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Synthesis of the Sialidase Inhibitor Siastatin B

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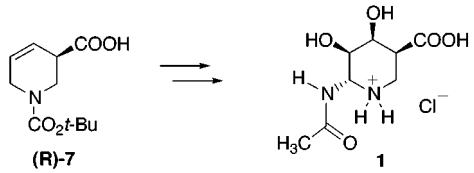
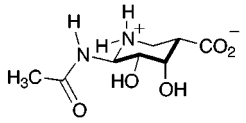
ABSTRACT



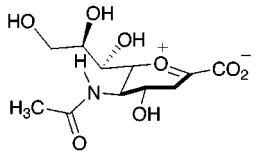
The resolved piperidinecarboxylate (*R*)-7 was converted to siastatin B (1) by an efficient and stereoselective sequence that includes a bromo-*β*-lactonization and an *N*-acyliminium azidation. Two analogues (3 and 4) of siastatin were also prepared.

Sialidases are enzymes that cleave *N*-acetylneuraminic acid from the nonreducing ends of glycoconjugates and thereby mediate a variety of cell surface recognition events.[1] Sialidase *inhibitors* have proven to be important tools for understanding and controlling sialidases:[2] the most prominent health-related application is the development of the various sialidase inhibitors (Zanamivir, Oseltamivir, RWJ-270201) that are currently in use and in clinical trials for the treatment of influenza virus infection.[3] As with any infection, however, the emergence of drug-resistant strains remains a concern.[4]

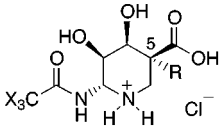
Siastatin B (**1**) is a broad spectrum sialidase inhibitor isolated from a *Streptomyces* culture and characterized as an unusual 6-acetamido-3-piperidinecarboxylate by Umezawa and co-workers in 1974.[5] The charge distribution in the zwitterion resembles that in the *N*-acetylneuraminate oxocarbenium ion **2**, the putative intermediate in the enzyme-catalyzed reaction,[6] and this may account for its effectiveness in binding sialidases. A synthesis of **1** from L-ribose was reported by Nishimura and co-workers in 1988,[7] and in the years since they have prepared a number of synthetic analogues and modified siastatin derivatives with an aston-



1: siastatin B



2: sialidase oxocarbenium intermedate



3: X = F, R = H
4: X = H, R = CH_2Ph

(1) Colman, P. M. *Protein Sci.* **1994**, *3*, 1687−1696. Taylor, G. *Curr. Opin. Struct. Biol.* **1996**, *6*, 830−837, and references therein.

(2) El Ashry, E. S. H.; Rashed, N.; Shobier, A. H. S. *Pharmazie* **2000**, *55*, 251−262. von Itzstein, M.; Thomson, R. T. *Top. Curr. Chem.* **1997**, *186*, 119−170. Luo, Y.; Li, S. C.; Li, Y. T.; Luo, M. *J. Mol. Biol.* **1999**, *285*, 323−332.

(3) Gubareva, L. V.; Kaiser, L.; Hayden, F. G. *Lancet* **2000**, *355*, 827−835, and references therein.

(4) McKimm-Breschkin, J. L. *Antiviral Res.* **2000**, *47*, 1−17, and references therein.

(5) Umezawa, H.; Aoyagi, T.; Komiyama, H.; Morishima, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 963−969.

(6) Yang; J. S.; Schenkman, S.; Horenstein, B. A. *Biochemistry* **2000**, *39*, 5902−5910. Horenstein, B. A.; Bruner, M. *J. Am. Chem. Soc.* **1998**, *120*, 1357−1362. Thomas, A.; Jourand, D.; Bret, C.; Amara, P.; Field, M. J. *J. Am. Chem. Soc.* **1999**, *121*, 9693−9702.

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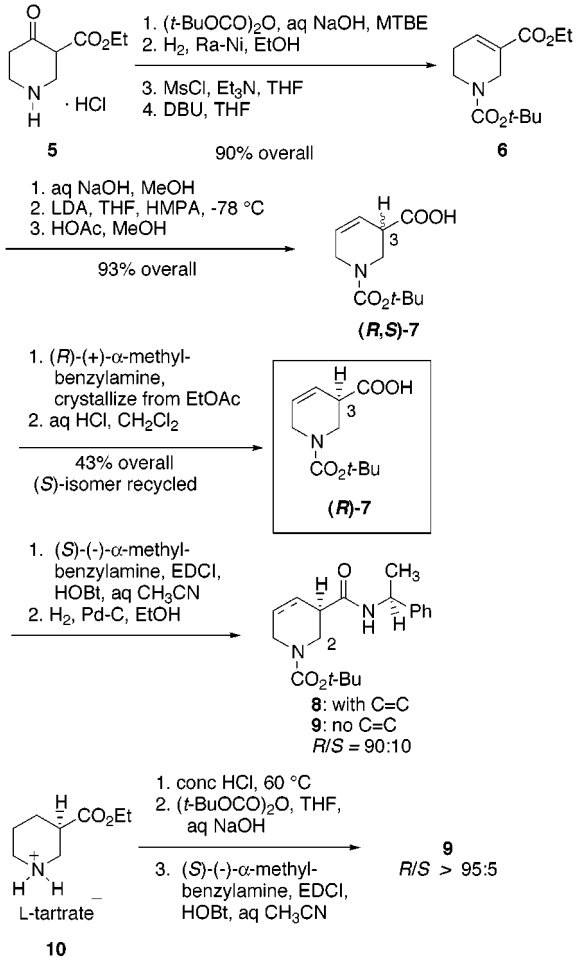
ishing array of biological and enzyme inhibitory activities.[8] One modification, the trifluoroacetamido analogue **3**, exhibits inhibition of bovine liver β-glucuronidase (IC_{50} 6.5×10^{-8} M)[9] and effectively suppresses tumor cell metastasis in mice.[10]

Our interest in the siastatin family of glycosidase inhibitors stems from their unusual mixed aminal structures, their diverse activities, the possibility of using them to improve the understanding of glycosidase binding and mechanism, and the challenge of devising a flexible synthesis from non-carbohydrate starting material. We report efficient and stereoselective syntheses of siastatin B (**1**, 13 steps) and its trifluoroacetamido analogue (**3**, 14 steps) from the resolved piperidine carboxylate **7** (Scheme 1). A racemic 3-*C*-benzyl analogue **4** was also prepared.

Scheme 1



Given that an easy route to unsaturated piperidine ester **6** (Scheme 1) from the commercially available keto-ester **5** has already been described,[11] the synthesis of siastatin reduces to three parts: (1) an effective deconjugation/resolution procedure, (2) the stereoselective functionalization of the piperidine ring, including introduction of the unusual mixed aminal, and then (3) careful deprotection. The deconjugation/resolution procedure is shown in Scheme 1.

Hydrolysis of ethyl ester **6** led to a crystalline unsaturated carboxylic acid, which was converted to its γ-extended enolate by treatment with LDA. Quenching with methanolic acetic acid gave the crystalline racemic β,γ-unsaturated carboxylic acid (*R*,*S*)-**7**. Several attempts to quench this enolate enantioselectively[12] were unsuccessful, but this remains a worthwhile objective. The deconjugated acid could be conveniently resolved by combining it with (*R*)-(+)-α-methylbenzylamine and then crystallizing the resulting salt from ethyl acetate. A second crystallization followed by breaking the salt with HCl afforded in 43% overall yield the unsaturated acid (*R*)-**7** with ee = 87%. The absolute configuration and minimum optical purity of (*R*)-**7** were established by converting it to an amide (**8**) of (*S*)-(−)-α-methylbenzylamine. The ^{1}H NMR spectrum of **8** exhibits signals for two diastereomers in the approximate ratio 12:1 (the dd signals of H-2 at 3.3−3.5 ppm are most easily integrated). Hydrogenation of **8** gave the amide **9** as a 12:1 mixture of diastereomers, and the major diastereomer of **9** was independently prepared from ethyl (*R*)-nipecotate, which is commercially available as its L-tartrate salt **10** (Scheme 1). Examination of the ^{1}H NMR spectrum of **9** prepared from **10** revealed no trace of the (*S*)-diastereomer, suggesting that no racemization takes place during the formation of the amide. Furthermore, amide formation from the racemic acid (*R*,*S*)-**7** gave a 1:1 mixture of **8** and its diastereomer, suggesting that preferential kinetic formation of the (*R*)-diastereomer is not occurring.

Bromolactonization of unsaturated acid (*R*)-**7** was envisioned as an effective tool for initial functionalization at C-4,5. Because literature methods for halocyclization to β-lactones[13] gave poor results, considerable effort was spent in trying to find an effective halonium source and solvent for this reaction. Prior conversion of (*R*)-**7** to its tetra-*n*-butylammonium carboxylate salt **11** allowed bromination under homogeneous conditions and at low temperature (Scheme 2). Thus, **11** was carefully dried and then treated

(7) Nishimura, Y.; Wang, W.; Kondo, S.; Aoyagi, T.; Umezawa, H. *J. Am. Chem. Soc.* **1988**, *110*, 7249−7250. Nishimura, Y.; Wang, W.; Kudo, T.; Kondo, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 978−986. This route proceeds in about 24 steps and 11% overall yield from L-ribose.

(8) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem.* **2000**, *8*, 343−352. Nishimura, Y. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1995; Vol 16, pp 75−121. Nishimura, Y. *Yuki Gosei Kagaku Kyokaishi* **1997**, *55*, 142−151.

(9) Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 2−11, and references therein.

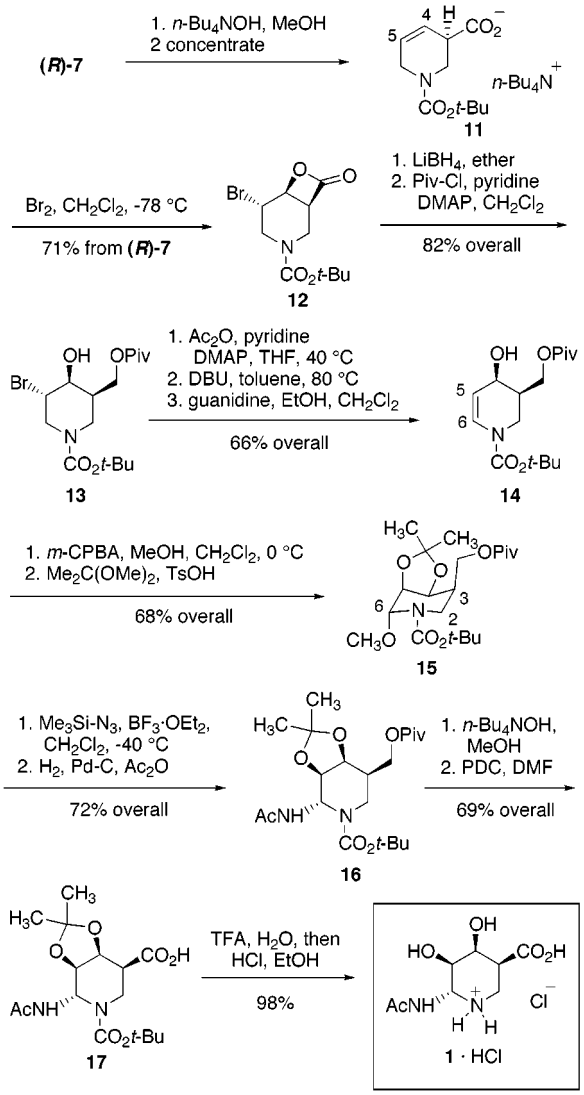
(10) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 101−107.

(11) Krogsgaard-Larsen, P.; Jacobsen, P.; Brehm, L.; Larsen, J. *Eur. J. Med. Chem.* **1980**, *15*, 529−535. Krogsgaard-Larsen, P.; Thyssen, K.; Schumberg, K. *Acta Chem. Scand.* **1978**, *B32*, 327−334. Krogsgaard-Larsen, P.; Hjeds, H. *Acta Chem. Scand.* **1974**, *B28*, 533−538. Allan, R. D.; Johnston, A. R. *Med. Res. Rev.* **1983**, *3*, 91−118.

(12) For a recent leading reference, see: Vedejs, E.; Kruger, A. W.; Lee, N.; Sakata, S. T.; Stec, M.; Suna, E. *J. Am. Chem. Soc.* **2000**, *122*, 4602−4607.

(13) Frederickson, M.; Grigg, R. *Org. Prep. Proc. Int.* **1997**, *29*, 35−62. Barnett, W. E.; Needham, L. L. *J. Org. Chem.* **1975**, *40*, 2843−2844. Ganem, B.; Holbert, G. W.; Weiss, L. B.; Ishizumi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6483−6491. Barnett, W. E.; Sohn, W. H. *J. Chem. Soc., Chem. Commun.* **1972**, 472. Mead, K. T.; Park, M. *Tetrahedron Lett.* **1995**, *36*, 1205−1208. Cook, C.; Chung, Y. *Arch. Pharm. Res.* **1981**, *4*, 133−135. Homsi, F.; Rousseau, G. *J. Org. Chem.* **1999**, *64*, 81−85.

Scheme 2



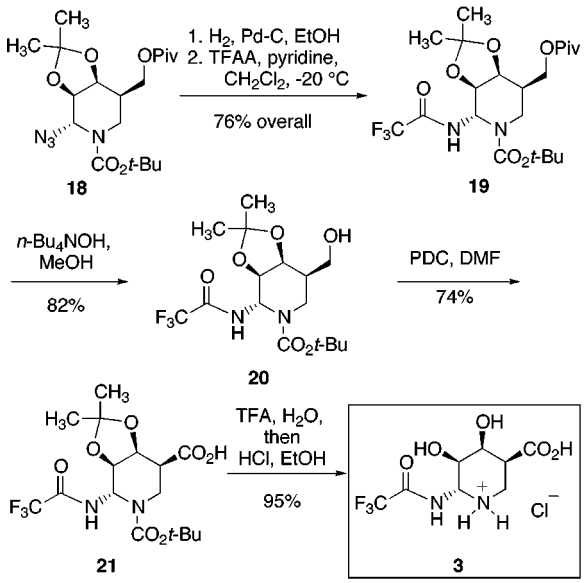
with bromine in dichloromethane solution at −78 °C, resulting in a 71% isolated yield of the bromo-β-lactone **12**. IR analysis of the crude reaction mixture indicated exclusive formation of the β-lactone (1840 cm^{-1}) without detectable γ-lactone. The lactone ring was reduced to the diol with lithium borohydride (no ring closure to the epoxide occurred under these conditions), and then the primary hydroxyl was selectively protected as its pivaloate (**13**).

For dehydrobromination of **13**, the derived acetate was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene, and then the acetate was cleaved with ethanolic guanidine.[14] The resulting allylic alcohol **14**, which is very acid-sensitive, is the appropriate substrate for a hydroxyl-directed *syn*-epoxidation at C-4,5. Several attempts to isolate a C-4,5 epoxide were unsuccessful, as were attempts to trap the epoxide with various azide nucleophiles. However, the product of methanolic *m*-chloroperoxybenzoic acid oxidation could be trapped as the ketal **15** by treating the crude oxidation mixture with 2,2-dimethoxypropane. The behavior of **14** under these conditions is perhaps analogous to pyranose glycal reactivity,[15] with the *N*-acylpiperidine nitrogen standing in for the pyranose ring oxygen.[16] Thus, **15** is analogous to a methyl glycoside. On the basis of its ^{1}H NMR spectrum (two BOC rotamers, $J_{H5-H6} \sim 0$ Hz, no H-2/H-3 *trans*-diaxial coupling), we assign a chairlike conformation to **15** in which the C-6 methoxy prefers an axial position to avoid allylic 1,3-strain with the *N*-BOC oxygens.

Exchange of methoxy for azido was accomplished by treating **15** with azidotrimethylsilane and boron trifluoride, with azido entering from the less hindered α-face. Hydrogenolytic reduction of the azide in the presence of acetic anhydride led directly to the acetamido product **16**. The pivaloate was cleaved with tetra-*n*-butylammonium hydroxide to expose the primary hydroxyl, which then was oxidized to the carboxylic acid (**17**) with pyridium dichromate.[17] Nearly quantitative deprotection of **17** was carried out in a one-pot procedure by sequentially adding trifluoroacetic acid (to remove the *N*-*tert*-butoxycarbonyl), adding water (to remove the acetonide), concentrating, adding ethanolic hydrochloric acid (to exchange the counterion), concentrating again, and finally adding hexane to solidify the hydrochloride of siastatin B (**1**·HCl). The ^{1}H and ^{13}C NMR spectra, and the optical rotation, $[\alpha]^{25}_D$ +52° (c = 0.25, H_2O), closely match the values reported for the natural product.[18] Although the optical purity of late synthetic intermediates was not checked, the small amount of unnatural enantiomer present in the resolved acid (*R*)-**7** had apparently been removed by crystallization at two intermediate stages along the synthetic route: the diol derived from **12** and the acetate derived from **13**.

The trifluoroacetamido analogue **3** was synthesized by modifying the siastatin route at the stage of the *N*-acetylation (Scheme 3). Thus, hydrogenation of azide **18** gave a primary

Scheme 3

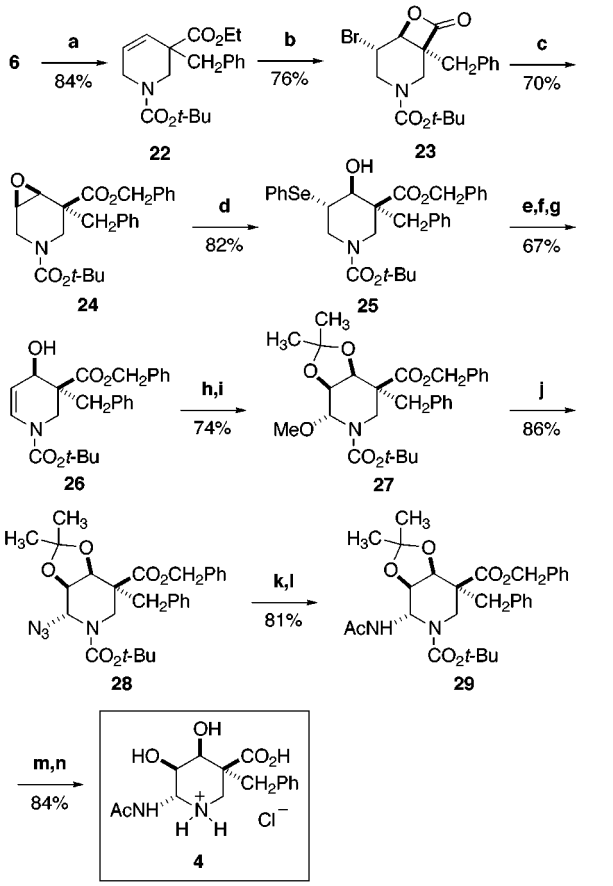


(14) Kunesch, N.; Miet, C.; Poisson, J. *Tetrahedron Lett.* **1987**, *28*, 3569−3572.

amine that was stable enough to survive until it could be trifluoroacetylated. The resulting trifluoroaceamide **19** was selectively deprotected at the pivaloate by treatment with methanolic tetrabutylammonium hydroxide. The trifluoroacetamide, which might have been expected to cleave under these conditions, likely survives because it is deprotonated instead. Oxidation and deprotection as before afforded (F_3)-siastatin B as its hydrochloride salt **3**, whose ^{1}H NMR spectrum and optical rotation, $[\alpha]^{25}_D$ +28° (c = 0.25, H_2O), closely match the literature values.[19]

The second analogue, 3-*C*-benzylsiastatin (**4**), was actually synthesized before **1** and **3** as a model for improving several of the transformations needed for the synthesis of the latter two. Unsaturated ester **6** was converted to its lithium γ-extended enolate, which reacted with benzyl bromide to afford the racemic ester **22** (Scheme 4). Treatment of **22** with tetrabutylammonium hydroxide led to the tetrabutylammonium salt of the corresponding carboxylate, which was directly bromocyclized at low temperature to give the bromo β-lactone **23**. Exposure of **23** to the lithium salt of benzyl alcohol caused alcoholysis of the β-lactone and subsequent ring closure to the epoxide (**24**). Isomerization of **24** to allylic alcohol **26** was accomplished by site-selective epoxide opening with sodium phenylselenylate[20] to give **25**, followed by acetylation, oxidation with spontaneous elimination of PhSeOH, and finally deacetylation. *Syn*-epoxidation of **26** and subsequent treatment with 2,2-dimethoxypropane provided the ketal **27**, and then azidation, reduction of the azide,[21] acetylation, hydrogenolysis of the benzyl ester, and finally deprotection gave the 3-*C*-benzyl analogue **4**. The steps leading to **22**, **23**, **27**, **28**, **29**, and **4** provided information valuable for application to the synthesis of **1** and **3**. In contrast to these routes, however, the route to **4** did not require reduction of the ester as there was no danger of elimination at C-3,4. Although it is structurally closely related to siastatin B, the 3-*C*-benzyl analogue **4** showed no inhibitory activity when evaluated against the commercial sialidase from *Salmonella tymphimurium*.

Scheme 4. Synthesis of 3-*C*-Benzylsiastatin[a]



[a] Reagents and conditions: (a) LDA, HMPA, −78 to 0 °C, then $PhCH_2Br$, −78 to 23 °C; (b) Bu_4NOH, MeOH, 23 °C, then Br_2, CH_2Cl_2, −78 °C; (c) $PhCH_2OH$, *n*-BuLi, THF, −40 °C; (d) PhSeSePh, $NaBH_4$, EtOH, 23 °C; (e) Ac_2O, pyridine, DMAP, THF, 40 °C; (f) 30% H_2O_2, *i*-Pr_2NH, THF, 0 to 23 °C; (g) Na_2CO_3, MeOH; (h) dimethyldioxirane, aqueous acetone; (i) $Me_2C(OMe)_2$, TsOH; (j) Me_3SiN_3, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −40 °C; (k) H_2, Lindlar catalyst, EtOH; (l) Ac_2O, pyridine, DMAP, THF; (m) H_2, 10% Pd−C, EtOH; (n) TFA, H_2O, then HCl, EtOH.

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Supporting Information Available: Experimental procedures and spectroscopic characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Review of *N*-acyliminum ion additions: Hiemstra, H.; Speckamp, W. N. In *Comprehensive Organic Synthesis: Selectivity, Strategy, and Efficiency in Modern Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: London; 1991; Vol. 2, pp 1047−1082.
(17) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *20*, 399−402.
(18) From ref 7, $[\alpha]^{20}_D$ +53° (c = 0.25, H_2O); from ref 5, [α] +57.2° (H_2O).
(19) From ref 10, $[\alpha]^{31}_D$ +27° (c = 0.22, H_2O).
(20) Sharpless, K. B.; Lauer, R. F. *J. Am. Chem. Soc.* **1973**, *95*, 2697−2699.
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