



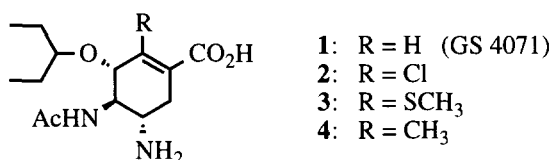
SYNTHESIS AND ACTIVITY OF C₂-SUBSTITUTED ANALOGS OF INFLUENZA NEURAMINIDASE INHIBITOR GS 4071

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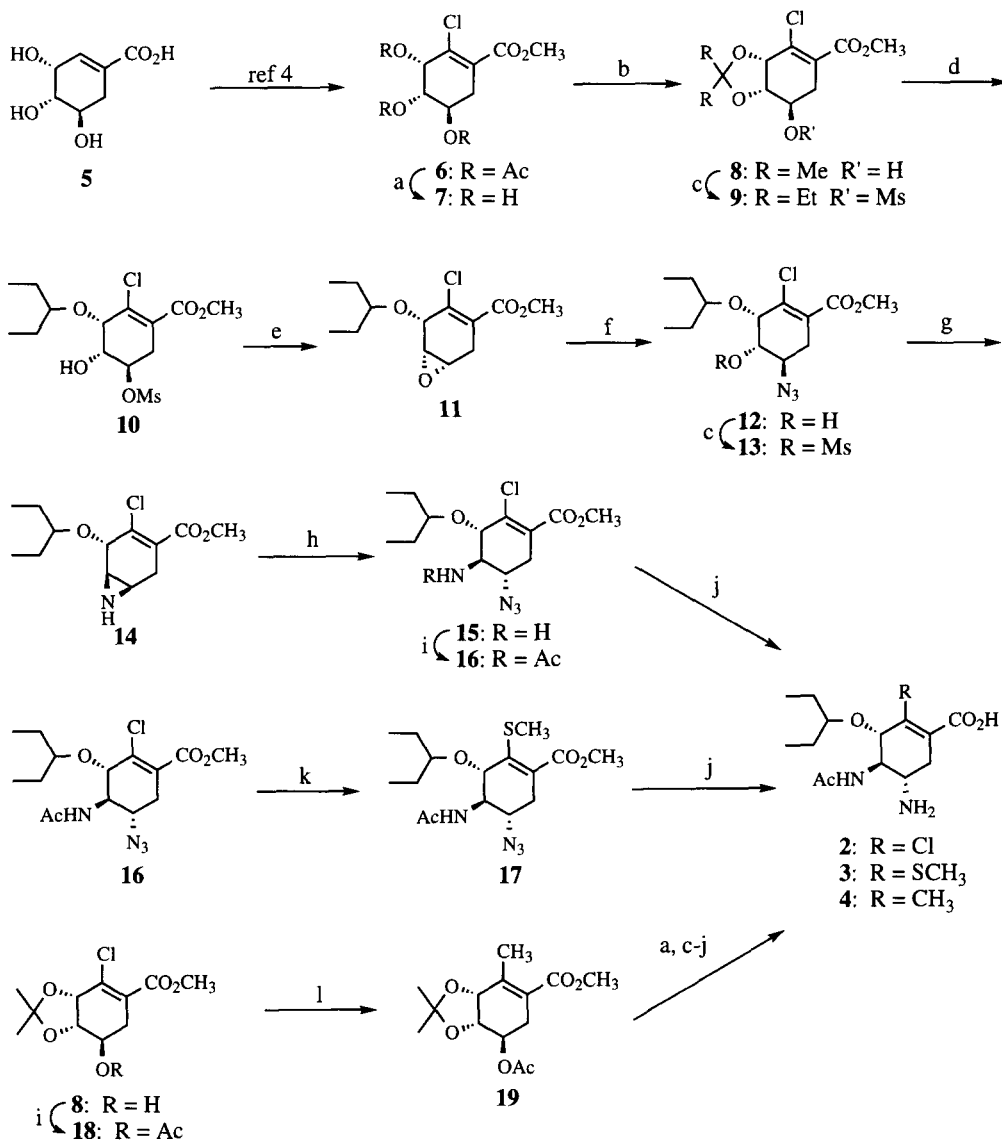
Abstract: The influence of C₂-substitution of GS 4071 on the influenza neuraminidase inhibitory activity was investigated. The introduction of lipophilic substituents (chloro, methyl, and methylthio) at the C₂ position resulted in a significant decrease of the activity. This result indicates that at the enzyme active site there is limited hydrophobic pocket for a group at the C₂ position of GS 4071. © 1997 Elsevier Science Ltd.

Influenza infection is the most serious respiratory illness both in term of morbidity and mortality.¹ In the search for a new anti-influenza agent, a potent influenza neuraminidase (NA) inhibitor GS 4071 (**1**) has recently been identified in our laboratory.² In addition, on the basis of in vitro and in vivo results, the ethyl ester of GS 4071 has been selected as a clinical trial candidate for the oral treatment and prophylaxis of influenza infection.³



As part of the study of the structure activity relationship of this series, C₂-substituted analogs of GS 4071 were investigated. Despite the presence of the highly lipophilic 3-pentyloxy group at the C₃ position, GS 4071 has a negative partition coefficient value (log P = -1.1), indicating that it is still a rather polar molecule. Therefore, we investigated the possibility of making the molecule less polar and then improving its pharmacological properties by introducing lipophilic functional groups at the C₂ position. To this end, C₂-chloro (**2**), C₂-methylthio (**3**), and C₂-methyl (**4**) analogs were prepared.

The synthesis of the C₂-chloro analog **2** was accomplished starting from the C₂-chloro shikimic acid derivative **6**, which was prepared from shikimic acid **5** by literature method⁴ (Scheme 1). Tetraester **6** was treated with a catalytic amount of sodium methoxide in methanol to give triol **7** in quantitative yield. Although the 3-pentyl ketal could be prepared directly from triol **7** in one step, a two-step procedure via acetone **8** gave a better yield. Thus, **7** was first treated with 2,2-dimethoxypropane in acetone in the presence of toluenesulfonic acid to give acetone **8**. Mesylation and transketalization of **8** in the presence of a catalytic amount of perchloric

Scheme 1^a

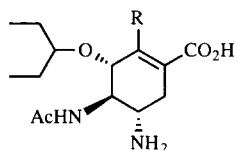
^aReagents: (a) CH₃ONa (5 mol %), CH₃OH, rt; (b) 2,2-dimethoxypropane, acetone, p-TsOH (2 mol %), reflux; (c) MsCl, Et₃N, CH₂Cl₂, rt; 3-pentanone, HClO₄ (2 mol %), 40 °C; (d) BH₃·Me₂S, TMSOTf, CH₂Cl₂, -20 °C; (e) KHCO₃, CH₃OH/H₂O, 50 °C; (f) NaN₃, NH₄Cl, CH₃OH/H₂O, reflux; (g) Ph₃P, THF, rt; Et₃N/H₂O, rt; (h) NaN₃, NH₄Cl, DMF, 70 °C; (i) Ac₂O, pyridine, rt; (j) Ph₃P, THF/H₂O, 50 °C; KOH; Dowex (H⁺); (k) CH₃SNa, CH₃CN, rt; (l) Me₂CuLi, Et₂O, -78 °C.

acid gave 3-pentyl ketal **9** in 94% yield. Reductive acetal cleavage of **9** resulted in a mixture of **10** and its regio-isomer in 70% yield (3:2 ratio), which could not be separated by chromatography on silica gel. When the mixture of **10** and its regio-isomer was treated with potassium hydrogen carbonate in methanol-water, the regio-isomer remained unreacted, and **10** reacted to give epoxide **11**, which was isolated by silica gel chromatography. Nucleophilic ring opening of epoxide **11** with sodium azide resulted in azido alcohol **12**. Mesylation of **12** gave **13**, which was then treated with triphenylphosphine in anhydrous tetrahydrofuran followed by triethylamine and water to give aziridine **14** in 75% yield. The ring opening of aziridine **14** with sodium azide gave **15** in 54% yield. Acetylation of **15** followed by reduction of the azido group and saponification of the methyl ester gave the C₂-chloro analog **2**.

The preparation of C₂-methylthio analog **3** was accomplished starting with intermediate **16**. Michael addition of the methylthio group to **16** followed by elimination of the chloro group resulted in **17**. Reduction of **17** followed by the saponification gave C₂-methylthio analog **3**.

The synthesis of C₂-methyl analog was also accomplished starting from intermediate **8**.⁵ Acetylation of **8** gave acetone **18**, which was then treated with methyl cuprate⁶ to give a mixture (1:1 ratio) of **19** and the reduced product in 70% yield. After removal of the acetyl group, **19** was converted to final product **4** through the same procedures used for the preparation of compound **2**.

Table 1. Influenza Neuraminidase Inhibition⁷



Compound	R	IC ₅₀ (nM)
1	H	1.4
2	Cl	3100
3	SCH ₃	3400
4	CH ₃	2300

The enzymatic neuraminidase inhibitory activity of the three compounds is shown in Table. The results show that the introduction of a lipophilic chloro, methyl or methylthio group at the C₂ position of GS4 071 resulted in an approximately 2000-fold decrease in the inhibitory activity. This result indicates that the enzyme active site contains a limited hydrophobic pocket capable of accommodating modifications at the C₂ position of GS 4071.

Neuraminidase enzyme assay: Neuraminidase enzyme activity was determined using minor modifications to the literature method.⁷ Influenza A/PR/8/34 (H1N1), purified on sucrose density gradients, was used as the source of enzyme and 2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid was used as substrate in a reaction buffer containing 33 mM MES, pH 6.5, and 4 mM calcium chloride. Virus was mixed with various inhibitor concentrations and incubated at room temperature for 30 min. before substrate was added to a final concentration of 10 μ M. Reactions were stopped after 8 min. at 37 °C with the addition of 1.5 volumes of 14 mM sodium hydroxide in 83% ethanol. Fluorescence was quantitated in a Perkin-Elmer fluorimeter (Model LS50B) with an excitation wavelength of 360 nm, emission wavelength of 448 nm, and slit widths of 2.5 nm.

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References and Notes

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5. An attempt at preparation of the C₂-methyl analogue **4** from intermediate **16** was unsuccessful. The treatment of **16** with methyl cuprate or higher order methyl cuprate only gave a reduced product (removal of chloro), while **17** resulted in the recovery of starting material.
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