Supplementary Information

Polyvalent C-glycomimetics based on L-fucose or D-mannose as potent DC-SIGN antagonists

Benedetta Bertolotti\(^a\), Ieva Sutkevičiute\(^{b,c}\), Martino Ambrosini\(^d\), Renato Ribeiro-Viana\(^{e,f}\), Javier Rojo\(^e\), Franck Fieschi\(^b\), Hana Dvořáková\(^g\), Martina Kašáková\(^a\), Kamil Parkan\(^a\), Martina Hlaváčková\(^a\), Kateřina Nováková\(^h\), and Jitka Moravcová\(^*\)

\(^a\). Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague, Czech Republic.
\(^b\). Institut de Biologie Structurale (IBS), Univ. Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France.
\(^c\). Present address: Laboratory for G Protein-Coupled Receptor Biology, Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.
\(^d\). Department of Molecular Cell Biology and Immunology, VU University Medical Center (VUmc), Amsterdam, The Netherlands.
\(^e\). Glycosystems Laboratory, Instituto de Investigaciones Químicas (IIQ), CSIC - Universidad de Sevilla, Sevilla, Spain.
\(^f\). Present address: Departamento Academico de Química, Universidade Tecnologica Federal do Paraná, Av dos Pioneiros, 3131. Londrina - PR - Brazil.
\(^g\). Laboratory of NMR, Central Laboratories, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague, Czech Republic.
\(^h\). The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

*contact: Jitka.Moravcova@vscht.cz

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1. NMR and MS identification of new compounds

\(^1\)H, \(^13\)C, COSY, HMQC and HMBC spectra were measured on a Bruker DPX-300, DRX-400, DRX-500, or Bruker Advance III 600 (Bruker Corporation, Germany) spectrometer. All spectra were acquired at 298 K. Chemical shifts are given in \(\delta\)-units (ppm) and are referenced to TMS. Coupling constants (\(J\)) are reported in Hz. Numbering of atoms for NMR signal assignment is placed in figures.

Low resolution ESI-MS was carried out using an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. ESI high resolution mass spectra were measured with a LTQ Velos Orbitrap XL (Thermo Fisher Scientific, UK) instrument equipped with LockSpray in ES+ and ES- modes with mobile phase of 80% methanol. MALDI high resolution mass analysis was carried out in positive reflectron mode using a MALDI TOF UltraflexXTremeTM MALDI TOF/TOF (Bruker Daltonics, Germany) instrument equipped with 1 kHz smartbeam II laser.

General comment on NMR

Due to the lack of triazolyl carbons signals in \(^13\)C NMR spectrum of all dendrimers (F-C-4, F-C-6, F-C-9, F-C-12, M-C-9, and M-C-12) we suppose the existence of some dynamic process resulted from conformational behaviour of C-pseudoglycosidic dendrimers. In the case of F-C-9, most of the carbon and some of the proton resonances were doubled although MS revealed that F-C-9 is a single compound with MW 3283. This observation could be explained by the presence of two F-C-9 stereoisomers.
General comment on MS
A molecular associate with Na\(^+\) was obviously the most abundant ion under both ESI and MALDI ionizations. Highly charged ions as well as associates of two molecules with H\(^+\)/Na\(^+\) were formed frequently. The C-pseudoglycosidic dendrimers gave molecular ions, which were further fragmented by elimination of one arm generating the loss of 255 (F-C-4, F-C-6, F-C-9, and F-C-12) or 271 (M-C-9, and M-C-12) mass.

2-(2,3,4,6-Tetra-O-acetyl-\(\alpha\)-l-fucopyranosyl)ethylazide (7)
C\(_{14}\)H\(_{21}\)N\(_3\)O\(_7\), MW 343; HRMS (ESI); \(^1\)H NMR (600.1 MHz, CDCl\(_3\)); \(^{13}\)C NMR (125 MHz, CDCl\(_3\))
2-\{(\alpha-L-Fucopyranosyl)ethylazide (2) \nC_{8}H_{15}N_{3}O_{4}, MW 217; HRMS (ESI); $^{1}{\text{H}}$ NMR (600.1 MHz, D$_{2}$O); $^{13}{\text{C}}$ NMR (125 MHz, D$_{2}$O) \n
![Graph with mass spectra and NMR spectra]
2-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)ethylazide (9)

C_{16}H_{23}O_{9}N_{3}, MW 401; HRMS (ESI); \[^1^H\text{NMR}\ (600.1\ \text{MHz, CDCl}_3); \[^{13}\text{C}\text{NMR}\ (125\ \text{MHz, CDCl}_3)\]
2-(α-D-Mannopyranosyl)ethylazide (1)

C$_8$H$_{15}$N$_3$O$_5$, MW 233; HRMS (ESI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendron F-C-Cl
$C_{42}H_{70}ClN_{17}O_{17}$, MW 1007; MS (ESI); HRMS (ESI); $^1$H NMR (600.1 MHz, $D_2$O); $^{13}$C NMR (125 MHz, $D_2$O)
Dendron F-C-N

C_{42}H_{70}N_{12}O_{15}; MW 1015; MS (ESI); HRMS (ESI); \textsuperscript{1}H NMR (600.1 MHz, D$_2$O); \textsuperscript{13}C NMR (125 MHz, D$_2$O)
Dendron M-C-Cl

C_{42}H_{71}ClN_{9}O_{20}, MW 1056; MS (ESI); HRMS (ESI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendron M-C-N

C_{12}H_{20}N_{12}O_{20}; MW 1063; MS (ESI); HRMS (ESI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendrimer F-O-4

C_{44}H_{92}N_{12}O_{26}, MW 1220; LRMS (ESI); \(^1\)H NMR (500 MHz, D\(_2\)O); \(^{13}\)C NMR (125 MHz, D\(_2\)O)
Dendrimer F-O-6

C<sub>76</sub>H<sub>122</sub>N<sub>18</sub>O<sub>37</sub>, MW 1878; LRMS (ESI); <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O); <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)

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[Graph of mass spectrum]

[Graph of NMR spectrum]

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**Dendrimer F-O-12**

C_{185}H_{300}N_{48}O_{84}, MW 4536; LRMS (ESI); $^1$H NMR (500 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)

\[2291.7 \text{ [M+2Na]}^{2+}, 1540.7 \text{ [M+3Na]}^{3+}, 1157.4 \text{ [M+4Na]}^{4+}\]
Dendrimer F-C-4

C_{40}H_{80}N_{12}O_{20}, MW 1157; MS (ESI); HRMS (ESI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendrimer F-C-6
$C_{76}H_{128}N_{21}O_{31}$, MW 1785; MS (MALDI); HRMS (MALDI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendrimer F-C-9
C_{141}H_{222}N_{36}O_{54}, MW 3283; MS (MALDI); HRMS (MALDI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendrimer F-C-12

C\textsubscript{185}H\textsubscript{300}N\textsubscript{48}O\textsubscript{72}, MW 4346; MS (MALDI); HRMS (MALDI); \textsuperscript{1}H NMR (600.1 MHz, D\textsubscript{2}O); \textsuperscript{13}C NMR (125 MHz, D\textsubscript{2}O)
Dendrimer M-C-9

C_{141}H_{222}N_{36}O_{63}, MW 3426; MS (MALDI); HRMS (MALDI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
Dendrimer M-C-12
C_{185}H_{200}N_{48}O_{84}, MW 4536; MS (MALDI); HRMS (MALDI); $^1$H NMR (600.1 MHz, D$_2$O); $^{13}$C NMR (125 MHz, D$_2$O)
2. SPR competition assay

2.1 General procedure

DC-SIGN extracellular domain (ECD) was expressed in *E.coli* as inclusion bodies, refolded and purified as described previously. SPR competition experiments were performed using Biacore 3000 instrument, at 25 °C as described previously. Briefly, using Biacore amino coupling kit and standard amino coupling procedure, interaction flow cell of sensor chip CM4 was covalently functionalized with BSA-Man1-3[Man1-6]Man (Man-BSA), while reference flow cell was EDC/NHS-activated and ethanolamine-deactivated carboxymethyl dextran. DC-SIGN ECD alone (20 µM) or in presence of increasing concentrations of test compounds was injected over reference (Fc1) and interaction surfaces (Fc2 and Fc3) at 20 µL/min flow rate. All samples were prepared in running buffer consisting of 25 mM Tris-HCl pH 8, 150 mM NaCl, 4 mM CaCl₂ and 0.005% surfactant P20. Binding of DC-SIGN ECD to Man-BSA surface was recorded in sensorgrams. After reference surface correction (Fc1-reference surface, activated/deactivated CM-dextran), DC-SIGN ECD binding responses for each injection were extracted and normalized to DC-SIGN ECD alone binding response. Normalized binding responses were plotted against compound concentration and IC₅₀ values were calculated from resulting competition curves using 4-parameter logistic model. Each run was repeated twice using two different ManBSA surfaces Fc2 (1912 RU) and Fc3 (1612 RU) within two days. ManBSA densities on Fc2 and Fc3 were different with higher density being on Fc3, however, the overall affinity of DC-SIGN to both Man-BSA surfaces was unchanged as Kₐ and Kᵤ values were comparable (Fig S1).


![Fig. S1 First titration of Man-BSA surfaces with DC-SIGN ECD. Running buffer 25 mM TRIS pH 8, 150 mM NaCl, 4 mM CaCl₂, 0.005% P20](image-url)
### 2.2 IC₅₀ values

**Table S1**

IC₅₀ values (µM) obtained in DC-SIGN inhibition assays (SPR) and valency-corrected factors β †

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<th>Acronym</th>
<th>Valency</th>
<th>IC₅₀ (µM)</th>
<th>SD (%)</th>
<th>Factor β</th>
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<tr>
<td>F-C-4</td>
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<td>212</td>
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<td>67</td>
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†L-fucose: IC₅₀ = 2,061 µM; D-mannose: IC₅₀ = 3,392 µM

2.4. Sensorgrams and inhibition curves
Fig. S2 Reference surface corrected sensorgrams

Fig. S3 Inhibition curves