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Deoxy-Mannose Derivatives as Potential Immune System Boosters in SARS-CoV-2

Infections

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Abstract: Coronaviruses such as SARS-CoV-2 are spherical infectious agents covered with out-pointing protein spicules. These spicules are coated with the host's glycans that display immunoevasion epitopes in the circulating medium of the infected body. The post-transcriptional construction of this external camouflaging glycan envelope provides the virus with an elusive protection from the immune system. The poly-mannose glycans are synthesized in the infected cells' endoplasmic reticulum and transferred onto the S-protein N-glycosylation sites of the spicules at the end of the virus reproduction cycle. The core structure of these glycans is composed of branched mannoses on an asparagine N-glycosylated chitobiose root. Deoxy-D-mannoses and derivatives such as fluoro- or chloro- substituted analogues possess the structural features to be potentially recognised and used by cytoplasm or endoplasmic reticulum oligo-mannose synthase enzymes during the construction of the dolichol-phosphate glycan biosynthesis. If incorporated onto the growing chitobiose rooted disaccharide, these residues would inhibit further growth of the terminal poly-mannose structure by introducing -OH glycosylation site deficiencies at key branching or elongating positions. These deficiencies would then prevent full growth of the oligo-mannose terminal glycan. The presence of shorter and incomplete glycan epitopes on the released virus spicules would leave a larger surface of the S-protein uncovered. Consequently, the virus antigenic sites would be more exposed (accessible) to the circulating antibodies of the host's immune system.

Keywords: Halo-deoxy-mannoses, polysaccharide, SARS-CoV-2, immune system, glycan shield, polymannose

1. Introduction

SARS-CoV-2 viral outbreak is at the origin of the unprecedented worldwide COVID -19 pandemic crisis of 2020, dramatically changing the lives of billions of people through sanitary and economic measures¹. The novelty of this virus has resulted in various back and forth political decisions, while the virus characteristics are being discovered². Research for drugs or vaccines is in full tilt and though there have been several leads, there is not yet a consensus on an effective cur. Studies of the virus structure have shown that its protein membrane is covered by a dense layer of out-pointing spicules (Fig.1), characteristic of previously encountered corona type viruses³ in the outbreak of SARS-CoV-1 in 2003 and MERS-CoV in 2012. SARS-CoV-2 is a RNA virus possessing numerous structural and non-structural proteins such as RNA polymerase⁴. It is highly contagious and is capable of crossing some species barriers⁵. The “stealth capacity” is enhanced by the presence of a very dense coverage of the host’s own glycans on the outer layer of its spicules. The glycan coverage provides immunoevasion by displaying familiar epitopes to the immune system and shielding the immunogenic sequences of the virus proteins.

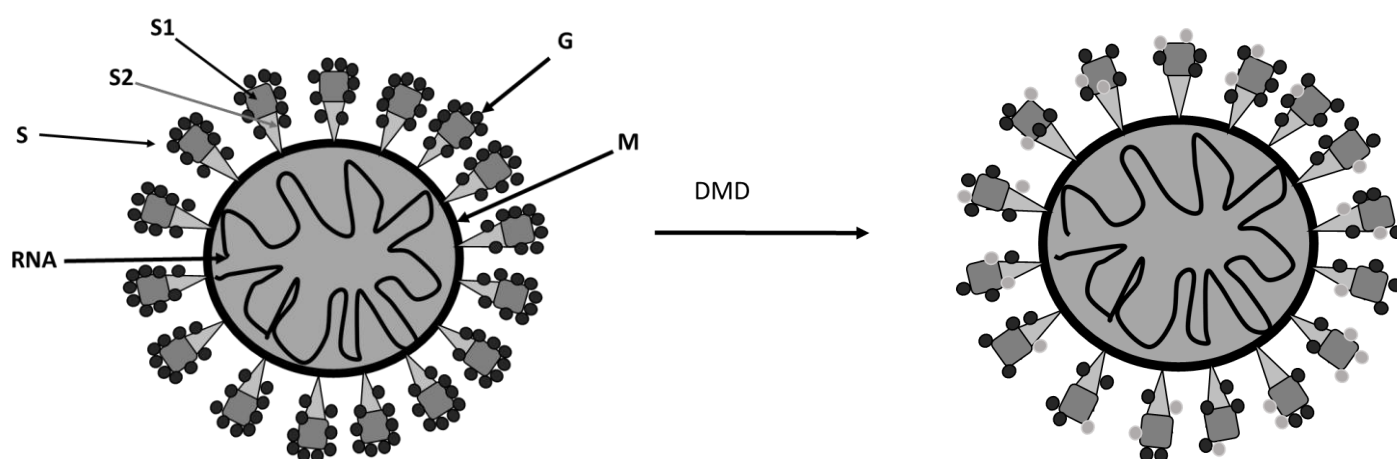
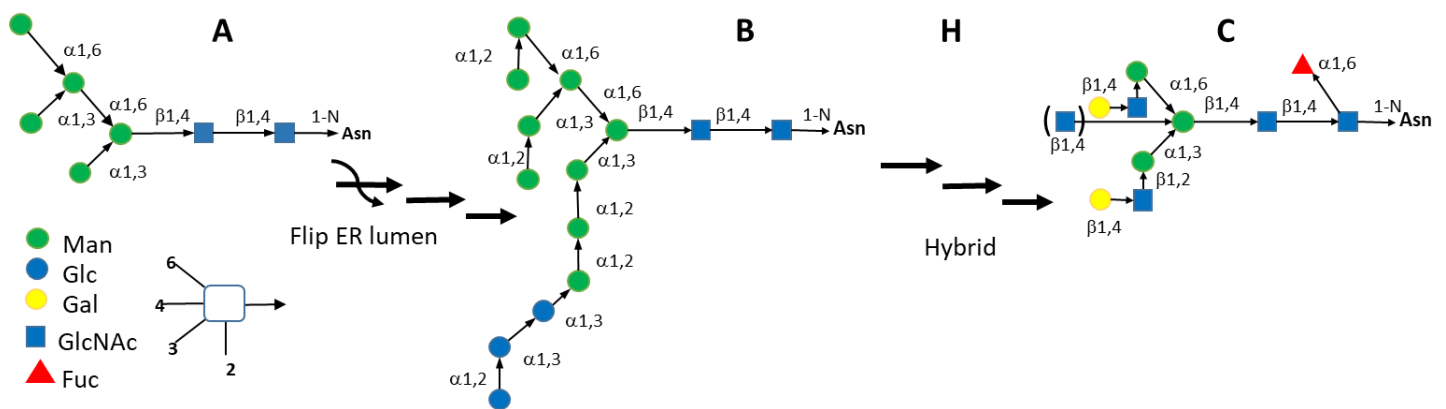


Figure 1 Schematic representation of a SAR-CoV-2 virus structure with the RNA nucleus surrounded by the membrane protein M from which the spikes S with the S1 (dark grey) and S2 (light grey) domains covered with glycans G (black dots) protrude; and the putative compromised glycan shield after inclusion of deoxy-mannose derivative DMD in the glycan structures, leaving S-protein antigenic sites uncovered.

Coronavirus spicules S are glycoprotein complexes composed of three S1 proteins on a S2 trimeric stem^{6,7,8}. The S1 proteins on the tip hold the receptor binding domains (RBD) that target angiotensin-converting enzyme-2 (ACE2) as well as other cell adhesion factors, such as CD26, Ezrin, cyclophilins and few others⁹. The RBD is key in the fusion process at the start of a new cell infection. Spike S-proteins possess numerous asparagine N-glycosylation sites¹⁰. These sites are heavily glycosylated with the host glycans within the infected cells by post-transcriptional modifications before the virus is released in the plasma. The glycosylation process results in the virus being able to evade the immunogenic response by shielding more than 40 % of the spikes' protein surface. The di-N-acetyl-glucosamine ((GlcNAc)₂ - chitobiose) -poly-mannosidic structures (**B** - Fig. 2) are built in a multistep N-glycosylation pathway within the endoplasmic reticulum (ER) prior to being N-glycosylated onto the asparagine sites.



Monomers & positions legend

Figure 2 Asparagine N-linked high-mannose glycans. **A**- Penta-mannose substituted chitobiose synthesized in the ER cytosol. **B**- High mannose polysaccharide synthesized in the ER lumen. **C**- Complex type polysaccharide bearing fucose and lactosamine on external mannoses and eventual glucosamine on the 4-position of the β -mannose elaborated further in the golgi. Hybrid polysaccharides **H** are intermediate structures built during the transformation of high mannose **B** to complex polysaccharides **C**.

These high-mannose glycans (**B Fig 2**) are composed of an asparagine N-glycosylated chitobiose root onto which a beta-mannose residue is attached in the 4-position of the second GlcNAc residue of the chitobiose root¹¹. The beta-mannose is substituted with two alpha-branched mannoses chains, one alpha 1-2 oligo-mannosyl chain in the 3- position of the beta-mannose and on the 6- position a more complex 6-alpha-mannose branched chain bearing alternating -2 and -6 alpha-mannose chains¹². This immunogenic oligosaccharide chain construction is built by sequential addition of two UDP-GlcNAc onto a dolichol diphosphate (Dol-PP) in the

cytoplasmic face of the endoplasmic reticulum (ER), anchored to the lipid bilayer¹³. Then a series of guanosine diphosphate mannoses (GDP-Man) is added onto specific positions by specialized mannosyl-transferases. Once the doubly beta branched oligo-mannose on the chitobiose has reached five mannoses (A- Fig.2), the heptasaccharide moiety flips towards the ER lumen where other specific mannosyl-transferases elaborates further alpha-mannose chain elongations by adding Dol-P-mannose onto the 2- and 6- positions of the mannose chains the Dol-PP-heptasaccharide until it reaches nine mannoses residues¹⁴. The growth of the dolichol-diphosphate glycanic complex can be completed by the addition of a final glucose mono-, di- or trimer (B- Fig.2). In the ER, some of the high-mannose envelope of the virus is subjected to glycosidase trimming actions, removing the external glucoses while the protein folds in the Calnexin/Calreticulin cycle¹⁵. In the cis-Golgi and Medial-Golgi other mannosidases cleave subsisting mannoses down to the doubly α -1,2 - α -1,6 branched mannotriose β -branched onto the Cell- NAc root core. Then a GlcNAc is added onto the α -1,2 branched mannose triggering the addition of a fucose residue to the second GlcNAc of the chitobiose root. This fucosylated heptasaccharide will be transformed into hybrid (H Fig.2) and finally complex type (C) by subsequent addition of GlcNAc, galactose and sialic acid by the action of the corresponding transferases. These oligosaccharidic structures are transferred to proteins by N-glycosylation of the asparagines. After the replication of their RNA and proteins within the infected cell, the viruses use the cell glycosylating machinery to cover the spikes S-protein with a dense layer of glycans through their numerous asparagine N-glycosylation sites. Once achieved, these specific glycans provide the virus with an efficient camouflage from the host's immune system. The three types –high mannose, hybrid and complex glycans– are susceptible to being transferred onto the virus spikes proteins within the cell; however, some envelope glycans found on immunodeficiency virions have been shown to be almost entirely high-mannose^{14,16}. Reducing the immunogenic coverage of the spikes by acting on the glycan structure would render the virus antigenic peptide sequences more accessible to antibodies (Fig. 1). This better access to antigenic sites could boost the immune system and help fight the infection.

Discussion

Sugar derivatives and sugar mimics have long been used as metabolism modifiers by interfering with enzymatic pathways. A plethora of chemically modified sugar or sugar-like molecules has been designed to

create inhibitors or reaction intermediate mimics that target specific enzymes¹⁷. In the case of immune system evading virions such as SARS-CoV-2, targeting the glycanic envelope of the virus can be done by interfering with the glycan construction process during the viral multiplication. Supplying glycosylating enzymes with a catalytic-site-fitting GDP-deoxy-mannose derivative instead of GDP-mannose during the polysaccharidic structure construction would result in oligosaccharidic structure lacking OH- groups in specific glycosylation positions. The incorporation of these deoxy-pseudo-mannoses onto the chitobiose-rooted growing glycan would prevent further growth of the poly-mannosyl structure. The introduction of -OH deficiencies at key branching or elongating positions on mannose would result in maimed polysaccharidic structures. The presence of shorter and incomplete poly-mannose terminated structure on the released virus' spicules could help better expose the antigenic surface protein to the circulating antibodies by rendering the virus-specific peptidic sequence more accessible to the host immune system. The three key positions (Fig. 3) involved in the construction of the polysaccharidic structures are:

- The C-3 position for the first β -mannose attached onto the second GlcNAc of chitobiose, and the second α -1,6 branched mannose (**A** – Fig. 2).
- The C-2 position for all α -C2- terminal mannose and oligo-mannosyl chains (**B** – Fig 2)
- The C-6 position for the first β -mannose and second 1-6-branched alpha-mannose.
- The position C-4 for the potential glucosamine addition on the complex type (**C** – Fig.2).

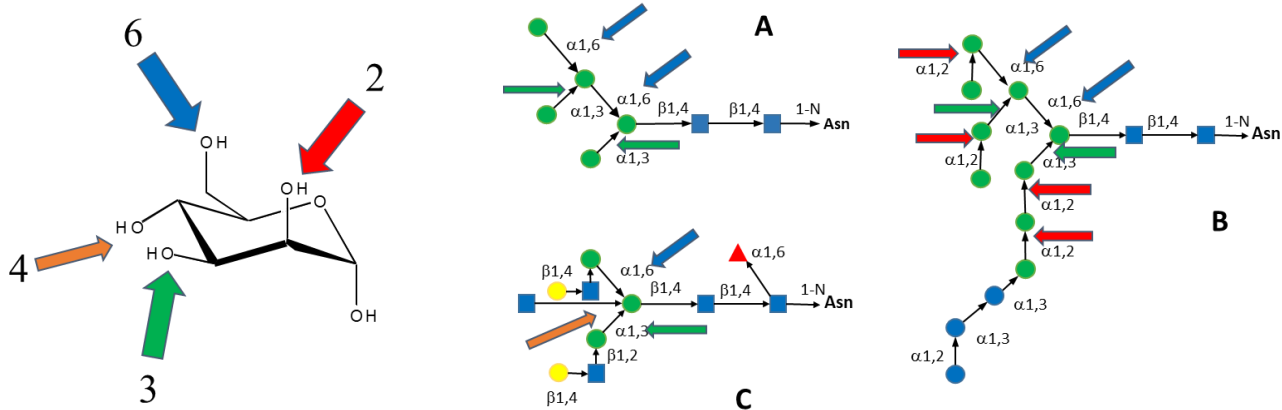


Figure 3 The four key -OH sites of mannose involved in the construction of polysaccharide structures. The position 3- and 6- involved in the construction of the core structure **A**, the 2- position involved in chain elongation during the construction of the high mannose structure **B** and the position 4- for the final glucosamine addition on the complex type polysaccharide **C** (For monomers type & positions legend see Fig 2).

Removal of mannose oxygen atoms or replacing them by a different atom on one or more of these three positions would result in deoxy-mannose derivatives: deoxy-2-mannose (formally deoxy-2-lyxohexose) **1** (Tab. 1), deoxy-3-mannose (formally deoxy-3-arabinohexose) **2** and deoxy-6-mannose (D-rhamnose) **3** and all combinations such as in Tyvelose (3,6-dideoxy-D-arabino-hexose) and other dideoxy mannose analogs. Incorporation of these deoxy-mannose analogues would render further elongation of the oligosaccharide on these positions impossible. However, such drastic changes in physico-chemical properties – size, shape, polarity, and electronic properties– of these deoxy-mannose analogues could impair the recognition and insertion of the derivative within the enzymes catalytic site. On another hand, deoxy-derivatives such as halogeno-sugars have been shown to present similar steric hindrance¹⁷ and electronic properties making them suitable analogues to the parent sugar molecules¹⁸. Fluorine in particular possess an atomic size comparable to oxygen and although it cannot mimic the -OH hydrogen-bond-giving feature, its electron-rich characteristics makes it an excellent electronegative hydrogen-bond-acceptor, as for the oxygen atom¹⁹. Most deoxy-fluoro-mannose derivatives have been synthesized²⁰ for a long time and several of them (i.e. **6**, **7** and **8**, Tab.1) are commercially available. The deoxy-hallo mannose derivatives substituted in position 2- have been proposed for cancer treatments²¹ and fluoro-substituted mannoses in 3- and 4- positions have been shown to inhibit or modify the normal glycosylation of proteins after their metabolization *in vivo* as GDP esters in yeast²². Although the chlorine atom has a somehow larger atomic radius than oxygen it has been used as a substitute for the oxygen atom as well. The most successful chloro-sugar analogues examples are probably the super sweet chlorinated sucrose derivatives that were discovered by accident in L. Hough's team^{24,25} at QEC King's college London, of which the exceptionally sweet trichloro derivative sucralose® has been commercialised now for more than 30 years with a current production of 15 000 tons /y.

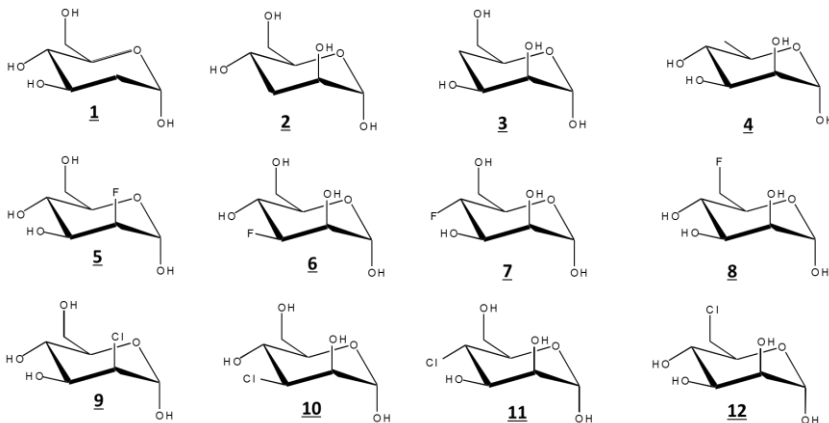


Figure 4. Haworth formulas of the mono-deoxy- and mono-deoxy-halo mannose analogues in position 2- (**1**, **5**, **9**), 3- (**2**, **6**, **10**), 4- (**3**, **7**, **11**), and 6- (**4**, **8**, **12**).

Table 1 lists the trivial, nomenclature names, synonyms and reference numbers of the mono-deoxy- and mono-deoxy-fluoro and -chloro mannose analogues that could be potential candidates for interfering with the glycan biosynthesis during the virus replication, by specifically blocking the full growth of poly-mannose structures.

Table 1

Compound	Subst. Pos	Repl. Atom	Formula	Trivial name	CH Nom.	IUPAC-Name	CAS
1	2	H	C6H12O5	2-deoxy-D-mannose	2-deoxy-D-arabinohexose	(3R,4S,5R)-3,4,5,6-tetrahydroxyhexanal	61-58-5 / 154-17-6
2	3	H	C6H12O5	3-deoxy-D-mannose	3-deoxy-D-arabinohexose	(2S,4S,5R)-3,4,5,6-tetrahydroxyhexanal	—
3	4	H	C6H12O5	4-deoxy-D-mannose	4-deoxy-D-lyxohexose	(2S,3S,5S)-2,5,6-tetrahydroxyhexanal	—
4	6	H	C6H12O5	6-deoxy-D-mannose	D-rhamnose	(2S,3S,4R,5R)-3,4,5,6-tetrahydroxyhexanal	634-74-2
5	2	F	C6H11FO5	2-fluoro-D-mannose	2-fluoro-2-deoxy-D-mannose	(2S,3S,4R,5R)-2-fluoro-3,4,5,6-tetrahydroxyhexanal	—
6	3	F	C6H11FO5	3-Fluoro-D-mannose	3-Fluoro-3-deoxy-D-mannose	(2R,3S,4R,5R)-3-fluoro-2,4,5,6-tetrahydroxyhexanal	87764-46-3
7	4	F	C6H11FO5	4-Fluoro-D-mannose	4-Fluoro-4-deoxy-D-mannose	(2S,3R,4R,5R)-4-fluoro-2-3,5,6-tetrahydroxyhexanal	87764-47-4
8	6	F	C6H11FO5	6-Fluoro-D-mannose	6-fluoro-D-rhamnose	(2S,3S,4S,5S)-6-fluoro-2,3,4,5-tetrahydroxyhexanal	—
9	2	Cl	C6H11ClO5	2-chloro-D-mannose	2-chloro-2deoxy-D-mannose	(2S,3S,4R,5R)-2-chloro-3,4,5,6-tetrahydroxyhexanal	74950-97-3
10	3	Cl	C6H11ClO5	3-Chloro-D-mannose	3-Chloro-3-deoxy-D-mannose	(2R,3S,4R,5R)-3-chloro-2,4,5,6-tetrahydroxyhexanal	—
11	4	Cl	C6H11ClO5	4-Chloro-D-mannose	4-Chloro-4-deoxy-D-mannose	(2S,3R,4R,5R)-4-chloro-2-3,5,6-tetrahydroxyhexanal	—
12	6	Cl	C6H11ClO5	6-chloro-D-mannose	6-Chloro-D-rhamnose	(2S,3S,4S,5S)-6-Chloro-2,3,4,5-tetrahydroxyhexanal	4990-81-2

In the laboratory, we have undertaken the task of synthesizing compounds **4**, **8**, **9** and **12** via alternative routes from literature. The results will be published elsewhere.

4. Conclusion

According to literature, glycans covering the external part of coronavirus spicules play a major role in evading the immune system response to viral infection. Studies on immune-challenging viruses have shown that these glycans are mostly poly-mannose or hybrid glycans. The host glycans are N-glycosylated to the asparagines of the spicules S-protein by post-transcriptional addition within the infected cells at the end of the virus reproductive cycle. Metabolism studies of deoxy-mannose in yeasts have shown the disruptive action of some deoxy-mannose derivatives on the normal glycosylation of proteins after their metabolization *in vivo* as GDP esters. Providing infected cells with deoxy-mannose derivatives could potentially prevent full growth of the polysaccharides lining of the spicule S-protein, uncovering some of the virus antigenic sites. Although deoxy-mannoses appear to be interesting to study for their glycan synthesis disruption potential, in the case of viral

spicules glycosylation during the replication in infected cells, they should be studied with extreme caution since they would also interfere with the normal glycosylation process of cellular metabolism.

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