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Synthesis of 6-Acetamido-5-amino- and -5-guanidino-3,4-dehydro-*N*-(2-ethylbutyryl)-3-piperidinecarboxylic Acids Related to Zanamivir and Oseltamivir, Inhibitors of Influenza Virus Neuraminidases

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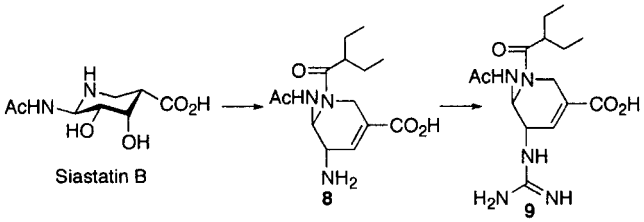
ABSTRACT



6-Acetamido-5-amino- and -5-guanidino-3,4-dehydro-*N*-(2-ethylbutyryl)-3-piperidinecarboxylic acids (8 and 9) have been synthesized starting from natural siastatin B, a bacterial neuraminidase inhibitor isolated from *Streptomyces* culture in a stereospecific fashion. These compounds are related to zanamivir and oseltamivir, inhibitors of influenza virus neuraminidases.

Two integral membrane glycoproteins, haemagglutinin (HA) and neuraminidase (NA), on the envelope of the surface of the influenza virus interact with receptors which contain terminal sialic acid residues of glycoproteins on the surface of the host cell.[1] Infection by the influenza virus begins with the attachment of HA to cellular receptors and subsequent fusion of viral and host cellular membranes.[2] NA is a glycosidase which cleaves the α-ketosidic bond linking a terminal neuraminic acid to the adjacent oligosaccharide residues of glycoproteins and glycolipids.[3] This cleavage is thought to facilitate transport of the virus through the mucus within the respiratory tract and to be involved in the elution of progeny virions from the infected cells and the prevention of self-aggregation of progeny virions.[4] Thus, NA plays a crucial role in the life cycle of the virus and it has been

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postulated that inhibitors of this enzyme should have antiviral properties.[5] Zanamivir (**1**) and oseltamivir (**2**) (Figure 1) are

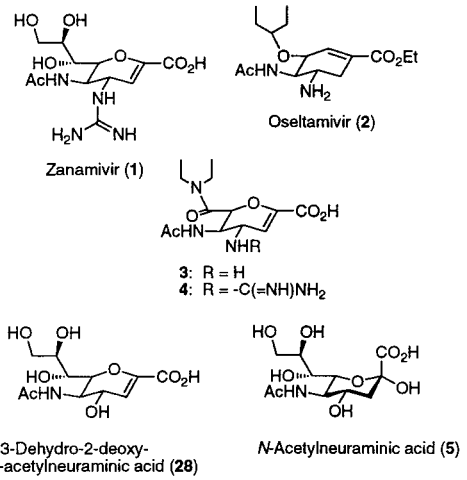

Figure 1. Representative inhibitors of influenze virus neuraminidases and *N*-acetylneuraminic acid.

potent inhibitors of neuraminidases from both influenza A and B viruses and are currently used for treatment of influenza viral infection.[6] This postulation was conceived through a rational drug design based on the crystal structure of influenza virus neuraminidase complexed with sialic acid.[6,7] These results revealed the presence of a large hydrophobic pocket as well as a hydrophilic pocket in the region corresponding to the glycerol substituent of *N*-acetylneuraminic acid (**5**). Several analogues based on **1** and **2** have been synthesized in the search for effective anti-influenza agents, and it appears that 4-amino- and 4-guanidino-4*H*-pyran-6-carboxamides (**3** and **4**) (Figure 1) also exhibit strong inhibition particularly against influenza virus A neuraminidases and show both in vitro and in vivo antiviral efficacy.[8]

In the course of our studies on the relationships between the structure and biological activity of siastatin B (**6**)[9] (Figure 2) isolated from a *Streptomyces* culture as an inhibitor of

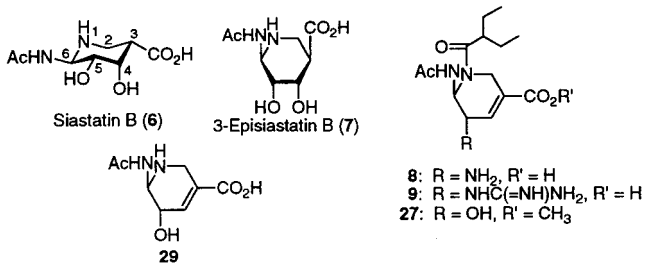

Figure 2. Siastatin B and its analogues.

bacterial neuraminidase, we demonstrated, in 1993, that 3-episiastatin B (**7**) (Figure 2) shows potent inhibitory activities against influenza virus neuraminidases and the influenza virus in vitro infectivities[10] while several analogues having the same equatorial carboxyl group as **6** act as inhibitors only for bacterial neuraminidases.[11] From the ^{1}H NMR spectrum, it was assumed that **7** exists in a boat conformation in an aqueous solution and also in a nonsolution by molecular modeling using PM3/MOPAC.[10,12] The lowest energy boat conformer of **7** was also superimposed onto the α-boat conformer of **5** (Figure 2) in a pocket of the active site residue of the crystal structure of influenza virus B/Beijing/1/87 neuraminidase complexed with **5** by a docking experiment using BIOCES/AMBER.[1e,10,12] Under these circumstances, we are interested in the synthesis of similarly functionalized analogues of **6** to **1** and **2** for development of a new class of influenza virus neuraminidase inhibitors. Here, we describe the synthesis of 6-acetamido-5-amino- and -5-guanidino-3,4-dehydro-*N*-(2-ethylbutyryl)-3-piperidinecarboxylic acids (**8** and **9**) together with 3,4-dehydro-4-deoxy-*N*-(2-ethylbutyryl)siastatin B (**27**) (Figure 2). First, we

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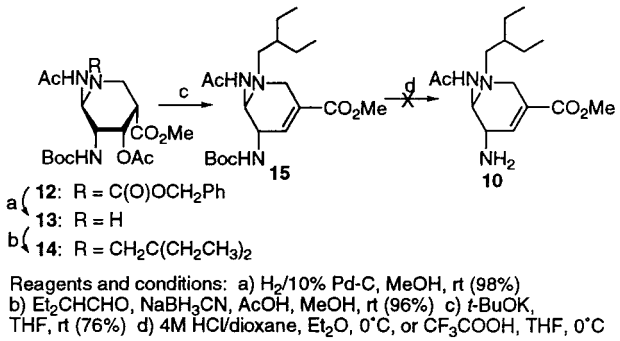
(13) The synthesis of **12** will be reported elsewhere.

(14) **8**: $[\alpha]^{23}_D$ +83.7° (*c* 0.37, H_2O); ^{1}H NMR (D_2O) δ 6.91 and 6.90 (total 1H, d each, $J_{4,5}$ = 5.9 Hz, H-4), 6.7 and 6.3 (total 1H, s each, H-6), 4.627 and 4.625 (total 1H, d each, $J_{2,2'}$ = 18.6 and 20.5, H-2), 4.2 and 4.1 (total 1H, d each, H-5), 4.1 and 3.9 (total 1H, d each, H-2′). **9**: $[\alpha]^{23}_D$ +60.1° (*c* 0.34, H_2O); ^{1}H NMR (D_2O) δ 6.6 and 6.7 (total 1H, d each, $J_{4,5}$ = 5.9 Hz, H-4), 6.5 and 6.1 (total 1H, s and d, $J_{6,2}$ = 1.5 Hz, H-6), 4.8 (1H, dd, $J_{2,2'}$ = 19.5 Hz, H-2), 4.4 and 4.3 (total 1H, d each, H-5), 3.8 (1H, d, H-2′). **20**: $[\alpha]^{23}_D$ +136.4° (*c* 0.27, H_2O); ^{1}H NMR (D_2O) δ 6.6 (1H d, $J_{4,5}$ = 5.9 Hz, H-4), 6.3 and 5.9 (total 1H, s and d, $J_{6,2}$ = 2.0 Hz, H-6), 4.6 and 4.5 (total 1H, d and dd, $J_{2,2'}$ = 18.6 Hz, H-2), 4.2 (1H, d, H-5), 3.9 and 3.6 (total 1H, d each, H-2′). **27**: $[\alpha]^{23}_D$ +146.8° (*c* 0.29, H_2O); ^{1}H NMR (CD_3OD) δ 6.99 and 6.98 (total 1H, dd each, $J_{4,2'}$ = 1.5 and $J_{4,5}$ = 5.9 Hz, H-4), 6.6 and 6.1 (total 1H, d each, $J_{6,2}$ = 1.0 and 1.5 Hz, H-6), 4.81 and 4.84 (total 1H, dd each, $J_{2,2'}$ = 20 Hz, H-2), 4.2 and 4.1 (total 1H, d each, H-5), 3.7 (1H, dt, H-2′). **12**: $[\alpha]^{23}_D$ +3.7° (*c* 0.45, MeOH). **13**: $[\alpha]^{23}_D$ +57° (*c* 0.45, MeOH). **14**: $[\alpha]^{23}_D$ −12.3° (*c* 0.40, MeOH). **15**: $[\alpha]^{23}_D$ +149.4° (*c* 0.55, MeOH). **19**: $[\alpha]^{23}_D$ +182° (*c* 0.71, MeOH). **21**: $[\alpha]^{23}_D$ +94.2° (*c* 0.3, MeOH), mp 149−150 °C. **23**: $[\alpha]^{23}_D$ +44.1° (*c* 0.49, MeOH). **24**: $[\alpha]^{23}_D$ +186.2° (*c* 0.44, MeOH). **25**: $[\alpha]^{23}_D$ +83.1° (*c* 1.25, $CHCl_3$). **26**: $[\alpha]^{23}_D$ +73.4° (*c* 0.47, MeOH).

attempted the synthesis of analogues (**10** and **11**) containing an *N*-alkyl side chain related to **1** and **2** (Scheme 1 and

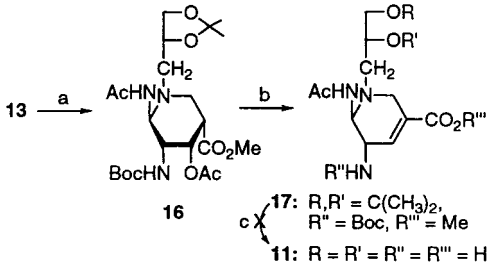
Scheme 1



Reagents and conditions: a) H_2/10% Pd-C, MeOH, rt (98%) b) Et_2CHCHO, $NaBH_3CN$, AcOH, MeOH, rt (96%) c) *t*-BuOK, THF, rt (76%) d) 4M HCl/dioxane, Et_2O, 0°C, or CF_3COOH, THF, 0°C

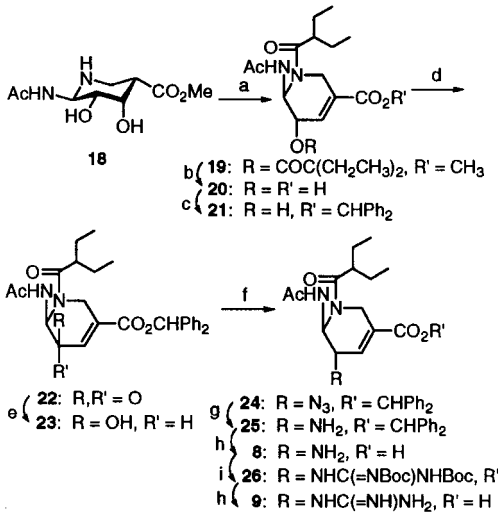
Scheme 2). Hydrogenolysis of the methyl ester **12**[13,14] prepared from **6** gave imine **13**, which was transformed into **14** by *N*-alkylation with 2-ethylbutyraldehyde and $NaBH_3$-CN in excellent yield. Elimination of the acetate group of **14** proceeded smoothly upon treatment with *t*-BuOK in THF to afford the α,*β*-unsaturated ester **15** in 76% yield. However, removal of the protecting *tert*-butoxycarbonyl group with an acid such as HCl in ether or trifluoroacetic acid in THF did not result in the desired amine **10** but gave the unresolved aromatized products. To confirm whether this kind of *N*-alkylation generally results in aromatization or not in the removal of protecting groups, we examined the preparation of **11** starting from amine **13**. Unfortunately, removal of the protecting groups of ester **17** was unsuccessful and gave the unresolved aromatized products. Therefore, we turned our attention to the synthesis of the imides (**8** and **9**) analogous to 4*H*-pyran-6-carboxamides (**3** and **4**), the alternative potent inhibitors particularly of influenza virus A neuraminidases (Figure 1 and Figure 2). This strategy was improved by shortening the number of steps in the above synthesis (Scheme 3). The desired α,*β*-unsaturated ester **19** was prepared in 84% yield by reaction of 2-ethylbutyryl chloride in pyridine and subsequent treatment with DBU in benzene starting from siastatin B methyl ester (**18**).[11b] Basic hydrolysis

Scheme 2



Reagents and conditions: D-1,2-*O*-isopropylidene-*sn*-glyceraldehyde, $NaBH_3CN$, AcOH, MeOH, rt (89%) b) *t*-BuOK, THF, rt (97%) c) 0.1M NaOH/MeOH, rt, then AcOH (pH 6)

Scheme 3



Reagents and conditions: a) (i) $Et_2CHCOCl$, Py, rt (ii) DBU, PhH (84%) b) 2M NaOH/H_2O-MeOH (1:1), rt (100%) c) Ph_2CN_2, MeOH, rt (87%) d) Dess-Martin periodinane, CH_2Cl_2, rt (85%) e) $NaBH_4$, MeOH, -10°C→10°C (94%) f) HN_3, Ph_3P, $EtO_2CN{=}NCO_2Et$, PhH, rt (70%) g) Ph_3P, CH_3CN, rt, then H_2O (72%) h) CF_3CO_2H, CH_2Cl_2 (98%) i) $(BocNH)_2C{=}S$, $HgCl_2$, Et_3N, DMF, rt (58%)

of **19** gave the α,*β*-unsaturated acid **20**, which was protected with an easily removable diphenylmethyl group to afford **21** in a good yield. After several unsuccessful attempts of direct epimerization of the hydroxy group, attention was directed to the general two-step epimerization by oxidation and reduction. The Dess−Martin oxidation[15] of **21** yielded enone **22** in 85% yield. Reduction of **22** with sodium borohydride was stereoselectively carried out to furnish allyl alcohol **23** in excellent yield. Introduction of an equatorial amino group was best achieved by Mitsunobu reaction[16] with HN_3 in benzene followed by treatment with Ph_3P in CH_3CN to give **25** in a good yield. Removal of a protecting group with CF_3CO_2H in CH_2Cl_2 resulted in the desired 4-amino product **8** in excellent yield. Compound **8** was further functionalized with a guanidino group upon treatment with *N*,*N*′-bis(*tert*-butyloxycarbonyl)thiourea in the presence of $HgCl_2$[17] to afford **26** in 58% yield. Compound **26** was straightforwardly converted into the 4-guanidino product **9** with acid. Compound **20** was also transformed into the α,*β*-unsaturated methyl ester **27** by treatment with TMSCl in MeOH in 75% yield.

A preliminary biological evaluation was carried out on compounds **8**, **9**, **20**, **27**, and the general standard inhibitor, 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid **28**. Unfortunately, compounds **8**, **9**, **20**, and **27** were found to be inactive when inhibitory activities of neuraminidases from influenza virus A/PR/8/34 (H1N1), A/Japan/307/56 (H2N2), A/Aichi/2/68 (H3N2), and B/Yamagata/16/88 and also from *Clostridium perfringens* (0% at 2.4×10^2 μM, compound **8**; 1.5×10^2 μM, compound **9**; 3.4×10^2 μM, compound **12**; and

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3.2×10^2 μM, compound **27**) were evaluated, while compound **28** was moderately active against these neuraminidases (IC_{50} 110, 27, 24, 2.4, and 24 μM, respectively).[18,19] The inactivities of **8**, **9**, **20**, and **27** can be explained from their ^{1}H NMR spectra. The small coupling constant ($J_{5,6}$ = 0 Hz) in comparison to the large coupling constant ($J_{5,6}$ = 6.4 Hz) of **29**[11] clearly indicates that these compounds preferentially adopt the half-chair conformation A (Figure 3) in solution as the conformational flip is generally seen in the imide- or carbamate-fashioned functionalization of imino group of **5**.[12,19,20] This is distinct from the normally preferred half-chair conformation B (Figure 3) which must be adopted upon binding of influenza virus neuraminidases as seen in **1**, **2**, **3**, **4**, and **28**.[6,7,8,21] Substitution around siastatin B (**6**) is in progress to probe the requirements of structural binding to neuraminidases.

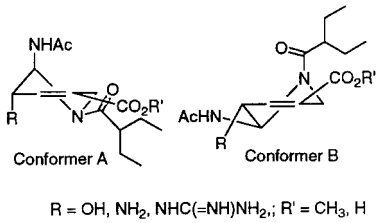

Figure 3. Two half-chair conformers of **8**, **9**, and **27**.

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