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STEREOSPECIFIC SYNTHESIS OF A GS 4104 METABOLITE: DETERMINATION OF ABSOLUTE STEREOCHEMISTRY AND INFLUENZA NEURAMINIDASE INHIBITORY ACTIVITY

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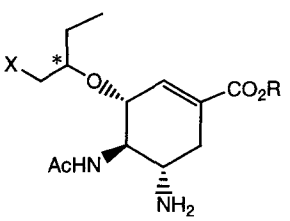
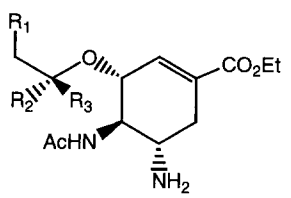
Abstract: The total synthesis for the determination of the absolute stereochemistry of a GS 4104 metabolite **3** is described. In addition, the influenza neuraminidase inhibitory activity of **3** and related intermediates are reported.

Compound **1** (GS 4071), which belongs to a class of new carbocyclic influenza neuraminidase inhibitors, has demonstrated potent in vitro and in vivo inhibitory activity against influenza A and B.[1,2] A new drug application for the corresponding ethyl ester prodrug **2** (GS 4104, oseltamivir) has been submitted to the U.S. Food and Drug Administration for approval as the only neuramindase inhibitor in pill form for the treatment of influenza infection. As part of preclinical studies of GS 4104, a metabolite was isolated from rat which was identified as **3** by ^{1}H NMR and MS analysis.[3] In order to confirm the absolute stereochemistry of the C_3 side chain of **3** total synthesis of both diastereomers **4** and **5** was required. In addition, the two mono hydroxy compounds **6** and **7**, which have been implicated as possible intermediates in the formation of **4** and **5** were required as reference standards. To this end, we describe the total stereospecific synthesis of these compounds as well as the influenza neuraminidase inhibitory activity of the parent compounds (Table 1).



1 R = H; X = Me GS 4071
2 R = Et; X = Me GS 4104 (oseltamivir)
3 R = Et; X = CO_2H



4 $R_1 = CO_2H$; R_2 = Et; R_3 = H
5 $R_1 = CO_2H$; R_2 = H; R_3 =Et
6 $R_1 = CH_2OH$; R_2 = Et; R_3 = H
7 $R_1 = CH_2OH$; R_2 = H; R_3 = Et

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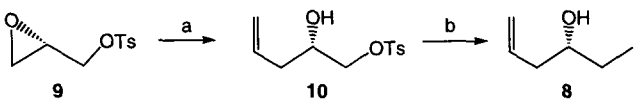
The synthesis requires the chiral alcohol **8** which is obtained from the readily available (*S*)-glycidyl tosylate **9** according to Scheme 1. Epoxide **9** is opened regiospecifically with vinyl magnesium bromide using catalytic dilithium tetrachlorocuprate at -40 °C to furnish **10** in 61% yield.[4] The hydroxy tosylate **10** was then treated with excess methyl magnesium bromide and 10 mol% copper iodide at low temperature to provide the desired alcohol **8** in 63% yield. The corresponding enantiomer of **8**, namely the (*S*) alcohol is obtained using the identical synthetic sequence but beginning from the (*R*)-glycidyl tosylate.

Scheme 1



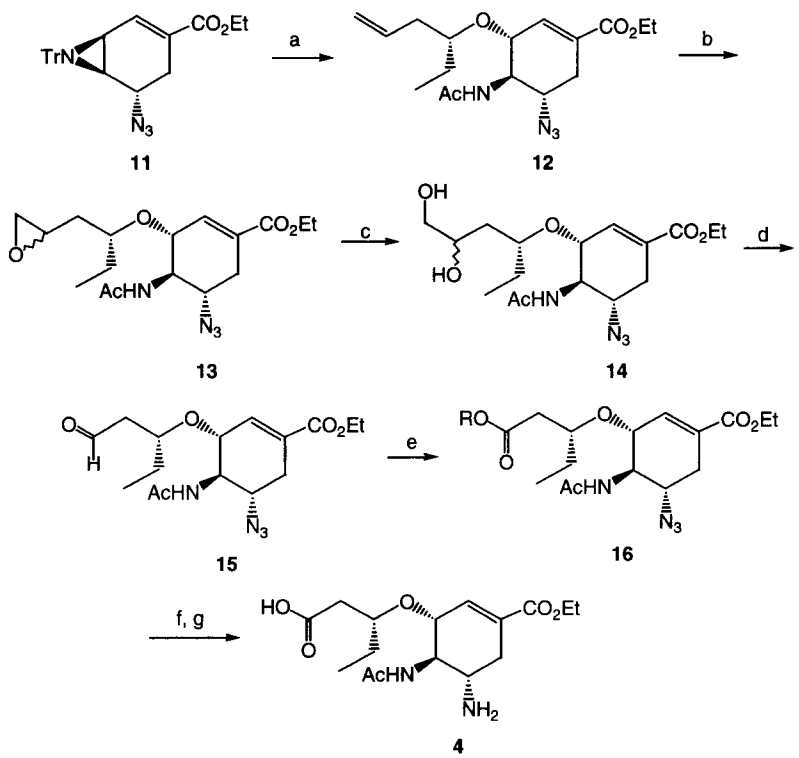
9 **10** **8**

Reagents: (a) vinylmagnesium bromide, Li_2CuCl_4, THF 61%;
(b) CH_3MgBr (2.2 equiv.),10% CuI, THF 63%.

The synthesis of metabolite **4** is shown in Scheme 2 and the synthesis of the corresponding diastereomer **5** is accomplished in the identical manner but utilizes the enantiomer of **8**. The known aziridine **11**[1] is opened in a regio- and stereospecific manner with excess **8** under BF_3•Et_2O catalysis at 75 °C. The crude reaction mixture is then treated directly with excess acetic anhydride in pyridine to provide the acetylated ester **12** in a 30% overall yield. The terminal vinyl group of **12** is then epoxidized with MCPBA in CH_2Cl_2 at room temperature to give **13** as a mixture of diastereomers in 78% yield. The epoxide **13** is then refluxed in 3:1 THF/water with catalytic perchloric acid to provide the diol **14** in 91% yield. The crude diol **14** is then cleaved to aldehyde **15** with silica gel supported sodium periodate in 97% yield.[5] Direct Jones oxidation of **15** to the carboxylic acid followed by treatment with diphenyl diazomethane in acetone furnishes diester **16** in 57% overall yield. The azido group of **16** was reduced with triphenylphosphine in THF/water to provide the amino diester in 93% yield, which was then treated with trifluoroacetic acid in the presence of anisole in CH_2Cl_2 to provide amino acid **4** in 84% yield after reverse phase chromatography and lyophilization.

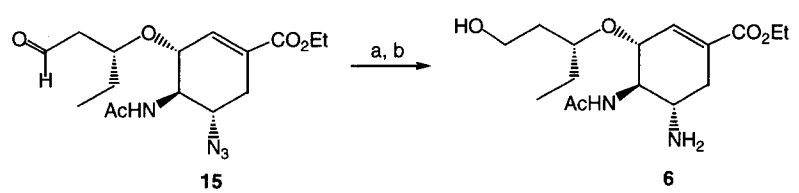
The hydroxyl compound **6** is synthesized according to Scheme 3. Reduction of aldehyde intermediate **15** with sodium cyanoborohydride in ethanol at pH 1–2 provided the primary alcohol in 61% yield which was treated with triphenylphosphine in THF/water to reduce the azide group to provide the amino ester **6** in 93% yield. The corresponding (*S*) diastereomer **7** is prepared in the identical manner. Comparison of the ^1H NMR spectrum of the two diastereomers **4** and **5** with that of the metabolite isolated from rat confirms the structure of the metabolite is indeed **4** and that the absolute stereochemistry of the C_3 side chain is the (*R*) configuration. In addition, co-elution studies by HPLC demonstrated that the two diastereomers **4** and **5** have markedly different retention times.[3]

Scheme 2



Reagents: (a) i. (4*R*)-1-hexen-4-ol (**8**), $BF_3{\bullet}Et_2O$, ii. Ac_2O, pyridine 30%; (b) MCPBA 78%; (c) $HClO_4$, THF, H_2O 91%; (d) $NaIO_4$, silica gel 97%; (e) i. CrO_3, H_2SO_4, ii. Ph_2CN_2, acetone 57%; (f) PPh_3, THF, H_2O 93%; (g) CF_3CO_2H, anisole 86%.

Scheme 3

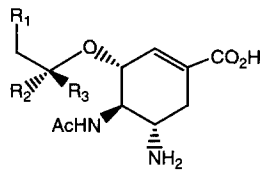


Reagents: (a) $NaCNBH_3$, EtOH, HCl 61%; (b) PPh_3, THF, H_2O 93%.

Saponification of ethyl esters **4**, **5**, **6** and **7** with aqueous KOH in THF followed by acidification and reverse-phase column chromatography furnishes the amino acids **17**, **18**, **19** and **20**, respectively, in good yields.[6] These compounds were then evaluated for their in vitro influenza A and B neuraminidase inhibitory activity by an enzymatic assay.[7] Their activities are summarized in Table 1.

Table 1. Influenza Neuraminidase Inhibition

Compound	enzyme IC_{50} (nM) Flu A[a]	FluB[b]
1	1	4
17	450	ND[c]
18	1150	ND[c]
19	30	85
20	40	400



17 $R_1 = CO_2H$; $R_2 = CH_2CH_3$; $R_3 = H$
18 $R_1 = CO_2H$; $R_2 = H$; $R_3 = CH_2CH_3$
19 $R_1 = CH_2OH$; $R_2 = CH_2CH_3$; $R_3 = H$
20 $R_1 = CH_2OH$; $R_2 = H$; $R_3 = CH_2CH_3$

[a]A/PR/8/34 (H1N1); [b]B/Lee/40; [c]not determined

Although not as potent as **1**, compounds bearing a terminal hydroxyl group at the 3-pentyl side chain, namely **19** and **20**, exhibit reasonable influenza neuraminidase inhibitory activity while compounds bearing a carboxylic acid group, compounds **17** and **18**, exhibit rather poor inhibition against influenza A. In summary, the absolute stereochemistry of the C_3 side chain of a metabolite isolated from rat in preclinical studies of GS 4104 was confirmed to possess the (*R*) configuration as shown in compound **4**.

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References and Notes

1. (a) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681 (b) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* **1998**, *41*, 2451.

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5. Daumas, M.; V-Quang, Y.; Vo-Quang, L.; Gaffic, F. L. *Synthesis* **1989**, 64.

6. All compounds gave satisfactory spectral and analytical data.

7. Details of enzymatic assay are found in reference 1.