Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2811–2814

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Convenient Synthesis and Evaluation of Enzyme Inhibitory Activity of Several *N*-Alkyl-, *N*-Phenylalkyl, and Cyclic Isourea Derivatives of 5a-Carba-α-DL-fucopyranosylamine

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Received 27 May 2002; accepted 24 July 2002

Abstract—Convenient synthesis and chemical modification of the potent α-L-fucosidase inhibitor, 5a-carba-α-DL-fucopyranosylamine (**1**), are described. Among seven N-substituted and three cyclic isourea derivatives newly prepared, the *N*-octyl derivative was found to be the strongest inhibitor of α-L-fucosidase (bovine kidney) more potent ($K_i = 0.016$ μM) than deoxyfuconojirimycin ($K_i = 0.031$ μM) with *p*-nitrophenyl-α-L-fucopyranoside as the substrate.

Mutant enzyme proteins have been found to be labile and rapidly degraded in somatic cells from patients with lysosomal storage diseases. They are stabilized and transported to lysosomes by competitive inhibitors of low molecular weight (chemical chaperones), expressing catalytic activity.[1] This phenomenon was confirmed for the mutant enzyme causing Fabry disease (α-galactosidase deficiency).[2] Very recently, *N*-octyl derivatives[3] of β-valienamine, an unsaturated 5a-carba-glucopyranosylamine, were shown[4] to elevate some mutant enzyme activities in human patients with β-galactosidase or β-glucosidase deficiency (Fig. 1).

5a-Carba-α-L-fucopyranosylamine[5] (**1**) and its β-anomer[6] **2** have been demonstrated to be very potent and specific inhibitors of α-L-fucosidase (bovine kidney), the effect of the former being essentially comparable to those of mammalian α-L-fucosidase, deoxyfuconojirimycin (DFJ),[7,8] the most powerful inhibitor identified. The present paper describes alternative synthesis of **1** and its chemical modification to prepare several *N*-alkyl, phenylalkyl and cyclic isourea derivatives (**20a**–**g** and **22a**–**c**), aimed at improving both the inhibitory potential and biochemical features in vivo. We expect that compounds of this group may find application in the near future in new therapeutic trial against genetic deficiency disorders.

Treatment of 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-5a-carba-β-DL-glucopyranosyl bromide[9] (**3**) with methanolic sodium methoxide at room temperature gave the 1,2:3,6-dianhydride,[10] which was then treated without isolation with NaH–benzyl bromide in DMF to give the benzyl ether **4** (65%). Reaction of **4** with sodium azide, followed by conventional tosylation, led to the azido tosylate **5** (75%), hydrogenation of which with Raney nickel in ethanol containing acetic anhydride gave the *N*-acetyl derivative **6** (80%). Nucleophilic cleavage of the anhydro ring followed by simultaneous *O*-debenzylation was effected by treatment with hydrobromic acid in acetic acid to give *N*-acetyl-3,4-di-*O*-acetyl-6-bromo-6-deoxy-2-*O*-tosyl-5a-carba-β-DL-glucopyranosylamine **7** (80%), dehydrobromination of which with silver fluoride in pyridine afforded the alkene **8** in 48% yield, together with **7** (46%) unchanged. Hydrogenation of **8** with 5% Pd/C proceeded selectively to give the 6-deoxy derivative **9** (90%), having an α-*ido* configuration. Treatment of **9** with excess methanolic sodium methoxide gave the 2,3-epoxide **10**, which was hydrolyzed with acid, followed by acetylation, to afford the *N*-acetyl-2,3,4-tri-*O*-acetyl-5a-carba-α-DL-fucopyranosylamine[11] **11** (80%). Removal of the protecting groups with 4 M

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PII: S0960-894X(02)00627-3

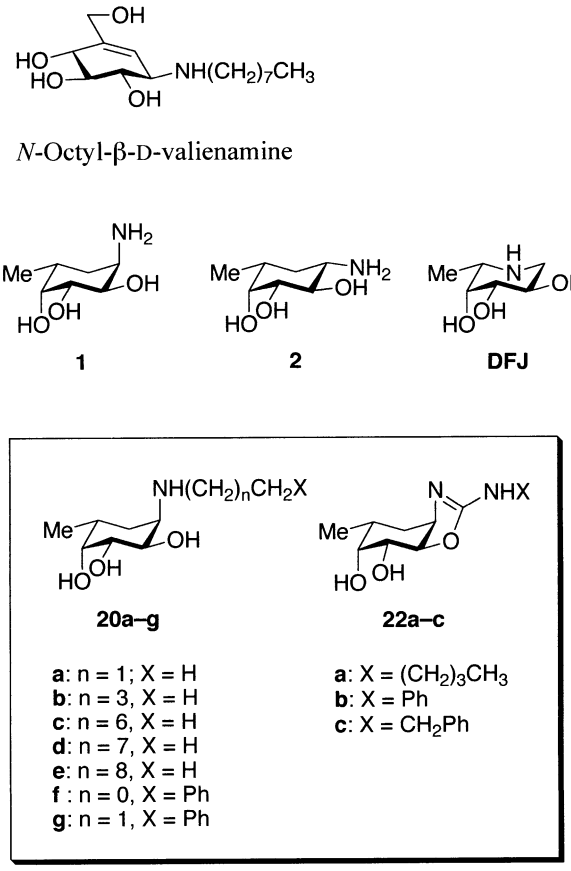
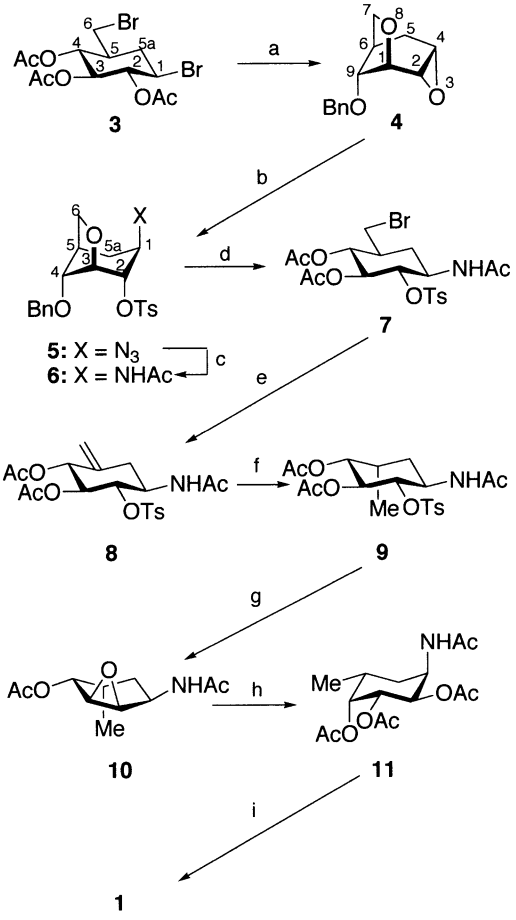


Figure 1.

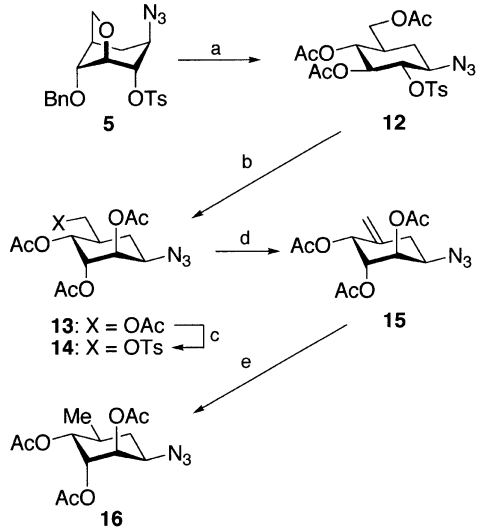
hydrochloric acid gave, after purification over a column of Dowex 50 W×2 (H^+) resin with aqueous 5% ammonia, the free base[11] **1** quantitatively. Some 3-substituted derivatives of **1** could be obtained using intermediates **8** or **9** (Scheme 1).

On the other hand, acetolysis of **5** with acetic acid–acetic anhydride–concd sulfuric acid (40:20:1, v/v) at 85 °C overnight gave the triacetate **12** (48%). Treatment of **12** with excess sodium acetate resulted in preferential inversion at C-2 and C-3 to produce, after acetylation, the 5a-carba-β-altropyranosyl azide derivative **13** (70%). *O*-Deacetylation of **13** under Zemplén conditions, followed by selective tosylation in pyridine, gave the 6-tosylate **14** (75%). An elimination reaction of **14** with DBU in toluene proceeded readily to give the alkene **15** (65%), which was hydrogenated with 5% Pd/C to give the undesired 2,3,4-tri-*O*-acetyl-6-deoxy-5a-carba-β-DL-altropyranosyl azide[12] **16** (80%) as the sole product. This sequence could, however, provide the 5-epimer of **1** (Scheme 2).

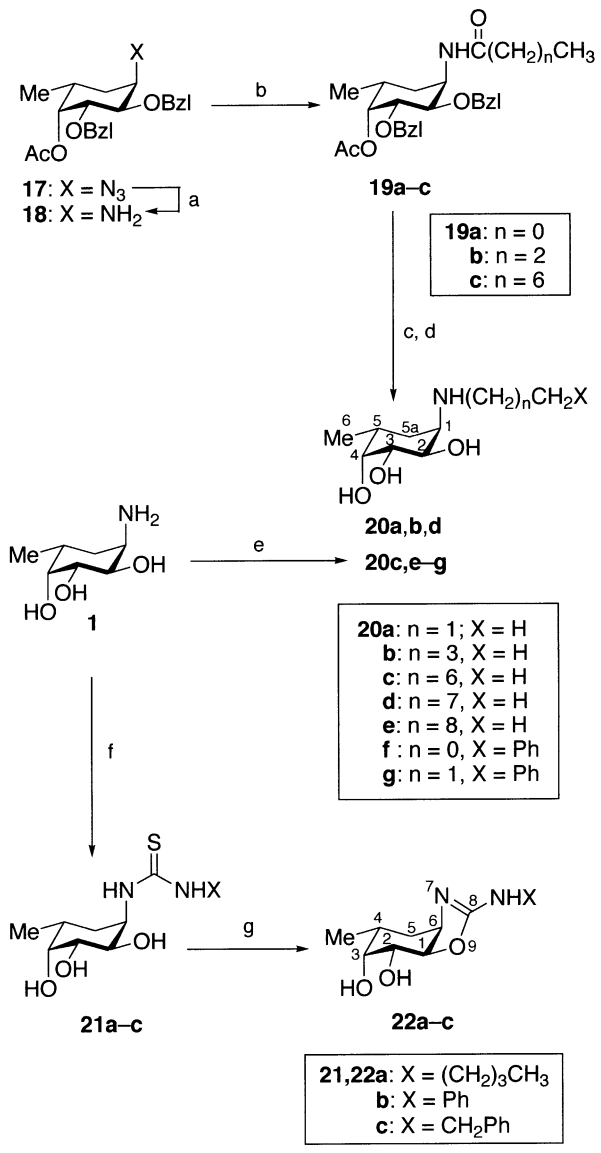
The *N*-alkyl derivatives[13] **20a**, **b**, **d** of **1** were initially prepared by lithium aluminum hydride reduction of the corresponding amides **19a–c** prepared from the protected amine **18** derived from the azide[11] **17**. Alternatively, direct reductive alkylation of **1** with the corresponding aldehydes proceeded smoothly on treatment with sodium cyanoborohydride in THF under slightly acidic conditions, leading to the *N*-alkyl and



Scheme 1. For convenience, the formulas depict only one enantiomer of the respective racemates. Reagents and conditions: (a) MeONa (4.5 molar equiv), MeOH, rt; NaH, BnBr, DMF; (b) NaN_3, (2 molar equiv), 90% aq 2-methoxyethanol, 100 °C; TsCl (4 molar equiv), pyridine; (c) H_2, Raney Ni, EtOH, Ac_2O; (d) 15% HBr–AcOH, 35 h, 85 °C; (e) AgF (2 molar equiv), pyridine; (f) H_2, PtO_2, EtOH; (g) MeONa, MeOH; (h) acetone, 10% H_2SO_4, 60 °C; Ac_2O, pyridine; (i) 4 M HCl, reflux, Dowex 50 W°2 (H^+) resin.



Scheme 2. For convenience, the formulas depict only one enantiomer of the respective racemates. Reagents and conditions: (a) AcOH/Ac_2O/H_2SO_4 (40:20:1, v/v), 30 h, 85 °C; (b) MeONa, MeOH; Ac_2O, pyridine; 10% H_2SO_4, acetone, 60 °C; Ac_2O, pyridine; (c) MeONa, MeOH; TsCl (2 molar equiv), pyridine; Ac_2O, pyridine; (d) DBU (4 molar equiv), toluene, rt; (e) H_2, PtO_2, EtOH.



Scheme 3. For convenience, the formulas depict only one enantiomer of the respective racemates. Reagents and conditions: (a) H_2, Raney Ni, EtOH; (b) RCOCl, pyridine; (c) LAH, THF, reflux; (d) 1 M HCl; MeOH, MS-4A, RCHO, $NaBH_3CN$, 20 h, rt; Ac_2O, pyridine; 4 M HCl, reflux; Dowex 50 W×2 (H^+) resin, 5% aq NH_3; (e) RNCS, 60% aq EtOH; (f) HgO (yellow), acetone/EtOH (1:1).

N-phenylalkyl derivatives **20c**, **e**–**g** in acceptable 45–70% yields (Scheme 3).

Furthermore, an attempt was made to improve inhibitory potential by incorporation of a cyclic isourea function with variable *N*-substituents,[14] thereby inducing change of the electron distribution and somewhat flattening the chair conformation without affecting the configurational arrangement of the two hydroxyl and methyl groups. Thus, the free amine **1** was treated with the corresponding alkyl and aryl isothiocyanates in aqueous 60% ethanol to yield the thioureas **21a**–**c** (~100%), which were treated with yellow mercuric oxide[15] in a mixture of acetone and ethanol to give the corresponding cyclic isoureas[16] **22a**–**c** (~100%).

Table 1. Enzyme inhibitory activity of compounds **1**, **2**, **20a**–**g**, and **22a**–**c**

Compd	Inhibitory activity, IC_{50} (K_i) (μM)	
	α-L-Fucosidase (bovine kidney)	β-Galactosidase (bovine liver)
1	9.3 (1.0)	NI
2	25 (2.9)	NI
20a	21 (0.18)	NI
20b	0.92 (0.074)	NI
20c	0.27 (0.048)	NT
20d	0.11 (0.016)	37
20e	0.24 (0.11)	NT
20f	1.0 (0.069)	NT
20g	0.24 (0.032)	NT
22a	140	NI
22b	7.6	NI
22c	72	NI
DFJ	0.41 (0.031)	NI

NI, no inhibitory activity was observed at 10^{-4} M; NT, not tested.

Biological Assay

Results of inhibition assay[17] of the newly prepared derivatives of **1** toward α-L-fucosidase (bovine kidney) and four other glycosidases are listed in Table 1. All compounds, except for **1**, **2**, and the reference compound DFJ, are racemic, and did not show any significant activity against either α-glucosidase (baker's yeast and rat intestine), α-mannosidase (Jack beans), or α-galactosidase (green coffee beans and rat liver). As shown within an only limited scope of racemic modifications[18] of the compounds tested, the inhibitory activity against α-L-fucosidase was dramatically increased by incorporation of alkyl and phenylalkyl groups into the amino function of the parent **1**. The changing the *N*-ethyl on **1** to a *N*-nonyl group clearly improved the inhibitory potential, reaching a maximum with an octyl chain: *N*-octyl-5a-carba-α-DL-fucopyranosylamine (**20d**) was shown to possess very strong and almost specific inhibitory activity[19] against α-L-fucosidase (bovine kidney), with *p*-nitrophenyl-α-L-fucopyranoside as substrate. The L-enantiomer should surely be evaluated as 5 times or more potent than DFJ, the strongest fucosidase inhibitor reported[7] so far. The results suggest that the catalytic site of the enzyme can tolerate addition of various sizes of aliphatic chain to the basic portion of the inhibitors involved in binding. Concerning chemical modification[8,20,21] of DFJ, *N*-alkylation was earlier found to result in rather a lowering of potential:[8] *N*-methyl DFJ has about one-fifth the potency of the parent. However, it is noteworthy that, the N-methyl derivative has any anti-(human immunodeficiency virus) activity,[8] although this is lacking with DFJ.

The octyl chain seems to act as a structurally efficient hydrophobic spacer, leading to proper electron-release to the nitrogen atom for docking at the active site of the enzyme. Interestingly, the results are in line with those observed for inhibition of glucocerebrosidase by *N*-alkyl-β-valienamines.[3] Thus, incorporation of *N*-alkyl functions could be clearly demonstrated to influence the activity of the inhibitors of this kind, suggesting that

there is much room for development of potent new forms by further modification.

Similar change of the potential has been observed for three cyclic isourea derivatives **22a**–**c**. The *N*-phenyl derivative **22b** showed good inhibitory activity, due to a possible stacking effect of the spacer phenyl group, indicating that a free hydroxyl group at C-2 is not always a minimum motif for attaining potential.[22]

The present work documents that an appreciable number of the derivatives tested have stronger inhibitory activity than the parent **1**. The *N*-octyl derivative **20d** has been shown to be a very strong and specific α-L-fucosidase inhibitor fully comparable to DFJ and other structurally related natural and synthetic inhibitors.[23] The inhibitory potentials of both enantiomers of **20c**–**e** clearly warrant assessment.

Acknowledgements

The authors sincerely thank Drs. A. Takahashi and A. Tomoda (Hokko Chemical Industry, Co. Ltd., Atsugi, Japan) for the biological assay, and Prof. Yoshiyuki Suzuki (International University of Health and Welfare, Otawara, Japan) for helpful discussions.

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12. ^{1}H NMR (300 MHz, $CDCl_3$) (inter alia): δ 4.79 (dd, 1H, $J_{3,4}=3.0$, $J_{4,5}=10.5$ Hz, H-4), 4.38 [ddd, 1H, $J_{1,2}$ 2.7, $J_{1,5a(ax)}$ 12.3, $J_{1,5a(eq)}=2.4$ Hz, H-1], 0.96 (d, 3H, *J* 6.4 Hz, Me).
13. The structures were confirmed on the basis of ^{1}H NMR spectra: for example ^{1}H NMR (300 MHz, CD_3OD) data for **20b**: δ 3.64 (dd, 1H, $J_{1,2}=4.9$, $J_{2,3}=10.2$ Hz, H-2), 3.63 (br d, 1H, $J_{3,4}=3.2$, $J_{4,5}=\sim 0$ Hz, H-4), 3.49 (dd, 1H, H-3), 2.86 (br s, 1H, H-1), 2.53 and 2.28 (2 ddd, each 1H, $J=7.5$, $J_{gem}=11.5$ Hz, $NHCH_2CH_2$), 1.72 (m, 1H, H-5), 1.48 [ddd, 1H, $J_{1,5a(eq)}=J_{5,5a(eq)}=\sim 3$, $J_{5agem}=14.6$ Hz, H-5a(eq)], 1.35–1.08 [m, 5H, H-5a(ax), $(CH_2)_2CH_3$], 0.76 (d, 3H, *J* 7.0 Hz, $CHCH_3$), 0.71 (t, 3H, $J=7.3$ Hz, CH_2CH_3); for **20d**: δ 3.83 (dd, 1H, $J_{1,2}=4.8$, $J_{2,3}=9.8$ Hz, H-2), 3.71 (br s, 1H, $J_{3,4}=J_{4,5}=\sim 0$ Hz, H-4), 3.48 (br d, 1H, H-3), 3.40 (br s, 1H, H-1), 2.91 (m, 1H, NCH_2CH_2), 1.74–1.44 (m, 3H, H-5, H-5a,5a), 1.25–1.05 [m, 12H, $(CH_2)_6CH_3$], 0.83 (br d, 3H, $J=6.6$ Hz, $CHCH_3$), 0.68 (m, 3H, CH_2CH_3). It is noteworthy that the chemical shifts of the signals due to H-1 and NCH_2CH_2 are shown to change appreciably according to the chain-length of *N*-alkyl group: for example in **20a**: δ 3.10, and δ 2.78 and 2.59, respectively.
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16. The structures were confirmed with reference to the ^{1}H NMR spectra of the corresponding di-*O*-acetyl derivatives: e.g., (1*RS*,2*RS*,3*RS*,4*RS*,6*RS*)-8-phenylamino-9-oxa-7-azabicyclo[4.3.0]non-7-ene-2,3-diol diacetate derived from **22b**: IR (neat): ν 1650 (C=N), 1750 (ester) cm^{-1}: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25–6.96 (m, 5H, Ph), 5.31 (t, 1H, $J_{2,3}=J_{3,4}=3.4$ Hz, H-3), 5.06 (dd, 1H, $J_{1,2}=7.3$ Hz, H-2), 4.58 (m, 2H, H-1, H-6), 2.47 [ddd, 1H, $J_{4,5(eq)}=6.1$, $J_{5(eq),6}=2.9$, $J_{5gem}=11.0$ Hz, H-5(eq)], 2.17 and 2.04 (2 s, each 3H, 2×Ac), 1.85 [ddd, 1H, $J_{4,5(ax)}=9.5$, $J_{5(ax),6}=5.1$ Hz, H-5(ax)], 0.97 (d, 3H, $J=6.6$ Hz, CMe).
17. Biological assays were carried out in a standard manner by Dr. Akihiro Tomoda (Hokko Chemical Industry, Co. Ltd.), to whom our thanks are due.
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