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Mendeleev Commun., 2009, **19**, 62–63
Mendeleev Communications

Monosialoside with multimer-like anti-influenza potency

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DOI: 10.1016/j.mencom.2009.03.002

Synthetic glycopeptides capable of assembling in aqueous solution demonstrated enhanced influenza virus blocking potency.

The influenza virus initiates infection by attachment to host cells. This attachment is mediated by the interaction of the virus surface glycoprotein hemagglutinin with cell-surface sialic receptors.[1] Molecules that sterically hinder the receptor-binding sites of virus by competitive interaction with hemagglutinin can be a perspective class of antiviral drugs.[2] Monomeric oligosaccharides are unable to compete effectively with the cell-surface oligosaccharides because of their low binding constants towards influenza virus hemagglutinin (~10^{-3} M) while the inhibitors of influenza virus receptor binding should demonstrate much higher affinity for virus. The way to increase the affinity lies in use of polyvalent inhibitors such as glycopolymers, glycodendrimers and star-like molecules.[2]

Recently, tetraantennary glycopeptides [Figure 1(*a*)] capable of forming submicron-size flat aggregates (tectomers) were synthesized. The surface of these tectomers is covered with carbohydrate groups, which allow a polyvalent interaction with influenza virus.[3] Such assembling glycopeptides demonstrated to be three orders of magnitude more active influenza virus blockers than their non-assembling analogues.

Assembling of the tetraantennary glycopeptides took place because the oligoglycine chains formed a so-called polyglycine II crystalline structure. Assembling occurred when the length of oligoglycine chains exceeded six glycine residues. In that case the system of intermolecular hydrogen bonds became so strong that water at room temperature could not destroy it. However, the area accessible for each carbohydrate group on surface of tectomer is rather small, ~20 Å^2 per group. On the one hand, this circumstance did not allow the incorporation of bulky sugars into the glycopeptide molecules to occur without sterical constraints both for their assembling and for interaction of the tectomers with carbohydrate recognizing proteins. On the other hand, not all of the carbohydrate groups on the surface could interact with the virus because of their high density.

With the view of further investigation of assembling glycopeptides, we have synthesized glycopeptides in new triantennary fashion [Figure 1(*b*)] possessing only one carbohydrate group, *i.e.*, formally incapable of oligovalent binding. Important gain of such molecular design is larger area accessible for the carbohydrate group on surface of tectomers comparing to the above tetraantennary glycopeptides.

The first step was the synthesis of a branched molecule representing a central fragment of glycopeptide, which could allow elongation peptide and non-peptide antennae independently of one another.

Pentaerythritol used as a starting material was transformed into a triamino alcohol as shown in Scheme 1. Amino groups

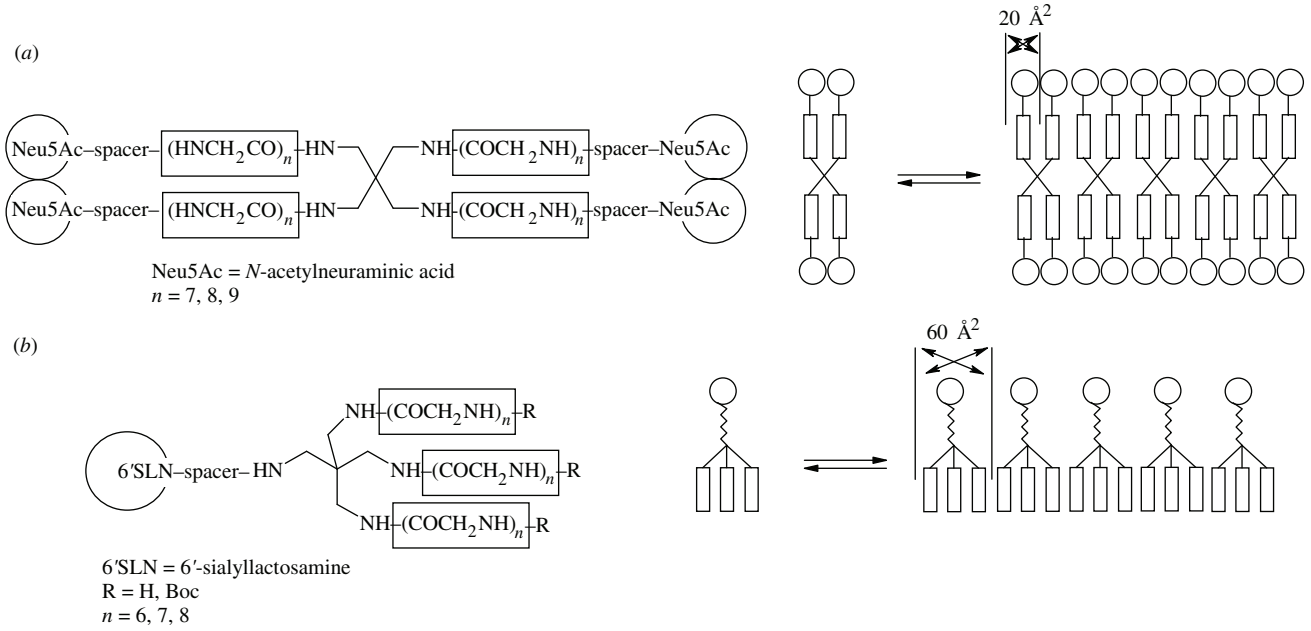
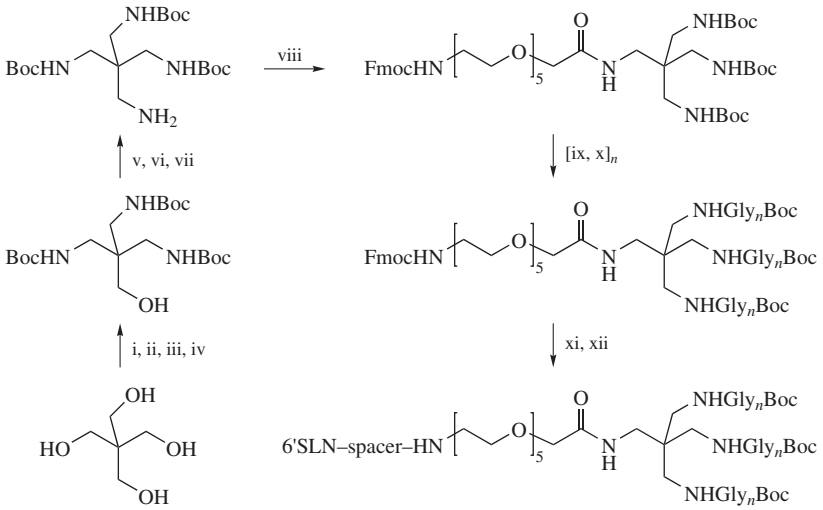


Figure 1 Branched glycopeptides and the most probable way of their assembling in aqueous solution.



Scheme 1 *Reagents and conditions*: i, HBr/AcOH, boiling, 18 h, then HBr/H_2SO_4, boiling, 8 h, 80%; ii, NaN_3, DMSO, 90 °C; iii, H_2, Pd/C, EtOH, 90%; iv, Boc_2O, EtOH, 75%; v, MsCl/Et_3N, CH_2Cl_2, 0 °C, 99%; vi, NaN_3, DMSO, 110 °C, 30%; vii, PPh_3, MeOH/H_2O, 85%; viii, $FmocNH(CH_2CH_2O)_5CH_2COOSu$, DMF, 50%; ix, CF_3COOH; x, $BocGly_2OSu$–Et_3N or BocGlyOSu–Et_3N, DMF, 67–98%; xi, piperidine, DMSO; xii, 6′SLN–spacer–COONp, DMSO, 60 °C, 45–70%.

were protected and the hydroxyl group was stepwise transformed into amino group, which was acylated by *N*-hydroxysuccinimide ester of Fmoc-protected aminoacid $FmocNH(CH_2CH_2O)_5CH_2$-COOSu. Oligoglycine antennae were elongated by stepwise N-acylation using *N*-hydroxysuccinimide esters of Boc-protected glycine or diglycine. After each step Boc groups were removed by treatment with trifluoroacetic acid and the obtained amine was elongated further. Thus, a set of branched peptides, $FmocNH(CH_2CH_2O)_5CH_2CONHCH_2C(CH_2NHGly_nBoc)_3$, where $n = 2$, 4, 6–8, was synthesized. After attachment of all glycine residues Fmoc group was removed from oligoethylene glycol spacer by treatment with piperidine (for peptides with $n = 6$–8), and glycopeptide was obtained by N-acylation using 6′SLN-$O(CH_2)_3NHCO(CH_2)_4COONp$, where 6′SLN is trisaccharide Neu5Acα2-6Galβ1-4GlcNAcβ-.

According to size-exclusion chromatography data, the glycopeptides assemble in aqueous solution, when the length of oligoglycine antennae reaches seven (Ultropac Column TSK G4000SW 7.5×300 mm, Sweden; elution with 2 M NaCl, 1 ml min^{-1}; UV detection at $\lambda = 195$ nm; retention times for monomers 8.1–8.6 min, for aggregates 3.7–3.9 min). The ratio of monomeric to aggregated forms depends on length of the peptide antennae and the nature of the end groups. Glycopeptides with terminal Boc groups assemble in water solution; those with terminal NH_2 groups do not assemble presumably because of intramolecular interaction between the amino groups and negatively charged sugar.

The Raman spectroscopy of the solid Boc-substituted peptide with $n = 7$ demonstrates that antennae adopt polyglycine II conformation (characteristic lines at 884, 1261, 1381, 1424 and 1651 cm^{-1}). The supramolecular organization of glycopeptides was investigated by atomic force microscopy. AFM studies show that the aggregates are thin, flat plates with height about

Table 1 Antiviral activity of triantennary glycopeptides related to 6′SLN.

Glycopeptide	Virus		
	NIB/23/89M (H1N1)	NIB/26/90M (H3N2)	B/NIB/48/90M (B)
R = Boc, $n = 6$	15	7.5	1
R = Boc, $n = 7$	30	7.5	4
R = H, $n = 7$	1	≤ 1	≤ 1
R = Boc, $n = 8$	10	7.5	4
6′SLN	1 (150)[a]	1 (150)[a]	1 (200)[a]

[a]Values of IC_{50}, mkM, for monomeric 6′SLN are given in parentheses.

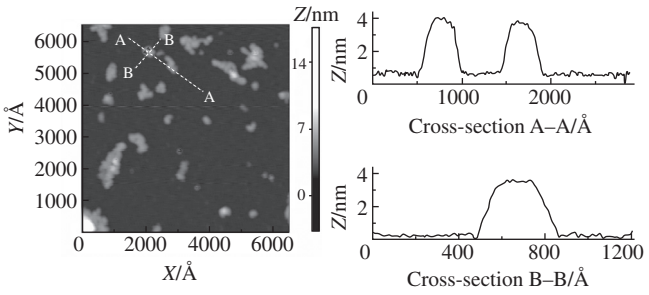


Figure 2 AFM image of the aggregates of triantennary glycopeptide ($n = 7$); solution of the glycopeptide (1 mg cm^{-3}) was placed on mica surface, kept for 1 min and then unadsorbed material was removed by a nitrogen stream.

3.5 nm and planar sizes of 20–500 nm (Figure 2), which can be considered as two-dimensional crystals similar to the tetraantennary tectomers.

The antiviral activity of glycopeptides was studied in fetuin binding inhibition test, where the synthesized glycopeptides inhibited the binding of fetuin peroxidase conjugate to virus immobilized on a plastic.[4] All the assembling glycopeptides show antiviral activity one order of magnitude higher than the activity of monomeric 6′SLN, while their non-assembling analogues are not active.

Thus, for triantennary glycopeptides we do not observe such dramatically increased virus-blocking activity (three orders of magnitude) as in case of tetraantennary ones.

This work was supported by the RAS Presidium programme ‘Molecular and Cell Biology’.

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Received: 2nd September 2008; Com. 08/3208