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SYNTHESIS OF POTENT β-D-GLUCOCEREBROSIDASE INHIBITORS: *N*-ALKYL-β-VALIENAMINES

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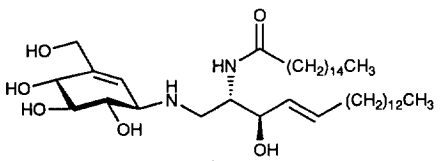
Abstract: Six homologous derivatives (*N*-butyl **3a**, hexyl **3b**, octyl **3c**, decyl **3d**, tetradecyl **3e** and stearyl **3f**) of β-valienamine were synthesized. All have been shown to be potent and specific inhibitors of β-glucocerebrosidase, and to have no potency against glucosylceramide synthase (mouse liver microsomes). Among them, the *N*-octyl derivative possesses the strongest activity (IC_{50} 3 x 10^{-8} M), being almost 10-fold more potent compared to the unsaturated 5a-carba-glucosylceramide **1**. Compounds **3b** and **3c** are also moderate inhibitors of α-glucosidase (Baker's yeast).

Keywords: Carbohydrate mimics, 5a-Carba-sugar derivatives, *N*-Alkyl glycosylamine analogues, glycosidase inhibitors, β-glucocerebrosidase inhibitors

Recent findings[1] of the potent and specific β-D-glucocerebrosidase inhibitors, 5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylceramides **1** (*E*- and *Z*-isomers) have stimulated us to develop a similar type of inhibitors by transforming β-valienamine **2** into some detrivatives with more simple structures. Extensive studies[2] on



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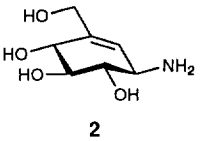
Unsaturated 5a-carbaglucosylceramide
(*E*- and *Z*-isomers)



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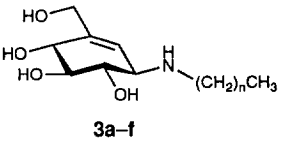
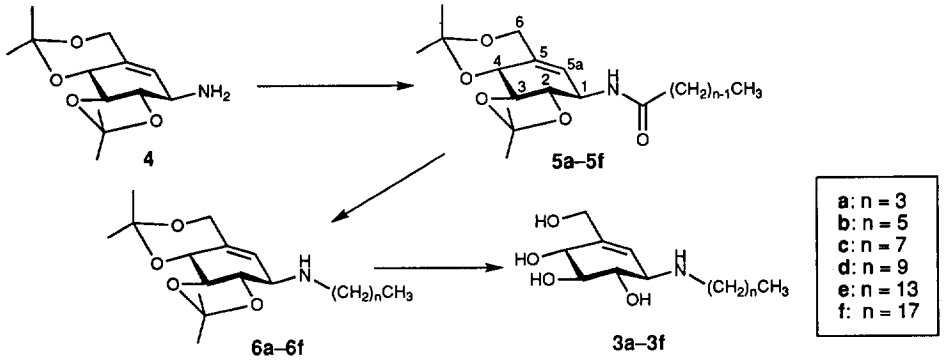


3a–f

n = 3, 5, 7, 9, 13, and 17



specific glucosylceramide synthase inhibitor PDMP (D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) and homologous series of compounds have so far been carried out in order to extend these potential from biological tools to therapeutic agents. Although 5a-carba-sugar analogues[1] of glycosylceramides were initially designed as inhibitors of glycosyl transferase, they have been shown to possess strong inhibitory-activity against β-glucocerebrosidase, but no potency against glucosylceramide synthase at all. Therefore, it has also become of interest to elucidate the structural features of these ceramide-analogues, which would specifically differentiate the inhibitory action against the two kind of enzymes.

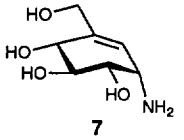
In this paper, in order to know inhibitory potency of 5a-carba-sugar analogues that mimic the potent glucosidase inhibitors[3] *N*-alkyl glucopyranosylamines, six homologous *N*-alkyl β-valienamines **3a–3f** were synthesized by the conventional procedure: conversion of the protected β-valienamine **4** into the corresponding amides, reduction with lithium aluminum hydride, and subsequent deprotection. Thus, acylation of di-*O*-isopropylidene-β-valienamine[4] (**4**) with *n*-octanoyl chloride (1.2 molar equiv.) in pyridine at room temperature produced the amide **5c**, quantitatively. Treatment of **5c** with an excess of lithium aluminum hydride (15 molar equiv.) in THF for 2 h at reflux temperature gave the protected amine **6c** in 85% yield. *O*-Deisopropylidenation was carried out with aqueous 80% acetic acid at 80°C for 4 h to give, after chromatography on silica gel with chloroform/methanol (3:1) as an eluent, the amine[5] **3c** as acetate in quantitative yield. Other homologous series of compounds[6] **3a**, **3b**, **3d** , **3e**, and **3f** were similarly prepapred as in the preparation of **3c**.

Inhibitory activity[7] of six compounds **3a–f** prepared in this work, along with those of ***E*-1**, conduritol B epoxide[3] **8**, and α- **7** and β-valienamines **2**, were listed in Table 1. Inhibitory activity against β-glucocerebrosidase seems to be largely depend on the length of *N*-alkyl chain. The activity of the most

Table 1. Inhibitory Activity of Six *N*-Alkyl-β-valienamines and Its Related Compounds against Three Enzymes

Compound	Inhibitory activity (IC_{50}, M)		
	β-Glucocerebrosidase	Glucocerebroside synthase	α-Glucosidase
5a-Carba-glucosylceramide ***E*-1**	3.0×10^{-7}	NI	NT
β-Valienamine **2**	NI	NI	1.0×10^{-4}
N-Butyl-β-valienamine **3a**	1.1×10^{-5}	NI	*
N-Hexyl-β-valienamine **3b**	3.0×10^{-7}	NI	5.0×10^{-5}
N-Octyl-β-valienamine **3c**	3.0×10^{-8}	NI	1.7×10^{-5}
N-Decyl-β-valienamine **3d**	7.0×10^{-8}	NT	NT
N-Tetradecyl-β-valienamine **3e**	1.2×10^{-7}	NT	NT
N-Stearyl-β-valienamine **3f**	3.0×10^{-7}	NI	*
α-Valienamine **7**	NI	NI	1.0×10^{-4}
Conduritol B epoxide **8**	1.1×10^{-6}	NI	*

*Activity less than IC_{50} 1.0×10^{-4} M; NI: No inhibitory activity observed at 1.0×10^{-4} M; NT: Not tested



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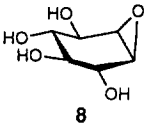

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potent *N*-octyl compound **3c** has been shown to be stronger than those of the analogues ***E*-1** and ***Z*-1** containing the natural ceramide hydrophobic portions and that of conduritol B epoxide[3] **8**, a well known irreversible inhibitor for this enzyme. These results demonstrated that the hydrophobic ceramide moiety can be replaced by a simple aliphatic chain without affecting the inhibitory activity, suggesting that this enzyme does not recognize so strictly the hydrophobic parts of the inhibitors. Since β-**2** and α-valienamines **7** have no activity against β-glucocerebrosidase, the *N*-alkyl chain with an appropriate length should be needed for exhibiting inhibitory-potency. Therefore, it is interesting to know the structure and inhibitory activity relationship of this kind of inhibitors by modifying the alkyl chains. Moreover, *N*-alkyl-β-valienamines are expected to act as specific competitive inhibitor, since valienamine moiety is seemed to mimic the transition state of the substrate for β-glucosidase reaction. On the other hand, compounds **3a–3f** completely lack inhibitory activity against glucocerebroside synthase. Then, our next targeted compounds should be the PDMP analogues, the morpholine parts of which being replaced by the β-valienamine residues.

References and Notes

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[5] Physical properties of compounds: compound **5c**, $[\alpha]_D^{24}$ -84° (*c* 0.8, $CHCl_3$), IR: 3280, 1650 cm^{-1} (amide); ^1H NMR (270 MHz, $CDCl_3$): δ 5.56 (d, 1 H, *J* 7.7 Hz, NH), 5.40 (s, 1 H, H-5a), 4.71 (dd, 1 H, *J* 7.7, 9.5 Hz, H-1), 4.63 (ds, 1 H, *J* 8.12 Hz, H-4), 4.45 and 4.16 (ABq, *J* 14.3 Hz, H_2-6), 3.79 (dd, 1 H, *J* 8.1, 9.5 Hz, H-3), 3.57 (t, 1 H, *J* 9.5 Hz, H-2), 2.20 (ddd, 1 H, *J* 5.1, 7.7, 14.7 Hz, H-2'), 2.15 (ddd, 1 H, *J* 5.1, 7.7, 14.7 Hz, H-2'), 1.63 (m, 2 H, H_2-3'), 1.55, 1.46, and 1.42 (3 s, 3, 6, 3 H, 2 CMe_2), 1.28 (m, 8 H, H_2-4', 5', 6', 7'), 0.87 (t, 3 H, *J* 6.6 Hz, Me). **6c**, $[\alpha]_D^{23}$ -46° (*c* 1.6, MeOH), IR: 3320 cm^{-1} (NH); ^1H NMR (270 MHz, $CDCl_3$): δ 5.46 (s, 1 H, H-5a), 4.62 (d, 1 H, *J* 7.7 Hz, H-4), 4.50 and 4.15 (ABq, *J* 13.9 Hz, H_2-6), 3.73 (dd, 1 H, *J* 7.7, 9.5 Hz, H-3), 3.50 (d, 1 H, *J* 8.4 Hz, H-1), 3.49 (dd, 1 H, *J* 8.4, 9.5 Hz, H-2), 2.72 (ddd, 2 H, *J* 5.1, 7.7, 14.3 Hz, H_2-1'), 1.56, 1.46, 1.42 (3 s, 3, 6, 3 H, 2 CMe_2), 1.30 (m, 12 H, H_2-2', 3', 4', 5', 6', 7'), 0.87 (m, 3 H, Me). **3c**, $[\alpha]_D^{22}$ -39° (*c* 0.6, MeOH), IR: 2920, 2858, 1630 cm^{-1} (NH_3^+); ^1H NMR (270 MHz, $CDCl_3/CD_3OD$ 2:1) δ 5.62 (s, 1 H, 5a-H), 4.22 and 4.17 (ABq, *J* 14.6 Hz, H_2-6), 4.16 (d, 1 H, *J* 7.7 Hz, H-4), 3.62 (t, 1 H, *J* 9.2 Hz, H-2), 3.58 (dd, 1 H, *J* 7.7, 9.2 Hz, H-3), 3.54 (d, 1 H, *J* 9.2 Hz, H-1), 2.99 (ddd, 1 H, *J* 7.5, 11.7 Hz, H-1'), 2.82 (ddd, *J* 7.5, 11.7 Hz, H-1'), 1.98 (s, 3 H, AcO^-), 1.66 (m, 14 H, H_2-2', 3', 4', 5', 6', 7'), 0.89 (t, 3 H, *J* 6.6 Hz, Me).

[6] All other compounds described herein gave the spectral data consistent with the assigned structures.

[7] Inhibitory activities (IC_{50}) listed in Table 1 were measured by the following procedures.
β-Glucocerebrosidase: The assay was performed with the fluorogenic substrate, NBD-glucosylceramide, with microsomal fraction of mouse liver in a total volume 0.2 ml containing glucocerebroside from Gaucher spleen, polyoxyethylene octylphenyl ether, sodium taurocholate and phosphate-citrate (pH 5.5) as reported[2].
Glucosylceramide synthase: UDP-glucose:ceramide glucosyltransferase was assayed with liver microsomes with slight modification of the method of Inokuchi and Radin (Inokuchi, J.-i.; Radin, N. S. *J. Lipid. Res.* **1987**, *28*, 565). Liposomes were prepared from *N*-octanoylsphingosine, dioleoyl phosphatidylcholine and brain sulfatide. The mixture (liposome and microsome) was incubated for 1 h with UDP-[^{3}H]glucose, β-NAD, DTT, EDTA, $MgCl_2$, and Tris-Cl (pH 7.4). The labeled GlcCer formed was isolated by partitioning between *t*-butyl methyl ether and 2-propanol aqueous Na_2SO_4 and counted without removing the precipitated protein.
α-Glucosidase: The assay was performed with *p*-nitrophenyl α-D-glucopyranoside (0.66 mM) as substrates in 0.1 M phosphate buffer, pH 6.8, with α-glucosidase from Baker's yeasts reported (Uchida, C.; Kimura, H.; Ogawa, S. *Bioorg. Med. Chem. Lett.* **1994**, *22*, 2643).

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