Preparation of the active isomer of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, inhibitor of murine glucocerebroside synthetase

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Abstract 1-Phenyl-2-decanoylamino-3-morpholino-1-propanol was previously shown to be an effective inhibitor of the glucosyltransferase in liver that forms glucosylceramide. Since the inhibitor consists of four isomers, it was important for further testing to determine which isomer was most effective and to devise a method for preparation of this isomer. The mixture of isomers was synthesized as described before and separated by crystallization into two diastereomers, differing in migration rate with thin-layer chromatography (TLC) and in retention time with high performance liquid chromatography (HPLC). The slower moving diastereomer, which proved to be the active inhibitor, was separated into its enantiomers by crystallization with dibenzoyltartaric acid isomers. The inhibitory activity resided in the less soluble salt formed with the D-tartaric acid compound. The optical isomers could be characterized by TLC as their (1R)-(−)-camphanate esters. Using a second synthetic route, starting with L-threo- and DL-erythro-1-phenyl-2-amino-1,3-propanediol, we tentatively established the active form of the inhibitor to be the D-threo (1S,2R) isomer. ^{13}C NMR spectroscopy supported the *threo* and *erythro* assignments. Kinetic analysis showed that it acted uncompetitively against UDP-glucose and by mixed competition against ceramide, with K_i of 0.7 μM. The DL-*erythro* and DL-*threo* compounds inhibited brain galactosylceramide synthetase to a small extent. Glucosylceramide glucosidase activity of liver was unaffected by the DL-*threo* mixture.—**Inokuchi, J., and N. S. Radin.** Preparation of the active isomer of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, inhibitor of murine glucocerebroside synthetase. *J. Lipid Res.* 1987. **28:** 565–571.

Supplementary key words inhibitor of glucocerebroside synthesis • inhibitors of galactocerebroside synthesis • resolution of DL amines with dibenzoyltartaric acids

UDP-glucose:N-acylsphingosine glucosyltransferase (EC 2.4.1.80), which makes glucosylceramide (GlcCer), appears to be present in many organisms. Its product serves as the precursor for a large number of glycolipids, such as lactosylceramide, globosides, hematosides, and gangliosides, and a variety of derivatives found only or primarily in cancer cells. In the human genetic disorder, Gaucher disease, GlcCer accumulates in liver and spleen because of the low level of glucosidase activity. The normal catabolism of the glucolipids is thereby slowed while their biosynthesis apparently continues unabated.

It appeared possible (1, 2) that administering an inhibitor of the glucosyltransferase to Gaucher individuals would slow the formation of GlcCer to a rate matching their hydrolytic capability and prevent further accumulation of the lipid. Stored GlcCer might be utilized by the body for anabolic processes and thus disappear. To test this possibility, we have synthesized compounds resembling ceramide (the transferase's lipoidal substrate) and GlcCer (3, 4). One of these compounds, 2-decanoylamino-3-morpholino-propiophenone, proved to be an inactivating inhibitor which could lower the level of GlcCer in the liver of mice injected with the compound (1). The instability of the compound and its inhibitory action on monoamine oxidase (5) led us to study a more active inhibitor (**Fig. 1**), 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP). This compound was shown, at the 40 μM level, to block the outgrowth of neurites in cultured retina (A. M. Heacock, B. W. Agranoff, and N. S. Radin, unpublished work). It also blocked glucolipid synthesis and produced distinctive phenotypic changes in cultured 3T3 cells (Y. Okada, N. S. Radin, and S. Hakomori, unpublished work). Injection of PDMP into mice showed that it could slow GlcCer synthesis, as shown by a lower level of the lipid in liver (to be reported).

Abbreviations: TLC, thin-layer chromatography; hR_f, $100 \times R_f$, as defined by E. Stahl; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; GlcCer, glucosylceramide or glucocerebroside; C-M-E, chloroform–methanol–diethyl ether in solvent mixtures; HPLC, high performance liquid chromatography; PAPD, 1-phenyl-2-amino-1,3-propanediol.

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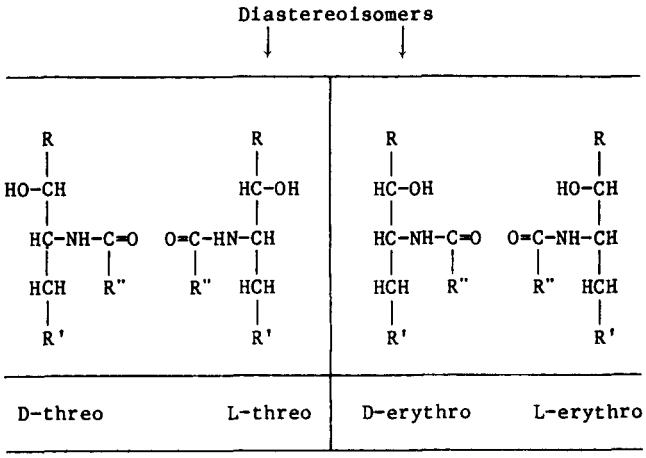

Fig. 1. Central structures of glucosylceramide and 1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Group R is a phenyl ring in PDMP and a pentadecenyl chain in GlcCer. Group R' is a morpholine ring in PDMP and a glucopyranoside ring in GlcCer. R" is the aliphatic chain of decanoic acid in PDMP; in GlcCer it is derived from a series of homologous fatty acids, C_{16-26}. The sphingols in naturally occurring GlcCer have the D-*erythro* configuration, so the enzymatically active form of PDMP differs sterically in the configuration of the hydroxyl group.

Inspection of the structure (Fig. 1) shows that two chiral centers are present, leading to four isomers (DL-*threo and* DL-*erythro*). TLC showed that the two diastereomeric forms could be separated (4). This report describes a procedure for large-scale separation of the two diastereomers and resolution of the enantiomers of the enzymatically active form, as well as their structural identification. Only one of the two enantiomers was active as an inhibitor. It is now possible to study the effectiveness of the active compound in vivo without fear of extraneous side effects from inactive isomers.

MATERIALS AND METHODS

TLC solvents

TLC was run with precoated silica gel 60 plates (E. Merck, #5763-7) 20 cm long, used without reactivation, and the spots were detected with 1% methanolic iodine. Solvent A was C-M-HOAc 90:10:10 (v/v/v); B was C-M-HOAc-acetonitrile 92:3:4:2, used without saturating the chamber.

High performance liquid chromatography

Separation of the *threo* and *erythro* forms was accomplished with a reverse phase column (Econosphere C8, 3μm particles, 4.6 × 100 mm, Alltech Associates, Deerfield, IL) protected by a C8 guard column. The solvent, 10 mM dibutylamine phosphate, pH 3 (Waters Associates, Milford, MA)-acetonitrile 60:40, was pumped at 1 ml/min. The effluent was monitored at 206 nm, the wavelength of maximal absorbance for PDMP.

Chemical synthesis and isomer separation

Most of the reagents were obtained from Aldrich Chemical, Milwaukee, WI. 2-Aminoacetophenone was acylated with decanoyl chloride in 95% yield, forming N-decanoyl-2-aminoacetophenone. The amidoketone (20 g, 68 mmol), paraformaldehyde (1.68 g, 55.4 mmol formaldehyde equivalent), morpholine (8.4 ml, 95.8 mmol), conc. HCl (2.3 ml), and 80 ml of 95% ethanol were refluxed for 15 hr (3, 5). The pH (wet indicator paper) was about 8.5. Sodium borohydride (9.6 g) in 200 ml of ethanol-water 75:25 and 16 ml of 1 mM NaOH was added dropwise to the stirred mixture over a 30-min period at 0°C. After overnight stirring at 22°C, the mixture was brought to pH 4 (wet indicator paper) with 1 N HCl and extracted with 2 × 150 ml of chloroform. The extract was washed with 300 ml of 10% NaCl, dried over sodium sulfate, and evaporated to an oil.

TLC of this product with solvent A revealed two spots of similar intensity, hRf 28 and 34, representing the *threo* and *erythro* forms. To separate the two forms, the oil was dissolved in 140 ml of chloroform, to which 280 ml of diethyl ether was added. Crystals slowly appeared on stirring at room temperature. After stirring at 4°C overnight, the resultant crystals were collected on a pressure filter and washed with 50 ml of cold C-E 1:2. The product was recrystallized from 75 ml of chloroform and 75 ml of ether as above, yielding 8.89 g of PDMP-I, the slower moving form of PDMP.HCl. Elemental analysis by Galbraith Laboratories: 63.75% C, 9.02% H, 6.39% N, 7.99% Cl, < 0.09% ash; 63.36% C, 9.25% H, 6.43% N, 8.13% Cl (theor. for the hemihydrate, mol wt 435). Based on the molecular weight for the hemihydrate, the yield of product was 20.4 mmol, 30% of the starting amidoketone. The compound did not lose weight on drying at 100°C over P_2O_5 under vacuum. The melting point was 155-156°C.

The material in the combined *filtrates* was crystallized from C-E 80:240 ml as above, yielding 6.5 g (22% yield based on the presumed anhydrous molecular weight of 427) of PDMP-Ia, corresponding to the faster of the two spots seen with solvent A. The melting point was 102-106°C.

TLC of PDMP-I, using a large sample (40 μg), showed that there was no visually detectable contamination with PDMP-Ia; however, the PDMP-Ia sample contained a trace of PDMP-I.

Resolution of optical isomers

Resolution of PDMP-I was accomplished by first converting 2.5 g (5.73 mmol) of the hydrochloride to the free base, by partitioning between 120 ml of chloroform -methanol-1 M ammonium hydroxide 8:4:3. The lower layer was washed several times with 70-ml portions of methanol-0.9% NaCl 1:1 to bring the pH of the aqueous layer to 7, then with 50 ml of water. The lower layer was evaporated to an oil, the residue was dissolved in ether, and the solution was dried further with sodium sulfate. Evapo-

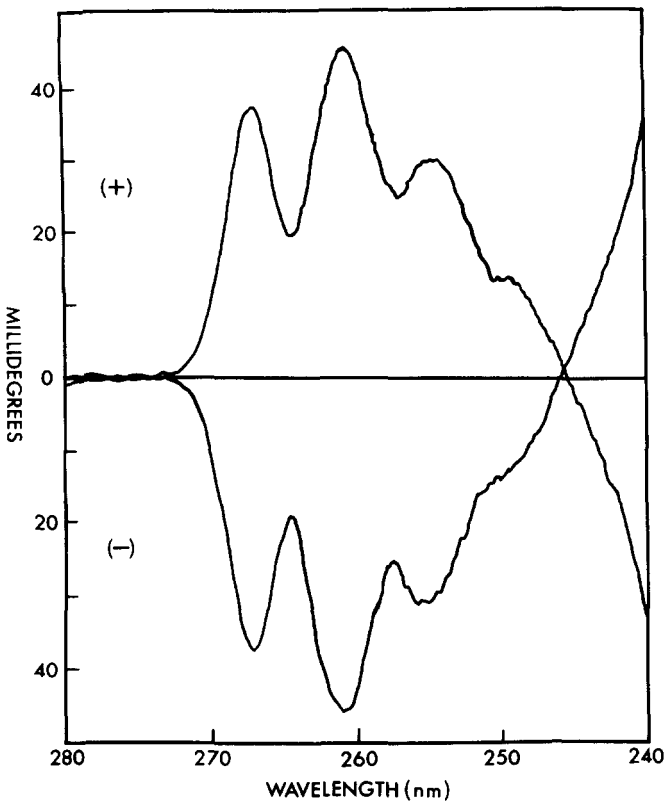

Fig. 2. Circular dichroism spectra of PDMP-II (upper curve) and PDMP-III (lower curve) enantiomers. The spectra were obtained with 9.2 mM solutions of the hydrochlorides in 2-propanol, using a 1-cm cuvette in a Jasco J-40C spectropolarimeter operating at a 2-nm bandwidth. The calculated $[\theta]_{261}$ is +484° for the largest peak of D-*threo* PDMP.

ration yielded 2.25 g (5.78 mmol) of an oil, which was dissolved in 80 ml of acetone. To this was added 2.19 g (5.81 mmol) of (+)-dibenzoyl-D-tartaric acid in 80 ml of acetone. Stirring at room temperature yielded some crystals and stirring was continued for no more than 4 additional hours. The crystals were collected on a pressure filter and washed with 2 × 10 ml of acetone; yield, 1.24 g (1.62 mmol, 55% of theory, assuming one molecule of water and one equivalent of each component). The pH of an aqueous solution of the salt was about 4.5, corresponding to an acid salt.

The combined filtrates were evaporated to dryness, dissolved in 80 ml of warm acetone, seeded with crystals from the first crop, and stirred for 5 hr. The second crop weighed 0.40 g (18%). Both crops melted at 158–159°C and produced only one iodine-positive TLC spot with solvent A (corresponding to PDMP-I). The base in the pooled crystals was designated PDMP-II. On drying as above, 2.05% of the weight was lost, corresponding to a monohydrate (2.35% theor.). Elemental analysis for the dried material: 65.08% C, 6.94% H, 3.57% N (found), 65.76% C, 7.00% H, 3.74% N (theor. for anhydrous, mol wt 748.9).

A similar procedure was used with another portion of PDMP-I and dibenzoyl-L-tartaric acid to yield the salt of the other optical isomer, PDMP-III. The melting point was the same as that of the enantiomeric salt, PDMP-II dibenzoyl tartrate.

The PDMP salts were converted to free bases by liquid/liquid partitioning as above against ammonium hydroxide (which brought all of the dibenzoyl tartrate into the water-rich layer) and then converted to the hydrochlorides with HCl.

Evaluation of the chiral purity

The camphanate ester was formed by dissolving 1–3 mg of PDMP • HCl and 2.5 equivalents of (1R)-(−)-camphanic acid chloride, 10% in pyridine. After 18 hr the excess chloride was decomposed by exposure to 50 μl of methanol for 1 hr. Rotoevaporation with toluene to remove the pyridine was followed by TLC with solvent B. This revealed two similar spots when PDMP-I was the starting material (hRf 30 and 33), while the product from the D-tartrate precipitation (PDMP-II) yielded only the upper spot and the product from L-tartrate precipitation (PDMP-

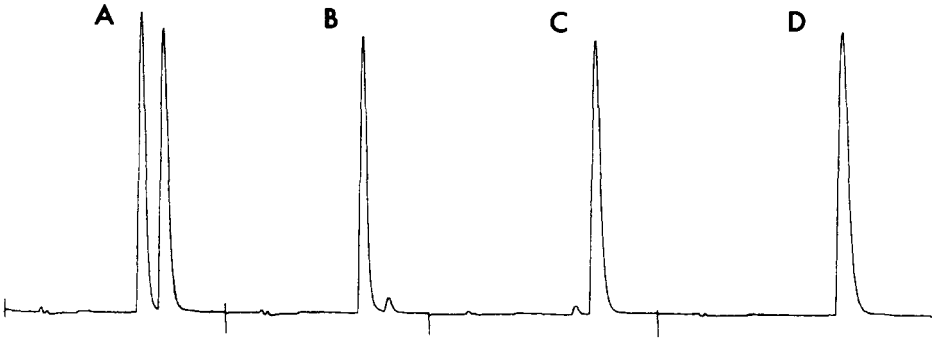

Fig. 3. Recordings from HPLC of PDMP preparations. The solvent was 10 mM dibutylamine phosphate, pH 3, and acetonitrile (60:40). Recorded at 206 nm, major peak heights were about 0.27 absorbance units. The sample in 3A consisted of 5 μg each of PDMP-I and Ia in 5 μl; 3B was derived from 5 μg of PDMP-Ia; 3C was from PDMP-I; and 3D was from PDMP-II.

III) yielded only the lower spot. The hRf for unesterified PDMP was 7. Semiquantitative TLC, run by mixing known amounts of the ester made from PDMP-I with 30 μg of the ester from PDMP-II showed the latter contained < 3% of the other isomer.

Chiral purity was also evaluated by circular dichroism (**Fig. 2**), which showed that the two enantiomers yielded very similar and symmetrically opposite spectra above 230 nm. The wavelengths of the peaks corresponded closely to those found by ultraviolet spectrophotometry.

Alternative synthetic route

(1R,2S)-2-amino-1-phenyl-1,3-propanediol (PAPD) (the L-*threo* compound) was from Research Organic/Inorganic Chemical Corp (Orange, CA) and the 1S,2S/1R,2R (or DL-*erythro*) isomer was a gift from Drs. Mildred Rebstock and H. M. Crooks of Parke, Davis & Co. The two compounds were N-acylated with decanoyl choloride, then tosylated with toluenesulfonyl chloride in pyridine at 0°C for 1 hr. Acetic anhydride (2.2 equiv., 3 hr, 25°C) was added to acetylate the secondary alcohol group. The excess reagents were decomposed with aqueous Na bicarbonate and the mixture was extracted with ethyl acetate. After further washing, the diester was heated with dry morpholine at 80°C for 20 hr. The resultant product was partitioned in chloroform–methanol–1 M HCl 8:4:3 and the lower layer was washed with methanol–saline 1:1, then with water. The residue from this was treated with NaOH in C–M to remove the O-acetyl group, converted to the hydrochloride by acid partitioning, and examined by TLC (solvent A).

Enzyme assays

UDP-glucose:ceramide glucosyltransferase (EC 2.4.1.80) was assayed with liver microsomes prepared by the method of Costantino-Ceccarini and Cestelli (6) from 24-day HSD-CFl mice (Harlan Industries). Liposomes were prepared from N-octanoyl sphingosine, lecithin, and Na cerebroside sulfate and incubated for 30 min with UDP[^{3}H]glucose, Tris-Cl$^-$, pH 7.4, DTE, EDTA, $MgCl_2$, and ATP (7). The labeled GlcCer formed was isolated by partitioning between chloroform, methanol, and aqueous NaCl and counted without removing the precipitated protein. The observed activity was proportional to the amount of enzyme, up to 120 μg of protein.

Galactosylceramide synthetase (EC 2.4.1.62) was assayed similarly with a mouse brain microsomal fraction, UDP[^{3}H]-galactose, and liposomes made from hydroxy ceramides (prepared from bovine brain cerebrosides), ethanolamine phospholipids (from bovine brain, Sigma Chemical, about 50% plasmalogen), and egg lecithin (6). The $MgCl_2$ concentration in the medium had to be reduced to 1.5 mM to prevent aggregation of the liposomes.

Glucocerebrosidase was assayed with an aqueous liver homogenate and [^{3}H]glucosylceramide (8).

Compounds to be tested as inhibitors were added to the incubation tubes in solution and the solvent was removed before addition of the other assay components.

Protein assay

Interfering contaminants were removed and the protein was determined colorimetrically by a combination of methods involving coprecipitation by trichloroacetic and phosphotungstic acids with RNA (9).

RESULTS

Characterization of PDMP isomers

Analytical HPLC of a mixture of the two diastereomers of PDMP resulted in two well-resolved peaks (**Fig. 3A**) with R_T of 5.5 and 6.4 min. Chromatography of PDMP-Ia alone showed that it was the source of the first peak and that it was contaminated by a small amount of the slower eluting isomer, 6.8% of the sample (Fig. 3B). Chromatography of PDMP-I alone showed that a small amount of the faster eluting isomer was present, 2.7% of the total (Fig. 3C). Chromatogram 2D, obtained from optically resolved PDMP-I (PDMP-II), showed only a single peak. PDMP-III, not shown, was also found to be pure and to exhibit the same R_T as PDMP-II. These observations are in agreement with those made by TLC.

Ultraviolet spectra of the resolved PDMP hydrochlorides II and III in isopropyl alcohol were similar and yielded four major peaks, three of which were relatively small. Measured at 9.2 mM, the latter molar absorptivities were 122 (263 nm), 169 (257 nm), and 71 (251 nm). The absorptivity and location of the major peak depended on the concentration: 5577 at 212 nm and 0.26 mM; 15,000 at 206 nm and 0.03 mM. Presumably this means that the compound self-associates at higher concentrations.

The product made by the alternative synthetic route from L-*threo*-PAPD yielded an iodine-positive spot corresponding to PDMP-I, the slower of the two PDMP spots. Evidently the heating with morpholine removed the O-acetyl group. This was confirmed by treating the product with NaOH in chloroform–methanol, which did not affect the hRf of the compound. The product made from *erythro*-PAPD yielded a spot corresponding to the faster of the two spots from PDMP (PDMP-Ia). From this it would appear that PDMP-I is the *threo* isomer and PMDP-Ia is the *erythro* isomer.

Further evidence for this conclusion comes from NMR spectroscopy, which was kindly run by Drs. A. Hara, T. Taketomi, and R. Irie (see Addendum).

To compare our resolved enantiomeric isomers, PDMP-II and PDMP-III, with the L-*threo*-PDMP preparation made by the alternative route, we purified the latter by preparative TLC on a 1-mm-thick silica gel, 20 × 20 cm plate, with solvent A. The band was extracted with C–M 2:1, the solution was evaporated to dryness, and the residue was dis-

TABLE 1. Effect of DL-*threo*- and DL-*erythro*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol on UDP-glucose:ceramide glucosyltransferase

Sample Tested	Observed Activity[a]	Specific Activity	Inhibition
	cpm	*nmol/hr/mg protein*	%
Controls	903;875	4.91	
PDMP-I 4 μM	664;626	3.56	27
PDMP-I 8 μM	464;496	2.65	46
PDMP-I 16 μM	324;365	1.91	61
PDMP-Ia 100 μM	493;	2.72	45
PDMP-Ia 200 μM	306;315	1.72	65
PDMP-Ia 500 μM	213;217	1.19	76

Enzyme was assayed with 60 μg of microsomal protein from livers of 23-day-old mice. Total radioactivity of UDP-glucose in each incubation tube was 193,100 cpm. The blank activity from incubation with boiled enzyme, including the radiation background, was about 39 cpm; this was subtracted from the observed readings.

[a]Duplicate incubations.

solved in 2 ml of chloroform and filtered. The residue from this purification was converted to the camphanate ester as above and found to give a spot corresponding to PDMP-III camphanate. It therefore appears likely that PDMP-II is the D-*threo* compound. However, the yield of L-*threo*-PDMP obtained through the tosylate route was low and the product contained a significant amount of byproducts, so this proof of structure needs further confirmation.

PDMP actions on glucosyltransferase

The PDMP-I preparation produced about 46% inhibition of ceramide glucosyltransferase at the level of 8 μM (**Table 1**). The same degree of inhibition was produced by 100 μM PDMP-Ia. Based on the HPLC analysis of diastereomer Ia, we can calculate that these incubation tubes contained 6.8 μM PDMP-I. This explains the observed amount of inhibitory activity in the *erythro* compound. A similar calculation explains the 65% inhibition seen with 200 μM PDMP-Ia. Thus it may be concluded that the *erythro* isomer is inactive toward this enzyme.

TABLE 2. Effect of separated and mixed optical isomers of *threo*-PDMP on glucosyltransferase activity

Sample Tested	Observed Activity[a]	Specific Activity	Inhibition
	cpm	*nmol/hr/mg protein*	%
Controls	821;885	4.87	
PDMP-II 2.5 μM	608;607	3.47	29
PDMP-II 5 μM	426;430	2.45	50
PDMP-III 2.5 μM	967;875	5.26	+ 8
PDMP-III 5 μM	888;879	5.04	+ 4
PDMP-II + III 2.5 μM	563;556	3.20	34
PDMP-II + III 5 μM	450;395	2.42	50
PDMP-I 5 μM	581;568	3.28	33
PDMP-I 10 μM	457;434	2.55	48

Assay conditions as in Table 1. The boiled enzyme blank activity was 41 cpm.

[a]Duplicate incubations.

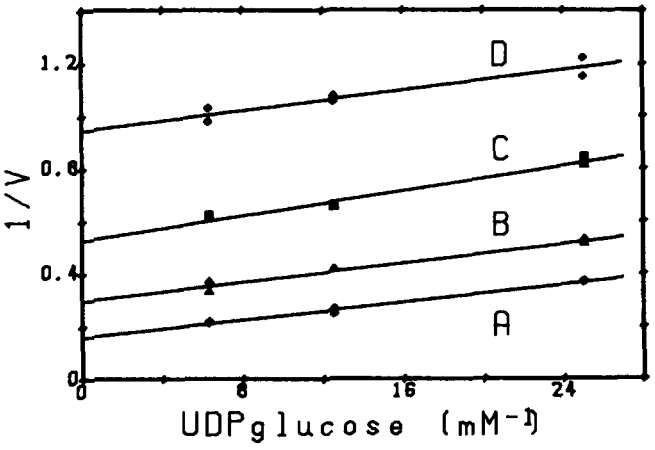

Fig. 4. Lineweaver-Burk plot of observed glucosyltransferase activities as a function of D-*threo*-PDMP and UDP-glucose concentrations. Line A, obtained without inhibitor; line B, with 3 μM inhibitor; line C, 10 μM inhibitor; line D, 20 μM inhibitor. Octanoyl sphingosine concentration was nominally 1 mM. V is the specific activity of the enzyme, nmol/hr per mg of protein.

Comparison of the optical isomers of PDMP-I (**Table 2**) showed that 5 μM PDMP-II produced about 50% inhibition while PDMP-III was completely inactive. Mixtures of isomers II and III produced the expected degrees of inhibition, showing that there is no unexpected mode of interaction between the two substances and the enzyme (e.g., a protective action of PDMP-III on the enzyme). Preparation PDMP-I yielded the same degrees of inhibition as the artificial mixtures of II and III, showing that the resolution process did not introduce an enzymatically inhibitory contaminant.

Kinetic analysis of the effect of PDMP-II showed that it was uncompetitive for UDP-glucose (**Fig. 4**). The ap-

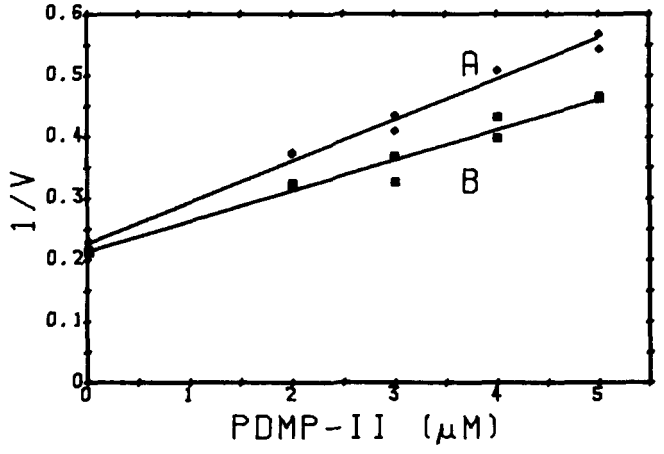

Fig. 5. Dixon plot of glucosyltransferase activities as a function of D-*threo*-PDMP and octanoyl-D-sphingosine concentrations. Line A, 0.6 mM ceramide; line B, 1 mM ceramide. V is the specific activity of the enzyme, nmol/hr per mg of protein. A Lineweaver-Burk plot of the same data clearly shows that the inhibition is both competitive and noncompetitive.

TABLE 3. Effect of DL-*threo*- and DL-*erythro*-PDMP on UDP-galactose:hydroxyceramide galactosyltransferase activity

Sample Tested	Observed Activity[a]	Specific Activity	Inhibition
	cpm	*nmol/hr/mg protein*	%
Controls	1000;998	1.90	
PDMP-I 10 μM	1038;1073	2.01	+ 6
PDMP-I 100 μM	848;772	1.54	19
PDMP-Ia 10 μM	1031;1078	2.01	+ 6
PDMP-Ia 100 μM	725;739	1.39	27

Tubes were incubated for 1 hr with 27 μg of brain microsomes from 23-day-old mice. Total radioactivity of UDP-galactose was 243,500 cpm/tube. The blank activity was 354 cpm.
[a]Duplicate incubations.

parent K_m for UDP-glucose was 55 μM. A study with two levels of octanoyl sphingosine as acceptor (**Fig. 5**) showed a mixed type of competition against ceramide with a K_i of 0.7 μM. (The extrapolation for the K_i value was made algebraically from a least squares calculation.)

Effects of PDMP on related enzymes

Both the DL-*erythro* and DL-*threo* isomers of PDMP showed weak inhibitory action on hydroxyceramide galactosyltransferase (**Table 3**). Glucocerebroside glucosidase was unaffected by PDMP-I at 10 to 40 μM.

Stability of PDMP

Initial attempts at chromatographic purification of PDMP with silica gel and a solvent consisting of chloroform, methanol, and acetic acid yielded impure products, evidently the result of chemical modication. Possibly the C-1 hydroxyl group, because of its benzylic character, is reactive under these conditions.

In water, however, PDMP seems to be quite stable. A 1-mM sample was stored at 41°C for 10 days in saline phosphate buffer, pH 7.4, and reexamined by TLC; no loss or new spot was seen.

DISCUSSION

The structural and enzymatic studies reported here indicate that only one of the four stereoisomeric forms of PDMP is active against UDP-glucose:ceramide glucosyltransferase. It is probably the 1S,2R (D-*threo*) isomer. This compound was made from readily available materials in satisfactory yield and is thus available for detailed investigations with small animals.

Comparison of the inhibitor structure (Fig. 1) with that of naturally occurring GlcCer shows that the phenyl group on C-1 corresponds to the long aliphatic chain of sphingosine, the hydroxyl group on C-1 corresponds to the hydroxyl on C-3 of sphingosine, the decanoylamino group on C-2 corresponds to the longer homologous group on C-2, and the morpholine on C-3 corresponds to the glucose group on sphingosine's C-1. Sphingols (10) have the *erythro* configuration (11), so it is unexpected that *erythro*-PDMP did not act as an inhibitor. Possibly the 3R configuration of the sphingol hydroxyl in ceramide serves, after binding to the glucosyltransferase, to reconfigure the enzyme to a catalytically active intermediate form. Similar striking differences between *threo* and *erythro* structures have been observed in previous studies of inhibitors of sphingolipid enzymes (2).

Our data from the kinetic study of PDMP-II's action on glucosyltransferase support the idea that the inhibitor binds stereospecifically to the ceramide-binding part of the active site and also at some modifying site on the enzyme molecule. The effectiveness of the basic nitrogen atom attached to C-3 in PDMP and other inhibitors (4) may indicate that this region of the inhibitor binds electrostatically to an acidic moiety in the enzyme's active region, presumably one that takes up the glucose from UDP-glucose.

ADDENDUM

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The ^{13}C NMR proton-decoupled spectra of the *threo*- and *erythro*-diasteroisomers were determined by power gated decoupling at 62.902 MHz with a Bruker AC-250 spectrometer (12). The chemical shifts (line 1 of **Table 4**) are distinctly different for the C-1 carbon atom of the propanol

TABLE 4. ^{13}C NMR chemical shifts (ppm) in the different moieties of *threo*- and *erythro*-DL-PDMP (upper table) and in three carbons of *threo*- and *erythro*-N-decanoyl-1-phenyl-2-amino-1,3-propanediol (bottom of table) measured by power gated decoupling at 62.902 MHz

Carbon Atom		PDMP-I (*threo*)	PDMP-Ia (*erythro*)
Propanol	C-1 CH	73.03	74.90
	C-2 CH	50.34	50.22
	C-3 CH_2	64.28	64.26
Acyl	C-1 C=0	176.52	176.14
	C-2 CH_2	36.70	36.79
	C-3 CH_2	32.45	32.45
	C-4 CH_2	29.95	29.92
	C-5 CH_2	29.90	29.89
	C-6 CH_2	29.83	29.84
	C-7 CH_2	29.66	29.61
	C-8 CH_2	25.99	25.96
	C-9 CH_2	23.18	23.18
	C-10 CH_3	14.27	14.27
Morpholine	CH_2-N	64.28	64.26
	CH_2-O	59.95	59.07
Phenyl	C-1	141.19	141.29
	C-2, C-6	126.52	127.24
	C-3, C-5	128.83	129.81
	C-4	128.37	128.79
		L-*threo*-DPAPD	DL-*erythro*-DPAPD
Propanediol	C-1	72.09	74.74
	C-2	56.85	56.26
	C-3	62.35	61.66

moiety, with PDMP-I showing a upfield shift compared to PDMP-Ia. Comparison of carbons 1, 2, and 3 of N-decanoyl-*threo*-1-phenyl-2-amino-1,3-propanediol (DPAPD) with those of the *erythro* compound (bottom of Table 4) show a similar relationship. A similar upfield shift had been observed for the C-3 carbon atom of *threo*-sphingosine (in sphingosyl phosphocholine) compared to the *erythro*-isomer (in glucosyl- and galactosyl sphingosine) (12). These observations confirm the conclusion drawn by the chemical method of characterization.

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