Siastatin B, a Potent Neuraminidase Inhibitor: The Total Synthesis and Absolute Configuration

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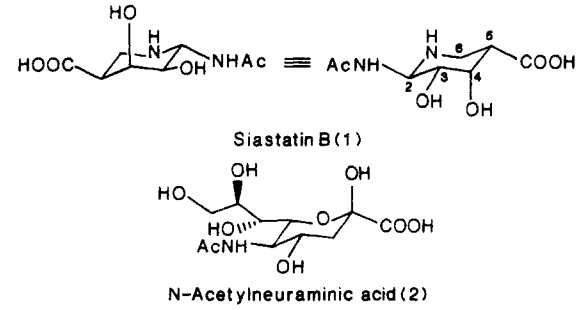
Neuraminidase (sialidase, *N*-acetylneuraminate glycohydrolase EC 3.2.1.18),[1] widely distributed among animal tissues and microorganisms, is involved in various biological functions such as immune response,[2] oncogenesis,[3] metastasis of tumors,[4] sperm penetration,[5] and viral infection,[6] etc.

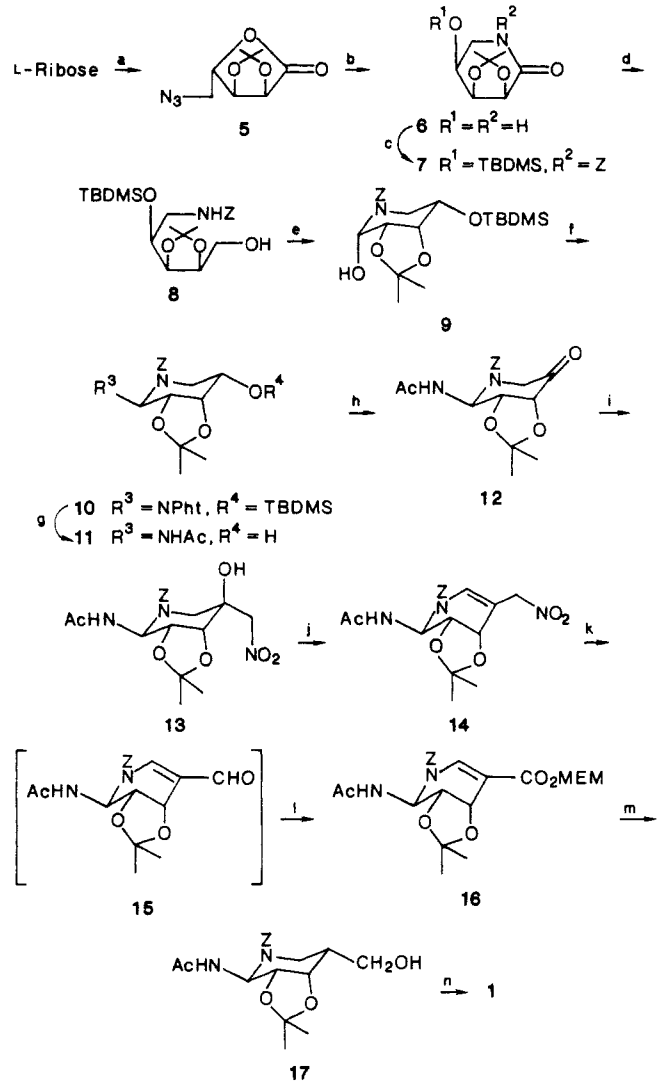
Siastatin B, a potent inhibitor of neuraminidase, was isolated by Umezawa et al.[7] in 1974 from a *Streptomyces* culture. Siastatin B inhibits neuraminidases isolated from various microorganisms, animal tissues, and viruses as well as β-glucuronidase and *N*-acetyl-β-D-glucosaminidase. The relative configuration of siastatin B was determined as 2(*S*/*R*)-acetamido-3(*S*/*R*),4-(*R*/*S*)-dihydroxypiperidine-5(*R*/*S*)-carboxylic acid by ^{1}H NMR and X-ray crystallographic studies.[7] However, its absolute configuration was unresolved. We speculated from its biological activity that the absolute configuration of siastatin B should be that shown in **1** by analogy with *N*-acetylneuraminic acid (**2**).

Here we wish to report the first total syntheses of siastatin B (**1**) and its enantiomer (**3**) based on a chiron strategy. Compound **1** has an unusual structure posessing the continuous –CH(NHAc)—NH—CH_2—CH(COOH)– constituent in a framework. It is distinct from glycohydrolase inhibitors belonging to the sugar analogues having a piperidine ring such as nojirimycin,[8] galactostatin,[9] and their congeners.[10] Our key intermediate for the synthesis of **1** was lactam **6**. The synthesis of **6** began with L-ribose which was transformed to 5-azido-5-deoxy-2,3-*O*-isopropylidene-L-ribonolactone (**5**), $[\alpha]^{22}_D$ –16.2° ($CHCl_3$), by protection of the 2,3-diol, azide formation, and oxidation[11] (Scheme I). Hydrogenation of the amide group of **5** and ring expansion[12] afforded crystalline **6**, $[\alpha]^{22}_D$ –16.4° ($CHCl_3$), mp 138–140 °C, in good yield. Stereospecific introduction of the hydroxyl group at C(2) was best achieved by hydride reduction of the protected lactam **7** to **8**, $[\alpha]^{22}_D$ +21° ($CHCl_3$), mp 164 °C, and Swern oxidation[13] to give aminal **9**, $[\alpha]^{22}_D$ +11° ($CHCl_3$), mp 106–107 °C. The ^{1}H NMR spectrum of **9** shows a proton of C(2) at δ 5.55 (a singlet with a small coupling, $J < 2$ Hz), clearly indicative of an equatorial hydrogen. Strikingly a single stereoisomer

Chart I



Scheme I[a]



[a] (a) *p*-TsOH, Me_2CO; CH_3SO_2Cl, C_6H_5N; NaN_3, DMSO; CrO_3/C_6H_5N, CH_2Cl_2, 89%; (b) H_2, Raney Ni, MeOH, 88%; (c) *t*-$BuMe_2SiCl$, imidazole, DMF; $PhCH_2OCOCl$, NaH, DMF, 99%; (d) $NaBH_4$, EtOH, 70%; (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 88%; (f) phthalimide, Ph_3P, DEAD, DMF, 100%; (g) NH_2NH_2, MeOH; Ac_2O, C_6H_5N; *n*-Bu_4NF, THF, 100%; (h) RuO_4, CH_2Cl_2/CCl_4, 99%; (i) CH_3NO_2, NaH, DME, 100%; (j) *p*-TsOH, Ac_2O; K_2CO_3, C_6H_6, 100%; (k) C_6H_5N, 38 °C, 80%; (l) $CH_3CH{=}C(CH_3)_2$/*t*-BuOH, $NaOCl_2$–NaH_2PO_4/H_2O; MEMCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 55%; (m) $NaBH_4$, 1:10 CF_3CH_2OH/THF, 75%; (n) PDC, DMF; H_2, 5% Pd/C, MeOH; 1 M aqueous HCl, then Dowex 50W-X4 (H^+ form) eluted with NH_4OH, 66%.

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0002-7863/88/1510-7249$01.50/0

controlled by an anomeric effect[14] results from this oxidation, whereas oxidation with CrO_3 in pyridine gives a 2:1 mixture of **9** and its epimer at C(2). Displacement of the axial hydroxyl group to the equatorial amino group proved troublesome until we discovered that the Mitsunobu reaction[15] (PPh_3, diethyl azodicarboxylate, phthalimide) in *N,N*-dimethylformamide gave the desired product **10**, $[\alpha]^{22}_D$ +26.4° ($CHCl_3$), mp 109 °C (dec), quantitatively. The stereochemistry at C(2) was established by its ^{1}H NMR spectrum which shows a proton of C(2) at δ 5.91 (d, J = 5 Hz), clearly indicative of an axial hydrogen. Replacement of the amino substituent and removal of the *tert*-butyldimethylsilyl group to **11**, $[\alpha]^{22}_D$ −20.4° ($CHCl_3$), and oxidation to **12**, $[\alpha]^{22}_D$ −56° ($CHCl_3$), were unexceptional.

Condensation of **12** with nitromethane was found to proceed smoothly to give **13** as a single stereoisomer, $[\alpha]^{22}_D$ +26° ($CHCl_3$), quantitatively. The stereochemistry at C(5) is tentatively assigned as **13**. Acetylation of **13** followed by base-catalyzed elimination of the acetoxy group afforded **14**, $[\alpha]^{22}_D$ +69° ($CHCl_3$), in a good yield. The structure of **14** was determined by its ^{1}H NMR spectrum which shows the methylene protons of the nitromethyl group at δ 4.78 and 5.16 (ABq, J = 15 Hz), a proton of C(4) at δ 4.71 (d, J = 6 Hz), and a proton of C(6) at δ 7.06 (S). Transformation of **14** to carboxylate **16**, $[\alpha]^{22}_D$ −4.1° (MeOH), mp 194–198 °C (dec), was achieved via α,β-unsaturated aldehyde **15** produced by simply warming **14** in pyridine. Compound **16** was also successfully produced from **13** by the stepwise successive sequences without isolation.

A problem arose, however, as catalytic reduction[16] of **16** accompanied by elimination of the hydroxyl group at C(4) and hydride reduction[17] of the double bond proceeded unfavorably and without chemoselectivity. To circumvent this problem, **16** was stereoselectively hydrogenated to α,β-saturated hydroxymethyl compound **17** ($NaBH_4$, 1:10 CF_3CH_2OH/THF), $[\alpha]^{22}_D$ +7.0° ($CHCl_3$). The ^{1}H NMR spectrum of **17** shows protons at δ 3.25 (t, J = 13 Hz, H-6 ax), 3.59 (dd, J = 5 and 13 Hz, H-6 eq), and 4.54 (dd, J = 3 and 8 Hz, H-4), clearly indicative of an axial hydrogen at C(5).

The remaining steps of the synthesis are rather straightforward. The carboxylic acid formed upon oxidation of **17** was converted by removal of protecting groups to crystalline **1**, $[\alpha]^{22}_D$ +56° (H_2O) (lit.[7] 57.2° (H_2O)), mp 135–136 °C (dec) (lit.[7] 137 °C), in 10.3% overall yield from L-ribose. Its spectral properties (IR, ^{1}H NMR, ^{13}C NMR, mass spectrum) were superimposable with those of the natural specimen.

The enantiomer **3** was also synthesized from D-ribonolactam by the same method used in the synthesis of **1**. Compound **3** was identical in all respects with the synthetic and the natural **1** except for the sign of the specific rotation.

Thus, the absolute configuration of siastatin B has been elucidated as the (2*R*,3*R*,4*S*,5*S*)-isomer **1**.

The synthetic **1** shows the same inhibitory effects as the natural one against neuraminidases prepared from *Cl. perfringens*, *Streptomyces*, rat mammary gland, rat mammary liver, chorioallantoic membrane (IC_{50} = 3, 10, 110, 170 and 55 μg/0.5 mL, respectively), β-glucuronidase (IC_{50} = 4 μg/0.5 mL), and *N*-acetyl-β-D-glucosaminidase (IC_{50} = 18 μg/0.5 mL).[7] In contrast, compound **3** shows only weak activities against neuraminidases mentioned above (IC_{50}: more than 100 μg/0.5 mL), whereas **3** demonstrates activity against β-glucuronidase (IC_{50} = 25 μg/0.5 mL).

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Observation and Substituent Control of Medium-Dependent Hot-Molecule Reactions in Low-Temperature Matrices

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Although reaction from vibrationally excited products is common in the gas phase, hot-molecule chemistry in condensed phases is rare.[2] Vibrational energy transfer to the medium is generally faster than chemically activated reaction. There are, however, a handful of cases where hot-molecule reactions of ground-state products of thermal[3-5] or photochemical[6-8] processes in various media have been proposed to explain unexpected products. Reaction from upper vibrational levels in electronic excited states has also been suggested in several instances[9-11] to rationalize wavelength dependence in condensed phase photochemistry.

There is spectroscopic evidence that vibrational relaxation in inert-gas matrices may be slow relative to solution. For example, Bondybey[12] and Rentzepis[13] have observed relatively long-lived hot bands (ca. 300 ps) in the fluorescence spectra of various aromatics in rare-gas matrices at 4 K. The higher energy vibrational emissions are absent in hydrocarbon matrices[14] or when the substrates are methylated.[12b] The surprising lifetimes have been attributed to mismatch of the vibrations of the aromatics and the low-energy lattice modes of the matrices, leading to poor energy transfer.[12,13] IR induced conformational interconversions in matrices are also well known.[5] The possibility of slow vibrational relaxation of photoproducts raises concerns for the observation of highly reactive intermediates with matrix-isolation techniques. We now wish to report *chemical evidence*, based on media and substituent effects, for the formation of vibrationally hot *photoproducts* in low-temperature matrices and their subsequent ground-state reactions.

We have reported that irradiation of **1** in toluene-d_8 at 77 K gives primarily 7-norbornadienone (**2**), characterized by ^{1}H NMR at −78° C.[15,16] A minor product, tentatively assigned as **3**, was also observed. On warming to ca. −60 °C, **2** cleanly gave benzene (and CO), with $\Delta G^{\ddagger}$ = 15 kcal/mol. Side product **3** polymerized

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