3.67 (4H, m, H-3, H-6b), 3.67-3.58 (108H, m, H-4, H-5, 104xCH₂O); ¹³C NMR (100 MHz, CD₃OD): δ = 145.1 (C-8), 126.2 (C-9), 100.8 (C-1), 75.0 (C-5), 72.6(C-3), 72.0 (C-2), 71.5 (CH₂O), 70.3 (C-10), 68.7 (C-4), 63.0 (C-6), 60.7 (C-7), 51.5 (C-11); HRMS (MALDI) m/z calcd for C₇₄H₁₄₀N₆O₃⁹Na₂: 1782.8952, found 1782.8946.

Compound 7

![Structure of Compound 7](image)

Obtained following the acetate deprotection to afford 7 (78% yield) as a colorless gum. [α]₂₀⁰₀⁺ = + 81 (C = 1, MeOH); ¹H NMR (400 MHz, D₂O): δ = 8.13-8.01 (8 H, s, H-9, H-9’), 5.02 (4 H, bs, H-1), 4.89-4.67 (16 H, m, H-7, H7’), 4.67-4.55 (18 H, m, H-11, H1’, H-2’, H-11’), 3.97 (21 H, m, H-2, H-10, H-4’, H-10’), 3.92-3.80 (12 H, m, H-3, H-6), 3.78-3.68 (8 H, m, H-4, H-5), 3.67-3.52 (36 H, m, H-3’, H-5’, H-6’, CH₂O), 3.41 (3 H, s, OCH₃); ¹³C NMR (100 MHz, D₂O): δ = 143.9, 143.8, 143.5 (C-8, C-8’), 125.7 (C-9), 99.5 (C-1), 98.4 (C-1’), 78.4, 74.5, 73.9 (C-3’, C-4’, C-5’), 73.0 (C-5), 70.6 (C-3), 70.3 (C-6’), 70.0 (C-2), 69.7-69.5 (CH₂O), 68.7 (C-10), 66.7 (C-4), 63.3 (C-2’), 63.2 (C-7’), 60.9 (C-6), 59.8 (C-7), 55.0 (OCH₃), 50.1 (C-11); HRMS (MALDI) m/z calcd for C₈₇H₁₄₄N₆₂O₄₂: 1098.4935, found 1098.4933.

Compound 8
Obtained following the acetate deprotection method 2 to afford 8 (65% yield) as a colorless gum. 

$[\alpha]_{D}^{20} + 68$ (C = 1, MeOH); $^{1}$H NMR (400 MHz, D$_2$O): $\delta$ = 8.08-8.01 (8 H, s, H-9, H-9’), 4.94 (4 H, bs, H-1), 4.82-4.65 (16 H, m, H-7, H7’), 4.61 (18 H, m, H-11, H1’, H-2’, H-11’), 3.94 (21 H, m, H-2, H-10, H-4’, H-10’), 3.92-3.80 (12 H, m, H-3, H-6), 3.78-3.68 (8 H, m, H-4, H-5), 3.67-3.52 (116 H, m, H-3’, H-5’, H-6’, CH$_2$O), 3.41 (3 H, s, OCH$_3$); 

$^{13}$C NMR (100 MHz, D$_2$O): $\delta$ = 143.9, 143.8, 143.6 (C-8, C-8’), 125.6, (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.6 (CH$_2$O), 68.7 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for C$_{119}$H$_{208}$N$_{24}$O$_{58}$ : 1451.2049, found 1451.2045.

**Compound 9**

Obtained following the acetate deprotection method to afford 9 (74% yield) as a yellowish solid.

$^{1}$H NMR (400 MHz, D$_2$O): $\delta$ = 8.18 (3 H, m broad, H-9), 5.20 (1 H, s broad, H-1’), 5.00-4.60 (m broad, 9 H, H-1, H-11), 4.20-4.07 (m broad, 3 H, H-6a), 4.04-3.86 (m broad, 9 H, 3xH-2, 3xH-6b, H-2’, H-3’, H-4’), 3.85-3.49 (21H, H-3, H-4, H-7, H-8, H-5’, H-6’), 3.17 (3H, s broad, H-5); 

$^{13}$C NMR (100 MHz, D$_2$O): $\delta$ = 144.4 (C-10), 125.6 (C-9), 99.7 (C-1), 96.7 (C-1’), 79.2 (C-3’), 77.8 (C-2’), 77.1 (C-4’), 73.0 (C-5), 70.6 (C-3’), 70.1 (C-2’), 69.8 (C-5’, C-4), 66.6 (C-8), 65.5 (C-6), 63.3 (C-6’), 60.9 (C-7), 50.2 (C-11).

**Compound 16.**

Obtained following the CuAAC method 1. The crude product was chromatographed on a silica gel column with 96/4 (DCM/MeOH) as eluent to afford 16 (88% yield) as a yellowish gum. $[\alpha]_{D}^{20} = (+)$ 42 (C = 1, CHCl$_3$); $^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.84 (1H, s, H-9), 5.31-5.24 (2H, m, H-3, H-4), 5.20 (1H, br s, H-2), 4.93 (1H, br s, H-1), 4.81 (1H, d, J = 10.5Hz, H-7a), 4.64 (1H, d, J = 10.5Hz, H-7b), 4.54 (2H, t, J = 4.7Hz, H-11), 4.26 (1H, dd, J = 5.0Hz, J = 12.2Hz, H-6a), 4.12-4.02 (2H, m, H-5, H-6b), 3.86 (2H, t, J = 4.7Hz, H-10), 3.71-3.55 (12 H, m, CH$_2$O), 2.11,2.08, 1.99, 1.94 (12H, 4 s, CH$_3$CO); 

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 170.7, 170.1, 169.9, 169.7
(COCH₃), 143.7 (C-8), 124.8 (C-9), 96.9 (C-1), 72.5, 70.6-70.3 (CH₂O), 69.5 (C-11), 69.5 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-6), 61.7 (CH₂OH), 61.0 (C-7), 50.5 (C-10), 20.9-20.6 (COCH₃); HRMS (ES+) m/z calcd for C₂₅H₄₀N₃O₁₄: 606.2510 found 606.2500.

Compound 18.

Obtained following the CuAAC method 1. The crude product was chromatographed on a silica gel column with 96/4 (DCM/MeOH) as eluent to afford 18 (73% yield) as a yellowish gum. $[\alpha]^2_{D} = +48$ (C = 1, CHCl₃); $^1$H NMR (400 MHz, CDCl₃): $\delta = 7.52$ (1H, s, H-10), 5.30 (1H, dd, $J_{3-2} = 3.4$Hz, $J_{3-4} = 10.0$ Hz, H-3), 5.25-5.20 (1H, m, H-4), 5.18 (1H, dd, $J_{2-3} = 1.8$Hz, $J_{2-1} = 3.4$Hz, H-2), 4.77-4.72 (3H, m, H-1, H-12), 4.32 (2H, t, $J = 7.0$Hz, H-9), 4.23 (1H, dd, $J_{6a-5} = 5.3$Hz, $J_{6a-6b} = 12.3$Hz, H-6a), 4.07 (1H, dd, $J_{6b-5} = 2.5$Hz, $J_{6b-6a} = 12.3$Hz, H-6b), 3.96-3.90 (1H, m, H-5), 3.68-3.58 (1H, m, H-7a), 3.44-3.36 (1H, m, H-7b), 2.12, 2.05, 2.00, 1.96 (12H, 4 s, CH₃CO), 1.92-1.83 (2H, m, H-8), 1.61-1.49 (2H, m, H-8), 1.36-1.27 (6H, m, H-8); $^{13}$C NMR (100 MHz, CDCl₃): $\delta = 170.7, 170.2, 170.1, 169.8$ (COCH₃), 147.9 (C-11), 121.6 (C-10), 97.6 (C-1), 69.8 (C-2), 69.2 (C-3), 68.5 (C-5), 68.4 (C-7), 66.4 (C-4), 62.6 (C-6), 56.5 (C-12), 50.3 (C-9), 30.2, 29.1, 28.7, 26.4, 26.0 (C-8), 20.9, 20.8, 20.74 (CH₃); HRMS (ESI) m/z calcd for C₂₄H₃₈N₆O₁₁Na: 544.2506 found 544.2504.

Compound 23.

Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 95/5 (DCM/MeOH) as eluent to afford 23 (92% yield) as a colorless gum. $[\alpha]^2_{D} = +29$ (C = 1, CHCl₃); $^1$H NMR (400 MHz, CDCl₃): $\delta = 7.76$ (2H, s, H-9), 5.32-5.28 (4H, m, H-3, H-4), 5.22 (2H, bs, H-2), 4.96 (2H, d, $J = 1.6$ Hz, H-1), 4.82 (2H, d, $J = 12.4$Hz, H-7a), 4.66 (2H, d, $J = 12.4$Hz, H-7b), 4.55 (4H, t, $J = 4.7$Hz, H-11), 4.29 (2H, dd, $J = 5.0$Hz, $J = 12.3$Hz, H-6a), 4.13-4.04 (4H, m, H-5, H-6b), 3.85 (4H, t, $J = 4.7$Hz, H-10), 3.64-3.57 (8H, m, CH₂O), 2.14, 2.12, 2.02, 1.96 (24H, 4 s, CH₃CO); $^{13}$C NMR (100 MHz, CDCl₃): $\delta = 170.8, 170.2, 170.0, 169.7$ (COCH₃), 143.4 (C-8), 124.3 (C-9), 96.9 (C-1), 70.6-69.6 (CH₂O), 69.5 (C-11), 69.4 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-4), 62.5 (C-6), 61.0 (C-7), 52.8 (C-10), 21.0, 20.9, 20.8, 20.7(COCH₃); HRMS (ESI) m/z calcd for C₄₃H₆₅N₆O₂₃Na: 1039.3608 found 1039.3615.
Compound 24.

Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 95/5 (DCM/MeOH) as eluent to afford 24 (90% yield) as a colorless gum. [α]_{D}^{20} = +36 (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.76 (2H, s, H-9), 5.29-5.25 (4H, m, H-3, H-4), 5.20 (2H, m, H-2), 4.94 (2H, d, J = 1.6 Hz, H-1), 4.80 (2H, d, J = 12.4Hz, H-7a), 4.65 (2H, d, J = 12.4Hz, H-11), 4.53 (4H, t, J = 4.7Hz, H-11), 4.27 (2H, dd, J = 5.0 Hz, J = 12.3Hz, H-6a), 4.11–4.02 (4H, m, H-5, H-6b), 3.87 (4H, t, J = 4.7Hz, H-10), 3.64-3.57 (40 H, m, CH₂O), 2.11, 2.09, 2.00, 1.94 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.7, 170.0, 169.9, 169.7 (COCH₃), 143.4 (C-8), 124.3 (C-9), 96.9 (C-1), 70.6 (CH₂O), 69.6 (C-11), 69.5 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-4), 62.5 (C-6), 61.0 (C-7), 50.4 (C-10), 20.9, 20.8, 20.7 (COCH₃); HRMS (ESI) m/z calcd for C₅₈H₉₂N₆O₃Na: 1391.5705 found 1391.5708.

Compound 25

Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford 25 (85% yield) as a white solid. [α]_{D}^{20} = +54 (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.78 (2H, s, H-9), 5.33-5.26 (4H, m, H-3, H-4), 5.22 (2H, bs, H-2), 4.95 (2H, bs, H-1), 4.82 (2H, d, J = 12.4Hz, H-7a), 4.67 (2H, d, J = 12.4Hz, H-7b), 4.55 (4H, t, J = 4.7Hz, H-11), 4.30 (2H, dd, J = 5.0Hz, J = 12.3Hz, H-6a), 4.13–4.05 (4H, m, H-5, H-6b), 3.88 (4H, t, J = 4.7Hz, H-10), 3.66-3.58 (72 H, m, CH₂O), 2.14, 2.11, 2.02, 1.96 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.1, 169.9, 169.8 (COCH₃), 143.4 (C-8), 124.4 (C-9), 96.9 (C-1), 70.7 (CH₂O), 69.5 (C-11), 69.5 (C-2), 69.2 (C-3), 68.7 (C-5), 66.2 (C-4), 62.5 (C-6), 61.1 (C-7), 50.4 (C-10), 20.9, 20.8 (COCH₃); HRMS (ESI) m/z calcd for C₇₄H₁₂₄N₆O₃₉Na: 1743.7802 found 1743.7811.

Compound 26
Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford 25 (70% yield) as a white solid. $\left[\alpha\right]_D^{20} = +43$ (C = 1, CHCl3); $^1$H NMR (400 MHz, CDCl3): $\delta = 7.80$ (2H, s, H-9), $5.32-5.28$ (4H, m, H-3, H-4), $5.23$ (2H, m, H-2), $4.97$ (2H, d, $J = 1.6$ Hz, H-1), $4.84$ (2H, d, $J = 12.4$Hz, H-7a), $4.67$ (2H, d, $J = 12.4$Hz, H-7b), $4.56$ (4H, t, $J = 4.7$Hz, H-11). $^13$C NMR (100 MHz, CDCl3): $\delta = 170.8, 170.1, 169.9, 169.8$ (COCH3), $143.4$ (C-8), $124.4$ (C-9), $97.0$ (C-1), $70.7$ (CH2O), $69.5$ (C-11), $69.5, 69.2, 68.7, (C-2, C-3, C-5), 66.2 (C-4), 62.5 (C-6), 61.1 (C-7), 50.4 (C-10), 20.9, 20.8, (COCH3); HRMS (ESI) m/z calcd for C90H156N6O47Na: 2095.9899 found 2095.9895.

Compound 30.

Obtained following the CuAAC method. This reaction afforded the compound 30 (84% yield) as a colorless gum. $\left[\alpha\right]_D^{20} = +16$ (C = 1, CHCl3); $^1$H NMR (400 MHz, CDCl3): $\delta = 7.93, 7.87, 7.78, 7.75$ (4H, s, H-9), $4.92$ (1H, d, $J = 11.3$Hz, H-7), $4.81$ (2H, bs, H-7), $4.73$ (1H, d, $J = 11.3$Hz, H-7), $4.72$ (1H, bs, H-2), $4.71$ (1H, d, $J = 3.2$Hz, H-1), $4.67$ (1H, d, $J = 11.3$Hz, H7), $4.58$ (1H, d, $J = 11.3$Hz, H-7), $4.56$ (2H, m, H-7), $4.53-4.48$ (6H, m, H-4, CH2O), $3.89-3.75$ (m, 13 H, H-3, H-5, H-6, CH2O), $3.36$ (m, 8 H, CH2N3), $3.30$ (3 H, bs, OCH3); $^13$C NMR (100 MHz, CDCl3): $\delta = 145.2-145.0$ (C-8), $124.4-124.0$ (C-9), $99.0$ (C-1), $79.8, 74.8, 74.5, (C-3, C-4, C-5), 70.8-69.4$ (CH2O), $69.2, 66.2, 64.9, 64.3$ (C-2, C-6, C-7), $54.9$ (OCH3), $50.8$ (CH2N3), $50.3-50.2$ (CH2N); HRMS (ESI) m/z calcd for C51H87N24O18: 1323.6630 found 1323.6626.

Compound 31.

Obtained following the CuAAC method. This reaction afforded the compound 31 (75% yield) as a colorless gum. $\left[\alpha\right]_D^{20} = +14$ (C = 1, CHCl3); $^1$H NMR (400 MHz, CDCl3): $\delta = 7.92, 7.86, 7.78, 7.75$ (4H, s, H-9), $4.94$ (1H, d, $J = 11.3$Hz, H-7), $4.82$ (2H, bs, H-7), $4.73$ (1H, d, $J = 11.3$Hz, H-7), $4.72$ (1H, bs, H-2), $4.67$ (1H, d, H-1), $4.64$ (1H, d, H7), $4.58$ (1H, d, H-7), $4.56$ (2H, m, H-7), $4.53-4.50$ (6H, m, H-4, CH2O), $3.89-3.75$ (m, 13 H, H-3, H-5, H-6, CH2O), $3.68-3.55$ (106 H, m, CH2O), $3.36$ (m, 8 H, CH2N3), $3.31$ (3 H, bs, OCH3); $^13$C NMR (100 MHz, CDCl3): $\delta = 144.9$ (C-8), $124.2-124.0$ (C-9), $98.9$ (C-1), $79.7, 74.8, 74.5, (C-3, C-4, C-5), 70.8-69.4$ (CH2O), $69.3, 66.1, 64.7, 64.1$
(C-2, C-6, C-7), 54.8 (OCH₃), 50.8 (CH₂N), 50.7, 50.1 (CH₂N); HRMS (ESI) [m+2H]/2z calcd for C₈₃H₁₅₂N₂₄O₃₄: 1014.5452 found 1014.5455.

Compound 32.

Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford 32 (78% yield) as a colorless gum. \([\alpha]_D^{20} = + 36\) (C = 1, CHCl₃); \(^1^H\) NMR (400 MHz, CDCl₃): δ = 7.93, 7.85, 7.78, 7.75, 7.74 (8H, s, H-9, H-9’), 5.26 (8H, m, H-3, H-4), 5.20 (4H, bs, H-2), 4.94 (4 H, J = 2.0, H-1), 4.91-4.60 (16 H, m, H-7, H-1’, H-2’, H7’), 4.54-4.45 (18 H, m, H-7, H-11, H-11’, H-7’), 4.28 (4 H, dd, J = 12.3, 5.2Hz, H-6a), 4.12-4.01 (9 H, s, OCH₃), 3.85 (20 H, m, H-10, H-3’, H-5’, H-6’, H-10’), 3.56 (32 H, m, CH₂O), 3.27 (3 H, s, OCH₃), 2.12, 2.09, 2.00, 1.94 (48 H, 4 s, CH₃CO); \(^1^3^C\) NMR (100 MHz, CDCl₃): δ = 170.7, 170.1, 169.9, 169.8 (COCH₃), 145.0, 144.9, 144.8, 143.3 (C-8, C-8’), 124.3, 124.1, 124.0 (C-9, C-9’), 99.0, 96.9 (C-1’), 96.9 (C-1), 79.8, 74.7, 74.7, (C-3’, C-4’, C-5’), 71.4 (C-6’), 70.6, 69.5, 69.4, 69.2, 68.8 (C-11, C-11’, CH₂O), 69.3, 69.2, 68.8, 66.2, 64.8 (C-2, C-3, C-4, C-5, C-7, C-7’), 64.2 (C-2’), 63.6 (C-7’), 62.5 (C-6), 61.0 (C-7’), 55.0 (OCH₃), 53.5, 50.4, 50.2 (C10, C-10’), 20.9, 20.8, 20.7, (COCH₃).

Compound 33.

Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford 32 (90% yield) as a colorless gum. \([\alpha]_D^{20} = + 18\) (C = 1, CHCl₃); \(^1^H\) NMR (400 MHz, CDCl₃): δ = 7.95, 7.88, 7.79, 7.72 (8H, s, H-9, H-9’), 5.39-5.29 (8H, m, H-3, H-4), 5.29-5.26 (4H, m, H-2), 4.87 (4 H, J = 2.0, H-1), 4.92-4.68 (18 H, m, H-7, H-1’, H-2’, H7’), 4.67-4.49 (18 H, m, H-7, H-11, H-11’, H-7’), 4.31 (4 H, dd, J = 5.2Hz, J =
12.3, H-6a), 4.16–4.04 (9 H, m, H-5, H-6b, H-4’), 3.93-3.80 (10 H, m, H-10, H-3’, H-5’, H-6’, H-10’), 3.60 (106 H, m, CH₂O), 3.30 (3 H, s, OCH₃), 2.14, 2.09, 2.03, 1.98 (48 H, s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.1, 170.0, 169.9 (COCH₃), 145.1-143.4 (C-8, C-8’), 124.4, 124.3, 124.2, 124.1 (C-9, C-9’), 99.1 (C-1’), 96.8 (C-1), 79.6, 74.7, 74.3 (C-3’, C-4’, C-5’), 71.3 (C-6’), 70.6-69.1 (C-11, C-11’, CH₂O), 69.2, 69.1, 68.8, 66.3, 64.3 (C-2, C-3, C-4, C-5, C-7, C-7’), 64.2 (C-2’), 63.6 (C-7’), 62.3 (C-6), 61.2 (C-7’), 55.3 (OCH₃), 52.9, 52.6 (C10, C-10’), 21.0, 20.8, 20.7, (COCH₃).

Compound 35

Copper sulfate (40 mg, 0.16 mmol) and sodium ascorbate (65 mg, 0.32 mmol) were added to a solution of propargyl dextran (20 mg, 72.4 µmol of monosaccharide unit) and 15 (125 mg, 0.30 mmol) in dioxane-H₂O (5 mL, 4–1). The mixture was irradiated at 80 °C for 2 x 45 min in a sealed vessel, with addition of copper sulfate sulfate (13.3 mg, 53.3 µmol) and sodium ascorbate sulfate (21.7 mg, 0.11 mmol) for the second run. Residual copper was removed with EDTA (94 mg, 0.32 mmol) for 2 h, and the mixture was poured into a NH₄Cl satd. solution (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was dried (MgSO₄), filtered and the solvent removed under reduced pressure. The crude product was dissolved in a small amount of CH₂Cl₂ and the product was precipitated with Et₂O (30 mL). The precipitate was collected by filtration, washed with Et₂O (50 mL) and precipitate twice from Et₂O, to afford 35 (64 mg, 54%) as a yellow solid.¹H NMR (500 MHz, CDCl₃): δ = 8.17-7.70 (3 H, br s, H-9), 5.18 (6 H, br, H-3, H-4) 5.00-4.41 (13 H, m broad, H-1, H-2, H-11, H-1’), 4.37-3.20 (27 H, br, H-6a, H-8, H-7, H-2’,3’,4’,5’,6’), 2.27-2.77 (36 H, m, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 171.2, 170.7, 170.0, 169.8 (CH₃CO), 145.4 (C-10), 124.4 (C-9), 97.7 (C-1, C-1’), 77.3 (C-3’), 69.3, 69.2, 68.9 (C-2, C-5’, C-3, C-5), 66.4 (C-4), 65.9 (C-11), 62.4 (C-6), 60.5 (C-7), 60.1 (C-6’), 49.7 (C-8), 21.1, 20.9, 20.8 (CH₃CO).
Materials and Methods

Cells

U937 cells (monocytic leukemia; ATCC® CRL-1593.2™, LGC Standards, UK) stably expressing full length DC-SIGN were generated by lentiviral transduction and propagated as already described elsewhere (Halary, Immunity, 2002). DC-SIGN expression was validated by flow cytometry with an APC-conjugated anti-human DC-SIGN mAb (clone DCN46) on a LSR II apparatus (BD Biosciences, Franklin Lakes, NJ). MRC-5 fibroblasts (RD Biotech, France) were propagated in DMEM, 2mM glutamine, 10% FCS (HyClone/GE Healthcare, Wauwatosa, WI). MRC-5 cells were used at 80-90% confluency for trans-infection assays.

Viral envelope glycoprotein binding assay

Parental or DC-SIGN-expressing U937 cells were resuspended in TBS, 1 mM CaCl₂, 2 mM MgCl₂, 0.1% bovine serum albumin (BSA) and then seeded in 96-well plates at 1.10⁵ cells per well. Biotin-labelled recombinant HCMV gB (2 µg/ml) was then added to cells for 20 min at 4°C. Cells were washed three times with cold TBS, 1 mM CaCl₂, 2 mM MgCl₂, 0.1% BSA and further stained with APC- or PE-conjugated streptavidin (BD Biosciences, Franklin Lakes, NJ) before being analyzed on a LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ) and the FlowJo software (Tree Star, Ashland, OR).

Trans-infection experiments

To assess the blocking properties of HM-based compounds described above in this section, a DC-SIGN-expressing U937 cell line and the parental U937 cells as a negative control were used in a trans-infection assay described elsewhere (Halary, Immunity, 2002). MRC-5 fibroblasts were seeded in flat-bottom 96-well plates the day before starting the trans-infection assay in DMEM, 2 mM glut, 10% FCS. Briefly, DC-SIGN-expressing U937 cells were incubated with inhibitors at indicated concentrations for 30 min at 4°C before adding a constant dose of AD-GFP (MOI=2), a AD169 genetically-modified CMV strain that allowed for the GFP expression in infected cells (REF
princeps). After being washed thoroughly, CMV-loaded cells were applied on a MRC-5 cell monolayer. A short centrifugation step (5’, x300g) was used to favour cell-to-cell contact and viral transmission which was assessed after 48h by counting the total number of GFP positive MRC-5 cells in each cell culture well through an automated processing described hereafter.

**Automated infected cell counting**

Absolute numbers of infected cells were obtained by analysing the total well area with a Cellomics® ArrayScan® VTI HCS Reader (Thermo Scientific) equipped with a x10 objective. For each field, phase-contrast and fluorescence (band-path filter 485nm±20nm) channels were recorded simultaneously. Image stitching was performed with the HCS Studio Cell Analysis Software (Thermo Scientific) and allowed to reconstruct an image of the whole well for all conditions. Digitalized images were processed with the “particles analysis” plugin from the Fiji software. Results were plotted as absolute numbers of infected cells (GFP positive cells) per well against the base 10 logarithm of the inhibitor concentrations. IC50 values were determined on the GraphPad Prism 5.0 software using a nonlinear regression analysis (GraphPad Software Inc., La Jolla, CA, USA).

**Cell viability assay**

Cell viability was determined by DAPI (4’,6-Diamidino-2-Phenylindole, Dihydrochloride) exclusion after incubation of U937 cells with all tested compounds for two hours at 37°C. Cells were then washed and resuspended into 300µL of PBS per condition. Ten microliters of a 1µM DAPI solution were extemporaneously to cells and immediately analysed on a LSR II flow cytometer.
Compound 1
Compound 2
Compound 3
Compound 4
Compound 6
Compound 7

[Diagram of compound 7]

[Graph with peaks labeled]
Compound 8
Compound 9

\[ R = \begin{align*} \text{HO} & \text{HO} \\ \text{O} & \text{O} \\ \text{O} & \text{N} \\ \text{N} \end{align*} \]
Compound 16
Compound 18
Compound 22
Compound 23
Compound 25
Compound 29
Compound 30
Compound 32
Trans-infection experiments

Mosaic of pictures showing whole culture wells of ADGFP-HCMV-infected fibroblasts (white shapes on GFP-channel pictures and black ones on the thresholded picture showing a condition where fibroblasts were cultured with ADGFP-HCMV-loaded DC-SIGN+ U937 cells (positive control of the trans-infection experiments). Similar pictures (all conditions are displayed in quadruplicates) show negative controls, in DC-SIGN+ U937 cells without ADGFP-HCMV or parental U937 cells with or w/o the virus (two upper panels).
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* IC<sub>50</sub><sup>Mon*o</sup> = expressed in mol of monomer; 95% confidence intervals from triplicates