A Sorbitol Dehydrogenase Inhibitor of Exceptional in Vivo Potency with a Long Duration of Action:
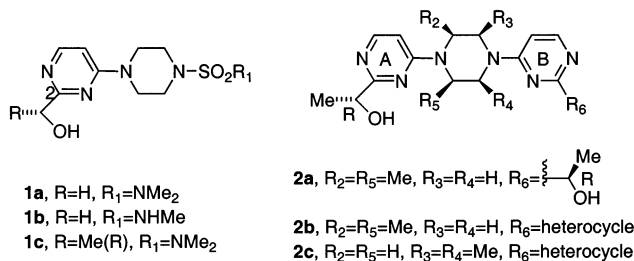## 1-(*R*)-{4-[4-(4,6-Dimethyl[1,3,5]triazin-2-yl)-2*R*,6*S*-dimethylpiperazin-1-yl]pyrimidin-2-yl}ethanol

Banavara L. Mylari,* Peter J. Oates,
William J. Zembrowski, David A. Beebe,
Edward L. Conn, James B. Coutcher,
Melissa T. O'Gorman, Michael C. Linhares, and
Gregory J. Withbroe

*Pfizer Global Research and Development,
Groton Laboratories, Groton, Connecticut 06340*

Received July 3, 2002

Abstract: We report here a novel sorbitol dehydrogenase inhibitor, **16**, that shows very high oral potency (50 μg/kg) in normalizing elevated fructose levels in the sciatic nerve of chronically diabetic rats and sustained duration of action (>24 h). Furthermore, **16** shows attractive pharmaceutical properties, including good solubility in simulated human gastric fluid, excellent Caco-2 Papp, moderate lipophilicity, and metabolic stability for achieving good oral absorption and long duration of action.

Giesen et al.[1] and Williamson et al.[2] have reported that inhibition of sorbitol dehydrogenase (SDH) can influence the early functional changes observed in experimental diabetes, including alterations in vascular albumin permeation, tissue blood flow, nerve conduction velocity (NCV), and renal hyperfiltration. In turn, such early functional changes may contribute to eventual pathology in affected diabetic tissues. Sorbitol dehydrogenase inhibitor (SDI), **1a**, was evaluated in normal and diabetic rats for its effects on NCV. As expected, it

1a, R=H, R₁=NMe₂
1b, R=H, R₁=NHMe
1c, R=Me(R), R₁=NMe₂

2a, R₂=R₅=Me, R₃=R₄=H, R₆=
2b, R₂=R₅=Me, R₃=R₄=H, R₆=heterocycle
2c, R₂=R₅=H, R₃=R₄=Me, R₆=heterocycle

caused significantly higher accumulation of sorbitol in the sciatic nerve of both normal and diabetic rats, 20× and 100×, respectively, over untreated normal rats.[3] However, in apparent contrast to expectations based on long-standing osmotic hypothesis,[4] **1a** showed no adverse effects on NCV in normal rats.[1] In chronically diabetic rats, apparently confounding results attributable to several possible factors, as iterated already,[5] have been obtained.[1,3,6−8] Because **1a** is a weak inhibitor with a very short plasma half-life in rats,[9] we have designed and synthesized more potent SDIs, with long

* To whom correspondence should be addressed. Address: 6 Quinley Way, Waterford, CT 06385. Phone: (860) 443-3085. Fax: (860) 715-4483. E-mail: blmylari@aol.com.
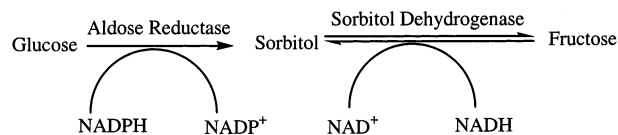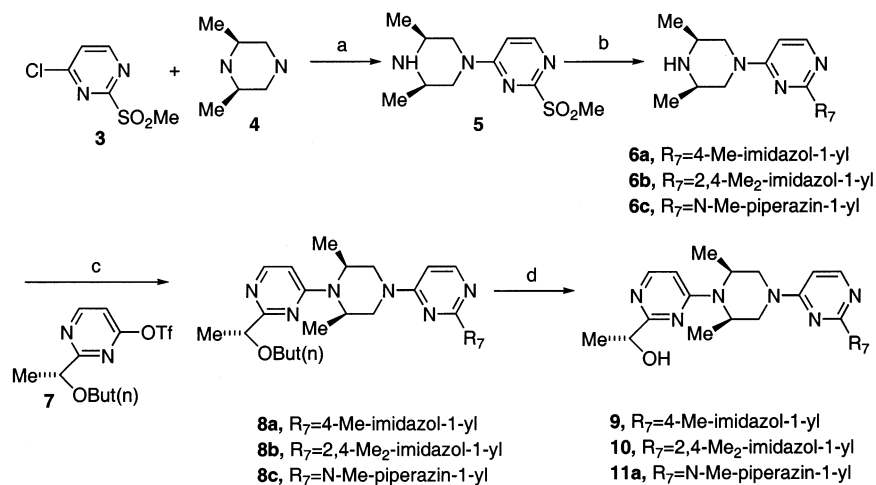
Figure 1.

serum half-lives, as better tools to help define the role of excess flux through SDH (Figure 1) in diabetic complications.

A seminal discovery in efforts to enhance SDI potency of **1a** was the rational design of **1c** with the (*R*)-hydroxyethyl side chain, which is the essential centerpiece of all potent SDIs in our pyrimidine series.[5] Because both SDIs **1a** and **1c** feature a metabolically labile *N,N*-dimethylsulfamoyl group,[10] a metabolically stable replacement for the *N,N*-dimethylsulfamoyl group became a focal point for discovery of highly potent inhibitors with longer serum half-lives. Other factors that govern oral in vivo potency in target tissues, e.g., peripheral nerve, include the inhibitor's extent of absorption into systemic circulation, protein binding, pK_a, and sufficient lipophilicity to efficiently partition from blood to tissues. Chu-Moyer et al.[11] have reported that replacement of the dimethylsulfamoyl moiety of **1c** by planar nitrogen-containing heterocycles provide potent SDIs with longer serum $t_{1/2}$. The most potent inhibitor from this effort was **2a** (*RR* diastereomer), which incorporates a strategic dimethylpiperazine linker with a second chiral hydroxyethyl side chain on the potentiating pyrimidine side chain. However, compounds with multiple chiral centers pose several challenges in pharmaceutical development, specifically, enantiomers and diastereomers issues relating to absorption, distribution, metabolism, elimination, analytical methods development, and synthesis. As it turns out, **2a** is rapidly metabolized by rats and a nearly equal mixture of all four possible optical isomers (*RR*, *SR*, *RS*, and *SS*) is found in systemic circulation.[12] As expected from our earlier experience,[5] the *RR* diastereomer was the most potent in vitro vs rat as well as vs human SDH, with the other three isomers being (2−80)× less potent than the *RR* diasetreomer.[12] Thus, in addition to the above-mentioned troublesome challenges posed by pharmacological agents with more than one chiral center, the unexpectedly complicated metabolism of **2a** in the test species precluded it from manifesting its full potency in vivo by generating major quantities of less potent metabolites. So, we set our objective to discover SDIs related to **2a** but with a single obligatory (*R*) chiral center at the pyrimidine 2-position[5] (ring A), in vivo potency significantly superior to **2a**, and a long duration of action similar to that of **2a**.[11] Herein, we report results of efforts culminating in the discovery of the title SDI, **16**, which has a single chiral hydroxyethyl side chain and shows unprecedented SDH inhibition potency in the sciatic nerve of chronically diabetic rats.

Our initial strategy was to investigate whether certain heterocycles with appropriate substituents could function as effective surrogates for the second chiral hydroxyethyl on the potentiating pyrimidine moiety in **2a** (ring B). Among SDIs with a single chiral hydroxy-

Scheme 1[a]

6a, R[7]=4-Me-imidazol-1-yl
6b, R[7]=2,4-Me[2]-imidazol-1-yl
6c, R[7]=N-Me-piperazin-1-yl

8a, R[7]=4-Me-imidazol-1-yl
8b, R[7]=2,4-Me[2]-imidazol-1-yl
8c, R[7]=N-Me-piperazin-1-yl

9, R[7]=4-Me-imidazol-1-yl
10, R[7]=2,4-Me[2]-imidazol-1-yl
11a, R[7]=N-Me-piperazin-1-yl

[a] Reagents and conditions: (a) CHCl[2], TEA, 40–60 °C; (b) excess NMe-piperazine or KOBu(t), DMF, room temp; (c) MeCH, TEA, 40–60 °C; (d) concentrated HCl, dioxane.

ethyl group, those with pendent piperazine methyl groups proximal to, rather than distal to, the chiral pyrimidine had already been found to be generally more potent in vivo.[13] So, we chose the hitherto unexplored pyrimidine **2b** (rather than **2c**) with pendent heterocycles R[6] as our first target.
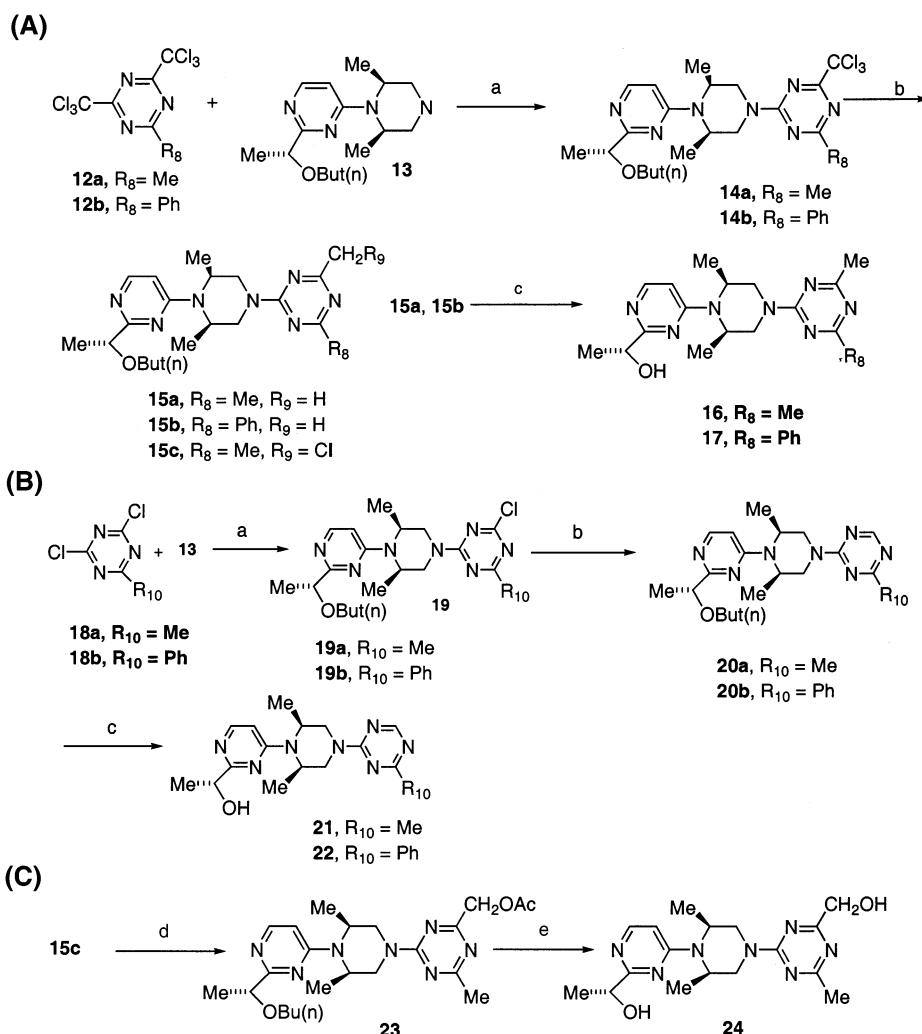
Biological Testing. Potential SDIs were screened for in vitro activity against rat and human recombinant SDH[14] and for oral in vivo activity in two streptozotocin diabetic rat models, acute and chronic, as measured by the ability of the inhibitor to lower sciatic nerve fructose that had become elevated in diabetic nerve.[5,11] The expedient acute model was used to also screen for potential racemization of the critical hydroxyethyl moiety, through the oxidoreduction pathway. Racemization was inferred if a more in vitro potent *R* enantiomer (as observed in our series) gave very nearly the same sciatic nerve fructose inhibition result as a less potent *S* enantiomer, when they were each administered, at the same dose. Because our objective was to block the flux through SDH in a complete and sustained manner, we focused attention on ED[90] values (90% inhibition of fructose elevation in the diabetic sciatic nerve) rather than on traditionally targeted ED[50] values, expressed as mpk (mg/kg), in the chronic model.

Results and Discussion.[15] Compounds **9**, **10**, and **11a** (see Scheme 1) were tested in the chronic model. The *S*-enantiomer of **11a**, **11b**, was prepared according to Scheme 1 by replacing **7** with its *S*-enantiomer. While all these SDIs were active in this model, **10** and **11a** were found to be nearly 2× more potent than **2a**, thus providing a new direction for SAR pursuit. Equally importantly, in contrast to the experience with **2a** but consistent with that of monochiral **1c**,[5] **11a** and **11b** gave distinctly different inhibition responses in the acute rat model when tested at 1 mpk. While **11a** gave >90% of fructose normalization in both the acute and chronic models, **11b** was essentially inactive in the acute model. If biological oxidoreduction as observed in the case of **2a** also prevailed with **11a**, nearly identical inhibition of nerve fructose would be expected, regardless of which enantiomer was administered. Thus, the strategy of mitigating metabolic complexity and concur-

rently gaining in vivo potency through structural modification was successful.

We then turned our attention to incorporate other reinforcing medicinal chemistry approaches to design even more in vivo potent SDIs. Inhibitor **2a** is dibasic with pK_a values of 6.8 and 6.0. At physiological pH of 7.4, 25% of **2a** would be protonated according to pH − pK_a = log [B/BH$^+$], i.e., 7.4 − 6.8 = log [B/BH$^+$]. Mindful of the unfavorable property of charged species to cross the cell membrane, the question was whether we could design SDIs with a pK_a sufficiently lower than 6.8 to enhance the ability of these inhibitors to partition from blood to the sciatic nerve tissue. A surrogate for the pyrimidine side chain with the potential to lower pK_a, which had not been fully exploited, was an *s*-triazine. With extensive SAR already in hand, we began to explore substitution at the two free sites on the *s*-triazine side chain. In addition, we were guided by the need to incorporate moderately lipophilic substituents on the *s*-triazine to accommodate the proposed hydrophobic site in the vicinity of the catalytic zinc metal complex in SDH as indicated by the enzyme structure model[5,16] and to address both aqueous solubility and protein binding. The title compound, **16**, was visualized as a critical target to test the design rationale. It was prepared according to Scheme 2A. Close-in analogues relevant to SAR around **16** (see Table 1), which include **17**, **21**, **22**, and **24**, were prepared according to Scheme 2A–C.

16 (MW, 343) was highly potent in vitro vs both human and rat SDHs At IC[90], it was 3× more potent than **2a** against rat SDH in a side-by-side determination (10 ± 0.6 vs 31 ± 4 nM). Strikingly, it was 40× more potent than **2a** in the chronic model (ED[90], 0.05 vs 2 mpk). It showed two pK_a values of 6.2 and 4.8, both significantly lower than those of **2a** (6.8 and 6.0) and was more lipophilic than **3** (log *P*, 2.0 vs 1.4), with a high solubility of >1.3 mg/mL in simulated gastric fluid. The reduced pK_a of **16** results in 6.3% (7.4 − 6.2 = log [B/BH$^+$]) circulating protonated species at physiological pH and thus reduces the extent of protonation by nearly 19% relative to **2a**. The higher lipophilicity of **16** could increase its penetrating power into the nerve tissue, which is wrapped by a fatty myelin sheath. While both

Scheme 2[a]

(A)

12a, R₈ = Me
12b, R₈ = Ph

14a, R₈ = Me
14b, R₈ = Ph

15a, R₈ = Me, R₉ = H
15b, R₈ = Ph, R₉ = H
15c, R₈ = Me, R₉ = Cl

16, R₈ = Me
17, R₈ = Ph

(B)

18a, R₁₀ = Me
18b, R₁₀ = Ph

19a, R₁₀ = Me
19b, R₁₀ = Ph

20a, R₁₀ = Me
20b, R₁₀ = Ph

21, R₁₀ = Me
22, R₁₀ = Ph

(C)

23

24

[a] Reagents and conditions: (a) NaHCO₃, DMF, room temp to 60 °C; (b) EtOH, TEA, Pd–C, H₂; (c) concentrated HCl, room temp; (d) NaOAc, HOAc, 80 °C; (e) aqueous KOH, MeOH.

16 and **2a** show very low rat plasma protein binding, **16** is relatively more protein-bound than **2a** (64% vs 42%), as would be expected from their log *P* values. The lipophilicity of **16** appears to be optimum in this series because the relatively less lipophilic **21**, the progressively more lipophilic **22**, and **17** are less potent than **16**. The least potent inhibitor, **17**, may have severely tested the permissive space within the SDH active site hydrophobic pocket. Furthermore, **24**, the most polar of all of these, is significantly less potent than **16**. As in the case of **11a**, **16** appeared stable to oxidoreduction of the chiral hydroxyethyl in the rat. A 2-fold difference in IC₅₀ values between **16** and **16a** was equally manifested by their chronic in vivo potencies. At 0.1 mpk, **16** and **16a** gave 96% and 56% fructose inhibition, respectively. These results, taken together with the observed lack of turnover of **16** by rat microsomes in vitro[17] to generate **16a**, are consistent with previous experience with **1c**[5] as well as with **11a**, regarding oxidoreduction of the monochiral hydroxyethyl. The aggregate contribution of favorable factors toward the striking in vivo potency enhancement of **16** over **2a**, underpinning our design rationale, includes enhanced in vitro potency of SDH inhibition, reduced in vivo generation of less potent diastereomer metabolites,

optimally increased log *P* to enhance nerve tissue penetration, and sufficiently lowered p*K*ₐ to curtail extent of charged species at physiological pH. Like other members of the series, **16** was highly selective (>2500-fold) for SDH compared to other dehydrogenases, including alcohol, lactate, and fructose dehydrogenases.

Pharmacokinetics of **16** was determined in both rats and dogs. It was well absorbed orally in both species. Its serum half-lives in rats and dogs were 7 and 10 h, respectively (similar to that of **2a**, 5.6 and 9.6 h, respectively). Consistent with its long serum half-life in rats, **16** at 0.05 mpk, like **2a** at 2 mpk,[11] showed sustained inhibition of sciatic nerve fructose in chronically diabetic rats (data not shown). It showed good movement across a Caco-2 cell monolayer via a transcellular mechanism, with $P_{app} > 10^{-5}$ cm/s, suggestive of the potential for very good oral absorption in humans.

In summary, we have described key aspects of a drug design rationale contributing to the remarkable optimization of in vivo potency, in progressing from **2a** to the title SDI, **16**. The preponderance of evidence suggests that, unlike **2a**, the monohydroxyethyl pyrimidines, e.g., **11a** and **16**, are not good substrates for rat liver microsomal oxidoreduction. Furthermore, **16** shows highly attractive druglike properties including high

Table 1. In Vitro and in Vivo Data of SDIs

	IC$_{50}$ (nM)		
no.	rat	human	ED$_{90}$,[a] mpk
2a	11[b]	10	2
9	11	13	1
10	7	4	1
11a	6	4	1
11b	32	c	c
16	4[b]	5	0.05
16a	9	16	d
17	39	43	e
21	5	7	0.3
22	10	7	1
24	20	c	f

[a] Chronic test, [b] Side-by-side comparison, [c] Not determined, [d] 56% at 0.05 mpk. [e] 81% at 1 mpk. [f] 65% at 1 mpk.

target enzyme potency/selectivity, low molecular weight, good solubility, low protein binding, modest lipophilicity, excellent Caco-2 P_{app} for good oral absorption, and sufficiently long plasma $t_{1/2}$, both in rats and dogs, to support sustained duration of action. This compound should allow powerful, long-acting in vivo inhibition of SDH and allow clarification of the role of the metabolic flux through SDH in the development of diabetic complications.

Supporting Information Available: Description of synthetic schemes and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Geisen, K.; Utz, R.; Grotsch, H.; Lang, J.; Nimmesgern, H. Sorbitol-Accumulating Pyrimidines: *Arzneim. Forsch.* **1994**, *44*, 1032−1043.

(2) (a) Williamson, J. R.; Chang, K.; Frangos, M.; Hasan, K. S.; Ido, Y.; Kawamura, T.; Nyengaard, J. R.; Van den Enden, M.; Kilo, C.; Tilton, R. G. Hypergylcemic Pseudohypoxia and Diabetic Complications. *Diabetes.* **1993**, *42*, 801−813. (b). Tilton, R. G.; Chang, K.; Nyengaard, J. R.; Van den Enden, M.; Ido, Y.; Williamson, J. R. Inhibition of Sorbitol Dehydrogenase. Effects on Vascular and Neural Dysfunction in Streptozotocin-Induced Diabetic Rats. *Diabetes* **1995**, *44*, 234−242.

(3) Oates, P. J. The Polyol Pathway and Diabetic Peripheral Neuropathy. In *The Neurobiology of Diabetic Neuropathy*; Tomlinson, D. R., Ed.; International Review of Neurobiology, Vol. 50; Academic Press: London, 2002; pp 325−392.

(4) (a) Kinoshita, J. H. A Thirty Year Journey in the Polyol Pathway. *Exp. Eye Res.* **1990**, *50*, 567−573. (b) Kinoshita, J. H.; Nishimura, C. The Involvement of Aldose Reductase in Diabetic Complications. *Diabetes Metab. Rev.* **1988**, *4*, 323−337.

(5) Mylari, B. L.; Oates, P. J.; Beebe, D. A.; Brackett, N. S.; Coutcher, J. B.; Michael S.; Dina, M. S.; Zembrowski, W. J. Sorbitol Dehydrogenase Inhibitors (SDIs): A New Potent, Enantiomeric SDI, 4-[2-(1*R*-Hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic Acid Dimethylamide. *J. Med. Chem.* **2001**, *44*, 2695−2700.

(6) Cameron, N. E.; Cotter, M. A.; Basso, M.; Hohman, T. C. Comparison of the Effects of Inhibitors of Aldose Reductase and Sorbitol Dehydrogenase on Neurovascular Function, Nerve Conduction and Tissue Polyol Pathway Metabolites in Streptozotocin-Diabetic Rats. *Diabetalogia* **1997**, *40*, 271−281.

(7) Ido, Y.; Chang, K.; Oates, P. J.; Mylari, B. L.; Williamson, J. R. Inhibitors of Sorbitol Dehydrogenase (SDI) and Aldose Reductase (ARI) Reverse Impaired Motor Nerve Conduction Velocity (MNCV) in Diabetic Rats. *Diabetes* **1999**, *48* (Suppl. 1), A150.

(8) Ng, T. F.; Lee, F. K.; Song, Z. T.; Calcutt, N. A.; Lee, A. Y.; Chung, S. S.; Chung, S. K.; Ng, D. T.; Lee, L. W. Effects of Sorbitol Dehydrogenase Deficiency on Nerve Conduction in Experimental Diabetic Mice. *Diabetes* **1998**, *47*, 961−966.

(9) Ballinger, W. E.; Day, W. W.; Beebe, D. A.; Oates, P. J.; Zembrowski, W. J.; Mylari, B. L. The Effect of Multiple Bolus Dosing vs Chronic Water Dosing with CP-166,572, a Potent Sorbitol Dehydrogenase Inhibitor, on Nerve Fructose Normalization in Diabetic Rats. Presented at the 7th North American ISSX Meeting, San Diego, CA, October 20, 1996.

(10) Avery, M.; Ballinger, W. E.; Zembrowski, W. J.; Inskeep, P. B.; Mylari, B. L.; Fouda, H. G. Microbore HPLC/MS of CP-166,572 Metabolites in the Rat. Presented at the American Society Mass Spectrometry Conference, Atlanta, GA, May 21, 1995.

(11) Chu-Moyer, M. Y.; Ballinger, W. E.; Beebe, D. A.; Berger, R.; Coutcher, J. B.; Day, W. W.; Li, J.; Mylari, B. L.; Oates, P. J.; Weekly, R. M. Orally-Effective, Long-Acting Sorbitol Dehydrogenase Inhibitors: Synthesis, Structure−Activity Relationships, and in Vivo Evaluations of Novel Heterocycle-Substituted Piperazino-Pyrimidines. *J. Med. Chem.* **2002**, *45*, 511−528.

(12) Chu-Moyer, M. Y.; Ballinger, W. E.; Beebe, D. A.; Coutcher, J. B.; Day, W. W.; Li, J.; Oates, P. J.; Weekly, R. M. SAR and Species/Stereo-Selective Metabolism of the Sorbitol Dehydrogenase Inhibitor, CP-470,711. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1477−1480.

(13) Mylari et al. Unpublished observation.

(14) Siegel, T. W.; Smith, S. R.; Ellery, C. A.; Williamson, J. R.; Oates, P. J. An Enzymatic Fluorimetric Assay for Fructose. *Anal. Biochem.* **2000**, *280*, 329−331.

(15) Complete experimental procedures for compounds discussed here can be found in Patents WO 0059510 and CAN 133:281794.

(16) Jeffery, J.; Jornvall, H. Sorbitol Dehydrogenase. *Adv. Enzymol.* **1988**, *61*, 47−106.

(17) We thank Mr. W. E. Ballinger of Pharmacokinetics, Dynamics & Metabolism Department within Pfizer Global Research and Development, for this result.