Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 14, pp. 1915-1920, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00341-7

SYNTHESIS AND BIOLOGICAL EVALUATION OF FOUR STEREOISOMERS OF PDMP-ANALOGUE, *N*-(2-DECYLAMINO-3-HYDROXY-3-PHENYLPROP-1-YL)-β-VALIENAMINE, AND RELATED COMPOUNDS

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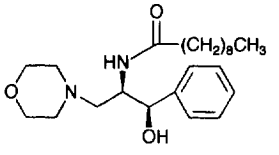
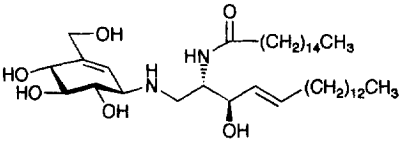
Abstaret: All stereoisomers with regard to C-1 and 2 of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) analogue containing unsaturated (β-valienamine) and saturated 5a-carba-β-D-glucopyranosylamine (β-validamine) residues in place of morpholine moiety were synthesized. Although PDMP is a potent and specific glucosylceramide synthase inhibitor, the former valienamine analogues (**4a–d**) have been shown to be strong glucocerebrosidase inhibitors (IC_{50} 3–7 $\times 10^{-7}$ M). The latter validamine analogues (**5a–d**) were also moderate glucocerebrosidase inhibitors (IC_{50} 5–20 $\times$ 10^{-6} M). A series of compounds synthesized lacked an inhibitory potency against the glucosyltransferase at all. Whereas the analogue **6a** composed of epimeric α-valienamine residue did not possess any potency against both enzymes. © 1997 Elsevier Science Ltd.

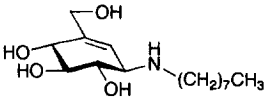
Very potent and specific glucosylceramide synthase inhibitor, PDMP (D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) **1** and homologues have so far been extensively studied[1–3] in order both to elucidate biosynthesis of glycosylceramides and glycolipids, and practically to extend these potential to therapeutic agents. We have previously synthesized several 5a-carba-sugar analogues[4,5] **2** (*E* and *Z*-isomers) of glycosylceramides to develop new type of glycosidase inhibitors connecting with glycosylceramide biosynthesis.



PDMP: 1
Glucosylceramide synthase inhibitor

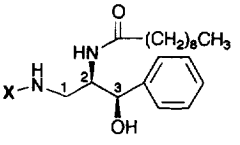


2
Glucocerebrosidase inhibitor



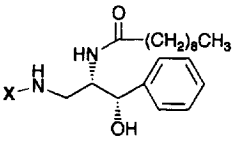
3
Glucocerebrosidase inhibitor

Both *E*- and *Z*-isomers of **2** are very potent and specific inhibitors of glucocerebrosidase. Also, the more simple analogue *N*-octyl-β-valienamine[6] **3** has been shown to possess about 10-fold higher potency compared to **2**, indicating the possibility of replacing a complex ceramide moiety by a simple aliphatic chain without affecting the inhibitory activity (in vitro). However, either inhibitors completely lack inhibition potential against glucosylceramide synthase. Therefore, we became interested in elucidation of the structure-inhibitory activity relationship, controlling a mode of the inhibitory action depending on the two enzymes involved in biosynthesis of glucosylceramide.



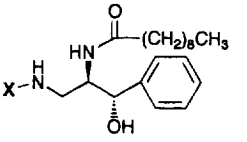
(2*R*,3*R*)

4a, 5a



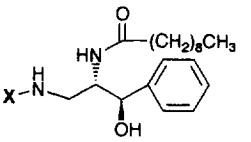
(2*S*,3*S*)

4b, 5b



(2*R*,3*S*)

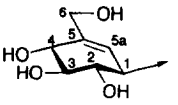
4c, 5c



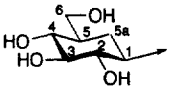
(2*S*,3*R*)

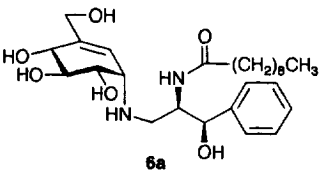
4d, 5d

4a–d: X =



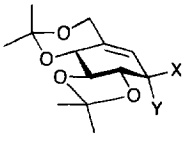
5a–d: X =



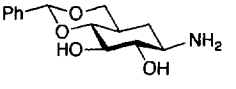


6a

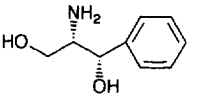
In this paper, the morpholine part of PDMP **1** was replaced with a β-valienamine residue, providing the target compound **4a**. Enzyme inhibitory activity of **4a** and its three stereoisomers **4b–d** with regard to the configurations of the 2-decanoylamino-3-phenylpropan-3-ol moiety initially seemed to shed a light on making clear the role of the morpholine moiety and giving a hint to design new type of inhibitors of glycosylceramide synthase. On the other hand, the analogues **5a–d** and **6** similar in kind, containing saturated β-validamine and 1-epimeric valienamine residues, respectively, were prepared to demonstrate a role of the unsaturated 5a-carba-β-D-glucopyranosylamine part.



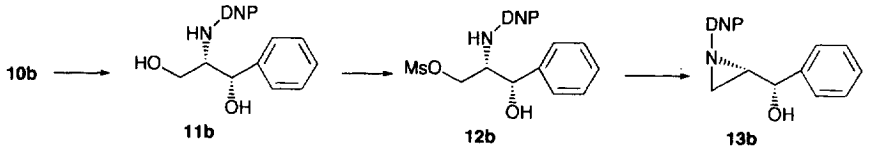
7: X = NH_2, Y = H
9: X = H, Y = NH_2



8

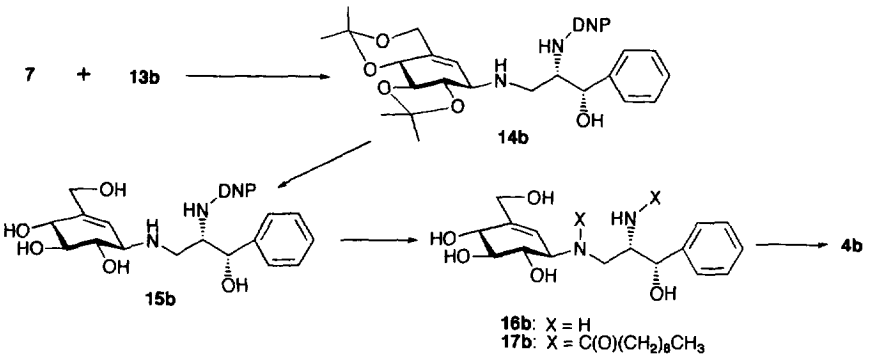


10b



11b **12b** **13b**

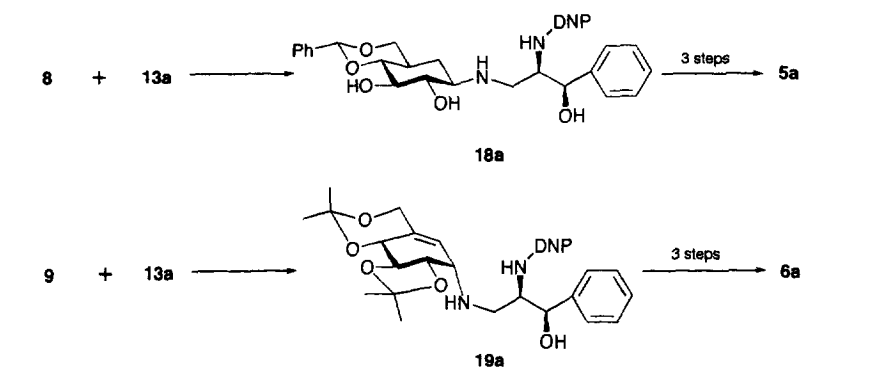
DNP = 2,4-dinitrophenyl



Incorporation of four stereoisomeric 2-decanoylamino-3-hydroxy-3-phenylprop-1-yl functions into β-valienamine by way of an imino bridge was carried out conventionally[5] by coupling of 2,3:4,6-di-*O*-isopropylidene-β-valienamine[7] **7** and newly prepared aziridines **13a–d**.

We described here a synthesis of (2*S*,3*S*)-isomer **4b** as a typical experiment using commercially available (1*S*,2*S*)-2-amino-1-phenyl-1,3-propanediol (**10b**). Treatment of **10b** with 2,4-dinitrophenylfluorobenzene gave the *N*-dinitrophenyl derivative[8] **11b** (97%). Selective sulfonylation of the primary hydroxyl group of **11b** with mesyl chloride (1.1 molar equiv.) in pyridine gave the mesylate **12b** (72%), which was subsequently treated with DBU (1.5 molar eq.) at 60°C in toluene to afford the aziridine[9] **13b**. Three stereoisomeric aziridines[10] **13a,c,d** were similarly prepared starting from the corresponding amino alcohols **10a,c,d** derived by hydrogenolysis of the known *N*-benzyloxycarbonyl derivatives.[11]

Condensation of **7** and **13b** proceeded cleanly in a sealed tube in 2-propanol (3 days, 120°C) to produce the coupling product[12] **14b** (77%). De-*O*-isopropylidenation of **14b** with aqueous 60% acetic acid (2 h, 60°C) gave the pentaol **15b** (86%), the *N*-protecting group of which was removed[13] (→ the free base **16b**) by treatment with Amberlite IRA-400 (OH^-) resin in acetone:MeOH:H_2O (3:5:2). Treatment of **16b** with an excess of decanoyl chloride in aqueous 30% NaOAc in THF:H_2O (3:2) for 2 h at room temperature gave the bisamide **17b**, which without isolation was subjected to a preferential hydrolysis of the tertiary amido group in aqueous M KOH-*t*-butanol (1:9) at 60°C for 12 h, affording desired compound[13] **4b** in 9% isolated yield based on **15b**.



Three stereoisomers[14] **4a,c,d** were similarly synthesized[15] from the coupling products **15a,c,d** in 9, 17, and 20% over-all yields, respectively.

The saturated analogues[16] **5a–d** were similarly obtained from the respective coupling products **18a–d** (~80%) of the protected validamine[5] **8** and the aziridines **13a–d**.

The 1-epimer[17] **6a** of **5a** was similarly prepared starting from the coupling product **19a** (85%) of the protected α-valienamine[18] **9** and the aziridine **13a**.

Table 1. Inhibitory activity (IC_{50}, M) of *N*-(2-decylamino-3-hydroxy-3-phenylprop-1-yl)-β-valienamines **4a–d** and its related saturated compounds **5a–d**, and the 1-epimer **6a** against two enzymes[19]

Compound	Inhibitory activity (IC_{50}, M)	
	β–Glucocerebrosidase	Glucosylceramide synthase
1	NI	2.3×10^{-5}
4a	7.0×10^{-7}	NI
4b	3.0×10^{-7}	NI
4c	7.0×10^{-7}	NI
4d	5.0×10^{-7}	NI
5a	1.0×10^{-5}	NI
5b	2.0×10^{-5}	NI
5c	7.0×10^{-6}	NI
5d	5.0×10^{-6}	NI
6a	NI	NI

NI: No inhibitory activity at 1.0×10^{-4} M.

All stereoisomers **4a–d** were demonstrated to be strong β-glucocerebrosidase inhibitors (Table 1). Whereas the saturated validamine-analogues **5a–d** were moderate inhibitors and the 1-epimer **6a** completely lost the potency, demonstrating that the 5a-carba-β-D-glucopyranosylamine moiety is indispensable to the glucocerebrosidase inhibitor and the unsaturated flattened half-chair conformation is certainly effective due to more resemblance to the postulated glucosyl cation formed during hydrolysis. Concerning inhibitory potency for the glucosylceramide synthase, all PDMP analogues synthesized have been shown to be inactive. Among four stereoisomers of PDMP, only D-*threo* isomer **1** possesses an inhibitory potency against the synthase. Therefore, the morpholine part presumably enhances a binding and/or an interaction existing between the synthase and the D-*threo*-2-decylamino-3-hydroxy-3-phenylpropane group, however, by contraries, the carba-sugar residue is likely to prevent it from funtioning as the synthase-inhibitor. The 2-decylamino-3-hydroxy-3-

phenylpropane moieties, independent of its absolute configuration, seem to play a role as a simple hydrophobic portion when the analogues act on β-glucocerebrosidase as inhibitors.

The present results conceivably suggest that an approach to design new potent inhibitors of the glycosyltransferases in future should be based on mimicking whole molecular features of the substrates, especially the ceramide hydrophobic parts, rather than the postulated structures of activated donor glycopyranosyl cations.

Acknowledgment

The authors thank Dr. M. Takebayashi (RIKEN, The Institute of Physical and Chemical Research, Frontier Research Program, Laboratory for Glycotechnology, Saitama, Japan) for measuring the HR-FAB-MS of our samples.

References and Notes

[1] Radin, N. S.; Shayman, J. A.; Inokuchi, J.-i.. *Adv. Lipid Res.* **1993**, *26*, 183.

[2] Inokuchi, J.-i.; Jimbo, M.; Momosaki, K.; Shimeno, H.; Nagamatsu, A.; Radin, N. S. *Cancer Res.* **1990**, *50*, 6731.

[3] Kyogashima, M.; Inoue, M.; Seto, A.; Inokuchi, J.-i. *Cancer Lett.* **1996**, *101*, 25, and references cited therein.

[4] Tsunoda, H.; Inokuchi, J.-i.; Yamagishi, K.; Ogawa, S. *Liebigs Ann. Chem.* **1995**, 279.

[5] Tsunoda, H.; Ogawa, S. *Liebigs Ann. Chem.* **1995**, 267.

[6] Ogawa, S.; Ashiura, M.; Uchida, C.; Watanabe, S.; Yamazaki, C.; Yamagishi, K.; Inokuchi, J.-i. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 929.

[7] Ogawa, S.; Uchida, C.; Shibata, Y. *Carbohydr. Res.* **1992**, *223*, 279.

[8] All new compounds described herein were homogeneous on TLC, elemental and ^{1}H NMR spectral analyses, being consistent with the assigned structures.

[9] Compound **13b**: $[\alpha]_D^{28}$ –326° (c = 0.53, $CHCl_3$), ^{1}H NMR (270 MHz, CD Cl_3) δ 8.85 (d, 1 H, J 2.6 Hz) and 8.24 (dd, 1 H, J 2.6, 9.2 Hz) (DNP), 7.52–7.33 (m, 5 H, phenyl), 7.14 (d, 1 H, J 9.2 Hz, DNP), 4.76 (t, 1 H, $J_{1,OH} = J_{1,2}$ = 5.1 Hz, H-1), 2.87–2.80 (m, 2 H, H-2, 3a), 2.55 (d, 1 H, $J_{1,OH}$ 4.8 Hz, OH), 2.20 (d, 1 H, $J_{2,3b}$ 5.9 Hz, H-3b).

[10] Compounds **13a**, $[\alpha]_D^{26}$ +434° (c = 0.53, $CHCl_3$); **13c**, $[\alpha]_D^{26}$ +112° (c = 0.87, $CHCl_3$); **13d**, $[\alpha]_D^{25}$ –102° (c = 1.1, $CHCl_3$).

[11] Oyamada, H.; Jimbo, M. *Jpn. Pat. Appl.* **JP 59576** (1996).

[12] Compound **14b**: $[\alpha]_D^{22}$ +95° (c = 1.1, $CHCl_3$), ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.14 (d, 1 H, J 8.1 Hz, N*H*DNP), 9.03 (d, 1 H, J 2.6 Hz) and 8.05 (dd, 1 H, J 2.6, 9.5 Hz) (DNP), 7.45–7.15 (m, 5 H, Ph), 6.75 (d, 1 H, J 9.5 Hz, DNP), 5.39 (s, 1 H, H-5a), 5.25 (d, 1 H, J 2.2 Hz, H-3'), 4.62 (d, 1 H, J 7.7 Hz, H-4), 4.48 (d, 1 H, J 13.9 Hz, H-6a), 4.16 (d, 1 H, J 14.7 Hz, H-6b), 4.07–3.96 (m, 1 H, H-2'), 3.74 (dd, 1 H, J 8.8, 8.1 Hz, H-3), 3.67–3.60 (m, 1 H, H-1), 3.46 (dd, 1 H, J 8.8, 9.2 Hz, H-2), 3.36 (dd, 1 H, J 5.7, 13.0

Hz, H-1'a), 3.23 (dd, 1 H, *J* 4.0, 13.2 Hz, H-1'b), 1.38–1.38 (4 s, 12 H, 4 × Me).

[13] Compound **4b**: $[\alpha]_D^{22}$ –49° (*c* = 0.87, MeOH), ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.43–7.19 (m, 5 H, Ph), 5.65 (s, 1 H, *J* 3.7 Hz, H-3'), 4.30–4.07 (m, 4 H, H-2', 4, 6a,b), 3.48 (t, 1 H, *J* 4.4 Hz, H-3), 3.47 (t, 1 H, *J* 6.05 Hz, H-2), 3.40–3.25 (m, 1 H, H-1), 3.04 (dd, 1 H, *J* 5.9, 12.1 Hz, H-1'a), 2.97 (dd, 1 H, *J* 7.7, 11.35 Hz, H-1'b), 2.13 (t, 2 H, *J* 7.3 Hz, $COCH_2$), 1.47–1.10 (m, 14 H, 7 × CH_2), 0.90 (t, 3 H, *J* 6.6 Hz, Me); HRMS *m/z* Calcd for $C_{26}H_{42}N_2O_6$: $[M+H]^+$, 479.3121. Found: 479.3114.

[14] Compounds **4a**, $[\alpha]_D^{27}$ –56° (*c* = 0.67, MeOH); **4c**, $[\alpha]_D^{25}$ –41° (*c* = 0.64, MeOH); **4d**, $[\alpha]_D^{25}$ –45° (*c* = 0.84, MeOH).

[15] The ease with which removal of the DNP protecting groups underwent in the presence of the basic resin seemed to be depend on the stereochemistry of C-2 and 3, the over-all yields of the free bases being largely influenced.

[16] Compound **5a**: $[\alpha]_D^{23}$–16° (*c* = 1.2, MeOH), ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.42–7.19 (m, 5 H, Ph), 4.91 (d, 1 H, *J* 2.2 Hz, H-3'), 4.32–4.23 (m, 1 H, H-2'), 3.76 (dd, 1 H, *J* 4.0, 10.6 Hz, H-6a), 3.57 (dd, 1 H, *J* 6.2, 10.6 Hz, H-6b), 3.22–3.09 (m, 4 H, H-2, 3, 4, 1'a), 2.97 (dd, 1 H, *J* 8.1, 11.7 Hz, H-1'b), 2.57 (m, 1 H, H-1), 2.12 (t, 2 H, *J* 7.5 Hz, $NHCOCH_2$), 2.08 (dt, 1 H, *J* 3.8, 3.8, 12.8 Hz, H-5a-eq), 1.05–1.02 (m, 16 H, H-5, 5a-ax, 7 × CH_2), 0.90 (t, 3 H, *J* 6.6 Hz, CH_3).
Compounds **5b**, $[\alpha]_D^{23}$ –2.6° (*c* = 1.3, MeOH); **5c**, $[\alpha]_D^{20}$ –9° (*c* = 1.6, MeOH); **5d**, $[\alpha]_D^{22}$ –16° (*c* = 1.2, MeOH).

[17] Compound **6a**: $[\alpha]_D^{22}$ +32° (*c* = 0.78, MeOH), ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.39–7.18 (m, 5 H, Ph), 5.83 (d, 1 H, *J* 3.3 Hz, H-5a), 4.94 (d, 1 H, *J* 3.3 Hz, H-3'), 4.26–4.13 (m, 3 H, H-6a, 6b, 2'), 3.96 (d, 1 H, *J* 6.2 Hz, H-4), 3.72 (dd, 1 H, *J* 6.6, 9.2 Hz, H-3), 3.59 (dd, 1 H, *J* 4.8, 9.5 Hz, H-2), 3.35–3.30 (m, 1 H, H-1), 2.93–2.88 (m, 2 H, H-1'a, 1'b), 2.11 (t, 2 H, *J* 7.5 Hz, $NHCOCH_2$), 1.51–1.11 (m, 14 H, 7 × CH_2), 0.90 (t, 3 H, *J* 6.8 Hz, CH_3).

[18] Ogawa, S.; Shibata, Y. *J. Chem. Soc., Chem. Commun.* **1988**, 605; Shibata, Y.; Ogawa, S. *Carbohydr. Res.* **1989**, *189*, 309.

[19] 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 phosphatecitrate (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, dioleoylphosphatidylcholine 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.

(*Received in Japan* 30 *April* 1997; *accepted* 19 *June* 1997)