Synthesis of Cluster Sialoside Inhibitors for Influenza Virus

Subramaniam Sabesan,* Jens Ø. Duus,† Peter Domaille,†
Sørge Kelm,‡ and James C. Paulson‡

Contribution No. 5802, Central Research and Development Du Pont Company, Wilmington, Delaware 19880-0328 Department of Chemistry and Molecular Biology Scripps Research Institute and Cytel Corporation, 11099 North Torrey Pines Road La Jolla, California 92037

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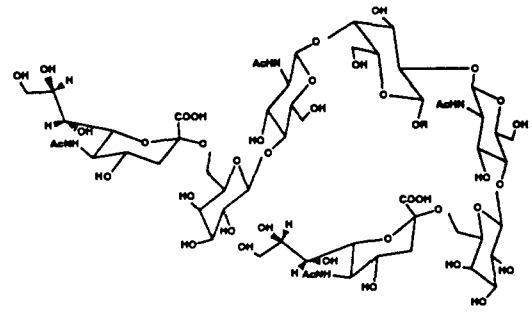
Influenza virus of human isolates binds via its hemagglutinins to the sialic acid residues of the trisaccharide αDNeuAc(2-6)-βDGal(1-4)βDGlcNAc fragments present on the cell surface glycoproteins[1,2] of the human erythrocytes. Due to the polyvalent and weak binding interaction between the carbohydrate and the hemagglutinin molecules,[3] the design of inhibitors for influenza virus has remained unsuccessful. Recently, polymeric sialosides have been shown to be effective inhibitors of viral hemagglutination.[4,5]

To probe the cluster carbohydrate effect[6] toward viral hemagglutinin binding, we have synthesized two heptasaccharides containing two αDNeuAc(2-6)βDGal(1-4)βDGlcNAc fragments (Figure 1, structures **2** and **3**). Even though the crystal structure of sialyllactose–hemagglutinin complex did not implicate a binding role for the aglycon attached to the sialic acid,[7] we decided to incorporate them on the basis of the hemagglutination inhibition assays,[3] which indicated a favorable binding role for the lactosamine attached to the sialosides. Our hope was that the bivalent trisaccharide ligands when anchored on sugar hydroxyls of a galactose residue would render the structures more rigid (consequently providing favorable entropic factors) as compared to the conventional linear polyol immobilized structures.[6] We also decided to vary the distance between two sialic acids (as seen in structures **2** and **3** of Figure 1) to establish the optimum distance requirements between the two receptor fragments for efficient binding.[8]

We used combined chemical (for the preparation of trisaccharides) and enzymatic (for the preparation of penta- and heptasaccharides) methods[8-10] to obtain these complex molecules (Scheme I). 5-(Methoxycarbonyl)pentyl β-D-galactopyranoside[9] (**4**) was used as the common starting material. For the chemical synthesis of the two trisaccharides **7** and **9**, two diols, namely, **5** and **8**, were needed. Allylation of the 3-hydroxyl of **4** (with di-*n*-butyltin oxide–tetraethylammonium bromide and allyl bromide)[11] followed by silylation of the 6-hydroxyl (with *tert*-butyldimethylsilyl chloride and imidazole) afforded **5**. Glycosylation of **5** with 2-deoxy-3,4,6-tri-*O*-acetyl-2-phthalimido-α,β-D-glucopyranosyl bromide (**6**) according to Lemieux, Takeda, and Chung's procedure[12] afforded a protected trisaccharide, from which the acetate and phthalimido protecting groups were removed according to the published procedure.[13] N-Acetylation with acetic anhydride in methanol gave trisaccharide **7**.

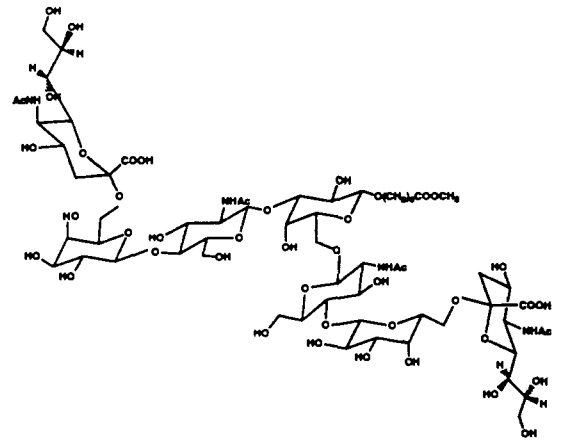
To obtain the other trisaccharide, diol **5** was converted to its dibenzoate followed by the simultaneous removal of the allyl and *tert*-butyldimethylsilyl groups to give **8**. Diglycosylation with bromide **6** and the removal of the protecting groups afforded trisaccharide **9**.

The trisaccharides **7** and **9** were converted enzymatically[14,15]



αDNeuAc(2-6)βDGal(1-4)βDGlcNAc(1-4)
\ βDGal-OR
αDNeuAc(2-6)βDGal(1-4)βDGlcNAc(1-2)/

2



αDNeuAc(2-6)βDGal(1-4)βDGlcNAc(1-6)
\ βDGal-OR
αDNeuAc(2-6)βDGal(1-4)βDGlcNAc(1-3)/

3

Figure 1. The two heptasaccharides containing bivalent receptor determinants (underlined) with random folding of sugar rings.

Table I. Inhibition of Influenza Virus Absorption to Erythrocytes by Cluster Sialosides

compd	concn for 50% inhibn,[a] mM	rel potency
MeαDNeuAc	1.90	1.0
monosialoside **1**	0.95	1.9
2,4-disialoside **2**	1.30	1.5
3,6-disialoside **3**	0.18	10.0

[a] Inhibition of influenza virus (A/Aichi/2/68) to resialylated erythrocytes was examined as described previously.[3b]

† Du Pont Company.
‡ Cytel Corporation.
§ Biochemisches Institut University of Kiel, Olshausenstrasse 40, 2300 Kiel, Germany.

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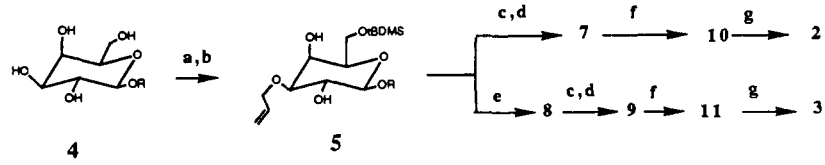
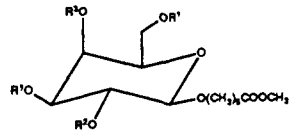
Scheme I[a]





7; R^1 = H, R^2 = R^3 = βDGlcNAc;

8; R^1 = H, R^2 = R^3 = PhCO

9; R^1 = βDGlcNAc, R^2 = R^3 = H

10; R^1 = H, R^2 = R^3 = βDGal(1-4) βDGlcNAc

11; R^1 = βDGal(1-4) βDGlcNAc, R^2 = R^3 = H

1; R^1 = H, R^2 = αDNeuAc(2-6)βDGal(1-4) βDGlcNAc, R^3 = βDGal(1-4) βDGlcNAc

2; R^1 = H, R^2 = R^3 = αDNeuAc(2-6)βDGal(1-4) βDGlcNAc

3; R^1 = αDNeuAc(2-6)βDGal(1-4) βDGlcNAc, R^2 = R^3 = H

[a] (a) n-Bu_2SnO–benzene, Et_4NBr, allyl bromide; (b) TBDMSCl–DMF; (c) 2-deoxy-3,4,6-tri-*O*-acetyl-2-phthalimido-α,β-D-glucopyranosyl bromide–AgOTf–collidine–CH_3NO_2; (d) NaOMe–MeOH; H_2NNH_2–MeOH; Ac_2O–pyridine; NaOMe–MeOH; (e) PhCOCl–pyridine; Ir(COD)-$[Ph_2Me]_2PF_6$–THF; 10% $HgCl_2$–90% aqueous acetone; (f) UDP-galactose–bovine galactosyltransferase; (g) CMP-NeuAc–2,6 sialyltransferase.

to the digalactosylated pentasaccharides **10** and **11** in one step using UDP-galactose and bovine galactosyltransferase (EC 2.4.1.22) followed by purification on a column of Bio Gel P2. Monogalactosylated intermediates were not seen in these reactions. The pentasaccharides were converted to the heptasaccharides **2** and **3** by using CMP-NeuAc and Galβ1,4GlcNAcβ 2,6 sialyltransferase[16] (EC 2.4.99.1). The disialosides were obtained as the major products along with minor monosialosides (**1**[17] was obtained as a byproduct in the enzyme reaction with **10**; even though **1** could be converted to **3**, we used it as a control in the binding assays). They were easily separated by adsorption on an ion-exchange column followed by successive elution with 5 and 50 mM phosphate buffer. The structural identities of **2** and **3** were confirmed by ^1H and ^{13}C NMR (^1H NMR spectra of the tri-, penta-, and heptasaccharides are provided in the supplementary material).

The inhibitory potencies of the synthetic disialosides were evaluated by titration of the synthetic compounds in a quantitative biological assay involving virus adsorption to resialylated erythrocytes as described by Pritchett et al.[3b] These kinetic inhibition assays are sensitive enough to reliably detect compounds that differ in inhibitory potencies by as little as 2-fold. As shown in Table I, the inclusion of neutral sugars as seen in the monovalent sialoside **1** makes it a 2-fold-better inhibitor than methyl α-D-sialoside. When the second sialic acid was added on the other lactosamine arm of **1** to give **2**, the inhibition efficiency increased only marginally to 1.5-fold, but weaker than the monosialoside **1**. However, when two sialic acids were placed in such a way as to keep them farthest apart as seen in compound **3**, then the inhibitory potency increased by as much as 10-fold.

This represents the first demonstration[18] of increased inhibitory potency by a synthetic, low molecular weight, bivalent sialoside and exemplifies the importance of cluster oligosaccharide effects for binding toward proteins.

Preparation of other heptasaccharides where the distance between the two sialic acids has been systematically varied, as well as the immobilization of the disialosides (via the linker arm attached to the anchoring galactose) on protein matrix such as bovine serum albumin, is in progress. We are also investigating the conformational properties of these molecules by 600-MHz NMR spectroscopy to understand the structure–inhibitory activity relationship.

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Supplementary Material Available: The 600-MHz ^1H NMR spectra of **2**, **3**, **7**, and **9–11** (7 pages). Ordering information is given on any current masthead page.

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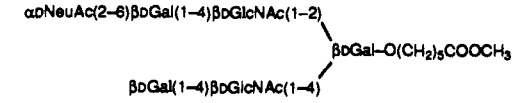

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Self-Assembled Multilayers of ω-Mercaptoalkanoic Acids: Selective Ionic Interactions

Stephen D. Evans,† Abraham Ulman,*
Kim E. Goppert-Berarducci, and Louis J. Gerenser

Corporate Research Laboratories and
Analytical Technology Division
Eastman Kodak Company
Rochester, New York 14650-2109

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Self-assembled (SA) monolayers are molecular assemblies that are *spontaneously* formed by the immersion of an appropriate substrate into a solution of an active surfactant.[1] This technique provides a potentially attractive route for the formation of systems ordered at the molecular level. That ionic interactions may serve as a useful vehicle for the production of multilayer films via self-assembly can be inferred from Lee et al.[2] and Smotkin et al.[3]

† Current address: Department of Physics, University of Leeds, Leeds, LS2 9JT United Kingdom.

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