(−)3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic Acid [(−)DRF 2725]: A Dual PPAR Agonist with Potent Antihyperglycemic and Lipid Modulating Activity

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(−)DRF 2725 (**6**) is a phenoxazine analogue of phenyl propanoic acid. Compound **6** showed interesting dual activation of PPARα and PPARγ. In insulin resistant db/db mice, **6** showed better reduction of plasma glucose and triglyceride levels as compared to rosiglitazone. Compound **6** has also shown good oral bioavailability and impressive pharmacokinetic characteristics. Our study indicates that **6** has great potential as a drug for diabetes and dyslipidemia.

Introduction

Diabetes mellitus is a polygenic disorder affecting a significant proportion of the people in industrialized nations. Insulin resistance is a basic etiological factor for type 2 diabetes[1] and is also linked to a wide spectrum of other pathophysiologic conditions including hypertension, hyperlipidemia, atherosclerosis, and obesity which are collectively called syndrome X or insulin resistance associated disorders (IRAD).[2] Despite intense research, the understanding of the molecular mechanism of insulin resistance is not very clear. Troglitazone (**1**),[3] rosiglitazone (**2**),[4] and pioglitazone (**3**)[5] (Figure 1) belong to the class of thiazolidinedione (TZD) antidiabetic agents that improve the blood glucose level in type 2 diabetes by an insulin sensitizing mechanism. The molecular mechanism of TZD is activation of peroxisome proliferator-activator receptor gamma (PPARγ),[6] a member of a family of ligand activated nuclear hormone receptors. Fibrates are another class of drugs that reduce triglyceride and increase HDL levels through activation of PPARα, which is present predominantly in the liver. WY 14,643 (**4**)[7] is a more potent PPARα specific activator (Figure 1), which is used in this study for comparative purpose. A drug that improves insulin sensitivity and effectively decreases hyperlipidemia would be useful for the management of diabetes and dyslipidemia. With this aim, a discovery program was initiated to find novel compounds that activate both PPARα and PPARγ receptors. A few β-aryl α-hydroxy propanoic acids, their derivatives, and their analogues have been reported[8] to be useful in the treatment of hyperglycemia and hyperlipidemia. Structure−activity relationship to provide novel β-aryl α-oxysubstituted alkylcarboxylic acids were investigated by substituting the methylaminobenzoxazole group of SB 213068 (**5**)[8] with different tricyclic ring systems. This lead to the discovery of **6**, a phenoxazine analogue of phenylpropanoic acid, which showed dual PPARα and PPARγ activity in in vitro transactivation assay and also interesting blood glucose and triglyceride lowering activity in experimental animal models.

Results and Discussion

Compound **6** is prepared from phenoxazine using a synthetic route shown in Scheme 1. Phenoxazine **7** upon reaction with *p*-bromoethoxy benzaldehyde **8**[9] gave benzaldehyde derivative **9**. Reacting **9** with triethyl 2-ethoxy phosphonoacetate afforded propenoate **10** as a mixture of geometric isomers. Reduction of **10** using magnesium methanol gave propanoate **11**, which on hydrolysis using aqueous sodium hydroxide gave propanoic acid **12** in racemic form. Resolution of **12** using (*S*)(+)-2-phenyl glycinol followed by hydrolysis using sulfuric acid afforded the propanoic acid **6** in (−) form.

In an in vitro transactivation assay, **6** showed dual activation of PPARα and PPARγ as shown in Table 1. On the other hand, the (+) isomer of **6** showed weak activation for both the isoforms (Table 1). As mentioned in the literature,[6,10] rosiglitazone and WY 14,643 showed PPAR isoform specific activation.

In genetically diabetic, insulin resistant, hyperlipidemic db/db mice, 9 days treatment with **6** showed 56% reduction in plasma glucose and 62% reduction in triglyceride as compared to 33% reduction in glucose and 16% reduction in triglyceride by rosiglitazone at the same dose. (Figure 2).

In pharmacokinetic studies in male wistar rats, **6** showed very good oral bioavailability and impressive pharmacokinetic characteristics (Table 2).

In conclusion, the transactivation assay results showed that **6** is a dual activator of PPARα and PPARγ. In insulin resistant db/db mice, the compound treatment showed much better antidiabetic and hypolipidemic activity than rosiglitazone. The pharmacokinetic profile of this molecule is also quite impressive. Thus treatment with **6** showed interesting blood glucose and triglyceride lowering activity through dual activation of PPARα and γ receptors. Consequently, **6**, which belongs to a different chemical class from thiazolidinediones, has great

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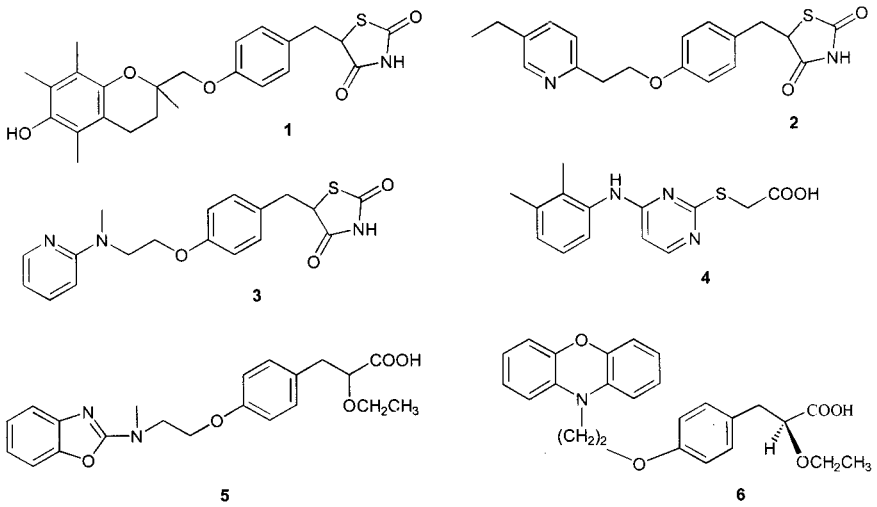
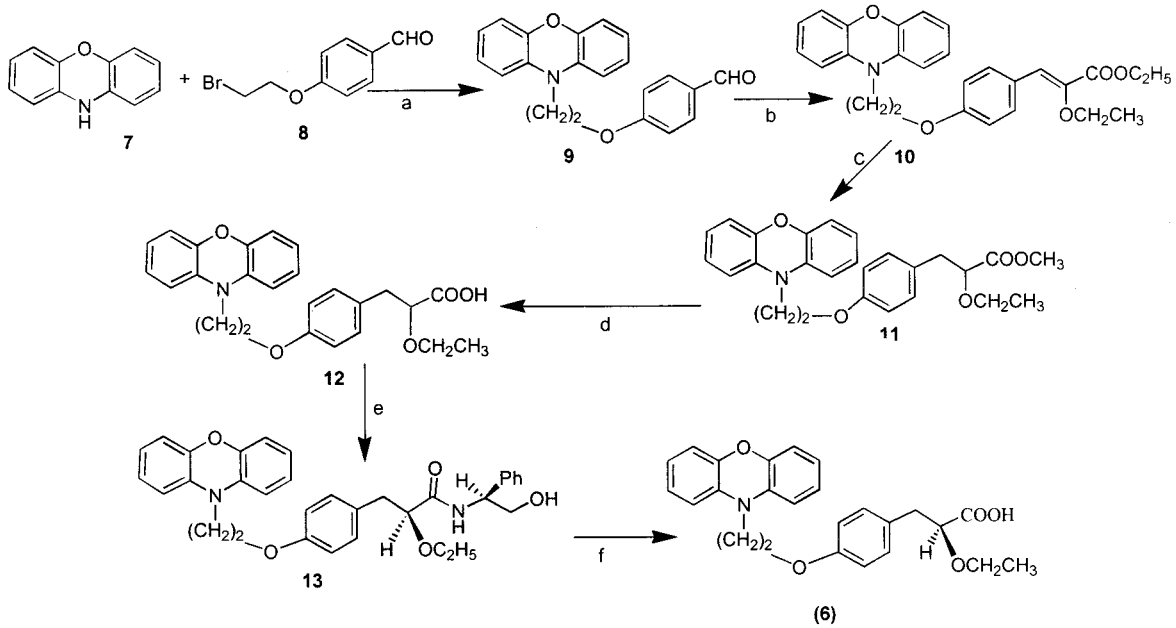


Figure 1. Structures of known PPAR activators and compound **6**.

Scheme 1[a]



[a] (a) NaH, DMF, 0−25 °C, 12 h; (b) triethyl 2-ethoxy phosphosphonoacetate, NaH, THF, 0−25 °C, 12 h; (c) Mg/CH_3OH, 25 °C, 12 h; (d) 10% aq NaOH, CH_3OH, 25 °C, 6 h; (e) (1) pivaloyl chloride, Et_3N, DCM, 0 °C, (2) (*S*)-2-phenyl glycinol/Et_3N; (f) 1 M H_2SO_4, dioxane/water, 90−100 °C, 80 h.

Table 1. PPAR Transactivation Study[a]

compd	PPARα fold activation (50 μM)	PPARγ fold activation (1 μM)
(−) isomer of **6**	10.87 ± 1.2	16.67 ± 1.24
(+) isomer of **6**	2.9 ± 0.3	1.23 ± 0.15
rosiglitazone	1.77 ± 0.07	18.4 ± 1.18
WY 14,643	12.5 ± 0.45	0.75 ± 0.07

[a] Values are expressed as mean ± SE ($n = 4$).

potential as a drug in the management of insulin resistance and dyslipidemia.

Experimental Section

Biology. Materials. The standard compounds, rosiglitazone (**2**) and WY 14,643 (**4**), were synthesized in-house using published procedures[4,7] and were found to be 99% pure. Plasma glucose and triglyceride levels were measured spectrophotometrically using commercially available kits (Point Scientific, USA). Carboxymethylcellulose (CMC) was obtained from LOBA Chemicals Pvt. Ltd., Mumbai, India.

PPAR Transactivation Assay. The reporter construct used for luciferase assay included (UASGAL4 × 5) response element upstream of pFR-Luc reporter under the Simian virus 40 early promoter. GAL4 fusions were made by fusing human PPARγ or PPARα ligand binding domain (amino acids: 174−475) to the C-terminal end of yeast GAL4 DNA binding domain (amino acids: 1−147) of pM1 vector. pAdVantage vector was used to enhance luciferase expression.

HEK 293T cells were transfected with relevant plasmids by Superfect as per the instruction manual.[11] Forty-two hours after transfection, cells were treated for 18 h with test compounds. DMSO (0.1%) was used as blank. Luciferase activity was determined as "fold activation" relative to untreated cells using the Luclite kit (Packard Instrument Co, Meriden, CT) in a Packard Top Count (Packard Instrument Co.)

Animal Experiments. Experiments were carried in accordance with internationally valid guidelines, with prior approval from Dr. Reddy's Research Foundation (DRF) animal ethics committee. Male C57 BL/Ks J-db/db mice were from the breeding stock of DRF animal house, generated from the breeding stock of Jackson Laboratories, ME. Animals were maintained under 12 h light and 12 h dark cycle at 25 ± 1 °C

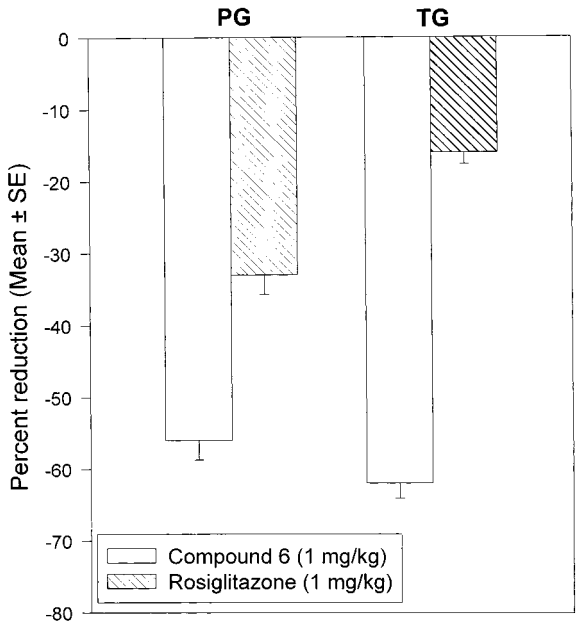


Figure 2. Comparative effect of compound **6** and rosiglitazone in db/db mice. Animals were treated for 9 days. All values are expressed as mean ± SE, $n = 5$.

Table 2. Pharmacokinetic Parameters of **6** in Male Wistar Rats[a]

pharmacokinetic parameter	oral	iv
dose	10 mg/kg	3 mg/kg
$AUC_{(0-\infty)}$ (μg h/mL)	71.40 ± 12.86	27.81 ± 5.85
C_{max} (μg/mL)	17.17 ± 4.22	18.33 ± 7.26[#]
T_{max} (h)	1.50 ± 0.58	-
K_{el} (h^{-1})	0.25 ± 0.0	0.15 ± 0.03
$T_{1/2}$ (h)	2.81 ± 0.06	4.69 ± 0.77
V_d (mL)	-	36.92 ± 19.9
Cl (mL/h)	-	21.32 ± 4.99
f (%)	77.02	-

[a] Each value is mean ± SD, $n = 4$; #C_0.

and were given standard chow supplied by National Institute of Nutrition, Hyderabad, India (NIN) and water ad libitum. db/db mice (8−9 weeks) were grouped according to blood sugar levels and treated with test compound at a dose of 1 mg/kg administered orally for 9 days. Animals in the control group received vehicle only (0.25% CMC, dose 10 mL/kg). Blood samples were collected from animals (in fed state) under mild ether anesthesia from the retro-orbital sinus 1 h after drug administration. Plasma samples were separated for glucose and triglyceride measurement. The percent reduction was calculated as per the formula:

$$1 - [(9\text{ day treated}/0\text{ day treated})/(9\text{ day control}/0\text{ day control})] \times 100$$

Pharmacokinetic studies were carried out in male Wistar rats (weight range 200−300 g) (NIN). For oral study, animals were fasted for 14 h and test compound was administered in 0.25% CMC suspension. For intravenous study, the compound was dissolved in a cocktail consisting of DMSO (6.6%), Cremophor ELP (6.6%), ethanol (3.3%), and saline (83.3%) and administered through tail vein. Blood samples were drawn from the retro-orbital sinus at 0.05, 0.5, 1, 2, 4, 6, 8, 10, and 21 h. The plasma was separated, processed with a suitable internal standard, extracted, and analyzed by a validated reverse phase HPLC method. The parameters were calculated based on noncompartmental model analysis. The oral bioavailability was calculated by the following formula:

$$f = [AUC_{(0-\infty)oral} \times \text{iv dose}] \times 100/[AUC_{(0-\infty)iv} \times \text{oral dose}]$$

Chemistry. Thin-layer chromatography was performed on precoated silica gel plates (F254, Merck). ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer and are reported as parts per million (ppm) from downfield to TMS. The mass spectra were recorded on a HP 5989A mass spectrometer. *p*-Bromoethoxy benzaldehyde[9] and triethyl-2-ethoxyphosphonoacetate were prepared according to the reported methods. Phenoxazine is procured from commercial sources. The chiral centers are fixed based on the optical rotation for appropriate compounds.

4-[2-(Phenoxazin-10-yl)ethoxy]benzaldehyde (9). To a suspension of sodium hydride (60% mineral oil, 7.86 g, 164 mmol) in dimethyl formamide (20 mL) was added a solution of phenoxazine (25 g, 136 mmol) in dimethyl formamide (250 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was heated at 60−80 °C for 1 h. The reaction mixture was cooled to 0 °C, and a solution of 4-(2-bromoethoxy)benzaldehyde (46.9 g, 204 mmol) in dry dimethyl formamide (100 mL) was added at 0 °C, and the stirring was continued for a further 12 h at ca. 25 °C. Water (250 mL) was added, and the mixture was extracted with ethyl acetate (2 × 150 mL). The combined organic extracts were washed with water (100 mL) and brine solution (100 mL), dried (Na_2SO_4), and filtered, and the solvent evaporated under reduced pressure. The residue was chromatographed over silica gel using a mixture of ethyl acetate and petroleum ether (1:9) as eluent to afford the title compound (25 g, 92%) as a greenish solid. ^1H NMR ($CDCl_3$, 200 MHz): δ 3.67 (t, $J = 6.2$ Hz, 1H), 4.05 (t, $J = 6.2$ Hz, 1H), 4.28 (t, $J = 6.3$ Hz, 1H), 4.38 (t, $J = 6.2$ Hz, 1H), 6.75 (m, 8H), 7.02 (m, 2H), 7.87 (m, 2H), 9.90 (s, 1H). Mass *m*/*z* (relative intensity) 331 (M^+, 49), 196 (100), 182 (23), 167 (10), 127 (7).

Ethyl (*E*/*Z*)-3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropenoate (10). A solution of triethyl-2-ethoxyphosphonoacetate (24.2 g, 90.0 mmol) prepared by the method of Grell and Machleid (*Ann. Chemie*, **1996**, *699*, 53) in dry tetrahydrofuran (30 mL) was added slowly to a stirred ice cooled suspension of sodium hydride (60% dispersion of oil) (4.32 g, 94.4 mmol) in dry tetrahydrofuran (100 mL), under a nitrogen atmosphere. The mixture was stirred at 0 °C for 30 min. followed by addition of a 4-[2-(phenoxazin-10-yl)ethoxy]-benzaldehyde (25.0 g, 75.5 mmol) in dry tetrahydrofuran (100 mL). The mixture was allowed to warm to room temperature and stirred at that temperature for further 12 h. The excess NaH was quenched with a few drops of cold water. The solvent was evaporated under reduced pressure and was distilled with water (150 mL) and extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), and filtered, and the solvent was evaporated under reduced pressure. The crude product was chromatographed over silica gel using a mixture of ethyl acetate and petroleum ether (1:9) as an eluent to afford the title compound (28.0 g, 83%) as a white solid, mp: 110−112 °C. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.16 and 1.38 (combined, 6H, isomeric $-OCH_2CH_3$ triplet signals), 3.89−4.05 (m, 4H), 4.14−4.31 (m, 4H), 6.06 (s, 0.36H, olefinic proton of *E* isomer), 6.66−6.95 (m, 10.6H), 7.75 (d, $J = 8.8$ Hz, 2H). Mass *m*/*z* (relative intensity): 445 (M^+, 50), 196 (100), 182 (29), 167 (7), 127 (5).

Methyl 3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoate (11). A mixture of ethyl (*E*/*Z*)-3-[4-[2-(phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropenoate (1.3 g, 2.9 mmol) and magnesium turnings (1.4 g, 58 mol) in dry methanol (50 mL) was stirred at 25 °C for 10 h. Water (100 mL) was added, and pH of the solution was adjusted to 6.5−7.5 with 2 N hydrochloric acid. The solution was extracted with ethyl acetate (3 × 75 mL). The organic layers were washed with water (50 mL) and brine (50 mL), dried (Na_2SO_4), and filtered, and solvent was evaporated under reduced pressure. The crude product was chromatographed over silica gel using a mixture of ethyl acetate and petroleum ether (2:8) as an eluent to afford the title compound (0.68 g, 52%) as a white solid, mp: 88−90 °C. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.16 (t, $J = 6.9$ Hz, 3H), 2.96 (d, $J = 6.6$ Hz, 2H), 3.22−3.40 (m, 1H), 3.51−3.66 (m, 1H), 3.68 (s, 3H), 4.00 (t, $J = 7.0$ Hz, 1H), 4.18

(m, 4H), 6.55−6.89 (m, 10H), 7.12 (d, J = 8.6 Hz, 2H). Mass m/z (relative intensity): 433 (M^+, 35), 196 (100), 182 (28), 167 (7), 127 (4). Purity by HPLC: 86.19%.

3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic Acid (12). To a solution of methyl 3-[4-[2-(phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoate (62 g, 139 mmol) in methanol (1000 mL) was added aqueous 10% sodium hydroxide (300 mL). The reaction mixture was stirred at ca. 25 °C for 6 h. The methanol was evaporated under reduced pressure, and water (200 mL) was added and acidified with 2 N hydrochloric acid. The mixture was extracted with ethyl acetate (3 × 500 mL). The combined ethyl acetate layers were washed with water (2 × 50 mL) and brine (500 mL), dried (Na_2SO_4), and filtered, and solvent was evaporated under reduced pressure. The residue was triturated with petroleum ether to afford the title compound (56 g, 96%) as a white solid, mp: 89−91 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.19 (t, J = 7.0 Hz, 3H), 2.90−3.18 (m, 2H), 3.41−3.62 (m, 2H), 3.90−4.10 (m, 3H), 4.18 (t, J = 6.2 Hz, 2H), 6.58−6.89 (m, 10H), 7.16 (d, J = 8.4 Hz, 2H), COOH proton is too broad to observe. Mass m/z (relative intensity): 419 (M^+, 26), 373 (5), 197 (16), 196 (100), 182 (38), 167 (5), 121 (16), 107 (37). Purity by HPLC: 98.52%.

[(2*S*),*N*(1*S*)]-3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxy-*N*-(2-hydroxy-1-phenylethyl)propanamide (13). To an ice cooled solution of 3-[4-[2-(phenoxazin-10-yl)ethoxy]-phenyl]-2-ethoxypropanoic acid (1.2 g, 2.9 mmol) and triethylamine (0.58 g, 5.8 mmol) in dry dichloromethane (25 mL) was added pivaloyl chloride (0.38 g, 3.19 mmol), and stirring was continued for further 30 min at 0 °C. A mixture of (*S*)(+)-2-phenyl glycinol (0.39 g, 2.9 mmol) and triethylamine (0.58 g, 5.8 mmol) in dichloromethane (20 mL) was added to the above reaction mixture at 0 °C, and stirring was continued for 2 h at 25 °C. Water (50 mL) was added and extracted with dichloromethane (2 × 50 mL). The organic extracts were washed with water (2 × 25 mL) and brine (25 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed over silica gel using a gradient of 40−60% ethyl acetate in petroleum ether as an eluent to afford first a diastereomer tentatively assigned as [2*R*,*N*(1*S*)]-3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxy-*N*-(2-hydroxy-1-phenylethyl)propanamide followed by [2*S*,*N*(1*S*)]-3-[4-[2-(phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxy-*N*-(2-hydroxy-1-phenylethyl)propanamide (0.5 g, 32%), mp: 139−141 °C. $[\alpha]_D^{25}$ = −13.3 (c = 1.00%, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.18 (t, J = 6.9 Hz, 3H), 2.05 (bs, 1H, D_2O exchangeable), 2.80−3.14 (m, 2H), 3.54 (q, J = 7.0 Hz, 2H), 3.85 (bs, 2H), 3.97 (m, 3H), 4.14 (t, J = 6.2 Hz, 2H), 4.92−5.01 (m, 1H), 6.62−6.85 (m, 9H), 7.02−7.20 (m, 5H), 7.26−7.30 (m, 3H), CONH is too broad to observe. Mass m/z (relative intensity): 538 (M^+, 65), 492 (6), 210 (8), 196 (100), 182 (12). Purity by HPLC: 99.4%.

(*S*)-3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic Acid (6). A solution of [(2*S*,*N*(1*S*)]-3-[4-[2-(phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxy-*N*-(2-hydroxy-1-phenyl)propanamide (0.45 g, 0.84 mmol) in a mixture of 1 M sulfuric acid (17 mL) and dioxane/water (1:1, 39 mL) was heated at 90 °C for 88 h. The pH of the mixture was adjusted to 3.0 by addition of an aqueous sodium hydrogen carbonate solution. The mixture was extracted with ethyl acetate (2 × 25 mL), and the organic extract was washed with water (50 mL) and brine (25 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed over silica gel using a gradient of 50−75% ethyl acetate in petroleum ether to afford the title compound (0.19 g, 54%) as a white solid, mp: 89−90 °C. $[\alpha]_D^{25}$ = − 12.6 (c = 1.0%, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.16 (t, J = 7.0 Hz, 3H), 1.42−1.91 (bs, 1H, D_2O exchangeable), 2.94−3.15 (m, 2H), 3.40−3.65 (m, 2H), 3.86−4.06 (m, 3H), 4.15 (t, J = 6.6 Hz, 2H), 6.63−6.83 (m, 10H), 7.13 (d, J = 8.5 Hz, 2H). Mass m/z (relative intensity): 419 (M^+, 41), 197 (15), 196 (100), 182 (35), 167 (7), 127 (6), 107 (19). Purity by HPLC: chemical purity: 99.5%; chiral purity: 94.6% (RT 27.5).

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