Expedited Articles

Sorbitol Dehydrogenase Inhibitors (SDIs): A New Potent, Enantiomeric SDI, 4-[2-1*R*-Hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic Acid Dimethylamide

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We report here on our medicinal chemistry and pharmacology efforts to provide a potent sorbitol dehydrogenase inhibitor (SDI) as a tool to probe a recently disclosed hypothesis centered on the role of sorbitol dehydrogenase (SDH) in the second step of the polyol pathway, under conditions of high glucose flux. Starting from a weak literature lead, **2**, and through newly developed structure−activity relationships, we have designed and executed an unambiguous synthesis of enantiomeric SDI, **6**, which is at least 10× more potent than **2**. Also, **6** potently inhibits SDH in streptozotocin-diabetic rat sciatic nerve. We have described an expedient synthesis of a key building template, **33**, for future research in the SDI area that may facilitate the discovery of even more potent SDIs with longer duration of action in vivo.

Sustained elevation of blood glucose is the hallmark of diabetes. Long-term diabetes results in maladies such as peripheral neuropathy, nephropathy, and retinopathy. In clinical practice these disorders could eventually lead to one or more devastating consequences which include lower limb amputation, end-stage renal failure, and loss of vision. In addition to human suffering, these complications impose a heavy burden both on individual and national medical budgets. Two landmark studies, the Diabetes Complications and Control Trial[1] and the United Kingdom Prospective Diabetes Study[2] have demonstrated both the benefits of lowering blood glucose through intensive insulin therapy and the strong connection between diabetic complications and chronic excess circulating blood glucose. Over the last several years, considerable effort has focused on developing an oral therapy that would protect vulnerable diabetic tissues from the detrimental effects of elevated blood glucose. One of the most investigated approaches has been to block excess glucose metabolism through the first step of the polyol pathway (Figure 1) via aldose reductase inhibitors[3] (ARIs).

Recently, Williamson et al.[4] have focused attention on another aspect of the polyol pathway. Excess glucose flux through the polyol pathway is proposed to create an imbalance in the cytoplasmic redox status (increased free NADH/NAD^+ ratio) because of rapid consumption of NAD^+ during the oxidation of sorbitol to fructose by sorbitol dehydrogenase (SDH) (Figure 1). They propose that the reductive stress ("pseudohypoxia") triggers a cascade of biochemical changes which may underlie

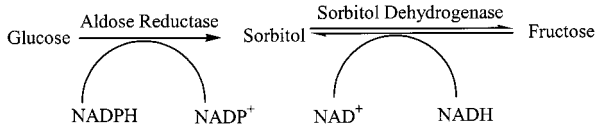

Figure 1.

many of the early functional changes observed in experimental diabetes, including alterations in vascular albumin permeation, tissue blood flow, and function, e.g., nerve conduction velocity (NCV). In turn, these early functional changes may contribute to eventual pathology in affected diabetic tissues.

A significant aspect of the pseudohypoxia theory is that it provides an exciting framework within which to explore the potential consequences of selectively blocking the second step of the polyol pathway with a specific sorbitol dehydrogenase inhibitor (SDI). Such an agent would accentuate sorbitol concentrations while tending to correct the tandem increases in NADH/NAD^+ and fructose levels in diabetic tissues. Obrosova et al.[5] confirm the significant inhibition of fructose in diabetic rats with SDI 157 (same as **1**, a prodrug of **2**), but did not detect any changes in cytosolic NAD^+/NADH. Seemingly confounding results have been obtained in NCV studies with a prototype SDI, **2**[6−9] (Chart 1), and genetically altered mice.[10] However, discrepant results with SDI **2** could be based on the use of different models and experimental protocols (for example, prevention vs reversal) and duration of dosing with **2**, which is a weak and short-lived SDI (vide infra). To provide a better tool for future studies, our initial thrust has been to address the SDI potency issue. Herein, we describe our efforts leading to the discovery of enantiomeric SDI, 4-[2-(1*R*-hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid

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Chart 1

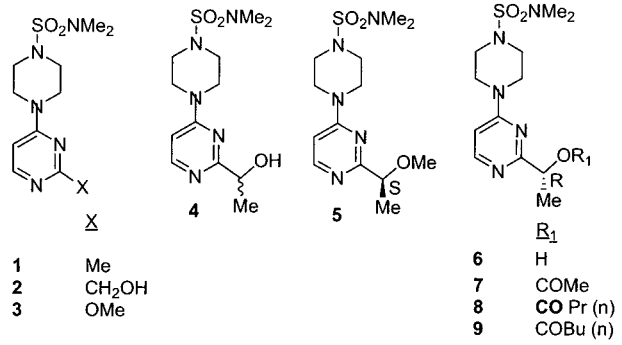
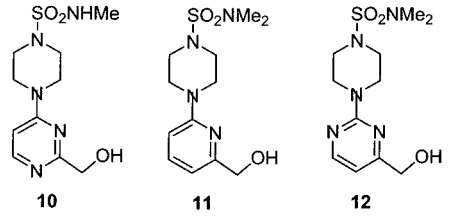


Chart 2

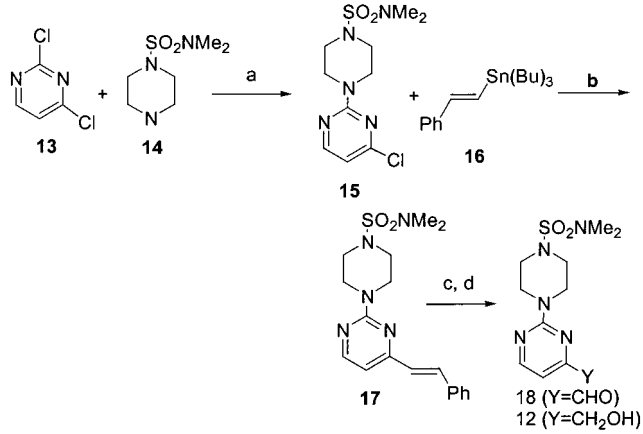


dimethylamide, **6**, which is significantly more potent than **2**, both in vitro and in vivo. We believe that the recently disclosed rat pharmacokinetic and metabolism results with **2**,[11,12] and our discovery of **6** coupled with development of a practical preparation procedure for a key building template, **33**, have set the stage for future clarification of the role of SDH in normal and diabetic tissues. These could include both potential benefits[6−9] and liabilities[6,13] of SDIs and examination of the potential consequences of sequentially blocking both AR and SDH, through ARI+SDI combinations.[14]

Geisen et al.[6] report that **2** inhibits sheep liver SDH and has a K_i of 92 μM for the oxidation of sorbitol to fructose and causes a dose-dependent increase in the sorbitol concentration in the sciatic nerve and in the erythrocytes of normal and streptozotocin-diabetic rats. We characterized the in vitro effects of **2** on both rat and human SDHs and the in vivo effects of **2** in our diabetic rat model. Both SDHs have been cloned,[15] and the recombinant enzymes have been made available to us.[16] We have confirmed that **2** is a moderately potent inhibitor of the rat SDH and that it also inhibits human SDH. Its IC_{50} values against the enzymes, respectively, are 225 and 246 nM. It is exquisitely selective for SDH. Against other dehydrogenases, including lactate, alcohol, fructose, and glyceraldehyde-3-phosphate, as well as aldose reductase, it showed no effect even at 50 μM (data not shown). The calculated oral ED_{50} from the dose−response for inhibition of nerve fructose in streptozotocin-diabetic rats is 14 mg/kg.

According to pharmacokinetic (PK) data[11] obtained with normal rats following oral administration of **2**, it has a very short $t_{1/2}$ (<30 min) both in serum and sciatic nerve. Rats fairly rapidly metabolize **2** to the corresponding *N*-monodemethylated compound,[12] **10** (Chart 2), which is a very weak SDI (IC_{50} > 10 μM). The excellent oral absorption of **2** as reflected in a high serum concentration and its efficient distribution into a highly protected nerve tissue are consistent with its pK_a (6.1), log *P* (0.55), high aqueous solubility (3 mg/mL), and very low protein binding (<1%).

Scheme 1[a]



[a] Reagents and conditions: (a) TEA/DMF; (b) AlBN, 80 °C; (c) $OsO_4/NaIO_4$, dioxane/H_2O, (d) $NaBH_4$/EtOH.

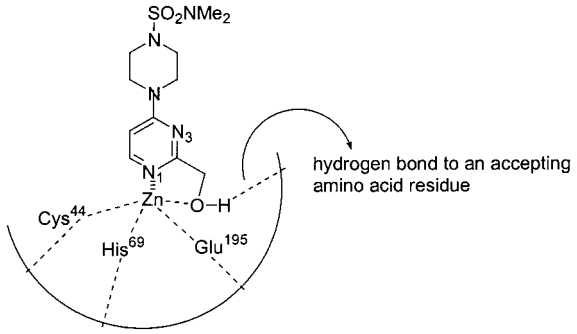


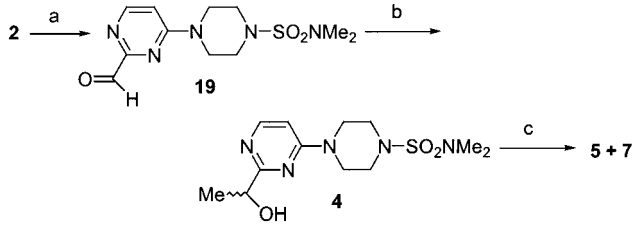
Figure 2.

SDH, like the well-studied liver alcohol dehydrogenase (ADH), is a zinc-containing enzyme.[17] It has been speculated that the hydroxymethyl group of **2** serves as a strong ligand to the zinc atom, mimicking the hydroxymethyl group of sorbitol.[18] While we agree with this speculation, we suggest that the proposed model is incorrect in regard to the depicted N_3 nitrogen ligand to the zinc atom, because neither **11**[19] nor **12** (Scheme 1) show any SDH inhibition activity, even at the highest dose tested, 10 μM. Also, it appears reasonable to expect the less sterically hindered N-1 atom of **2** to be a better co-ligand than the N-3 atom. The crucial role of the hydroxymethyl group as a cooperative zinc ligand is entirely congruent with the extremely poor SDI activities of **1** and **3** (IC_{50} > 10 μM).

On the basis of the above observations and the results of the site-directed mutation on structure−function carried out by Hoog et al.,[20a,b] we propose a revised model shown in Figure 2. Definitive proof for this model will have to await data from X-ray crystal structure studies on SDH and complexes of SDH−SDI.

Because the specific conformation of the hydroxymethyl in **2** would be expected to have a significant impact on its zinc-liganding strength, we examined the effect of chirality at the hydroxymethyl carbon on SDH inhibition potency. As a test case, we first prepared **4** (racemate) to make sure that it is at least as potent an SDI as **2**. It was prepared by oxidation of **2**, under Swern conditions, to the corresponding aldehyde **19** and then allowing it to react with methyl Grignard (Scheme 2). It was found that **19** was at least as potent as **2** with an IC_{50} of 173 nM. Spurred by this finding, **19** was

Scheme 2[a]



[a] Reagents and conditions: (a) $(COCl)_2$/DMSO; (b) MeMgBr/THF; (c) lipase P30/DME.

subjected to a lipase-mediated resolution of enantiomers, using lipase P30. Reaction monitoring showed the formation of a less polar compound. Separation of the reaction mixture gave **7**, which showed optical activity with a positive rotation. Base hydrolysis of **7** gave **6**. A second component isolated from the reaction mixture was **5** with an optical rotation equal in magnitude, but of opposite sign relative to **6**.

To ascertain the absolute configuration of the hydroxyethyl group of **6**, we embarked on its total synthesis starting from the methyl ester of *R*(+) lactic acid, **20** (Scheme 3). Through the first four steps, **20** was converted to the known amide **21**.[21] Dehydration of **21** using P_2O_5 gave the corresponding nitrile, **22**, which served as a precursor to the desired amidine **15**, via the intermediate imidate, **23**. A cyclo-condensation reaction of **24** with **25** gave **26**, which was converted to the corresponding sulfonate ester **27**. Displacement of the sulfonate moiety by **14** gave **28**. Demethylation of **28** gave the dextrorotatory **6**, thus unambiguously establishing the *R* configuration for the chiral center in **6**. To be sure that the chiral center integrity was maintained through the synthetic steps, we reacted **4**, **5** (Scheme 2), and **6** (Scheme 3) with *R*-α-methoxy-α-trifluoromethylphenyl acetyl chloride and obtained a

Table 1. In Vitro and in Vivo Data for SDIs

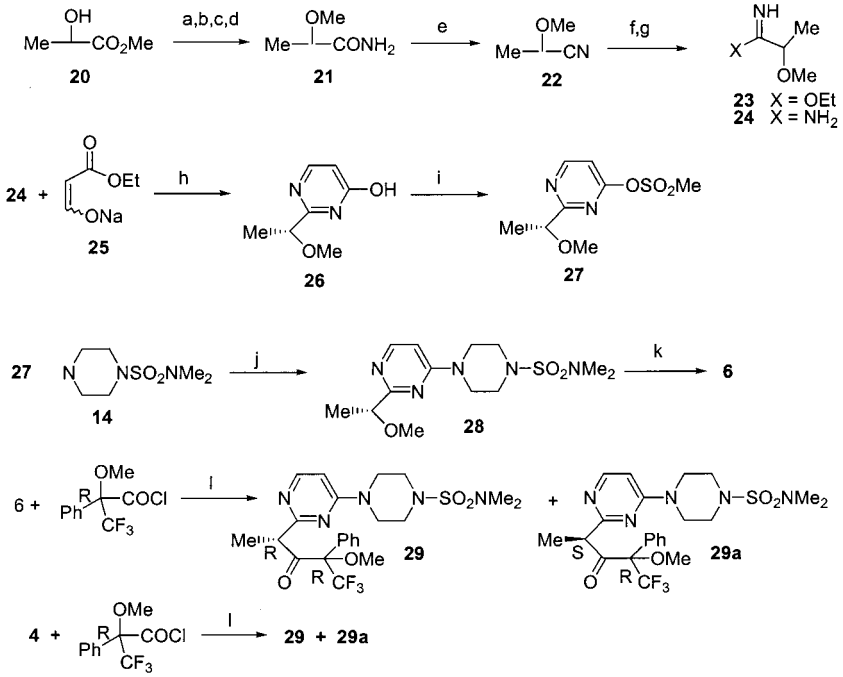
entry	IC_{50} (h-SDH) (nM)	ED_{50}[a] (mg/kg)
2	246 ± 35	14.1 ± 1.6
4	173 ± 50	2.4 ± 0.08
5	486 ± 24	9.0 ± 1.4
6	27 ± 4	1.6 ± 0.1

[a] For inhibition of diabetic rat sciatic nerve fructose accumulation.

mixture of *RR* and *RS* diastereomeric esters, **29** and **29a**. The structural assignment as well as enantiomeric purity of these esters was determined from their NMR spectra, by monitoring the signal for the methyl group attached to the chiral carbon. All the esters showed a pair of doublets at 1.61 and 1.68 ppm. The ratio of the area under the signals for **4**, **5**, and **6**, respectively, was 1:1 (as expected), 97:3, and 4:96. Results of in vitro testing of the *R* and *S* enantiomers are shown in Table 1. The *R* enantiomer, **6**, was found to be a significantly more potent inhibitor (>10×) than the *S* enantiomer, **5**, as well as **2** against h-SDH.

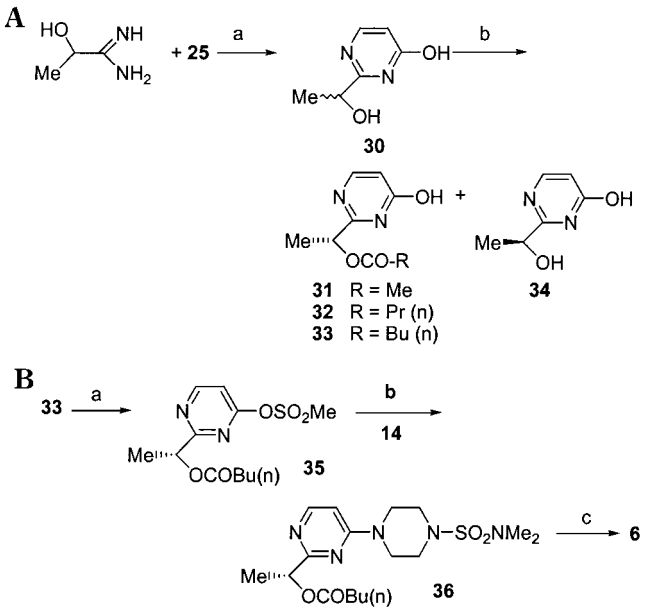
At this juncture, we needed a more expedient synthetic route to prepare larger supplies of **6** for testing in our diabetic rat models. We therefore prepared racemic 2-(1-hydroxyethyl)-3*H*-pyrimidin-4-one, **30**, to investigate the possibility of P30 lipase-mediated enantiospecific acylation (Scheme 4A). On the basis of an earlier result with **4**, we expected that reaction of **30** with lipase P30 would, selectively or exclusively, give an acylated alcohol of *R* configuration. We are pleased to report the experimental design and execution of the successful approach. Pyrimidone **30** is freely soluble in water. Reaction of **30** with the lipase using acetic anhydride as the acyl donor gave the (*R*)-acetate, **31**, which was also water soluble. So, we had to resort to cumbersome separation procedures to isolate the (*R*)-

Scheme 3[a]



[a] Reagents and conditions: (a) Ag_2O/MeI; (b) aq KOH; (c) $SOCl_2$; (d) NH_3; (e) P_2O_5; (f) HCl/EtOH; (g) NH_3; (h) H_2O; (i) $ClSO_2Me$; (j) THF/Et_3N; (k) BBr_3; (l) Py-CH_3CN.

Scheme 4[a]



[a] Reagents and conditions for part A: (a) H_2O/rt; (b) $CH_2{=}CH{-}O{-}(C{=}O){-}R$/dioxane, 50 °C. For part B: (a) $ClSO_2Me$; (b) THF/TEA; (c) HCl (conc).

acetate. We thought that the water solubility of pyrimidone **30** could be exploited to our advantage if we could make the acyl derivative sufficiently lipophilic for facile and complete extraction by organic solvents, which would avoid the need for any separation procedures. Use of propionic anhydride gave the propionate, **32**, which was still water soluble. Surprisingly, it turned out that butyric anhydride was the optimum choice not only because it led to the butyrate, **33**, with the desired criteria (easily and completely extracted by ethyl acetate) but also because the rate of acylation by higher anhydrides including the next homologue, valeric anhydride, was extremely slow. Now using the butyric anhydride procedure, we have prepared **33** in kilogram quantities. This easy access to **33** has provided an efficient route to **6** (Scheme 4B), and has opened up opportunities for further SAR development in this chemical family.

In our diabetic rat model, **6** was found to be more potent than either **5** or **2** in inhibiting sciatic nerve fructose accumulation. Thus we have provided a SDI that is ~10× more potent than the literature lead, **2**, both in vitro and in vivo. While **5** is less potent in vitro than **2**, it is nevertheless slightly more potent in vivo than **2**. This can be attributed to the higher lipophilicity of **5**, which could result in its more efficient nerve tissue penetration. We have not yet determined the serum half-life of **6**. However, the finding[12] that **2** is fairly rapidly metabolized by the rat suggests that **6** would undergo a similar metabolic fate. Compound **6** is certainly a more potent tool for further understanding the effects of SDIs. However, we are now focusing on discovery of even more potent SDIs with a longer serum half-life, and the progress we achieve in this endeavor will be the subject of future publications.

Conclusion

SDI **2** inhibits the accumulation of fructose in the sciatic nerve of severely diabetic rats. Design of more potent SDIs based on extant literature on alcohol dehydrogenase and on our SAR data around **2** strongly suggests that the pyrimidine N-1 atom and the oxygen atom of the hydroxymethyl group of **2** function as key ligands to the zinc atom in SDH. This rationale led to the synthesis of the enantiospecific inhibitor, **6**, which is substantially more potent in vitro than **2** against human SDH and also more potent than **2** in inhibiting nerve fructose production in diabetic rats. This new and more potent SDI could serve as a tool to further probe the second step of the polyol pathway. To address the rapid N-demethylation of the sulfamoyl moiety by rats, we are now pursuing other modifications of **6**, based on the versatile pyrimidine template, **33**. We have developed a highly practical route for the preparation of **33**, which facilitated in vivo evaluation of **6** as well as has laid a strong foundation to pursue structure−activity relationships for discovery of highly potent in vivo SDIs with longer serum half-life.

Experimental Section[22]

4-(2-Formyl-pyrimidin-4-yl)-piperazine-1-sulfonic Acid Dimethylamide (19). To a solution of 4-[2-hydroxy-methyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide (12.12 g, 40.2 mmol) in methylene chloride (100 mL) was added a solution of oxalyl chloride (3.9 mL, 44.2 mmol), and the reaction mixture was cooled to −78 °C. To this was added a solution of DMSO (6.27 mL, 88.4 mmol) in methylene chloride (20 mL) dropwise so as to keep the reaction temperature from rising above −70 °C. After 2 h, triethylamine (28.0 mL, 88.4 mmol) was added dropwise, and the reaction was allowed to come to room temperature. The reaction mixture was diluted with water (200 mL), and the collected organic layer was washed with saturated sodium bicarbonate solution. The washed organic layer was collected, dried over sodium sulfate, and filtered, and the filtrate was evaporated to a solid. The solid was triturated with ether (20 mL), and the mixture was filtered to obtain the title compound (10.84 g, 94%): mp 124−127 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.88 (s, 6H), 3.31 (m, 4H), 3.99 (m, 4H), 6.4 (d, J = 8 Hz, 1H), 8.2 (d, J = 8 Hz, 1H), 9.35 (s, 1H).

4-[2-(1*RS*-Hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic Acid Dimethylamide (4). To a −5 °C cooled solution of 4-(2-formyl-pyrimidin-4-yl)-piperazine-1-sulfonic acid dimethylamide (419 g, 14 mmol) in dry tetrahydrofuran (100 mL) was added an ether solution of methylmagnesium bromide (7.6 mL, 23.1 mmol). After 20 min, the reaction was allowed to warm to room temperature and then refluxed for 30 min. After cooling the reaction to room temperature, it quenched with saturated ammonium chloride solution and then extracted with ethyl acetate (2 × 50 mL). The organic layer was collected, dried over sodium sulfate, and filtered, and the filtrate was evaporated to a pale yellow solid (4.21 g, 95%): mp 115−116 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 1.48 (d, J = 7 Hz, 6H), 2.83 (s, 3H), 3.31 (m, 4H), 3.74 (m, 4H), 4.70 (q, J = 8 Hz), 1H), 6.40 (d, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H); MS (CI) M^{+1}316.

***R*-4-(4-Dimethylsulfamoyl-piperazin-1-yl)-pyrimidin-2-ylmethyl Acetate (7) and 4-[2-(1*S*-Hydroxy-ethyl)-pyrimidin-4-yl]-piperzine-1-sulfonic Acid Dimethylamide (5).** To a solution containing 4-[2-(1*RS*-hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide (**4**, 0.9 g, 2.86 mmol), dimethoxyethane (5.7 mL), and vinyl acetate (10.5 mL) was added Lipase P30 (90 mg, 10%), and the reaction mixture was stirred at room temperature for 14 days. It was filtered, and the filtrate was evaporated to brown oil, which was chromatographed over silica gel. Elution with a 95:5 mixture of ethyl acetate and methanol and evaporation of the eluent gave the title compound (220 mg, 43%): mp 107−109 °C, $[\alpha]_D$ +41.9 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.55(d, J = 6H, 6H), 2.2(s, 3H), 2.9(s, 6H), 3.3 (m, 4H), 3.8(m, 4H), 6.35(q, J = 8 Hz,1H), 6.4(d, 1H), 8.25(d, 1H). Subsequent

elution fractions were combined and evaporated to obtain **5** (45 mg, 9%): $[\alpha]_D$ +15.9 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) 1.48 (d, J = 7 Hz, 6H), 2.83 (s, 3H), 3.31 (m, 4H), 3.74 (m, 4H), 4.70 (q, J = 8 Hz), 1H), 6.40 (d, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H); MS (CI) M^{+1} 316.

(*R*)-2-Methoxy-propionitrile (22). A mixture of 2-methoxy-propionamide, phosphorus pentoxide (4.13 g, 29.1 mmol), and dry sand (3.0 g) was heated to 160 °C in a distillation apparatus, and the distillate was collected (1.01 g, 44%): $[\alpha]_D$ +139.2 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.5 (d, J = 8 Hz, 3H), 3.45 (s, 3H), 4.15 (q, J = 8 Hz, 1H).

(*R*)-2-Methoxy-propionimidic Acid Ethyl Ester Hydrochloride (23). Hydrogen chloride gas was passed into an ice-cold solution of 2-methoxy-propionitrile (9.91 g, 116.5 mmol) in anhydrous ethanol (100 mL) until the solution was saturated with the gas. The reaction was stored in a refrigerator overnight. The excess ethanol was removed under vacuum to obtain the title compound as a deliquescent solid (100%, 19.5 g); $[\alpha]_D$ +46.4 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.4 (d, J = 8 Hz, 3H), 3.4 (s, 1H), 3.7 (q, J = 8 Hz, 2H), 4.8 (q, J = 8 Hz, 1H), 6.2 (b, 1H).

(*R*)-2-Methoxy-propionamidine Hydrochloride (24). A solution of the 2-methoxy-propionimidic acid ethyl ester hydrochloride in ethanol was cooled in an ice bath, gaseous ammonia was passed into the solution until the reaction was saturated with ammonia, and the reaction was stirred overnight. Excess ethanol was removed to obtain a syrupy liquid, which was triturated with diethyl ether (100 mL). The resulting solid was filtered to collect the title compound (12.2 g): mp 60−75 °C; $[\alpha]_D$ +45.4 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.4 (d, J = 8 Hz, 3), 3.4 (s, 3H), 3.7 (q, J = 8 Hz, 1H), 5.8 (b, 1H), 6.5 (b, 1H).

(*R*)-2-Methoxymethyl-pyrimidine-3*H*-4-one (26). A mixture of (*R*)-2-methoxy-propionamidine hydrochloride (10.0 g, 72.15 mmol), sodium 2-ethoxycarbonyl ethenolate (**25**, 19.93 g, 144.3 mmol), and water (50 mL) was stirred at room temperature for 24 h. The reaction was neutralized by addition of sufficient concentrated HCl to adjust the pH to around 7.0 and extracted with methylene chloride. The extract was dried over anhydrous sodium sulfate, evaporated to dryness, and the residue was chromatographed over silica gel. Elution with a mixture of 95:5 methylene chloride and methanol, and evaporation of the eluent gave a thick viscous oil (1.7 g): $[\alpha]_D$ +76.4 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.51 (d, J = 8 Hz, 3H), 3.44 (s, 3H), 4.29 (q, J = 8 Hz, 1H), 7.91 (d,1H), 11.00 (b, 1H).

(*R*)-2-(1-Methoxyethyl)-pyrimidin-4-yl-methanesulfonate (27). To an ice-cold solution of (*R*)-2-methoxymethyl-4-hydroxy-pyrimidine (1.69 g, 10.95 mmol) in methylene chloride (10 mL) was first added triethylamine (1.7 mL, 12.05 mmol) and then mesyl chloride (1.38 g, 12.05 mmol). The reaction was stirred at ice temperature for 20 min and later allowed to come to room temperature. After 1 h, the reaction was quenched with saturated aqueous sodium bicarbonate solution, the organic layer was collected, and it was washed with water (10 mL), dried over sodium sulfate, and filtered, and the filtrate was evaporated to an oil (2.11 g, 83%): $[\alpha]_D$ +69.2 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.52 (d, J = 8 Hz, 3H), 3.38 (s, 3H), 3.62 (s,3H), 4.53 (q, J = 8 Hz, 1H), 7.00 (d, 1H), 8.80 (d, 1H).

(*R*)-4-[2-(1-Methoxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic Acid Dimethylamide (28). To a solution of the above sulfonate (2.11 g, 9.1 mmol) in tetrahydrofuran (20 mL) was added *N*,*N*-dimethylsulfamoyl piperazine (**14**, 1.94 g, 10 mmol) followed by triethylamine (1.4 mL, 10 mmol). The reaction was refluxed for 15 h and evaporated to an oily residue. This was extracted with ethyl acetate (20 mL), and the extract was washed first with a saturated aqueous solution of sodium bicarbonate and then with water (10 mL). The ethyl acetate extract was dried over sodium sulfate and filtered, and the filtrate was evaporated to a crude product, which was chromatographed over silica gel. Elution with a mixture of 9:1 ethyl acetate and methanol and evaporation of the solvents gave the title compound (1.75 g, 59%): mp 65−70 °C; $[\alpha]_D$ +54.4° (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48(d, 3H), 2.89(s,6H), 3.31(m, 4H), 3.37(s, 3H), 3.78(m, 4H), 4.34(q, J = 8 Hz, 1H), 6.41(d, J = 7.5 Hz, 1H), 8.30(d, J = 7.5 Hz, 1H).

4-[2-(1*R*-Hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic Acid Dimethylamide (6). Method 1. To an ice-cold solution of 4-[2-(1-methoxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide (1.75 g, 5.31 mmol) in methylene chloride (53 mL) was added boron tribromide (10.6 mL, 10.6 mmol), and the reaction mixture was stirred for 1 h. The mixture was allowed to warm to room temperature and was quenched with a saturated aqueous solution of sodium bicarbonate. The methylene chloride layer was washed with water (20 mL), dried over sodium sulfate, and filtered, and the filtrate was evaporated to a solid residue, which was crystallized from a mixture of isopropyl ether and methylene chloride to obtain the title compound as a white solid (642 mg, 38%): mp 103−105 ºC; $[\alpha]_D$ +16.1° (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) 1.48 (d, 6H), 2.83 (s, 3H), 3.31 (m, 4H), 3.74 (m, 4H), 4.70 (q, 1H), 6.40 (d, 1H), 8.21 (d, 1H); MS (CI) M^{+1} 316.

Method 3. *R*-1-[4-(4-Dimethylsulfamoyl-piperazin-1-yl)-pyrimidin-2-yl]-ethyl butyrate (230 mg, 0.60 mmol) was combined with concentrated hydrochloric acid (5.0 mL) and stirred at ambient temperature for 6 h and diluted with water, the pH of the solution was adjusted to 9.0 with 6 N aqueous sodium hydroxide, and it was extracted twice with ethyl acetate. The extract was washed once with water, dried over magnesium sulfate, and filtered, and the filtrate was concentrated to an oil which was purified by flash chromatography (9:1 methylene chloride:methanol). Evaporation of the eluent gave an oil which was crystallized from isopropyl ether to obtain the title compound as a white solid identical to that obtained by method 1.

Preparation of 31. (*R*,*S*) 2-Hydroxyethyl 4-hydroxy pyrimidine (2.1 g, 15.07 mol) was added to dioxane (63 mL) containing vinyl acetate (4.3 g, 50 mol), and the mixture was heated to 50 °C. To the resulting solution was added lipase P30 (0.21 g), and the heating was continued for 24 h. The reaction mixture was filtered, and the filtrate was evaporated to obtain a thick syrupy liquid residue. The residue was chromatographed over silica gel and eluted with a mixture of 95:5 methylene chloride and methanol. Evaporation of the collected eluent gave the title compound as a colorless liquid (0.97 g, 92%): $[\alpha]_D$ +39.9° (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.61 (d, J = 7 Hz, 3H), 2.2 (s, 3H), 5.65 (q, J = 7 Hz, 1H), 6.35 (d, J = 6 Hz, 1H), 7.97 (d, J = 6 Hz, 1H), 11.94 (s, 1H).

Preparation of 33. (*R*,*S*) 2-Hydroxyethyl 4-hydroxy pyrimidine (21.75 g, 155.2 mmol) was added to dioxane (650 mL) containing vinyl butyrate (17.72 g, 310 mmol), and the mixture was heated to 50 °C. To the resulting solution was added lipase P30 (4.35 g), and the heating was continued for 24 h. The reaction mixture was filtered, and the filtrate was evaporated to obtain a thick syrupy liquid residue. The residue was partitioned between methylene chloride (300 mL) and water (600 mL), and the methylene chloride layer was collected, dried over anhydrous sodium sulfate, and then filtered. The filtrate was evaporated to obtain the title compound as a colorless liquid (9.35 g, 86%): $[\alpha]_D$ +29.5 (c = 1, methanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, J = 8 Hz, 3H), 1.65 (m, 5H), 2.4 (m, 2H), 5.65 (q, J = 8 Hz, 1H), 6.45 (d, J = 8 Hz, 1H), 8.0 (d, J = 8 Hz, 1H).

***R*-1-[4-(4-Dimethylsulfamoyl-piperazin-1-yl)-pyrimidin-2-yl]-ethyl Butyrate (36)**. To a solution of (*R*)-1-[4-piperazin-1-yl)-pyrimidin-2-yl]-ethyl butyrate (1.49 g, 5.0 mmol), triethylamine (0.61 g, 6.0 mmol), and tetrahydrofuran (20.0 mL) was added *N*,*N*-dimethylsulfamoyl chloride (0.86 g, 6.0 mmol) at ambient temperature and stirred for 2 h. The mixture was diluted with water and extracted twice with ethyl acetate. The ethyl acetate extract was washed once with water (10 mL), dried over magnesium sulfate, and filtered, and the filtrate was concentrated to obtain the title compound as an oil (0.72 g, 87%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H), 1.55(d,

6H), 1.55 (d, 3H), 1.6−1.7 (m, 4H) 2.42 (t, $J = 8$ Hz, 2H), 3.2 (m, 1H), 3.45 (m, 4H), 3.74 (m, 4H), 5.4 (q, $J = 8$ Hz, 1H), 6.4 (d, $J = 8$ Hz, 1H), 8.1 (d, $J = 8$ Hz, 1H).

Supporting Information Available: Supplementary information for the preparation of other new compounds described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) After we completed our work on **11**, a publication by Varlet and co-workers disclosed both synthesis (same as ours) and SDH inhibition activity of **11** relative to **2**. For details, see *Heterocycles* **2000**, *53*, 797−804.

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