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Synthesis and Anti-Influenza Evaluation of Polyvalent Sialidase Inhibitors Bearing 4-Guanidino-Neu5Ac2en Derivatives

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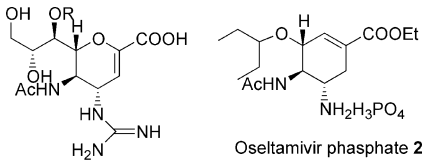
Abstract—We synthesized polyvalent sialidase inhibitors bearing 4-guanidino-Neu5Ac2en analogues on the polyglutamic acid back bone, via a spacer of alkyl ether at the C-7 position. These multivalent conjugates **9** and **10** showed enhancement of antiviral activity against infuenza A virus and more potent efficacy in vivo relative to a monomeric sialidase inhibitor.

Influenza A and B viruses have two major membrane-associated surface glycoproteins, hemagglutinin (HA) and sialidase (NA), which are both essential for infectivity. There are typically about 600 trimeric HA and about 50 copies of NA tetramer units on the surface of each virus particle. Therefore, both HA and NA are attractive potential targets in the search for anti-influenza drugs. Influenza virus HA binds to the sialic acid moiety of glycoproteins and glycolipids on cell-surface receptors, thereby initiating the process of attachment of the virus to a cell and subsequent infection.[1] The strength of the binding of a virus particle to the cell membrane is dependent on the interaction of multiple copies of the influenza HA with multiple sialic acid groups on the cell surface. Using this concept of polyvalent interaction, several groups have reported the synthesis of macromolecules containing two or more sialic acid derivatives as HA inhibitors.[2]

Although some strong HA inhibitors have been discovered, none of these polyvalent macromolecules has been shown to prevent influenza infection in vivo. Influenza virus sialidase promotes virus release from infected cells and facilitates virus spread within the respiratory tract.[3] Several potential specific inhibitors of this enzyme have been developed[4] and two (zanamivir **1**[5] and oseltamivir phosphate **2**[6] in Fig. 1) have been approved for human use. However, in contrast to the situation with HA synthetic studies, few macromolecular or polymeric sialidase inhibitors have been reported.[7] One of them demonstrated that the polymeric compound **5** in Figure 2 was more active than the simple monomeric ligand **4** in a plaque reduction assay, particularly when calculated on the basis of the molar concentration of the monomeric sialidase inhibitor, but there has been no evaluation in detail.

Therefore, as part of our study[8] of the structure activity relationship of 7-*O*-alkyl ether derivatives **3** related to zanamivir, we were interested in the 4-guanidino-7-alkoxy-7-deoxy-Neu5Ac2en analogues attached to polymers as multivalent sialidase inhibitors. Herein, we report the synthesis of a series of polymeric or multivalent sialidase inhibitors **9** and **10** carrying 4-guanidino-Neu5Ac2en analogues via a linker of alkyl ether at their C-7 position and their biological activity.



Oseltamivir phasphate **2**

1 R : H (Zanamivir)

3 R : Alkyl

Figure 1.

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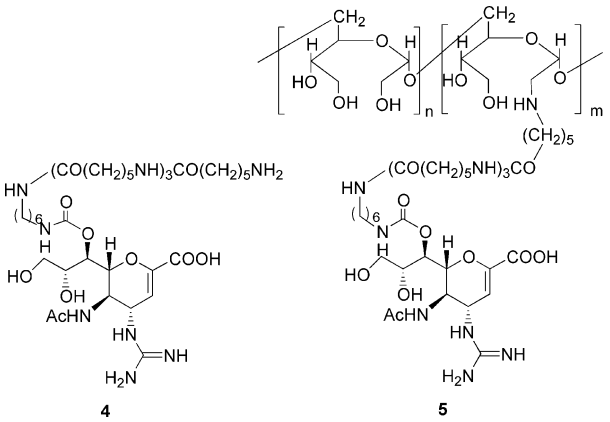


Figure 2. Recent polymeric sialidase inhibitors.

provide the activated polyglutamic acid **7**. Subsequent condensation of activated polyglutamic acid **7** with the terminal amine linked with 4-guanidino-Neu5Ac2en analogues **8a**, **8b**, or **8c** via a spacer of alkyl ether at the C-7 position were performed under the appropriate conditions by varying quantities of the amine from 0.05 to 1.00 equiv per activated carboxylic acid, then quenching with ammonia. Subsequent dialysis with water afforded polymers **9** having various ratios (p/q) of the number of side chains containing a sialidase inhibitor to the total number of side chains in the polyglutamic acid. These ratios were determined by ^{1}H NMR analysis. Polymers **10** were prepared by sequential reaction of activated polyglutamic acid with their terminal amine **8a** or **8b** and with the different amines such as *n*-pentyl amine or benzyl amine.

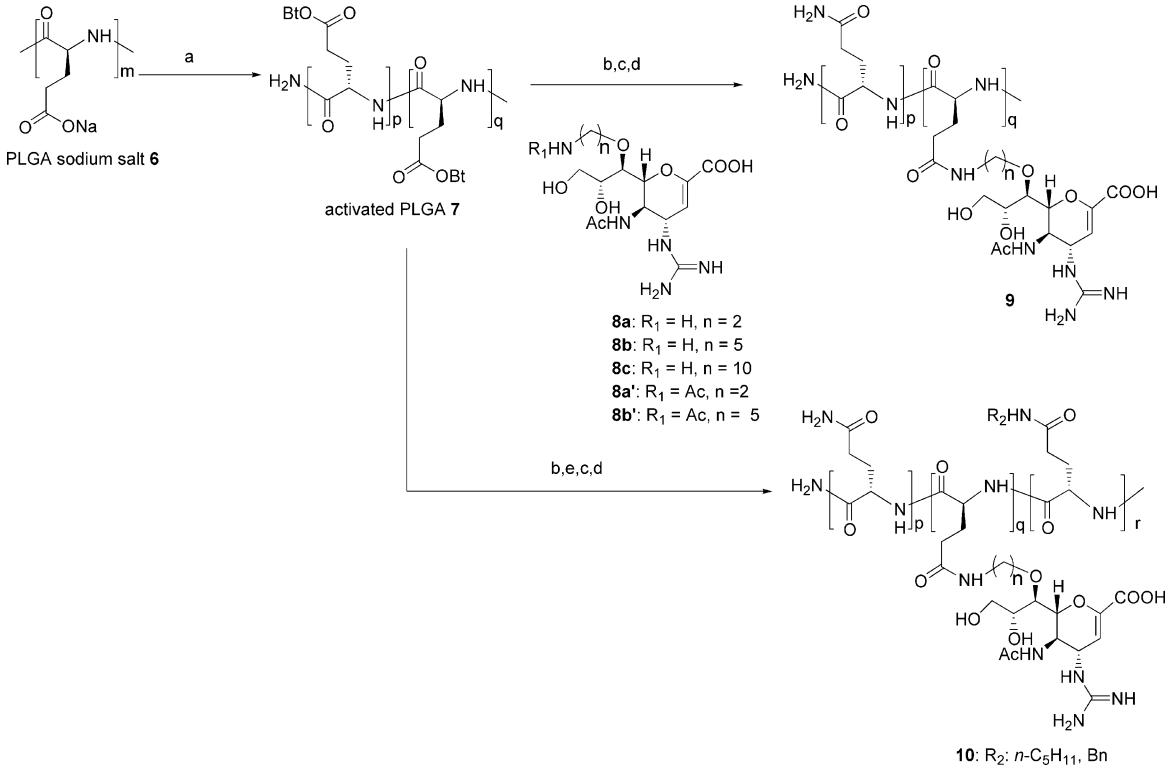
Chemistry

We chose poly-L-glutamic acid (PLGA) **6** as a polymer back bone because PLGA possesses some advantages as a drug carrier such as biodegradability, high water solubility, the presence of multiple carboxyl groups that are easily modified chemically, low toxicity, and low immunogenicity.[8]

Synthesis of macromolecules possessing the sialidase inhibitory activities was shown in Scheme 1. The synthesis of 4-guanidino-Neu5Ac2en analogues **8a**, **8b**, and **8c** attached to the polymer was described previously.[9] Polyglutamic acid which is commercially available (M_r 50,000–70,000) was treated with WSC and HOBt to

Biological Activities

The influenza A virus sialidase inhibitory[10] and plaque reduction activities[11] of the polymers are summarized in Table 1. The sialidase inhibitory activities of all polymers prepared against influenza A virus sialidase were less potent than that of zanamivir. However, the evaluation of the plaque reduction assay against influenza A virus demonstrated that all of them were much more active than monomeric sialidase inhibitors, **8a′**, **8b′**, and zanamivir, regardless of their ratios (p/q) and lengths of linker. In particular, it was found that the polymeric compounds **9a** and **9c** were approximately 100-fold more efficient than zanamivir, when calculated on the basis of the molar concentration of monomeric sialidase



Scheme 1. Syntheses of polyvalent sialidase inhibitors. Reagents and conditions: (a) WSC (1.1 equiv), HOBt (2.2 equiv); (b) **8** (0.05 ~ 1.00 equiv), pyridine; (c) aq NH_3; (d) dialysis with H_2O (20–30% over all yield from **6**; (e) RNH_2 (0.5 equiv) (10–20% over all yield from **6** to **10**).

Table 1. Sialidase inhibitory and plaque reduction activities of compounds **9** and **10** (IC_{50}, nM)[a]

	n	R	p/q/r[b]	Sialidase inhibitory assay	Plaque reduction assay
				A/PR/8/34	A/Yamagata/32/89
Zanamivir				11–29 (1.0)[c]	1.3–27 (1.0)[c]
8a′	2			22 (1.47)	5.5 (0.2)
8b′	5			90 (6.0)	5.9 (1.3)
9a	2		10:1	114 (5.2)	0.066 (0.0077)
9b	2		3:1	364 (22.8)	0.17 (0.020)
9c	5		10:1	161 (7.3)	0.072 (0.0085)
9d	10		10:1	62 (2.1)	0.076 (0.023)
10a	2	*n*-C_5H_{11}	7:1:4	585 (36.6)	0.10 (0.029)
10b	2	Bn	7:1:4	324 (20.0)	0.15 (0.042)
10c	5	*n*-C_5H_{11}	8:1:2	113 (10.3)	0.32 (0.25)
10d	5	Bn	8:1:2	63 (5.73)	0.028 (0.018)

[a]Relative to the monomeric sialidase inhibitor content.
[b]The ratio was determined by ^1H NMR.
[c]Since IC_{50} values varied depending on experiments, the relative potencies of the compounds to zanamivir are shown in the parentheses, based on the IC_{50} values of zanamivir as a reference. IC_{50} values of zanamivir in enzyme inhibition and plaque reduction were 11–29 and 1.3–27 nM, respectively.

Table 2. Survival rates of infected mice[a] administered compound **9c**[b] and zanamivir[b]

	No. of survivors/total no. of mice	
	At 10 days after infection	At 20 days after infection
Zanamivir	1/8	0/8
9c∗	7/7	7/7

[a]Mice were infected with influenza A/PR/8/34 (H1N1) virus.
[b]Compound **9c** and zanamivir were administered intranasally at doses of 0.3 μmol/kg once beginning 24 h prior to infection. The concentration of 0.3 μmol of **9c** was calculated based on the molar concentration of the monomeric sialidase inhibitor.
[c]$p = 0.0009$ versus zanamivir (Log-rank test).

inhibitor units **8a′** and **8b′**. This enhancement of antiviral activity compared to monomeric sialidase inhibitor could be due to entropically enhanced binding of the polymer (acting as a polyvalent inhibitor) with the viral sialidase. In addition, the unsubstituted back bone (PLGA) had no activity against influenza A virus in the sialidase inhibitory and plaque reduction assays. Furthermore, the efficacy of intranasally administered polymeric sialidase inhibitor **9c** was tested in the influenza virus infected mouse model on the basis of the survival rate for treated and infected mice relative to that for control mice. Compound **9c** was administered intranasally once beginning 24 h prior to infection. It was found that **9c** was much more effective than zanamivir as shown in Table 2 (7/7 survived in the **9c**-treated infected group while there were no survivors in the case of zanamivir). This in vivo efficacy is the first example for polymeric sialidase inhibitors.

In summary, we prepared macromolecules which were polyglutamic acid amides carrying sialidase inhibitors, 4-guanidino-Neu5Ac2en analogues, via a spacer of alkyl ether at the C-7 position. All polymers exhibited less potent enzymatic inhibitory activity against the influenza A virus, however, much more efficacy in a plaque reduction assay and on the mouse model by intranasal administration than zanamivir.

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