Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3461–3463

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Glycosidase Inhibitory Activity of Some *N*-Substituted 6-Deoxy-5a-carba-β-DL- and L-galactopyranosylamines

Seiichiro Ogawa,* Shigeo Fujieda, Yuko Sakata, Masahiro Ishizaki, Seiichi Hisamatsu and Kensuke Okazaki

Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

Received 14 May 2003; accepted 14 July 2003

Abstract—Chemical modification of 5a-carba-β-DL-fucopyranosylamine (**3**) generated six *N*-substituted derivatives **9a–f**, among which *N*-octyl **9b**, decyl **9c**, and phenylbutyl ones **9f** were found to be very strong β-galactosidase as well as β-glucosidase inhibitors. The inhibitory activity appeared attributable to D-enantiomers from biological assays of prepared L-enantiomers. Therefore, 6-deoxy-5a-carba-β-D-galactopyranosylamine (**D-3**) might be a promising lead compound for further design of new carba sugar-type β-galactosidase inhibitors.

Recently, the *N*-octyl derivative[1] **2** of 5a-carba-α-DL-fucopyranosylamine[2] (**1**) has been demonstrated to be very strong α-L-fucosidase inhibitor (bovine kidney) with *p*-nitrophenyl-α-L-fucopyranoside, essentially comparable with deoxyfuconojirimycin (**DFJ**) in this regard. Although the oxocarbenium ion transition-state analogues **4**α,β of **1** have been shown to possess a weak activity, contrary to expectation, it is of interest to note that the β-anomer[3] **3** has high activity against α-L-fucosidase. These results are in contrast with the α-glucosidase inhibitory activity relationship[4] observed between a ground-state mimic of 5a-carba-α-D-glucopyranosylamine, validamine, and a transition-state mimic of its unsaturated derivative, valienamine (Fig. 1, Scheme 1).

In the present communication, chemical modification of the β-anomer **3** was carried out by incorporation of hydrophobic alkyl and phenylalkyl portions into the amino group by direct treatment of a carba-sugar epoxide **7**, a newly prepared versatile precursor, with alkyl and phenylalkyl amines. For convenience, rough screening of the activity of several racemic compounds was performed to hopefully allow estimation of the approximate enantiomeric contribution to the activity, based on comparison of data observed for D- and/or L-enantiomers. The antipode of 5a-carba-β-L-fucopyranosylamine should be 6-deoxy-5a-carba-β-D-galactopyranosylamine. Therefore, inhibitory activity of the racemic modification of 5a-carbahexopyranosylamine should be a sum of those of its components. This consideration is based on the assumption that each enantiomer does not interfere with the others competitive inhibitory action. For example, with racemic DL-**9c**,

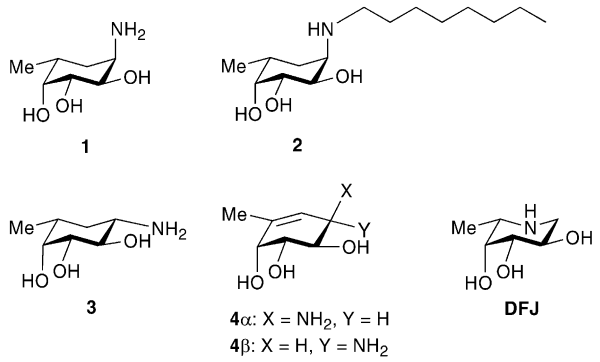
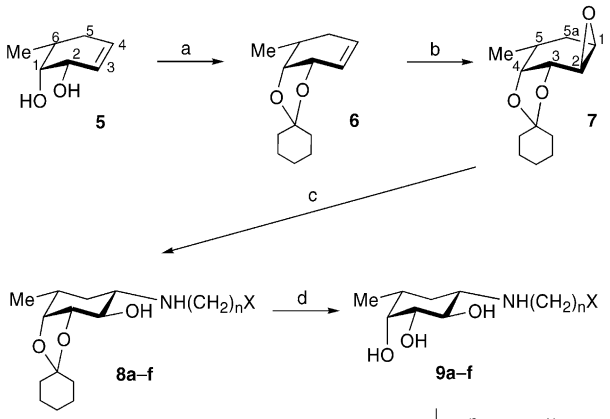


Figure 1. 5a-Carba-α and β-fucopyranosylamines and deoxyfuconojirimycin (DFJ).

*Corresponding author. Tel.: +81-45-563-1141; fax: +81-45-563-0446; e-mail: ogawa@bio.keio.ac.jp

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00742-X



	n	x
8a, 9a	5	Me
8b, 9b	7	Me
8c, 9c	9	Me
8d, 9d	11	Me
8e, 9e	2	Ph
8f, 9f	4	Ph

Scheme 1. For convenience, the formulas depict only one enatiomer (L-series) of the respective racemates. Reagents and conditions: (a) 1,1-dimethoxycyclohexane (4 molar equiv), $TsOH{\cdot}H_2O$ (0.2 molar equiv), DMF, rt; (b) *m*CPBA (1.3 molar equiv), phosphate buffer, CH_2Cl_2, 0 °C; (c) a molar equiv of alkyl or phenylalkyl amine, 2-propanol, 120 °C; (d) 80% aq AcOH, 80 °C; Dowex 50W×2 (H^+) resin, 1% aq NH_3.

activity (IC_{50} 2.7 μM) against L-fucosidase should be largely due to the L-enantiomer, and high inhibition toward β-galactosidase (IC_{50} 0.2 μM) and β-glucosidase (IC_{50} 2.0 μM) would be probably attributable to the D-enantiomer (Table 1). This conclusion is partially supported by data obtained for newly synthesized optically active L-**9c**.

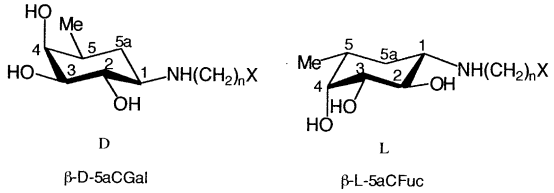
As indicated by *N*-substitution of the α-anomer **1**, chemical modification of the β-anomer **3** might be expected to increase its potential. Protection of (1*RS*,2*RS*)-6-methyl-3-cyclohexene-1,2-diol[4] (**5**) with a cyclohexylidene group (→**6**, 85%) and subsequent epoxidation with *m*-chloroperbenzoic acid gave selectively the β-epoxide[5] **7** (90%). Reaction of **7** with alkyl amines or phenylalkylamines proceeded almost regio-selectively to afford the respective individual *N*-substituted carbaglycosylamines[6] **8a–f** with a β-*galacto* configuration in ~85% yield. Both selectivity of epoxidation and regioselectivity of cleavage of the epoxide ring appeared to be improved by incorporation of a cyclohexylidene group at C-1 and -2.[7] The products were deprotected with aqueous acetic acid and the amine acetates obtained were purified by passage through an acidic resin column with 1% aqueous ammonia, giving the free bases[8] **9a–f** quantitatively.

Enzyme Inhibitory Activity

Four *N*-alkyl and two *N*-phenylalkyl derivatives **9a–f** of **3** were assayed for activity against seven glycosidases: α-galactosidase (green coffee beans), β-galactosidase (bovine liver), α-glucosidase (Baker's yeast), β-glucosidase (almond), β-glucosaminidase (bovine kidney), α-mannosidase (Jack beans), and α-fucosidase (bovine kidney) in a standard manner.[9] All compounds were shown not to be inhibitors of glycosidases other than β-galactosidase, β-glucosidase, and α-fucosidase, as listed in Table 1. Compared to the corresponding derivatives[1] of the α-anomers, they possessed about one tenth of the inhibitory activity against α-fucosidase and the potential was thought to be mainly due to β-L-fucose-mimicking enantiomers, as verified by assaying some prepared L-enantiomers.

It is interesting to note that all the tested compounds possess very strong activity toward both β-galactosidase and β-glucosiadase. Their high potential could be demonstrated to be largely due to the respective D-enantiomers, that is, *N*-alkyl-6-deoxy-5a-carba-β-D-galactopyranosylamines. In fact, the L-enantiomers, *N*-alkyl-5a-carba-β-L-fucopyranosylamines had decreased activity toward these two enzymes as shown for **9b**, **9c**, and **9f** in Table 1. Compound DL-**9e** has been demonstrated to be

Table 1. Inhibitory activity of some *N*-alkyl and -phenylalkyl derivatives **9a–f** of 6-deoxy-5a-carba-β-DL- and L-galactopyranosylamines[a]



Compd	*n*	X	Inhibitory activity, IC_{50} K_i (μM)					
			α-Fucosidase (bovine kidney)		β-Galactosidase (bovine liver)		β-Glucosidase (almond)	
			DL	L	DL	L	DL	L
9a	5	Me	8.2	NT	3.7	NT	0.73	NT
9b	7	Me	5.5	1.8	0.7 (0.11)	3.7	1.5 (3.2)	14.7
9c	9	Me	2.7	0.7	0.2 (0.009)	1.0	2.0 (0.4)	16.6
9d	11	Me	NT	1.2	NT	3.0	NT	3.6
9e	2	Ph	7.5	NT	5.7	NT	0.38 (0.057)	NT
9f	4	Ph	5.1	1.2	0.9 (0.046)	2.4	1.4 (1.2)	10.2

NT, not tested.
[a]Compounds **9a** and **9e** are racemic, and compound **9d** is an L-enantiomer.

very strong inhibitor possessing characteristic pH-dependent activity against β-glucosidase (almond): K_i 0.39 μM at pH 5.5; K_i 0.057 μM at pH 6.8. These results would indicate that optically active D-**9e** is a very potent β-glucosidase inhibitor fully compatible with isofagomine[10]) (K_i 0.11 μM, at pH 6.8) and calystegine[11] (K_i 0.75 μM, pH independent). Therefore, 6-deoxy-5a-carba-β- D-galactopyransylamine might be a promising lead compound for development of new carba sugar-type β-galactosidase inhibitors. Preliminary experiments[12] suggest that *N*-alkyl-5a-carba-β-D-galactopyranosylamines are moderate β-galactosidase inhibitors, so that the hydrophobic area conferred in by the 5-methyl branching on the cyclohexane ring is likely to enhance binding potential at the active site of enzymes.

Acknowledgements

The authors sincerely thank Drs. Atsushi Takahashi and Akihiro Tomoda (Hokko Chemical Industry, Co. Ltd., Atsugi, Japan) for the biological assays, Prof. Naoki Asano (Hokuriku University, Kanazawa, Japan) for providing us with a sample of calystegine, Prof. Yoshiyuki Suzuki (International University of Health and Welfare, Otawara, Japan) for helpful discussions, and Ms. Miki Kanto for her assistance in preparing this manuscript.

References and Notes

1. Ogawa, S.; Mori, M.; Takeuchi, G.; Doi, F.; Watanabe, M.; Sakata, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2811.
2. Ogawa, S.; Sekura, R.; Maruyama, A.; Yuasa, H.; Hashimoto, H. *Eur. J. Org. Chem.* **2000**, 2089.
3. Ogawa, S.; Watanabe, M.; Maruyama, A.; Hisamatsu, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 749.
4. Takeuchi, M.; Kamata, K.; Yoshida, M.; Kameda, Y.; Matsui, K. *J. Biochem.* **1990**, *108*, 42. Kameda, Y.; Takai, N.; Asano, N.; Matsui, K. *Chem. Pharm. Bull.* **1990**, *38*, 1970.
5. Other than compound **6** the di-*O*-benzyl and 1,2-*O*-isopropylidene derivatives were subjected to conventional conditions for epoxidation. Exclusive selectivity was obtained for **6**.
6. Data for compound **7**: $[\alpha]_D^{24}+19°$ (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 4.31 (br d, 1H, $J_{3,4}=5.6$ Hz, H-3), 3.98 (br d, 1H, $J_{3,4}=5.6$ Hz, H-4), 3.23 (br s, H-1), 2.97 (br d, 1H, $J_{2,3}=3.2$ Hz, H-2), 1.84 [m, 3H, H-5, H-5a(ax), 5a(eq)], 1.78–1.25 (m, 10H, C_6H_{10}), 1.04 (d, 3H, $J_{5,6}=4.4$ Hz, Me).
7. Diequatorial cleavage of the epoxide ring of **7** proceeded very slowly but regioselectively due to steric hindrance of the bulky 1,2-*O*-cyclohexylidene group.
8. For example, data for compound **9b**: $[\alpha]_D^{24}+21°$ (*c* 1.4, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 3.80 (br s, 1H, H-4), 3.59 (dd, 1H, $J_{1,2}=J_{2,3}=9.5$ Hz, H-2), 3.21 (br d, 1H, $J_{2,3}=9.5$ Hz, H-3), 2.75 (dt, 1H, $J=7.1$ Hz, $J_{gem}=11.0$ Hz, $NHCH_2$), 2.39–2.51 (m, 2H, H-1, $NHCH_2$), 1.59–1.70 [m, 2H, H-5, H-5a(eq)], 1.29–1.36 [m, 7H, $(CH_2)_3CH_3$, H-5a(ax)], 1.04 (d, 3H, $J_{5,6}=6.5$ Hz, H-6), 0.88 (t, 3H, $J=6.6$ Hz, CH_2CH_3); for **9c**: $[\alpha]_D^{24}+1.4°$ (*c* 0.8, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 3.61 (br s, 1H, H-4), 3.45 (dd, 1H, $J_{1,2}=9.5$ Hz, $J_{2,3}=9.3$ Hz, H-2), 3.21 (br d, 1H, $J_{2,3}=9.3$ Hz, H-3), 2.73 (dt, 1H, $J=7.3$ Hz, $J_{gem}=11.5$ Hz, $NHCH_2$), 2.46–2.60 (m, 2H, H-1, $NHCH_2$), 1.45–1.57 [m, 4H, $NHCH_2CH_2$, H-5, H-5a(eq)], 1.21–1.34 [m, 15H, $(CH_2)_7CH_3$, H-5a(ax)], 1.05 (d, 3H, $J_{5,6}=6.3$ Hz, Me); for **9e**: ^1H NMR (300 MHz, CD_3OD): δ 7.16–7.31 (m, 5H, Ph), 3.69 (br s, 1H, H-4), 3.50 (dd, 1H, $J_{1,2}=9.7$ Hz, $J_{2,3}=9.6$ Hz, H-2), 3.30 (br d, 1H, $J_{2,3}=9.6$ Hz, H-3), 2.82–3.06 [m, 4H, $NH(CH_2)_2$], 2.53 [ddd, 1H, $J_{1,2}=9.7$ Hz, $J_{1,5a(ax)}=11.9$ Hz, $J_{1,5a(eq)}=2.2$ Hz, H-1], 1.59–1.68 [m, 2H, H-5, H-5a(eq)], 1.39 [m, 1H, H-5a(ax)], 1.02 (d, 3H, $J_{5,6}=6.6$ Hz, H-6); for **9f**: $[\alpha]_D^{24}+0.4°$ (*c* 1.5, MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 7.04–7.17 (m, 5H, Ph), 3.60 (br s, 1H, H-4), 3.38 (dd, 1H, $J_{1,2}=9.3$ Hz, $J_{2,3}=9.5$ Hz, H-2), 3.18 (br d, 1H, $J_{2,3}=9.5$ Hz, H-3), 2.29–2.69 [m, 5H, $NH(CH_2)_2$, H-1], 1.45–1.62 [m, 6H, $(CH_2)_2Ph$, H-5, H-5a(eq)], 1.24 [br dd, $J_{1,5(aax)}=8.7$ Hz, $J_{gem}=12.6$ Hz, H-5a(ax)], 0.92 (d, 3H, $J_{5,6}=6.5$ Hz, H-6).
9. Dr. Akihiro Tomoda (Hokko Chemical Industry, Co. Ltd.) carried out the biological assays applying a standard approach.
10. (a) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1778. (b) Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998**, *120*, 3007.
11. Asano, N.; Kato, A.; Oseki, K.; Kizu, H.; Matsui, K. *Eur. J. Biochem.* **1995**, *229*, 369.
12. *N*-Octyl and decyl derivatives have been shown to be moderate β-galactosidase (bovine liver) inhibitors: IC_{50} 2.3 and 22 μM, respectively: Ogawa, S.; Sakata, Y.; Fujieda, S. Unpublished results.