The First L-Iduronic Acid-Type 1-*N*-Iminosugars Having Inhibitory Activity of Experimental Metastasis

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Tumor cell adhesion to various basement membrane components and degradation of extracellular matrix and basement membranes are an important step of tumor metastasis. β-D-Glucuronidase and α-L-iduronidase are known to degrade the mammalian glycosaminoglycans, the major constituents of endothelial basement membranes.[1,2] Heparanase (end-β-glucuronidase) activity was also proved to correlate with lung colonization abilities of murine B16 melanoma cells by extracellular matrix degradation and to be inhibited by heparanase inhibitors.[3]

Siastatin B (**1**) was isolated as an inhibitor of neuraminidase and β-glucuronidase from *Streptomyces* culture,[4] and the 6-epimer of **1** has recently been shown to be an inhibitor for heparanase.[5] This discovery stimulated interests in the synthesis of specific glucuronidase inhibitors for antimetastasis of tumor cells and led to highly potent β-glucuronidase inhibitors **2** and **3**. They showed the inhibition of invasion of B16 variant (B16 BL6) and Lewis lung carcinoma (3LL) cells through reconstituted basement membrane and the significant suppression of experimental and spontaneous pulmonary metastasis of B16 BL6 and/or 3LL cells in mice.[6] Compounds **2** and **3**, which structurally resemble glucuronic acid (**4**) as a 1-*N*-iminosugar,[7] probably mimic **4** in binding to β-glucuronidase and strongly inhibit the enzymatic reaction. We speculated from these facts and results that if the metabolism of α-L-iduronide as well as β-D-glucuronide of basement membranes was responsible for tumor metastasis, L-iduronic acid-type 1-*N*-iminosugars should inhibit tumor metastasis. Here, we report the first L-iduronic acid-type 1-*N*-iminosugars **6**, **7**, and **8** having experimental antimetastatic activity in mice.

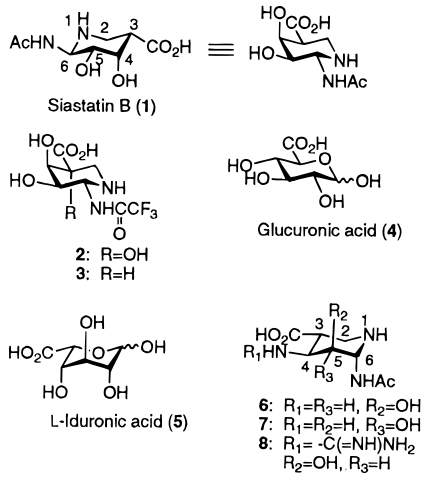
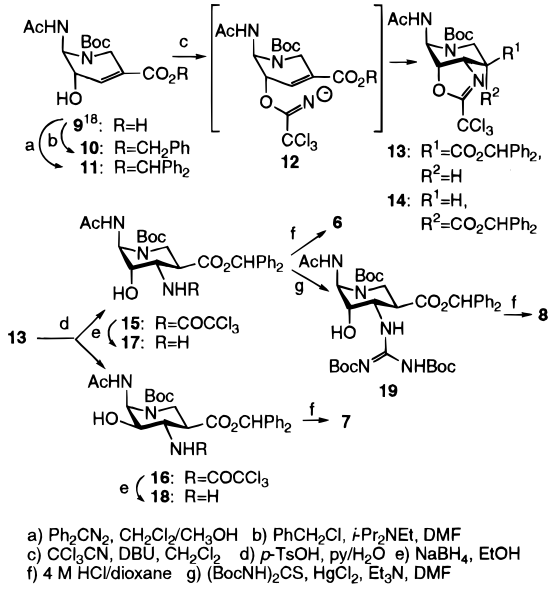


Figure 1.

Scheme 1



In order to synthesize 1-*N*-iminosugar corresponding to L-sugar, the configurational inversion of the carboxyl group of **1** was examined. After unsuccessful attempts of epimerization of the carboxyl group of some ester derivatives of **1** with bases and a nonstereoselective epimerization by a conjugated Michael addition of the alcohol to the α,β-unsaturated ester **10** with base,[8] attention was directed to the intramolecular Michael addition of *O*-imidate[9] to α,β-unsaturated ester **11**. Compound **11** underwent smoothly *cis* oxyamination to give the oxazoline **13** in 76% yield and a trace amount of its epimer **14**. The intermediate imidate anion **12** from reaction with CCl_3CN underwent efficient conjugate addition without the use of an electrophile to trigger oxazoline formation. Hydrolysis of **13** (p-$CH_3C_6H_4SO_3H$, C_6H_5N/H_2O)[10] gave **15** and **16** in yields of 77 and 9%, respectively. Reductive cleavage of the trichloro-

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(1) (a) Klein, U.; von Figura, K. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 569. (b) Thuberg, L.; Baeckstroem, G.; Wasteson, Å.; Robinson, H. G.; Oegren, S.; Lindahl, U. *J. Biol. Chem.* **1982**, *257*, 10278. (c) Oldberg, A.; Heldin, C.-H.; Wasteson, Å.; Busch, C.; Hoeoek, M. *Biochemistry* **1980**, *19*, 5755. (d) Oosta, G. M.; Favreu, L. V.; Beeler, D. L.; Rosenberg, R. D. *J. Biol. Chem.* **1982**, *257*, 11249. (e) Nakajima, M.; Irimura, T.; Di Ferrante, N.; Nicolson, G. L. *J. Biol. Chem.* **1984**, *259*, 2283.

(2) (a) Matalon, R.; Cifonelli, J. A.; Dorfman, A. *Biochem. Biophys. Res. Commun.* **1971**, *42*, 340. (b) Bach, G.; Friedman, R.; Weissman, B.; Neufeld, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2048. (c) Roden, L. In *The Biochemistry of Glycoproteins and Proteoglycans*; Lennarz, W. G., Ed.; Plenum Press; New York, 1980; pp 267−371. (d) Takagaki, K.; Nakamura, T.; Majima, M.; Endo, M. *J. Biol. Chem.* **1988**, *263*, 7000.

(3) (a) Irimura, T.; Nakajima, M.; Nicolson, G. L. *Biochemistry* **1986**, *25*, 5322. (b) Nakajima, M.; Irimura, T.; Nicolson, G. L. *J. Cell. Biochem.* **1988**, *36*, 157. (c) Keren, Z.; Leland, F.; Nakajima, M.; Legrue, S. J. *Cancer Res.* **1989**, *49*, 295. (d) Nakajima, M.; DeChavigny, A.; Johnson, C. E.; Hamada, J.-I.; Stein, C. A.; Nicolson, G. L. *J. Biol. Chem.* **1991**, *266*, 9661.

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

(5) Takatsu, T.; Takahashi, M.; Kawase, Y.; Enokita, R.; Sakaida, Y.; Kagasaki, T.; Kinoshita, T.; Tanzawa, K. Annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry. *Nippon Nogei Kagaku Kaishi* **1995**, *69*, 187 (abs. 2Aa 15).

(6) (a) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 963. (b) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 101. (c) Nishimura, Y.; Satoh, T.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 840.

(7) (a) Nishimura, Y. In *Stereoselective synthesis and transformation of siastatin B, a novel glycosidase inhibitors, directed toward new drugs for viral infection and tumor metastasis*; Atta-ur-Rahman, Ed.; Studies in Natural Products Chemistry; Elsevier: Amsterdam, 1995; Vol. 16, pp 75−121. (b) Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett.* **1995**, *36*, 1767. (c) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1778.

(8) (a) Nishimura, Y.; Kudo, T.; Umezawa, Y.; Kondo, S.; Takeuchi, T. *Nat. Prod. Lett.* **1992**, *1*, 39. (b) Nishimura, Y.; Umezawa, Y.; Kondo, S.; Takeuchi, T.; Mori, K.; Kijima-Suda, I.; Tomita, K.; Sugawara, K.; Nakamura, K. *J. Antibiot.* **1993**, *46*, 1883.

(9) Alonso, R. A.; Burgey, C. S.; Rao, B. V.; Vite, G. D.; Vollerthun, R.; Zottola, M. A.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1993**, *115*, 6666.

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acetyl group with $NaBH_4$[11] then gave **17** and **18** in good yields. Thus obtained **17** and **18** were smoothly converted into **6** and **7** by treatment with acid, respectively. The guanidine moiety is an important feature in many biological active compounds, especially in binding to the enzyme such as influenza viral *N*-acetylneuraminidase.[12,13] The major isomer **17** was then converted to the bis-Boc-protected guanidine **19** ($(BocNH)_2CS$, $HgCl_2$)[14] in excellent yield. Compound **19** was straightforwardly transformed into **8** by treatment with acid. The $^6C_3$-conformations as well as stereochemistries of **6**, **7**, and **8** were established by ^1H NMR spectra.[15]

As expected, all analogues did not inhibit D-glycosidases[16] (IC_{50} > 0.39 mM). These results indicate that the analogues having $^6C_3$-conformation are significantly distinct from the hitherto known analogues[7] of **1** having $^3C_6$-conformation on the specificity against D-sugar hydrolases. These analogues were then assayed for anti-invasive and antimetastatic activities of tumor cells.[17] Since the inhibitory activity of siastatin B analogues for experimental pulmonary metastasis is well proportional to that for spontaneous pulmonary metastasis,[6c] the experimental metastasis assay was employed for this study. As expected, pulmonary colonization after intravenous transplantation of B16 BL6 cells into the tail veins of mice was significantly suppressed dose-dependently by *in vitro* pretreatment with **6**, **7**, and **8** (44 and 31% inhibition at 172 μM of **6** and **7** (P < 0.05), respectively; 91 and 97% inhibition at 90 and 150 μM of **8** (P < 0.001), respectively).[15] Compounds **6**, **7**, and **8** had no significant effects on cell growth at the concentrations used in this study (data not shown). Compound **8** was also inhibitory against B16 BL6 cell invasion through reconstituted basement membranes (59% inhibition at 870 μM (P < 0.01)).[15] These facts indicate that the antimetastatic effect of **8** is due to its anti-invasive rather than antiproliferative activity. On the other hand, molecular modeling[15] using PM3 in MOPAC revealed that **8** superimposes well on L-iduronic acid (**5**) and has the hydroxyl and carboxyl groups lying in the same region of space as those of **5**, with the acetamide and guanidino moiety also being topographically equivalent to the hydroxyl moieties of **5**. It is highly likely that in contrast with β-glucuronidase inhibitors **2** and **3**, compound **8** mimics L-iduronic acid in binding to L-iduronidase and inhibits the enzymatic hydrolysis. These findings suggest that the metabolism of α-L-iduronide of the basement membranes of a normal cell and/or tumor cell may participate in human melanoma metastasis and that melanoma cells may secrete not only heparanase but also α-L-iduronidase.

The present study shows that the L-iduronic acid-type 1-*N*-iminosugars are a promising candidate of new drugs for tumor metastasis.

Supporting Information Available: Experimental procedures and characterization data for new compounds, biological data for **6**, **7**, and **8**, and PM3/MOPAC optimized structures of **8** and α-L-iduronic acid (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) Gent, P. A.; Gigg, R.; May, S.; Conant, R. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2748.
(11) Weygand, F.; Frauendorfer, E. *Chem. Ber.* **1970**, *103*, 2437.
(12) Lednicer, D.; Mitscher, L. A. *The Organic Chemistry of Drug Synthesis*; Wiley: New York, 1977; Vol. 1, pp 1−432 and 1980; Vol. 2, pp 1−482.
(13) (a) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418. (b) Woods, J. M.; Bethell, R. C.; Coates, J. A. V.; Healy, N.; Hiscox, S. A.; Peason, B. T.; Ryan, D. M.; Ticehurst, J.; Tilling, J.; Walcott, S. M.; Penn, C. R. *Antimicrob. Agents Chemother.* **1993**, *37*, 1473. (c) Colman, P. M. *Protein Sci.* **1994**, *3*, 1687.
(14) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.
(15) See supporting information.
(16) Inhibitory activities against Baker's yeast α-glucosidase,[16a] almond β-glucosidase,[16b] Jack beans α-mannosidase,[16c] snail β-mannosidase,[16c] *Escherichia coli* α- and β-galactosidase,[16d] bovine liver β-glucuronidase,[16e] chicken liver α-*N*-acetylgalactosaminidase,[16f] and bovine epididymis β-*N*-acetylglucosaminidase[16g] were assayed by similar methods described in references. All enzymes were purchased from Sigma Chemical Company. (a) Halvorson, H. O.; Fllias, L. C. *Biochim. Biophys. Acta* **1958**, *30*, 28. (b) Kobayashi, A. *Agr. Biol. Chem.* **1962**, *26*, 303. (c) Li, Y.-T. *J. Biol. Chem.* **1967**, *242*, 5474. (d) Craven, G. R.; Steers, E.; Jr.; Anfinsen, C. B. *J. Biol. Chem.* **1965**, *240*, 2468. (e) Stahl, P. P. D.; Fishman, W. H. In *β-D-Glucuronidase. β-D-Glucuronide glucuronosohydrolase, EC 3.2.1.31*; Voight, K. D., Ed.; Methods of Enzymatic Analysis; Academic Press: New York, 1974; Vol. 4, pp 246−256. (f) Uda, Y.; Li, S.-C.; Li, Y.-T.; McKibbin, J. M. *J. Biol. Chem.* **1977**, *252*, 5194. (g) Tarentino, A. L.; Maley, F. In *β-N-Acetylglucosaminidase from Hen Oviduct.*; Ginsburg, V.; Ed.; Methods in Enzymology; Academic Press: New York, 1972; Vol. 28, pp 772−776.
(17) α-L-Iduronidase is now not available for us.
(18) Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1993**, *46*, 300.