A Substrate Mimetic Approach for Influenza Neuraminidase Inhibitors

Joon Won Jeong, Jae Kuk Kim, Byoung Young Woo, Bok Ju Song, Deok-Chan Ha,† and Zaesung No*

Medicinal Science Division, Korea Research Institute of Chemical Technology, Daejeon 305-343, Korea
†Department of Chemistry, Korea University, Seoul 136-701, Korea
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Over recent years increased understanding of the influenza virus replication cycle has allowed investigators to identify several potential molecular targets for drug design. The crystal structures of two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), expressed by both influenza A and B viruses have been determined and well characterized. These results have encouraged basic research for the development of specific and potent inhibitors of HA and NA.[1,2]

The infection cycle of influenza virus starts in the surface epithelial cells of the respiratory tract. HA mediate the binding of virus to the host cell *via* terminal sialic acid residue in glycoconjugates and the process of endocytosis. NA, a glycohydrolase, removes the sialic acid from glycoconjugates and facilitates the release of the virus particles from the infected cell surfaces during the budding processes and this prevents aggregation of virions by removing sialic acid residues from viral glycoproteins.[3]

Inhibition of viral replication step should be an effective method to control and potentially to eradicate viruses from infected tissues. Therefore, inhibition of the viral neuraminidase should prevent the influenza virus replication and NA has been considered to be a suitable target for designing drugs against influenza viruses.

Since the discovery that *N*-acetylneuraminic acid **1** (NANA) had inhibitory activity against NA, a novel analog, 2,3-didehydro-2-deoxy-N-acetylneuraminic acid **2** (DANA), was synthesized with 1,000 times more activity than NANA.[4] DANA is considered as a transition state-like analogue binding to the active site of NA.[2b,5] On the basis of structural information generated from the X-ray crystallographic study, rationally designed guanidino analogue zanamivir **3** was also active and launched with the name of Relenza®. However, because of its poor oral bioavailibility and rapid excretion, zanamivir is administered only by nasal inhalation.[5,6] Recently, a new class of compounds having a chemically versatile carbocyclic ring in place of the dihydropyran ring of DANA was developed as another transition-state mimic by Gilead Sciences. In this series oseltamivir **4** (Tamiflu®) was highly active with good oral bioavailibility, which has been in clinical use since 1999.[7]

Contrary to this progress, we designed compound **5** for the comparative study of a substrate-mimetic approach to the transition-state mimics. For this study a carbocyclic ring was

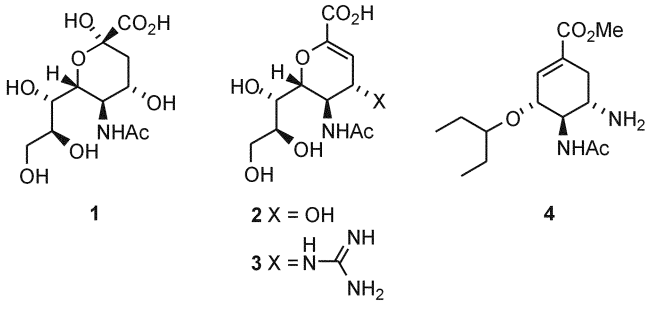
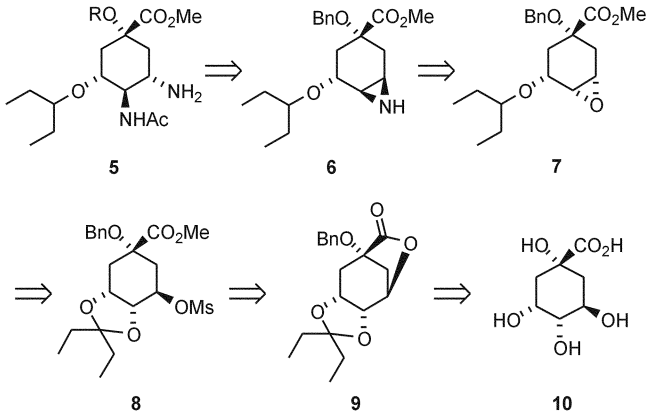
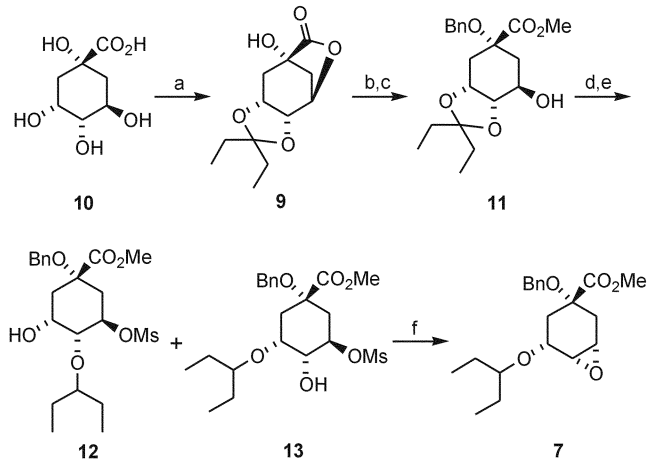


Figure 1



Scheme 1. Retrosynthetic analysis.

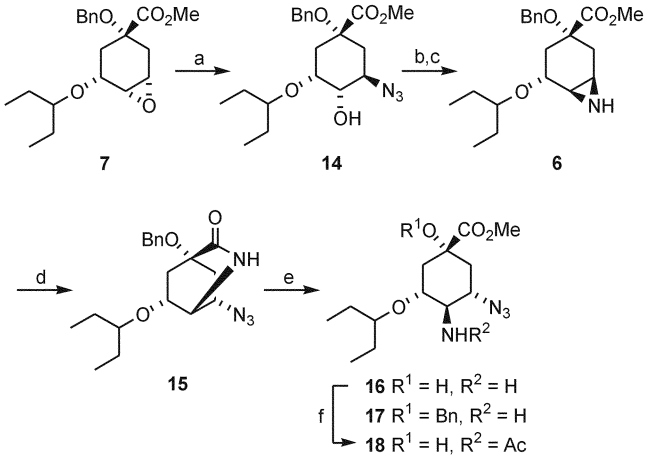


Scheme 2. Reagents and conditions: (a) 3-pentanone, DMF, PhH, 110 °C, then Dowex 50WX2 (H^+) resin, 110 °C, 83%; (b) BnBr, NaH, DMF, -20 °C to 0 °C, 85%; (c) NaOMe, MeOH, 0 °C to r.t., 96%; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C, 95%; (e) $BH_3 \cdot SMe_2$, TMSOTf, CH_2Cl_2, -40 °C to 0 °C, 97%; (f) DBU, THF, r.t., 80%.

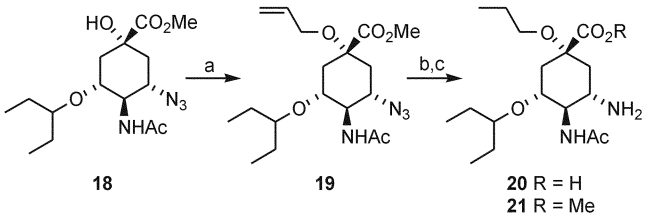
introduced to increase the chemical stability of **5**, and the same groups as in oseltamivir **4** were adapted for the other side chain functionality of this cyclohexane ring except the anomeric carbon. Based on the hydrolysis mechanism of the neuraminidase,[8] we designed the anomeric sp^3 carbon connected to the alkoxy derivatives by ether bond.

As proposed in our retrosynthetic analysis, aziridine **6** was chosen as a key synthetic intermediate and the regioselective ring-opening of **6** using azide ion was planned to construct all the requisite functional groups and chiral centers (Scheme 1).[7b] Its precursor, epoxide **7** could be derived from the regioselective cleavage of ketal **8**. We envisioned that lactone **9** was generated from the commercially availible (–)-quinic acid *via* ketalization and lactonization. For the synthesis of epoxide **7**, (–)-quinic acid **10** having common structural features of target **5** was ketalized using 3-pentanone in the presence of Dowex 50WX2 (H^+) ion resin with concomitant lactonization to give **9** (Scheme 2).[9] Alcohol **9** was converted to benzyl ether and the resulting lactone was reacted with sodium methoxide to render methyl ester **11**. After mesylation of **11**, the ketal was reduced with borane dimethylsulfide in the presence of TMSOTf to afford a 20 : 1 inseparable mixture of **13** and **12**. Then, the mixture was treated with DBU to give epoxide **7** and the remained **12**.

The epoxide ring-opening reaction of **7** was carried out using sodium azide in the presence of ammonium chloride to furnish azide **14** exclusively by the steric influence of the 3-pentyl group (Scheme 3). For the introduction of amino group to C-4 position, aziridine ring-opening was attempted. The hydroxyl group of **14** was mesylated and the resulting azide was subjected to reduction with triphenylphosphine in the presence of triethylamine and water to furnish aziridine **6** *via* nucleophilic attack of amino group. To prepare azido amine **16**, the aziridine ring-opening of **6** was carried out with the same method used in the epoxide ring-opening of **7**. However, successive nucleophilic attack of the generated



Scheme 3. Reagents and conditions: (a) NaN_3, NH_4Cl, aq MeOH, 80 °C, 98%; (b) MsCl, DMAP, Et_3N, CH_2Cl_2, r.t., 82%; (c) PPh_3, THF, r.t., then Et_3N, H_2O, r.t., 75%; (d) NaN_3, NH_4Cl, aq MeOH, 80 °C, 74%; (e) HCl, MeOH, r.t., 74% for **16** and 5% for **17**; (f) Ac_2O, Et_3N, CH_2Cl_2, r.t., 94%.



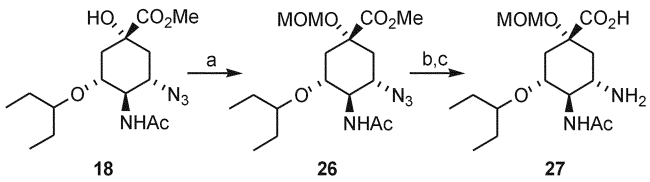
Scheme 4. Reagents and conditions: (a) allyl bromide, Bu_4NI, KHMDS, DMF, 30 °C, 89%; (b) $LiOH·H_2O$, aq MeOH, r.t., 97%; (c) H_2, 10% Pd/C, MeOH, r.t., 88%.

amine to the ester group occurred in aqueous condition to furnish lactam **15**. Methanolic HCl for ring-opening of the lactam **15** furnished the desired intermediate **16** with a small amount of benzyl compound **17**. Finally, azido amine **16** was acetylated with acetic anhydride in the presence of triethylamine to furnish the key intermediate **18** for syntheses of target molecules.

Tertiary alcohol **18** was converted to allyl ether **19** using allyl bromide and KHMDS in the presence of *n*-Bu_4NI (Scheme 4). Next, saponification of methyl ester of **19** with lithium hydroxide followed by hydrogenation using palladium charcoal for reduction of azido group and terminal olefin afforded the target molecule **20**. Reduction of **19** using the same hydrogenation condition was performed to give ester **21**. Also, benzyl ether **24** and alcohol **22** were synthesized from **17** and **18** *via* similar procedures. The enzymatic inhibitory activities of these carboxylic acids and esters were determined as shown in Table 1.

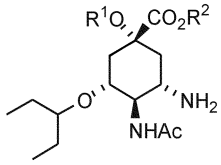
Other types of ether having different small alkyl group were also synthesized. Alcohol **18** was converted to methoxymethyl ether **26** using dimethoxymethane and phosphorous oxide, and the following saponification and reduction furnished **27** (Scheme 5). With the same procedure, ethoxymethyl ether **28** and methoxyethyl ether **29** were also prepared.

As shown in Table 1, carboxylic esters **21**, **23**, and **25** had much less inhibitory activities relative to α-hydroxy carboxylic acid **22**. The ether group on anomeric carbon also affected the inhibitory activity significantly and simple ether **20** or alkoxyalkyl ethers **27**, **28**, and **29** were more effective inhibitors than the aromatic benzyl ether derivatives **24** and **25**. The best results in terms of the inhibitory activity were attained from simple *n*-propyl ether **20** and methoxymethyl ether **27**. Those results may indicate that the enzymatic surroundings corresponding to the bond-breaking and bond-forming anomeric center is very sensitive to the lipophilicity



Scheme 5. Reagents and conditions: (a) $CH_2(OMe)_2$, P_2O_5, CH_3Cl, r.t., 89%; (b) $LiOH·H_2O$, aq MeOH, r.t., 98%; (c) H_2, 10% Pd/C, MeOH, r.t., 72%.

Table 1. Influenza neuraminidase inhibitions[10]



Compd	R^1	R^2	IC_{50} (μM)
22	H	H	261
23	H	Me	10762
24	Bn	H	5740
25	Bn	Me	6228
20	*n*-Pr	H	75
21	*n*-Pr	Me	1100
27	CH_2OMe	H	56
28	CH_2OEt	H	219
29	CH_2CH_2OMe	H	219

of the attached pharmacophores.

In summary, a series of analogs as a substrate mimetic approach for the neuraminidase inhibitor were prepared and their inhibitory activities were determined. Though they were less active than we expected, the selective recognition of the ligands by the enzyme was promising. We are now in progress for further optimization of the side chains of **5**.

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