ATP-Citrate Lyase as a Target for Hypolipidemic Intervention. Sulfoximine and 3-Hydroxy-β-lactam Containing Analogues of Citric Acid as Potential Tight-Binding Inhibitors

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Received July 7, 1992

Citric acid analogues (±)-**12a,b** and (±)-**17a,b**, where one of the primary carboxylates has been replaced by a sulfoximinoyl and a 3-(3-hydroxy-β-lactamyl) moiety, respectively, have been synthesized and evaluated as inhibitors of ATP-citrate lyase. The design of these inhibitors was based on methionine sulfoximine and tabtoxinine β-lactam, potent, tight-binding inhibitors of glutamine synthetase. Both ATP-citrate lyase and glutamine synthetase employ phosphate–carboxylate anhydrides as a method for carboxylate activation during catalysis. Only one diastereomer, (±)-**12a**, displayed weak, reversible inhibition, while the remaining citrate analogues (±)-**12b** and (±)-**17a,b** were inactive against the lyase. No time-dependent inactivation of the enzyme was observed.

A major advance in therapy for the treatment of hypercholesterolemia has come with the discovery and commercial development of clinically effective inhibitors of HMG-CoA reductase, a key regulatory enzyme of mammalian cholesterol biosynthesis.[1] These agents have been shown to significantly reduce plasma levels of low-density lipoprotein cholesterol.[2] They may therefore reduce the mortality and morbidity associated with coronary heart disease.[3] In addition, evidence is accumulating that suggests HMG-CoA reductase inhibitors may reverse or even prevent the formation of atheromatous plaque.[4] If proven in a clinical setting, this would have an enormous impact on human health. However, the long-term treatment of hypercholesterolemia with existing HMG-CoA reductase inhibitors is not without adverse effect[5] and the discovery of other lipid-lowering agents having an improved therapeutic index continues. Such novel agents may be identified through the design of second and third generation HMG-CoA reductase inhibitors[1b,c]

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(1) (a) Grundy, S. M. HMG-CoA Reductase Inhibitors for the Treatment of Hypercholesterolemia. *N. Engl. J. Med.* **1988**, *319*, 24–33. (b) Lee, T.-J. Synthesis, SARs and Therapeutic Potential of HMG-CoA Reductase Inhibitors. *Trends Pharm. Sci.* **1987**, *8*, 442–446. (c) Kathawala, F. G. HMG-CoA Reductase Inhibitors: An Exciting Development in the Treatment of Hyperlipoproteinemia. *Med. Res. Rev.* **1991**, *11*, 121–146.

(2) (a) Bilheimer, D. W.; Grundy, S. M.; Brown, M. S.; Goldstein, J. L. Mevinolin and Colestipol Stimulate Receptor-Mediated Clearance of Low Density Lipoprotein From Plasma in Familial Hypercholesterolemia Heterozygotes. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4124–4128. (b) Illingworth, D. R.; Sexton, G. J. Hypocholesterolemic Effects of Mevinolin in Patients With Heterozygous Familial Hypercholesterolemia. *J. Clin. Invest.* **1984**, *74*, 1972–1978. (c) Illingworth, D. R. Mevinolin Plus Colestipol in Therapy For Severe Heterozygous Familial Hypercholesterolemia. *Ann. Inter. Med.* **1984**, *101*, 598–604. (d) The Lovastatin Study Group. A Multicenter Comparison of Lovastatin and Cholestyramine Therapy for Severe Primary Hypercholesterolemia. *J. Am. Med. Assoc.* **1988**, *260*, 359–366.

(3) (a) The Lipid Research Clinics Coronary Primary Prevention Trial Results I. Reduction in Incidence of Coronary Heart Disease. *J. Am. Med. Assoc.* **1984**, *251*, 351–364. (b) The Lipid Research Clinics Coronary Primary Prevention Trial Results II. The Relationship of Reduction in Incidence of Coronary Heart Disease to Cholesterol Lowering. *J. Am. Med. Assoc.* **1984**, *251*, 365–364.

(4) (a) Follath, F.; Drexel, H. Is Regression of Atherosclerosis by Drugs Possible? *Schweiz. Med. Wochenschr.* **1991**, *121*, 1803–1806. (b) Senaratne, M. P.; Thomson, A. B.; Kappagoda, C. T. Lovastatin Prevents the Impairment of Endothelium Dependent Relaxation and Inhibits Accumulation of Cholesterol in the Aorta in Experimental Atherosclerosis in Rabbits. *Cardiovasc. Res.* **1991**, *25*, 568–578. (c) Ross, R. Mechanisms of Atherosclerosis—A Review. *Adv. Nephrol.* **1990**, *19*, 79–86. (d) Ross, R. The Arterial Wall and Atherosclerosis. *Annu. Rev. Med.* **1979**, *30*, 1–15.

(5) (a) Brocard, J. J.; Keller, U.; Oberhansli, A.; Riesen, W. F. Effects and Side Effects of a 1-Year Treatment of Primary Hypercholesterolemia with Simvastatin. *Schweiz. Med. Wochenschr.* **1991**, *121*, 977–983. (b) Kojima, M.; Hockwin, O.; Rao, G. S.; Garcia, J. Investigations of the Presence of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in Lens of Various Animal Species. *Lens Eye Toxic Res.* **1990**, *7*, 605–623. (c) Behrens-Baumann, W.; Thiery, J.; Fieseler, H. G.; Seidel, D. Pravastatin—Ocular Side Effects After a Two Year Follow-Up. *Lens Eye Toxic Res.* **1990**, *7*, 311–318. (d) Steiner, A.; Weisser, B.; Vetter, W. A. Comparative Review of the Adverse Effects of Treatments for Hyperlipidemia. *Drug Saf.* **1991**, *6*, 118–130. (e) Ditschuneit, H. H.; Dreyer, M.; Dammann, H. G.; Ditschuneit, H. Pravastatin, Cholestyramine and Gemfibrozil in Long-Term Therapy of Primary Hypercholesterolemia. An Open Randomized Comparative Study. *Med. Klin.* **1991**, *86*, 142–148. (f) Bilheimer, D. W. Long-Term Clinical Tolerance of Lovastatin and Simvastatin. *Cardiology*, **1990**, *77* (Suppl 4), S58–S65. (g) Seidel, D. The HELP System: An Efficient and Safe Method of Plasma Therapy in the Treatment of Severe Hypercholesterolemia. *Ther. Umsch.* **1990**, *47*, 514–519. (h) Darioli, R.; Bovet, P.; Brunner, H. R.; Bercher, L. Evaluation of Tolerance, Efficacy and Safety of 3-Year Simvastatin Use in The Treatment of Primary Hypercholesterolemia. *Schweiz. Med. Wochenschr.* **1990**, *120*, 85–91. (i) Hunninghake, D. B.; Mellies, M. J.; Goldberg, A. C.; Kuo, P. T.; Kostis, J. B.; Schrott, H. G.; Insull, W., Jr.; Pan, H. Y. Efficacy and Safety of Pravastatin in Patients with Primary Hypercholesterolemia II. Once-Daily Verses Twice-Daily Dosing. *Atherosclerosis* **1990**, *85*, 219–227. (j) Smith, P. F.; Eydelloth, R. S.; Grossman, S. J.; Stubbs, R. J.; Schwartz, M. S.; Germershausen, J. I.; Vyas, K. P.; Kari, P. H.; MacDonald, J. S. HMG-CoA Reductase Inhibitor-Induced Myopathy in the Rat: Cyclosporine A Interaction and Mechanism Studies. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1225–1235. (k) Israeli, A.; Raveh, D.; Arnon, R.; Eisenberg, S.; Stein, Y. Lovastatin and Elevated Creatine Kinase: Results of Rechallenge. *Lancet* **1989**, *1*, 725–728.

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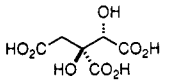
or through the design of inhibitors of other enzymes involved in cholesterol biosynthesis[6a-i] and regulation.[6j,k,l]

In this context, we became interested in literature reports describing the lipid-lowering action of (−)-hydroxycitrate 1.[7] This analogue of citric acid 2 inhibits cholesterol biosynthesis in the human cell line HepG2. It also causes



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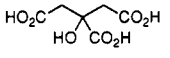


2

an increase in HMG-CoA reductase enzyme levels and an up-regulation of the LDL receptor activity, analogous to mevinolin.[8] The manifestation of the biological properties of 1 have been ascribed to the specific inhibition (K_i = 0.15 μM) of ATP-citrate lyase (E.C. 4.1.3.8), an enzyme positioned upstream from HMG-CoA reductase in the mammalian cholesterol biosynthesis pathway.[9] ATP-citrate lyase catalyzes a reversible retro-Claisen reaction utilizing ATP, 2, and CoA-SH as substrates to yield ADP, inorganic phosphate (P_i), oxaloacetate 3, and acetyl-CoA 4 (Scheme I).[10,11] Acetyl-CoA is the donor of two-carbon units required for de novo lipogenesis and cholesterogenesis.[12] Given the action of 1 in vivo, ATP-citrate lyase was viewed as a potential target for hypolipidemic intervention. More potent inhibitors of the enzyme could lead to a novel and therapeutically beneficial class of lipid-lowering agents.[13,14] An ATP-citrate lyase inhibitor would be expected to inhibit both fatty acid and cholesterol biosynthesis.[12] It may therefore have an advantage over a pure cholesterogenesis inhibitor in light of the implication of hypertriglyceridemia in coronary heart disease.[15]

The overall mechanism by which ATP-citrate lyase catalyzes the retro-Claisen reaction has been recently revised[11] and is presented in Scheme I. The human enzyme is initially phosphorylated at histidine 765[16] by ATP-Mg^{2+} to form a phosphoenzyme complex. Subsequent transfer of phosphate to the *pro-S* carboxylate of 2 occurs, producing a noncovalent, citrate phosphate anhydride i in the active site. Direct attack by enzyme bound CoA-SH results in the formation of a second noncovalent but tightly bound intermediate, citryl-CoA ii. Finally, fragmentation of ii via a retro-aldol type cleavage ensues, releasing 3 and 4 from the active site.

The formation of i during ATP-citrate catalysis is reminiscent of the carboxylate kinase activity displayed by glutamine synthetase.[17] In the reaction catalyzed by the synthetase, phosphorylation of the γ-carboxylate of

(6) (a) Swaminathan, S.; Pinkerton, F. D.; Schroepfer, G. J., Jr. Inhibitors of Sterol Synthesis. 3β,25-Dihydroxy-5α-cholest-8(14)-en-15-one, an Active Metabolite of 3β-Hydroxy-5α-cholest-8(14)-15-one. *J. Med. Chem.* **1992**, *35*, 793–795. (b) Greenspan, M. D.; Yudkovitz, J. B.; Chen, J. S.; Hanf, D. P.; Chang, M. N.; Chaing, P. Y.; Chabala, J. C.; Alberts, A. W. The Inhibition of Cytoplasmic Acetoacetyl-CoA thiolase by a Triyne Carbonate (L-660,631). *Biochem. Biophys. Res. Commun.* **1989**, *163*, 548–553. (c) Greenspan, M. D.; Yudkovitz, J. B.; Lo, C. Y.; Chen, J. S.; Alberts, A. W.; Hunt, V. M.; Chang, M. N.; Yang, S. S.; Thompson, K. L.; Chaing, Y. C. Inhibition of Hydroxymethylglutaryl-Coenzyme A Synthesis by L-659,699. *Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7488–7492. (d) Tomoda, H.; Kumagai, H.; Tanaka, H.; Omura, S. F-244 Specifically Inhibits 3-Hydroxy-3-methyl-glutaryl Coenzyme A Synthase. *Biochem. Biophys. Acta* **1987**, *922*, 351–356. (e) Thompson, K. L.; Chang, M. N.; Chiang, Y.-C. P.; Yang, S. S.; Chabala, J. C.; Arison, B. H.; Greenspan, M. D.; Hanf, D. P.; Yudkovitz, J. Synthesis of δ-Lactone, Oxetane and Azetidinone Analogs From the Naturally Occurring β-Lactam L-659,699. The preparation of a novel HMG CoA Synthase Inhibitor. *Tetrahedron Lett.* **1991**, *32*, 3337–3340. (f) Biller, S. A.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Marretta, J.; Scott, W. A.; Ciosek, C. P., Jr. Squalene Synthetase Inhibition: Progress Towards a New Approach for the Regulation of Cholesterol Biosynthesis. *Int. Congr. Ser.—Excerpta Med.* **1990**, *905*, 213–216. (g) Tanaka, R. D.; Lee, L. Y.; Schafer, B. L.; Freudenberger, J. S.; Kratunis, V. J.; Mosley, S. T. Molecular Cloning of Mevalonate Kinase and Its Novel Role in Regulation of Cholesterol Biosynthesis. *Int. Congr. Ser.—Excerpta Med.* **1990**, *905*, 205–208. (h) Tuck, S. F.; Patel, H.; Safi, E.; Robinson, C. H.; Lanosterol 14α-Demethylase: Effects of P45014DM Inhibitors on Sterol Biosyntheis Downstream of Lanosterol. *J. Lipid Res.* **1991**, *32*, 893–902. (i) Mayer, R. J.; Adams, J. L.; Bossard, M. J.; Berkhout, T. A. Effects of a Novel Lanosterol 14α-Demethylase Inhibitor on the Regulation of 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase in Hep G2 Cells. *J. Biol. Chem.* **1991**, *266*, 20070–20078. (j) Sliskovic, D. R.; White, A. D. Therapeutic Potential of ACAT Inhibitors as lipid Lowering and Anti-Atherosclerotic Agents. *Trends Pharmacol. Sci.* **1991**, *12*, 194–199. (k) Field, F. J.; Albright, E.; Mathur, S. Inhibition of Acylcoenzyme A:Cholesterol Acyltransferase Activity by PD128042: Effect on Cholesterol Metabolism and Secretion in CaCo-2 Cells. *Lipids* **1991**, *26*, 1–8. (l) Sundseth, S. S.; Wasman, D. J. Hepatic P-450 Cholesterol 7α-Hydroxylase. Regulation In Vivo at the Protein and mRNA Level in Response to Mevinolate, Diurnal Rhythm, and Bile Acid Feedback. *J. Biol. Chem.* **1990**, *265*, 15090–15095 and references cited therein.

(7) (a) Pullinger, C. R.; Gibbens, G. F. Regulation of Cholesterol Synthesis in the Liver and Mammary Gland of the Lactating Rat. *Biochem. J.* **1983**, *210*, 625–632. (b) Mathisa, M. M.; Sullivan, A. C.; Hamilton, J. G. Fatty Acid and Cholesterol Synthesis From Specifically Labeled Leucine by Isolated Rat Hepatocytes. *Lipids* **1981**, *16*, 739–743. (c) Sullivan, A. C.; Triscari, J.; Hamilton, J. G.; Kritchevsky, D. Hypolipidemic Activity of (−)-Hydroxy-citrate. *Lipids* **1977**, *12*, 1–9. (d) Lowenstein, J. M.; Brunengraber, H. In *Methods Enzymology*; Lowenstein, J. M., Ed.; Academic Press: New York, 1981; Vol. 72, pp 486–497. (e) Sullivan, A. C.; Triscari, J.; Hamilton, J. G.; Miller, O.; Wheatley, V. R. Effects of (−)-Hydroxycitrate Upon the Accumulation of Lipid in the Rat. I. Lipogenesis. *Lipids* **1973**, *9*, 121–128. (f) Sullivan, A. C.; Triscari, J.; Hamilton, J. G.; Miller, O. Effects of (−)-Hydroxycitrate Upon the Accumulation of Lipid in the Rat. II. Appetite. *Lipids* **1973**, *9*, 129–134.

(8) Berkhout, T. A.; Havekes, L. M.; Pearce, N. J.; Groot, P. H. E. The Effect of (−)-Hydroxycitrate on the Activity of the Low-Density-Lipoprotein Receptor and 3-Hydroxy-3-methylglutaryl-CoA Reductase Levels in the Human Hepatoma Cell Line Hep G2. *Biochem. J.* **1990**, *272*, 181–186.

(9) Sullivan, A. C.; Singh, M.; Srere, P. A.; Glusker, J. P. Reactivity and Inhibitor Potential of Hydroxycitrate Isomers with Citrate Synthetase, Citrate Lyase, and ATP-Citrate Lyase. *J. Biol. Chem.* **1977**, *252*, 7583–7590. (b) Cheema-Dhadli, S.; Halperin, M. L.; Leznoff, C. C. Inhibition of Enzymes Which Interact With Citrate by (−)-Hydroxycitrate and 1,2,3-Tricarboxybenzene. *Eur. J. Biochem.* **1973**, *38*, 98–102. (c) Sullivan, A. C.; Hamilton, J. G.; Miller, O. N.; Wheatley, V. R. Inhibition of Lipogenesis in Rat Liver by (−)-Hydroxycitrate. *Arch. Biochem. Biophys.* **1977**, *150*, 183–190. (d) Rudney, H.; Sexton, R. C. Regulation of Cholesterol Biosynthesis. *Annu. Rev. Nutr.* **1986**, *6*, 245–272.

(10) (a) Houston, B.; Nimmo, H. G. Purification and Some Kinetic Properties of Rat Liver ATP-Citrate Lyase. *Biochem. J.* **1984**, *224*, 437–443. (b) Plowman, K. M.; Cleland, W. W. Purification and Kinetic Studies of the Citrate Cleavage Enzyme. *J. Biol. Chem.* **1967**, *242*, 4239–4247. (c) For further discussion, see: Walsh, C. *Enzyme Reaction Mechanisms*; W. H. Freeman and Company: San Francisco, 1979; pp 765–768 and references therein.

(11) Wells, T. N. C. ATP-Citrate Lyase From Rat Liver. Characterisation of the Citryl-Enzyme Complexes. *Eur. J. Biochem.* **1991**, *199*, 163–186.

(12) (a) Srere, P. A. In *Advances in Enzymology*; Meister, A., Ed.; John Wiley & Sons: New York, 1975; Vol. 43, pp 85–101. (b) Linn, T. C.; Srere, P. A.; Walsh, C. *Biochemistry* **1981**, *20*, 3719–3723.

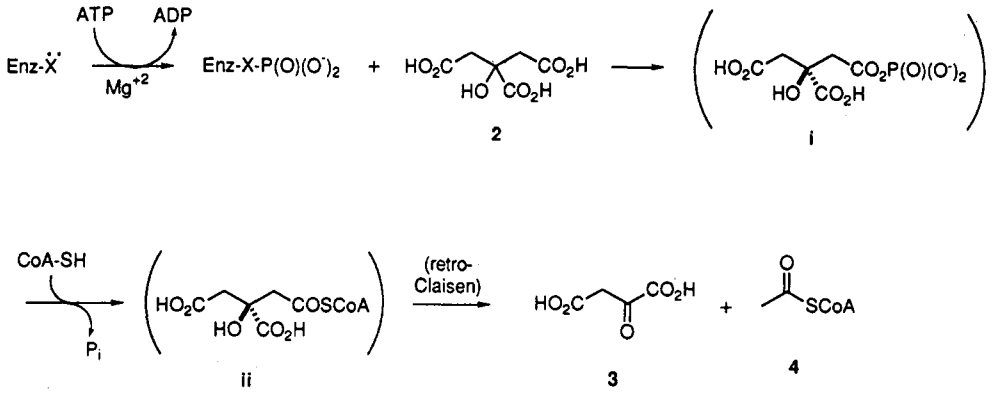
(13) Saxty, B. A.; Novelli, R.; Dolle, R. E.; Kruse, L. I.; Reid, D. G.; Camilleri, P.; Wells, T. N. C. Synthesis and Evaluation of (+)- and (−)-2,2-Difluorocitrate as Inhibitors of Rat-Liver ATP-Citrate Lyase and Porcine-Heart Aconitase. *Eur. J. Biochem.* **1992**, *202*, 889–896.

(14) Dolle, R. E.; Novelli, R.; Saxty, B. A.; Wells, T. N. C. Wells, Kruse, L. I.; Camilleri, P.; Eggleston, D. Preparation of (±)-(Erythro)- and (±)-(Threo)-2-Vinyl Citric Acids as Potential Mechanism-Based Inhibitors of ATP-Citrate Lyase. *Tetrahedron Lett.* **1991**, *32*, 4587–4590.

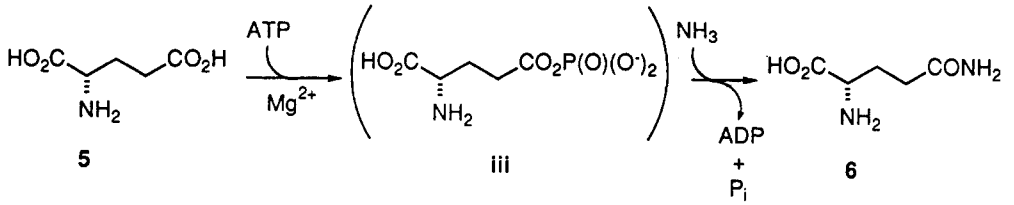
(15) (a) NIH Consensus Development Conference Summary. *Arteriosclerosis* **1984**, *4*, 296–301. (b) Havel, R. J. Role of Triglyceride-Rich Lipoproteins in Progression of Atherosclerosis. *Circulation* **1990**, *81*, 694–696. (c) International Committee for the Evaluation of Hypertriglyceridemia as a Vascular Risk Factor. Hypertriglyceridemias: Risk and Management. *Am. J. Cardiol.* **1991**, *68*, 1A–42A.

(16) (a) Elshourbagy, N. A.; Near, J. C.; Kmetz, P. J.; Sathe, G. M.; Southan, C.; Strickler, J. E.; Gross, M.; Young, J. F.; Wells, T. N. C.; Groot, P. H. E. Rat ATP Citrate-Lyase. Molecular Cloning and Sequence Analysis of a Full-Length cDNA and mRNA Abundance as a Function of Diet, Organ, and Age. *J. Biol. Chem.* **1990**, *265*, 1430–1435. (b) Elshourbagy, N. A.; Near, J. C.; Kmetz, P. J.; Wells, T. N. C.; Groot, P. H. E.; Hughes, S. A.; Franklin, M.; Gloger, I. S. Cloning and Expression of a Human ATP-Citrate Lyase cDNA. *Eur. J. Biochem.* **1992**, *204*, 491–499.

Scheme I. Revised Mechanism of ATP-Citrate Lyase Catalysis[11]

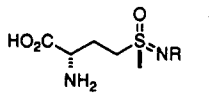


Scheme II. Mechanism of Glutamine Synthetase Catalysis[17]

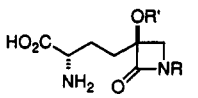


glutamate **5** yields a mixed carboxylate–phosphate anhydride iii which upon attack by enzyme-bound ammonia generates glutamine **6** (Scheme II). Well-known irreversible inhibitors of glutamine synthetase include methionine sulfoximine (**7**)[17a,18] and β-tabtoxinine (**8**).[19] Although **7** and **8** have little intrinsic inhibitory activity, these agents are phosphorylated by glutamine synthetase, producing **9**[18b,d] and **10** (or **11**)[19,20] which mimic the enzyme-bound γ-carboxylate phosphate anhydride iii and are the slow, tight-binding, essentially irreversible inhibitory species.

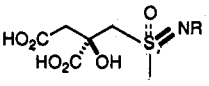
By analogy to the glutamine synthetase inhibitors **7** and **8**, citric acid analogs **12a,b** and **13a,b** were conceived as potential ATP-citrate lyase inhibitors. In structures **12a,b** and **13a,b**, one of the primary carboxylate groups has been replaced by a sulfoximinoyl and a 3-(3-hydroxy-2-oxoazetidinyl) moiety. Enzyme-mediated phosphorylation of **12a,b** and **13a,b** could furnish **14a,b** and **15a,b** (or **16a,b**). These would mimic the enzyme-bound intermediate i and be tight-binding inhibitors. During the course of this work, the synthesis of **13a,b** was abandoned due to the observed instability of **17a,b** in aqueous buffer ($t_{1/2}$ < 12 h; pH 4–8, 25 °C). Thus in this study, we describe the synthesis and enzyme evaluation of (±)-**12a,b** and (±)-**17a,b** as potential ATP-citrate lyase inhibitors.



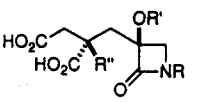
7: R = H
9: R = P(O)(O⁻)₂



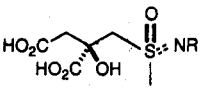
8: R = R' = H
10: R = H; R' = P(O)(O⁻)₂
11: R = P(O)(O⁻)₂; R' = H



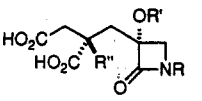
(±)-**12a**: R = H
(±)-**14a**: R = P(O)(O⁻)₂



(±)-**13a**: R = R' = H; R" = OH
(±)-**15a**: R = H; R' = P(O)(O⁻)₂; R" = OH
(±)-**16a**: R = P(O)(O⁻)₂; R' = H; R" = OH
(±)-**17a**: R = R' = H; R" = H



(±)-**12b**: R = H
(±)-**14b**: R = P(O)(O⁻)₂



(±)-**13b**: R = R' = H; R" = OH
(±)-**15b**: R = H; R' = P(O)(O⁻)₂; R" = OH
(±)-**16b**: R = P(O)(O⁻)₂; R' = H; R" = OH
(±)-**17b**: R = R' = H; R" = H

Chemistry

Preparation of Sulfoximines (±)-12a and (±)-12b. The synthesis of the diastereomeric pairs of sulfoximines (±)-**12a** and (±)-**12b** is presented in Scheme III. The reaction of NaSMe with methyl 4-chloroacetoacetate (**18**) in MeOH afforded methyl sulfide **19** in 55% yield. Elaboration of the ketone carbonyl in **19** to the hydroxy acid function present in **20** was carried out using cyanohydrin/hydrolysis conditions similar to those reported by Beach.[21] Benzylation of **20** (2.3 equiv each of K_2CO_3 and BzBr, DMF, 60 °C, 18 h; **20** → **21**) followed by oxidation

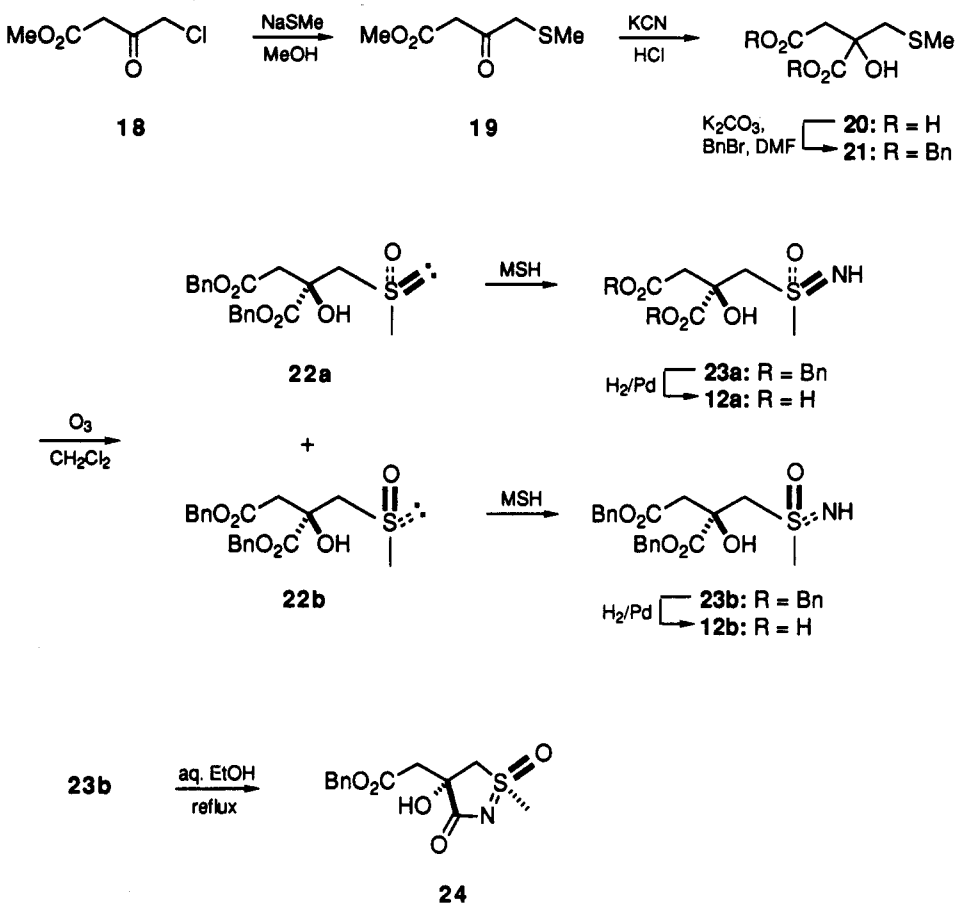
(17) (a) Meister, A. In *The Enzymes*, 3rd ed.; Boyer, P., Ed.; Academic Press: New York, 1974; Vol. 10, pp 699–807. (b) Meek, T. D.; Villafranca, J. J. Kinetic Mechanism of *Escherichia Coli* Glutamine Synthetase. *Biochemistry* **1980**, *19*, 5513–5519.

(18) (a) Rando, R. Mechanisms of Action of Naturally Occurring Irreversible Enzyme Inhibitors. *Acc. Chem. Res.* **1975**, *8*, 281–288. (b) Ranzio, R.; Rowe, W.; Meister, A. Mechanism of Inhibition of Glutamine Synthetase by Methionine Sulfoximine. *Biochemistry* **1969**, *8*, 1066–1075. (c) Ranzio, R.; Rowe, W.; Meister, A. Inhibition of Glutamine Synthetase by Methionine Sulfoximine. Methionine Sulfoximine Phosphate. *Biochemistry* **1969**, *8*, 2674–2680. (d) Manning, J.; Moore, S.; Rowe, W.; Meister, A. Identification of L-Methionine S-Sulfoximine as the Diastereoismer of L-Methionine SR-Sulfoximine That Inhibits Glutamine Synthetase. *Biochemistry* **1969**, *8*, 2681–2685.

(19) (a) Stewart, W. W. Isolation and Proof of Structure of Wildfire Toxin. *Nature* **1971**, *229*, 174–178. (b) Uchytil, T. F.; Durbin, R. D. Hydrolysis of Tabtoxins by Plant and Bacterial Enzymes. *Experientia* **1980**, *36*, 301–302. (c) Langston-Unkefer, P. L.; Macy, P. A.; Durbin, R. D. Inactivation of Glutamine Synthetase by Tabtoxinine-β-lactam. Effects of Substrates and pH. *Plant Physiol.* **1984**, *76*, 71–74.

(20) Although the mechanism of inhibition of glutamine synthetase by tabtoxinine is not known, formation of the N- or O-phosphorylated forms, **10** or **11**, as the inhibitory species is postulated.[19] The mechanism of inhibition of glutamine synthetase by methionine sulfoximine, via formation of **9**, is known and has been studied in detail.[18b-d]

Scheme III. Synthesis of (±)-12a,b[a]



[a] All compounds are racemic. Only one stereoisomer is shown for clarity.

with ozone in the presence of Sudan III[22] gave a 1:1 mixture of diastereomeric sulfides **22a** and **22b** (stereochemical assignment vida infra) in 40% overall yield from **20**. Separation of the diastereomers was achieved by silica gel chromatography (**22a**: mp 88–89 °C; R_f 0.48 (silica, 10% MeOH–Et_2O); **22b**: mp 69–70 °C; R_f 0.45 (silica, 10% MeOH–Et_2O)). Conversion of sulfoxide **22a** (**22b**) to sulfoximine **23a** (**23b**) occurred smoothly upon exposure of **22a** (**22b**) to *O*-(mesitylsulfonyl)hydroxylamine (MSH)[23] (2.0 equiv of MSH, CH_2Cl_2, 25 °C, 18 h). The oxidative amination reaction was conveniently monitored by ^1H NMR by following the disappearance of the S(O)*Me* proton absorption (DMSO δ 2.58) and the appearance of the S(O)(NH)*Me* proton absorption (DMSO δ 2.90), as the R_f of **22a** (**22b**) and **23a** (**23b**) did not differ. Target sulfoximines (±)-**12a** (mp 103–105 °C (MeOH)) and (±)-**12b** (mp 138–139 °C (MeOH)) were obtained when **23a** and **23b** were respectively subjected to an ambient atmosphere of H_2 over a Pd catalyst (10% Pd/C, MeOH, 2 h).

The assignment of relative stereochemistry for the series **22a,b**, **23a,b**, and **12a,b** was unequivocally deduced from the X-ray crystallographic analysis of the cyclic sulfoximine **24** (ORTEP drawing, Figure 1). In attempts to find a suitable recrystallization solvent for **23b**, it was discovered that **23b** underwent facile conversion to **24** in hot aqueous EtOH. The formation of **24** from **23b** is not surprising in light of the nucleophilic character of the sulfoximine nitrogen.[24]

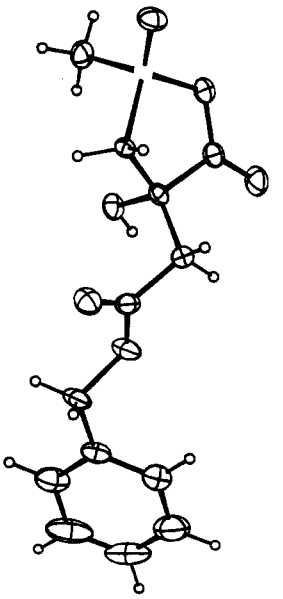


Figure 1. Molecular structure of cyclic sulfoximine **24** as determined by X-ray crystallography (ORTEP diagram).

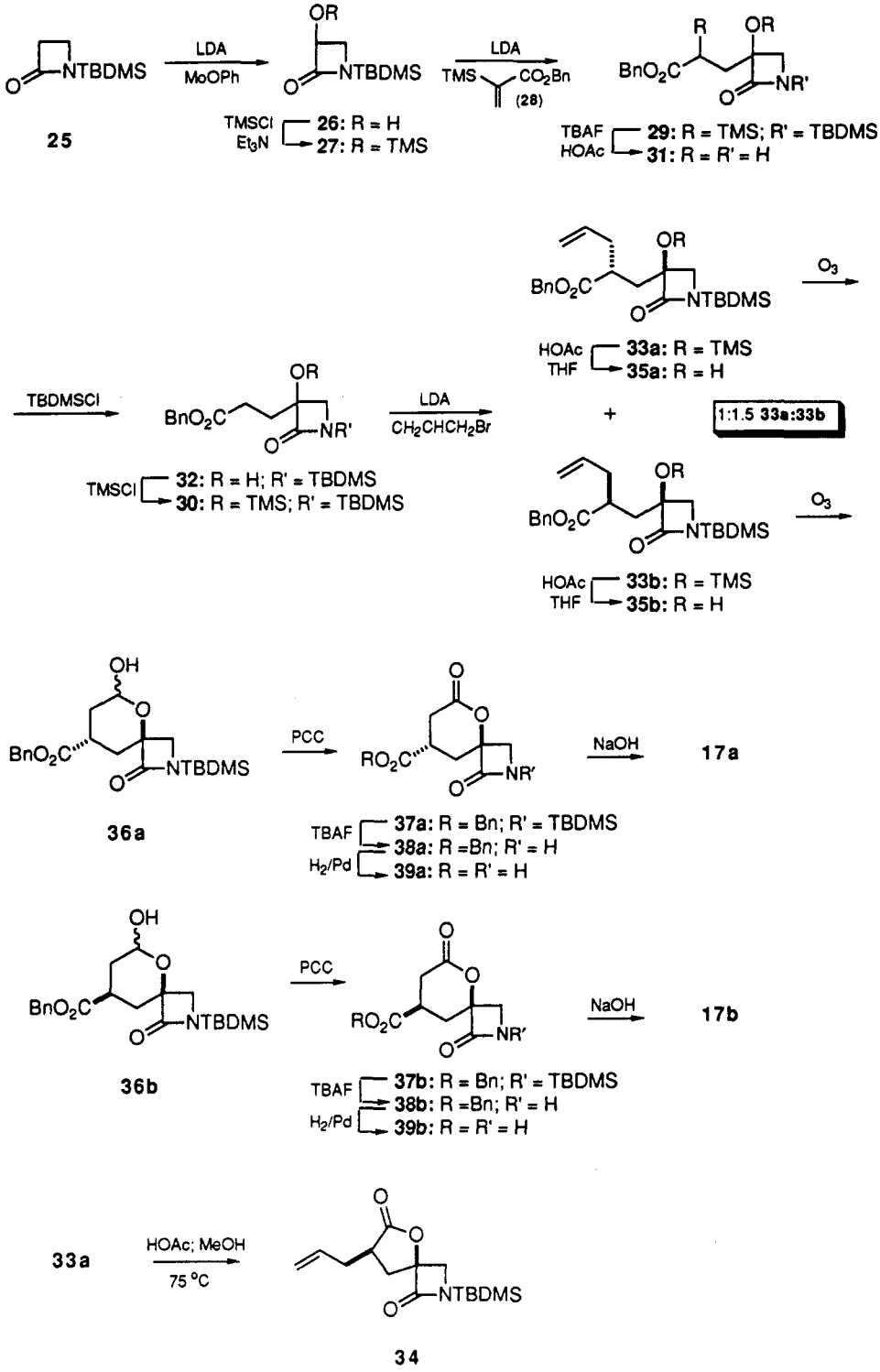
Preparation of β-Lactams (±)-17a and (±)-17b. The synthesis of β-lactams (±)-**17a** and (±)-**17b** was initiated by hydroxylation of azetidinone **25**[25] (1.1 equiv of LDA,

(21) Beach, R. L.; Aogaichi, T.; Plaut, G. W. E. Identification of D-threo-α-Methylisocitrate as Stereochemically Specific Substrate for Bovine Heart Aconitase and Inhibitor of TPN-Linked Isocitrate Dehydrogenase. *J. Biol. Chem.* **1977**, *252*, 2702–2709.

(22) Veysoglu, T.; Mutscher, L.; Swayze, J. K. A Convenient Method for the Control of Selective Ozonations of Olefins. *Synthesis* **1980**, 807–810.

(23) Tamura, Y.; Minamikawa, J.; Somoto, K.; Fujii, S.; Ikeda, M. Synthesis and Some Properties of O-Acyl and O-Nitrophenylhydroxylamines. *J. Org. Chem.* **1973**, *38*, 1239–1241.

Scheme IV. Synthesis of (±)-17a,b[a]



[a] All compounds are racemic. Only one stereoisomer is shown for clarity.

THF, −78 °C; then 1.2 equiv of MoO_5–pyridine–HMPA (MoOPh))[26] followed by silylation of the nascent hydroxyl group (TMSCl, Et_3N; **26** → **27**) in 55% overall yield from **25** (Scheme IV). Michael addition of **27** to benzyl acrylate did not occur in good yield; however, 1,4-addition to benzyl 2-(trimethylsilyl)acrylate (**28**) (prepared from 2-(trimethylsilyl)acrylic acid[27] and NaH, $PhCH_2Br$) proceeded smoothly to furnish **29** as a 3.6:1 mixture of

(24) (a) Levenson, C. H.; Meyer, R. B., Jr. Design and Synthesis of Tetrahedral Intermediate Analogues as Potential Dihydroorotase Inhibitors. *J. Med. Chem.* **1984**, *27*, 228–232. (b) Hwang, K.-J. N-(Trimethylsilyl)methylphenylsulfoximine: A Convenient Intermediate for the Preparation of Functionalized Sulfoximines. *J. Org. Chem.* **1986**, *51*, 99–101 and references cited therein.

(25) Ogilvie, W. W.; Durst, T. Oxidation of 3-Alkylidene-β-lactams. A Preparation of 3-Alkenyl-3-hydroxy-β-lactams. *Can. J. Chem.* **1988**, *66*, 304–309.

(26) Vedejs, E.; Engler, D. A.; Telschow, J. E. Transition-Metal Peroxide Reactions. Synthesis of α-Hydroxycarbonyl Compounds from Enolates. *J. Org. Chem.* **1978**, *43*, 188–196.

(27) Ottolenghi, A.; Fridkin, M.; Zilkna, A. Synthesis and Polymerization of α-Trimethylsilylacrylic Monomers. *Can. J. Chem.* **1963**, *41*, 2977–2982.

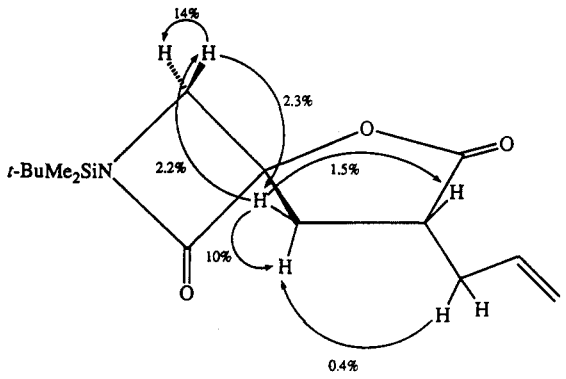


Figure 2. ^{1}H NMR (NOE) structural assignment of spiro lactam **34**.

diastereomers (74% yield).[28] Selective removal of the α-TMS group in the presence of the N- and O-silyl protecting groups in **29** to yield **30** was not possible. Thus, a three-step deprotection–reprotection sequence (**29** → **31** → **32** → **30**) using standard reagents and reaction conditions was carried out to provide key intermediate **30**. To complete the construction of the carbon skeleton present in (±)-**17a,b**, intermediate **30** was alkylated with allyl bromide (1.1 equiv of LDA, 1.2 equiv of CH_2CHCH_2-Br, −78 °C, 1 h; 65%). A 1:1.5 mixture of diastereomers **33a**:**33b** was obtained and a portion of the mixture was separated by HPLC (silica, 38:1 hexane–EtOAc). Relative stereochemistry as depicted for **33a**, and hence for **33b**, was readily determined after extensive NOE experiments were carried out on the rigid spirocycle **34** (Figure 2). Spirocycle **34** was derived from the minor diastereomer **33a** following *O*-trimethylsilyl deprotection and spontaneous spiro-lactonization in MeOH containing HOAc.

The remaining 1:1.5 mixture of **33a**:**33b** was subjected to sequential O-desilylation (9:1 MeOH–HOAc, 25 °C, 18 h; **33a** (**33b**) → **35a** (**35b**), and ozonolysis (O_3, CH_2Cl_2, −78 °C; then PPh_3 −78 °C → 25 °C; **35a** (**35b**) → **36a** (**36b**). The spirolactols **36a** (32%) and **36b** (50%) so obtained were conveniently separated by column chromatography (silica gel, 30% Et_2O–hexane). Oxidation of **36a** and **36b** with PCC (1.5 equiv of PCC, NaOAc, CH_2Cl_2) furnished lactones **37a** and **37b**, respectively. Remaining transformations including (1) N-desilylation (2 equiv each of Bu_4-NF and HOAc, CH_2Cl_2; **37a** (**37b**) → **38a** (**38b**); 90%), (2) debenzylation (1 atm H_2, 10% Pd/C, THF; **38a** (**38b**) → **39a** (**39b**); 85%), and (3) chemoselective lactone hydrolysis (4.1 equiv of aqueous NaOH; **39a** (**39b**) → **17a** (**17b**); 100%) led to the successful completion of the synthesis of (±)-**17a** and (±)-**17b**. These β-lactams showed limited stability in aqueous solution over a narrow pH range ($t_{1/2} \sim 12$ h, pH 4–8, 25 °C) as determined by ^{1}H NMR (D_2O).

Results and Discussion

One of the early steps in the ATP-citrate lyase mediated conversion of citrate **2** to acetyl-CoA **4** is the activation of the *pro-S* carboxylate of **2** as a mixed carboxylate–phosphate anhydride (Scheme I). The activation results in the formation of a noncovalently bound citryl phosphate intermediate. By analogy with the well-known inhibitors

Table I. Kinetic Results of Potential Inhibitors Evaluated against Rat ATP-Citrate Lyase

compd	reversible binding[a] K_i (μM)	ATPase activity[a] V_{max} (rel)	ATPase activity[a] K_m (μM)
2		1	110
12a	250	0.25	1300
12b	>10000	<0.003	nd[b]
17a	>9000	nd	nd
17b	>20000	nd	nd

[a] Reversible binding was measured by the inhibition of the carbon–carbon bond cleavage activity. ATPase activity was measured using a pyruvate kinase coupled assay. See the Experimental Section for details. [b] Not determined.

7[18] and **8**[19,20] of glutamine synthetase, an enzyme which also employs carboxylate–phosphate activation as part of its catalytic mechanism,[17] sulfoximines (±)-**12a** and (±)-**12b** and β-lactams (±)-**17a** and (±)-**17b**, were designed as potential tight-binding (irreversible) inhibitors of ATP-citrate lyase.

The results of the kinetic analysis of compounds (±)-**12a,b** and (±)-**17a,b** against enzyme are presented in Table I. Only one pair of diastereomeric sulfoximines, (±)-**12a**, exhibited inhibitory activity against enzyme, while (±)-**12b** and (±)-**17a,b** were devoid of activity. The inhibition by (±)-**12a** was weak and reversible ($K_i = 250\ \mu M$). Neither irreversible inactivation of enzyme nor carbon–carbon bond cleavage of (±)-**12a** was observed (data not shown). Modest ATP-ase activity was also seen for (±)-**12a** when (±)-**12a** was substituted for citrate as a substrate. The K_m for (±)-**12a** was 10-fold greater relative to citrate and the relative V_{max} was 25% that of normal substrate. The source of the ATP-ase activity displayed by ATP-citrate lyase is a consequence of the formation of a discrete phosphoenzyme intermediate[10,11,16] as the first step in catalysis (Scheme I). The ATP-ase activity observed for (±)-**12a** is not a measure of the extent (if at all) (±)-**12a** is phosphorylated by ATP-citrate lyase, but demonstrates nonproductive ATP → ADP hydrolysis in the presence of (±)-**12a**.

It is highly probable that only one antipode of (±)-**12a** has affinity for enzyme given the precedent for the rigorous stereochemical binding requirement for **7** and **8** to glutamine synthetase.[18,19,28b] If this is taken into account, the K_m for (+)-**12a** (or (−)-**12a**) comes within 6-fold that of **2**. This would suggest that the sulfoximinoyl functional group can mimic, to some extent, the carboxylate group in the active site of the enzyme, probably in terms of its hydrogen-bonding ability. However, the size of the sulfoximinoyl group may preclude it from being considered a generic, neutral carboxylate isoster.

It is evident that carboxylate–phosphate anhydride traps of the sulfoximine and β-lactam type cannot be exploited as inhibitors of ATP-citrate lyase. Insight as to why (±)-**12a,b** and (±)-**17a,b** do not function as inhibitors of ATP-citrate lyase while **7** and **8** are potent inhibitors of glutamine synthetase[18,19] may be gleaned upon considering the difference in mechanism by which the two enzymes form their respective carboxylate–phosphate intermediate i and iii (Schemes I and II). For glutamine synthetase, formation of iii occurs by *direct* transfer of a phosphate from ATP to glutamate in the presence of Mg^{2+}.[17] The metal cofactor, iii, and so-produced ADP all remain bound to enzyme and it is not until the attack of NH_3 on iii that Mg^{2+} and products ADP, P_i, and **6** are released from the active site, completing the catalytic cycle (Scheme II). The binding

(28) For the alkylation of the lithium enolate of **27** with other electrophiles, see: (a) Dolle, R. E.; Hughes, M. J.; Li, C.-S.; Kruse, L. I. Preparation and Alkylation of N-*t*-Butyldimethylsilyl-3-trimethylsilyloxyazetidin-2-one. *J. Chem. Soc., Chem. Commun.* **1989**, 1448–1449. (b) Dolle, R. E.; Li, C.-S.; Novelli, R.; Kruse, L. I.; Eggleston, D. Enantiospecific Synthesis of (−)-Tabtoxinine β-Lactam. *J. Org. Chem.* **1992**, *57*, 128–132.

of 7, ATP, and Mg^{2+} to glutamine synthetase generates phosphorylated form 9, yielding a slow, tight-binding ternary inhibitor complex with Mg^{2+} and ADP.[17a,18] Only one stereoisomer, (*S,S*)-7, is a potent, tight-binding inhibitor, while the other three diastereomers are reversible inhibitors.[17a,18d]

In the case of ATP-citrate lyase, formation of i occurs by *indirect* transfer of a phosphate from ATP to 2, via histidine 765 in the presence of Mg^{2+}, as the very first step in the catalytic cycle is the phosphorylation of enzyme with ATP (Scheme I).[10,11,16] Hence, the formation of an analogous slow, tight-binding ternary complex between **14a,b** (if formed), Mg^{2+} and ADP is not possible. It may be for this reason that (±)-**12a** (or (±)-**14a**) is a weak, reversible inhibitor and (±)-**12b**, (±)-**17a**, and (±)-**17b** are inactive.[29-31]

Experimental Section

General Procedures. Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Analytical samples were homogeneous by TLC performed on silica gel plates. Elemental analyses (C, H, N) of new compounds are within 0.4% of the theoretical values. NMR spectra were determined on Bruker AM 250 or AM 360 spectrometers using tetramethylsilane (TMS) as the internal standard. IR spectra were recorded on a Perkin-Elmer Model 298 instrument. Low-resolution and high-resolution (EI) mass spectrums were recorded on a VG-70-250-SEQ instrument. All structural assignments were consistant with IR, NMR, and mass spectra.

4-(Methylthio)-3-oxobutanoic Acid Methyl Ester (19). Sodium hydride (63.8 g, 1.60 mol, 60% oil dispersion) was added portionwise to a precooled (0 °C) solution of MeSH (96.2 g, 2.0 mol) in MeOH (2.0 L). Following the addition of the NaH and stirring from 30 min, a solution of methyl 4-chloroacetoacetate (18) (200 g, 1.3 mol) in MeOH (150 mL) was added over a 30-min period at 0 °C. The formation of NaCl (white precipitate) was immediately noted. The reaction mixture was stirred at 25 °C for 12 h and most of the MeOH (1500 mL) was then removed by fractionation at ambient pressure. The solution was filtered and the filtrate was diluted with brine (1500 mL) and extracted with diethyl ether. The organic extract was dried ($MgSO_4$) and concentrated in vacuo, yielding a brown oil which was distilled under high vacuum to give sulfide **19** (114.6 g. 55%): oil; bp_{1mm} 98–100 °C; R_f 0.5 (50% Et_2O–hexane); IR (neat) 2955, 2920, 1750, 1710, 1625, 1440, 1325 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.75 (s, 3 H, OMe), 3.69 and 3.32 (singlets, 2 H each, CH_2), 2.07 (s, 3 H, SMe); mass spectrum *m/z* (rel intensity) 162 (M^+, 22), 130 (20), 88 (23), 61 (100). Anal. ($C_6H_{10}O_3S$) C, H.

2-Hydroxy-2-[(Methylthio)methyl]butanedioic Acid (20). Ester **19** (114.5 g, 0.71 mol) was added to a stirred suspension of KCN (229.5 g, 3.5 mol) in diethyl ether (500 mL) at 0 °C. Concentrated HCl (295 mL, 12 M solution) was then added to the vigorously stirred suspension at a rate of 1–1.5 mL/min during which time the reaction mixture was slowly warmed to 25 °C. After the heterogeneous reaction mixture was stirred vigorously for 6 h at 25 °C, the suspension was allowed to settle and the diethyl ether was decanted from the residue. The residue was washed with diethyl ether (2 × 100 mL), and the organic extracts were combined. To this diethyl ether solution was added concentrated HCl (1500 mL, 12 M solution) and the mixture was stirred for 30 min and then left to stand for 12 h (precipitation of NH_4Cl was observed).

Diethyl ether was added to the mixture until a phase separation was observed and then the biphasic solution was stirred for 30 min. The solution was filtered, and the diethyl ether and acid were completely removed in vacuo. The residue so obtained was dissolved in HCl (900 mL, 3 N aqueous solution) and the solution was heated to 100 °C for 3 h. The aqueous acid was removed under high vacuum (1 mm) at 50 °C, and the residue was azeotroped with water (3 × 100 mL) to remove residual HCl. The brown amorphous solid which remained was dried (P_2O_5) for 12 h at 40 °C in vacuo. The residue was extracted with hot EtOAc (4 × 100 mL). The extracts were combined, filtered, and decolorized with charcoal, and the solvent was removed in vacuo to give analytically pure diacid **20** (42.7 g. 68%): mp 119–120 °C; IR (KBr disc) 3440, 3100–2800, 1740, 1700, 1455, 1220, 1100 cm^{-1}; ^1H NMR (DMSO) δ 2.65 (m, 4 H, CH_2) 2.12 (s, 3 H, SMe); mass spectrum *m/z* (rel intensity) 194 (M^+, 100). Anal. ($C_6H_{10}O_5S$) C, H.

2-Hydroxy-2-[(methylthio)methyl]butanedioic Acid Dibenzyl Ester (21). Powdered K_2CO_3 (76 g, 0.55 mol) was added to solution of diacid **20** (42.7 g, 0.22 mol) in anhydrous DMF (2.1 L). Benzyl bromide (62.6 mL, 0.53 mol) was then added by cannula and the reaction mixture was stirred at 60 °C for 18 h. The reaction mixture was diluted with water (6 L) and was extracted with diethyl ether (3 × 300 mL). The organic extracts were combined, washed with water and brine, and dried ($MgSO_4$). The solvent was removed in vacuo to give a yellow viscous oil which was purified by chromatography (30% Et_2O–hexane) yielding dibenzyl ester **21** (49.5 g, 61%): oil; R_f 0.55 (50% Et_2O–hexane); IR (neat) 3500, 3065, 2955, 1758, 1608, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (m, 10 H, Ar), 5.10 (m, 4 H, CH_2Ar), 3.94 (s, 1 H, OH), 2.84 (m, 4 H, CH_2S and CH_2CO), 2.14 (s, 3 H, SMe); mass spectrum *m/z* (rel intensity) 465 (20), 375 (25), 181 (12), 91 (100). Anal. ($C_{20}H_{22}O_5S$) C, H.

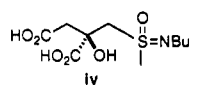
[*S*-(2*R,*S**)]-2-Hydroxy-2-[(methylsulfinyl)methyl]butanedioic Acid Dibenzyl Ester (22a) and the [*S*-(2*R**,*R**)]-Diastereomer (22b).** A solution of sulfide **21** (49.2 g, 130 mmol) in CH_2Cl_2 containing a trace of Sudan III was cooled to −78 °C. Ozone was passed into the deep red solution until a colorless solution resulted. The solution was purged with N_2 for 10 min and then the solution was warmed to 25 °C. The solvent was removed in vacuo and the residue was purified by chromatography (5% MeOH–Et_2O) to give the diastereomeric sulfoxides **22a** (13.2 g, 34%) and **22b** (11.9 g, 31%). For **22a**: mp 88–89 °C (Et_2O); R_f 0.48 (10% MeOH–Et_2O); IR (KBr disc) 2930, 2855, 1745, 1727, 1460, 1380 cm^{-1}; ^1H NMR (DMSO) δ 7.34 (m, 10 H, Ar), 6.30 (s, 1 H, OH), 5.08 and 5.04 (singlets, 2 H each, CH_2Ar), 3.15 (m, 4 H, CH_2), 2.58 (s, 3 H, SMe); ^{13}C NMR (DMSO) δ 171.8 and 169.1 (C=O), 135.8, 135.4, 128.2, 127.9, 127.9, 127.8 and 127.7 (Ar), 73.0 (COH), 68.5 and 65.6 (CH_2Ar), 61.2 ($C(O)CH_2$), 41.7 (CH_2S), 39.3 (SCH_3); mass spectrum *m/z* (rel intensity) 391 (M^+, 100) 301 (15), 185 (28), 91 (64). Anal. ($C_{20}H_{22}O_6S$) C, H.

For **22b**: mp 69–70 °C (Et_2O); R_f 0.45 (10% MeOH–Et_2O); IR 2925, 2855, 1750, 1735, 1455, 1380, 1230, 1000 cm^{-1}; ^1H NMR (DMSO) δ 7.35 (m, 10 H, Ar), 6.12 (s, 1 H, OH), 5.02 (s, 4 H, CH_2Ar), 3.34 and 3.24 (doublets, 1 H each, J = 12.0 Hz, $C(O)CH_2$), 3.16 and 2.78 (doublets, 1 H each, J = 12.0 Hz, CH_2S), 2.58 (s, 3 H, SMe); ^{13}C NMR (DMSO) δ 171.8, 168.7, 135.7, 135.8, 128.4, 128.3, 128.0, 127.9, 72.7, 66.6, 65.8, 63.2, 44.2, 39.7; mass spectrum *m/z* (rel intensity) 391 (M + H, 42) 301 (12), 277 (9), 185 (77), 93 (100). Anal. ($C_{20}H_{22}O_6S$) C, H.

[*S*-(2*R,*S**)]-2-Hydroxy-2-[(*S*-methylsulfonimidoyl)methyl]butanedioic Acid Dibenzyl Ester (23a) and the [*S*-(2*R**,*R**)]-Diastereomer (23b).** A solution of sulfoxide **22a** (12.1 g, 31.1 mmol) in CH_2Cl_2 (100 mL) was cooled to 0 °C and treated with freshly prepared *O*-(mesitylsulfonyl)hydroxylamine[23] (13.4 g, 62.2 mmol). The solution was stirred at 25 °C for 18 h when amination was judged complete by ^1H NMR (DMSO) δ 2.58 (S(O)*Me*; δ 2.90 (S(O)(NH)*Me*). The reaction mixture was washed with saturated aqueous $NaHCO_3$ and brine and dried $MgSO_4$. Removal of the solvent in vacuo and purification of the residue by chromatography (5% MeOH–Et_2O) gave sulfoximine **23a** (6.9 g, 55%): mp 95–96 °C (5% MdOH–Et_2O); R_f 0.48 (10% MeOH–Et_2O); IR (KBr disc) 3290, 2930, 2855, 1740, 1455, 1380, 1065, 1015 cm^{-1}; ^1H NMR (DMSO) δ 7.34 (m, 10 H, Ar), 6.39 (s,

(29) We did not attempt to prepare the sulfoximine phosphonates **14a,b**.

(30) Sulfoximine iv was also found inactive (K_i > 10 mM); unpublished results.



iv

(31) For another unsuccessful attempt of employing carboxylate–phosphate anhydride traps (β-lactam type) as an enzyme inhibitor, see: Greenlee, W. J.; Springer, J. P.; Patchett, A. A. Synthesis of an Analogue of Tabtoxinine as a Potential Inhibitor of D-Alanine:D-alanine Ligase (ADP Forming). *J. Med. Chem.* **1989**, *32*, 165–170.

1 H, OH), 5.04 (m, 4 H, CH_2Ar), 4.01 (s, 1 H, NH), 3.65 and 3.55 (doublets, 1 H each, J = 12.0 Hz, $C(O)CH_2$), 3.03 and 2.92 (doublets, 1 H each, J = 12.0 Hz, CH_2S), 2.96 (s, 3 H, SMe); ^{13}C NMR (DMSO) δ 171.4 and 168.6 (C=O), 135.6, 135.4, 128.2, 128.1, 127.9 and 127.8 (Ar), 73.2 (COH), 66.6 and 65.7 (CH_2Ar), 62.1 ($C(O)CH_2$), 44.6 (SMe), 43.3 (CH_2S); mass spectrum m/z (rel intensity) 406 (M + H, 8), 391 (8), 316 (32), 185 (62), 93 (100). Anal. ($C_{20}H_{23}NO_6S$) C, H, N.

Sulfoximine **23b** (5.5 g, 49%) was prepared from sulfoxide **22b** (10.8 g, 28 mmol) in an identical manner as described for the preparation of **23a**. For **23b**: mp 95–96 °C (5% MeOH–Et_2O); R_f 0.45 (10% MeOH–Et_2O); IR (KBr disc) 3345, 2925, 2855, 1755, 1740, 1460, 1380 cm^{-1}; ^1H NMR (DMSO) δ 7.34 (m, 10 H, Ar), 6.37 (s, 1 H, OH), 5.06 (m, 4 H, CH_2Ar), 3.84 (s, 1 H, NH), 3.64 and 3.58 (doublets, 1 H each, J = 2.0 Hz, $C(O)CH_2$), 3.05 and 2.95 (doublets, 1 H each, J = 12.0 Hz, CH_2S), 2.98 (s, 3 H, SMe); ^{13}C NMR (DMSO) δ 171.3, 168.7, 135.6, 135.5, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 126.4, 126.2, 73.1, 66.5, 65.6, 61.4, 60.1, 44.9, 42.7; mass spectrum m/z (rel intensity) 406 (M + H, 98), 316 (28), 270 (12), 181 (20), 91 (100). Anal. ($C_{20}H_{23}NO_6S$) C, H, N.

[*S*-(1*R-*trans*)]-4,5-Dihydro-4-hydroxy-1-methyl-3-oxo-3*H*-1λ⁴-isothiazole-4-acetic Acid Benzyl Ester (24).** Cyclic sulfoximine **24** was obtained in quantitative yield upon six recrystallizations of **23b** from 50% aqueous EtOH. For **24**: mp 171–172 °C dec; IR (KBr disc) 3300, 2980, 2880, 1715, 1685, 1280, 1210, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (m, 5 H, Ar), 5.20 (s, 2 H, CH_2Ar), 3.80 (m, 2 H, $C(O)CH_2$), 3.45 (s, 3 H, SMe), 3.10 and 2.90 (doublets, 1 H each, J = 15.0 Hz, CH_2S); mass spectrum m/z (rel intensity) 298 (M + H, 12), 262 (2), 210 (100), 163 (24). Anal. ($C_{13}H_{15}NO_5S$) C, H, N.

[*S*-(2*R,*S**)]-2-Hydroxy-2-[(*S*-methylsulfonimidoyl)methyl]butanedioic Acid (12a) and the [*S*-(2*R**,*R**)]-Diastereomer (12b).** A solution of benzyl ester **23a** (2.03 g, 5 mmol) in MeOH (50 mL) was stirred under ambient H_2 pressure in the presence of 10% Pd/C (1.0 g). After 2 h the hydrogenation reaction was judged complete by TLC (10% MeOH–Et_2O) and the solution was filtered to remove the catalyst. The filtrate was reduced to half its original volume and cooled to 0 °C. Diacid **12a** (860 mg, 76%) which crystallized from the solution was collected and dried (0.1 mm, 35 °C): mp 103–105 °C (MeOH); R_f 0.1 (10% MeOH–Et_2O); IR (KBr disc) 2930, 2855, 1720, 1615, 1461, 1375, 1245 cm^{-1}; ^1H NMR (DMSO) δ 3.60 (m, 2 H, CH_2S), 3.16 (s, 3 H, SMe), 2.85 and 2.65 (doublets, 1 H each, J = 12.0 Hz, $C(O)CH_2$)); ^{13}C NMR (DMSO) δ 173.4 and 170.6 (COOH), 72.4 (COH), 61.5 (CH_2S), 44.5 (SMe), 43.3 ($C(O)CH_2$); mass spectrum m/z (rel intensity) 226 (M + H), 12), 185 (72), 93 (100). Anal. ($C_6H_{11}NO_6S$) C, H, N.

Diacid **12b** (480 mg, 45%) was prepared from diester **23b** (1.92 g, 4.7 mmol) in an identical manner as described for the preparation of **12a** from **23a**. For **12b**: mp 138–139 °C (MeOH); R_f 0.1 (10% MeOH–Et_2O); IR 2925, 2854, 1710, 1610, 1460, 1275 cm^{-1}; ^1H NMR (DMSO) δ 3.62 and 3.48 (doublets, 1 H each, J = 12.0 Hz, CH_2S), 3.00 (s, 3 H, SMe), 2.87 and 2.84 (doublets, 1 H each, J = 12.0 Hz, $C(O)CH_2$); ^{13}C NMR (DMSO) δ 173.3, 170.8, 72.4, 61.2, 44.6, 42.0; mass spectrum m/z (rel intensity) 226 (M + H, 15), 185 (75), 93 (100). Anal. ($C_6H_{11}NO_6S$) C, H, N.

1-(*tert*-Butyldimethylsilyl)-3-hydroxy-2-azetidinone (26). To a stirred solution of diisopropylamine (5.2 mL, 37 mmol) in THF (100 mL) at 0 °C was added *n*-butyllithium (23 mL of a 1.5 M solution in hexanes, 34.5 mmol). The solution was stirred for 10 min at 0 °C and then was cooled in a dry ice–acetone bath to −78 °C. To this solution of LDA was added azetidinone **25**[25] (5.3 g, 28.6 mmol) as a solution in THF (30 mL) over a period of 10 min. After the mixture was stirred for an additional 30 min, oxodiperoxymolybdenum (pyridine) hexamethylphosphoramide (MoOPH)[26] (18.6 g, 43 mmol) was added in one portion. The reaction mixture was allowed to warm to 0 °C, quenched with saturated aqueous $NaHSO_3$ (40 mL), and partitioned between diethyl ether (200 mL) and brine (50 mL). The organic phase was separated and washed with water (50 mL) and brine (50 mL). The combined aqueous phases were back-extracted with diethyl ether (50 mL), and the combined organic phase was dried (Na_2SO_4), filtered through Celite, and concentrated under reduced pressure. The residue so obtained was purified by chromatography (50% hexane–Et_2O) followed by vacuum sublimation (150 °C at 1 mm) to give alcohol **26** (3.7 g; 64%): white solid; mp 52–55 °C; IR (KBr disc) 3280, 2926, 2856, 1745, 1465, 1254 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.95 (m, 1 H, CHOH), 4.16 (br s, 1 H, OH), 3.49 (t, 1 H, J = 5.8 Hz, CHH'N), 3.19 (dd, 1 H, J = 6.3, 2.5 Hz, CHH'N), 0.94 (s, 9 H, $SiCMe_3$), 0.24 (singlets, 3 H each, $SiMe_2$); mass spectrum m/z (rel intensity) 202 (M + H, 100). Anal. ($C_9H_{19}NO_2Si$) C, H, N.

1-(*tert*-Butyldimethylsilyl)-3-[(trimethylsilyloxy]-2-azetidinone (27). To a stirred solution of **26** (2.42 g, 12 mmol) in CH_2Cl_2 (50 mL) was added sequentially triethylamine (2.5 mL, 18 mmol) and chlorotrimethylsilane (2.2 mL, 17 mmol). The reaction mixture was stirred for 1.5 h and then the mixture was partitioned between water (50 mL) and diethyl ether (150 mL). The organic phase was washed with a mixture of brine (50 mL) and saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by bulb to bulb distillation (150 °C, 1 mm) to give β-lactam **27** (3.15 g; 96%): oil; IR ($CHCl_3$ solution) 2958, 2890, 1745, 1471, 1313, 1254 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.88 (dd, 1 H, J = 5.2, 2.6 Hz, CHOTMS), 3.44 (t, 1 H, J = 5.6 Hz, CHH'N), 3.11 (dd, 1 H, J = 6.0, 2.6 Hz, CHH'N), 0.95 (s, 9 H, $SiCMe_3$), 0.23 and 0.19 (singlets, 15 H total, SiMe); mass spectrum m/z (rel intensity) 258 (10), 230 (5), 216 (5), 116 (100). Anal. ($C_{12}H_{27}NO_2Si_2$) C, H, N.

Benzyl 2-(Trimethylsilyl)acrylate (28). To a stirred suspension of NaH (800 mg, 27 mmol; 80% oil dispersion) in DMF (15 mL) was added a solution of 2-(trimethylsilyl)acrylic acid[27] (3.6 g, 24 mmol) in DMF (10 mL). After hydrogen evolution had ceased, benzyl bromide (4.15 g, 24 mmol) was added. The reaction mixture was stirred at 25 °C for 1 h and then partitioned between diethyl ether (200 mL) and water (100 mL). The organic phase was washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (10% Et_2O–hexane) followed by bulb to bulb distillation (150 °C, 1 mm) to give ester **28** (4.1 g, 72%): oil; IR (thin film) 3068, 2957, 2899, 1719, 1594, 1456, 1275, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 6.84 and 6.05 (doublets, 1 H each, J = 2.9 Hz, CH_2), 5.20 (s, 2 H, CH_2Ar), 0.17 (s, 9 H, $SiMe_3$); mass spectrum m/z (rel intensity) 234 (8), 219 (15), 91 (100). Anal. ($C_{13}H_{18}O_2Si$) C, H.

1-(*tert*-Butyldimethylsilyl)-2-oxo-α-(trimethylsilyl)-3-[(trimethylsilyl)oxy]-3-azetidinepropanoic Acid Benzyl Ester (29). To a stirred solution of LDA (50 mL of a 0.5 M solution in THF–hexanes) at −78 °C was added **27** (4.35 g, 15.9 mmol) as a solution in THF (5 mL). The solution was stirred for 10 min prior to the addition of **28** (3.78 g, 15.9 mmol). The reaction mixture was stirred for a further 10 min and quenched with saturated aqueous NH_4Cl (30 mL). The reaction mixture was allowed to warm to 25 °C and partitioned between diethyl ether (150 mL) and water (30 mL). The organic phase was washed wtih brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue so obtained was purified by chromatography (10% Et_2O–hexane) to give ester **29** as a 3.6:1 mixture of diastereomers (5.94 g; 74%): oil; IR (thin film) 2950, 2890, 2860, 1745, 1715, 1465, 1250, 1185, 840 cm^{-1}; ^1H NMR ($CDCl_3$) (major isomer) δ 7.35 (m, 5 H, Ar), 5.10 (s, 2 H, CH_2Ar), 3.13 and 3.05 (doublets, 1 H each, J = 6.2 Hz, CH_2N), 2.52 (dd, 1 H, J = 14.4, 10.7 Hz, $CHSiMe_3$), 2.07 (d, 1 H, J = 10.7 Hz, CHH'), 1.71 (d, 1 H, J = 14.4 Hz, CHH'), 0.93 (s, 9 H, $SiCMe_3$), 0.20, 0.17, and 0.02 (singlets, 24 H total, SiMe); mass spectrum m/z (rel intensity) 492 (5), 259 (80), 169 (25), 91 (95), 73 (100).

3-Hydroxy-2-oxo-3-azetidinepropanoic Acid Benzyl Ester (31). To a stirred solution of **29** (7.8 g, 15.4 mmol) in THF (100 mL) were added glacial acetic acid (3.6 mL, 63 mmol) and tetrabutylammonium fluoride (22 mL of a 1 M solution in THF; 22 mmol). The reaction mixture was stirred for 2 h and poured onto silica gel (~35 g) and the solvent was evaporated under reduced pressure. The white powder so obtained was purified by chromatography (Et_2O) to give ester **31** (3.3 g; 87%): oil; IR (thin film) 3320, 2970, 2800, 1740, 1450, 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 6.3 (br s, 1 H, NH), 5.12 (s, 2 H, CH_2Ar) 4.8 (br s, 1 H, OH), 3.34 and 3.31 (doublets, 1 H each, J = 5.7 Hz, CH_2N), 2.68 (t, 2 H, J = 7.3 Hz, $COCH_2CH_2$), 2.19 (t, 1 H, J = 7.3 Hz, $COCH_2CH_2$); mass spectrum m/z (rel intensity) 140 (13), 106 (25), 91 (100).

1-(*tert*-Butyldimethylsilyl)-3-hydroxy-2-oxo-3-azetidinepropanoic Acid Benzyl Ester (32). To a stirred solution of **31** (4.6 g, 18.5 mmol) in DMF (50 mL) was added triethylamine

(5.2 mL, 37.1 mmol) and *tert*-butyldimethylsilyl chloride (5.6 g, 37.1 mmol). The reaction mixture was stirred for 40 min and partitioned between diethyl ether (500 mL) and water (100 mL). The organic phase was washed with water (2 × 200 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (50% Et_2O–hexane) to give alcohol **32** (6.18 g; 92%): oil; IR (thin film) 2950, 2920, 2855, 1745, 1720, 1460, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.12 (s, 2 H, CH_2Ar), 4.49 (br s, 1 H, OH), 3.25 and 3.21 (doublets, 1 H each, J = 7.8 Hz), 2.65 (m, 2 H, $COCH_2CH_2$), 2.16 (t, 2 H, J = 9.5 Hz, $COCH_2CH_2$), 0.93 (s, 9 H, $SiCMe_3$), 0.22 and 0.21 (singlets, 3 H each, $SiMe_2$).

1-(*tert*-Butyldimethylsilyl)-3-[(trimethylsilyl)oxy]-2-oxo-3-azetidinepropanoic Acid Benzyl Ester (30). To a stirred solution of **32** (6.18 g, 17 mmol) in CH_2Cl_2 (100 mL) were added triethylamine (7 mL, 50 mmol) and chlorotrimethylsilane (5 mL, 40 mmol). The reaction mixture was stirred for 2 h and partitioned between diethyl ether (500 mL) and water (200 mL). The organic phase was separated, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (10% Et_2O–hexane) followed by bulb to bulb distillation (250 °C, 0.3 mm) to give ester **30** (6.35 g; 86%): oil; IR (thin film) 2960, 2930, 2890, 1745, 1470, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.12 (s, 2 H, CH_2Ar), 3.21 and 3.16 (doublets, 1 H each, J = 6.2 Hz, CH_2N), 2.64 (ddd, 1 H, J = 17.0, 8.9, 6.9 Hz, $COCHH'CH_2$), 2.52 (ddd, 1 H, J = 17.1, 9.1, 6.8 Hz, $COCHH'CH_2$), 2.21 (m, 2 H, $COCH_2CH_2$), 0.94 (s, 9 H, $SiCMe_3$), 0.23 and 0.21 (singlets, 3 H, each, $NSiMe_2$), 0.18 (s, 9 H, $OSiMe_3$); mass spectrum m/z (rel intensity) 435 (8), 420 (15), 392 (10), 144 (45), 91 (100). Anal. ($C_{22}H_{37}NO_4Si_2$) C, H, N.

[*S*-(α*R,3*R**)]-1-(*tert*-Butyldimethylsilyl)-2-oxo-α-2-propenyl-3-[(trimethylsilyl)oxy]-3-azetidinepropanoic Acid Benzyl Ester (33a) and the [*S*-(α*S**,3*R**)]-Diastereomer (33b).** To a stirred solution of LDA (4.4 mL of a 0.5 M solution in THF–hexanes) at −78 °C was added **30** (435 mg, 1 mmol) as a solution in THF (2 mL). The solution was stirred for 20 min and then was transferred by cannula into a stirred solution of allyl bromide (0.5 mL, 5.6 mmol) in THF (3 mL) which was also precooled to −78 °C. The reaction mixture was allowed to warm to 25 °C, quenched with saturated aqueous NH_4Cl (10 mL), and partitioned between diethyl ether (50 mL) and water (20 mL). The organic phase was washed with brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue so obtained was purified by chromatography (10% Et_2O–hexane) to give a 1:1.5 diastereomeric mixture of esters **33a:33b** (312 mg; 65%) which was used directly in the subsequent transformation. A small portion of the isomeric mixture was separated by HPLC (25 mm prep silica column, eluant 39:1 hexane–ethyl acetate) to give **33b** (eluting first) and **33a**. For **33a** (minor isomer): IR ($CHCl_3$ solution) 3020, 2980, 1740, 1510, 1420, 1205 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.68 (ddt, 1 H, J = 18.0, 10.0, 7.0 Hz, $CH{=}CH_2$), 5.14 and 5.05 (doublets, 1 H each, J = 12.4 Hz, CH_2Ar), 5.02 (d, 1 H, J = 18 Hz, $CH{=}CH_2$), 5.01 (d, 1 H, J = 10 Hz, $CH{=}CH_2$), 3.18 (s, 2 H, CH_2N), 2.68 (m, 1 H, O_2CCHCH_2), 2.36 (m, 2 H, O_2CCHCH_2), 2.25 (dt, 1 H, J = 14.0, 7.0 Hz, $CHH'CH{=}CH_2$), 1.77 (dd, 1 H, J = 14.3, 4.2 Hz, $CHH'CH{=}CH_2$), 0.96 (s, 9 H, $SiCMe_3$), 0.22 and 0.20 (singlets, 3 H each, $NSiMe_2$), 0.18 (s, 9 H, $OSiMe_3$); mass spectrum m/z (rel intensity) 475 (1), 227 (18), 183 (100), 130 (25), 91 (95); high-resolution mass spectrum calcd for $C_{25}H_{41}NO_4Si_2$ 475.2574, found 475.2527.

For **33b** (major isomer): IR ($CHCl_3$ soln) 2950, 2925, 2855, 1735, 1460, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 7.32 (m, 5 H, Ar), 5.7 (ddt, 1 H, J = 17.0, 10.0, 7.0 Hz, $CH{=}CH_2$), 5.11 and 5.09 (doublets, 1 H each, J = 12.4 Hz, CH_2Ar) 5.03 (d, 1 H, J = 7.0 Hz, $CH{=}CH_2$), 5.01 (d, 1 H, J = 10.1 Hz, $CH{=}CH_2$), 3.13 and 3.01 (doublets, 1 H each, J = 6.2 Hz, CH_2N), 2.9 (ddt, 1 H, J = 9.5, 7.0, 3.3 Hz, O_2CCHCH_2), 2.40 and 2.30 (doublet of triplets, 1 H each, J = 14.0, 7.0 Hz, O_2CCHCH_2), 2.17 (dd, 1 H, J = 14.3, 9.4 Hz, $CHH'CH{=}CH_2$), 1.91 (dd, 1 H, J = 14.2, 3.2 Hz, $CHH'CH{=}CH_2$), 0.92 (s, 9 H, $SiCMe_3$), 0.20 and 0.17 (singlets, 3 H each, $NSiMe_2$), 0.16 (s, 9 H, $OSiMe_3$); mass spectrum m/z (rel intensity) 475 (1), 460 (4), 279 (5), 227 (15), 183 (100); high-resolution mass spectrum calcd for $C_{25}H_{41}NO_4Si_2$ 475.2574, found 475.2527.

[*R*-(4*S-*cis*)]-2-(*tert*-Butyldimethylsilyl)-5-oxo-2-azaspiro-[3.4]octane-1,6-dione (34).** A solution of **33a** (10 mg, 21 μmol) in methanol (1 mL) containing glacial acetic acid (100 ml) was stirred for 18 h at 25 °C and was then concentrated under reduced pressure at 50 °C. The residue was purified by chromatography (50% Et_2O–hexane) to give spiro-lactone **34** (4.6 mg; 74%): oil; IR ($CHCl_3$ soln) 2950, 2930, 2860, 1785, 1750, 1600, 1460 1250, 1100 cm^{-1}; ^1H NMR (C_6D_6) δ 5.65 (m, 1 H, $CH{=}CH_2$), 5.05 (d, 1 H, J = 15.6 Hz, $CH{=}CH_2$), 5.04 (d, 1 H, J = 11.5 Hz, $CH{=}CH_2$), 3.14 and 2.74 (doublets, 1 H each, J = 6.6 Hz, CH_2N), 2.65 (m, 1 H, $CHH'CHCO$), 2.21 (m, 3 H, $CH_2CH{=}CH_2$ and $CHH'CHCO$), 1.78 (dd, 1 H, J = 12.9, 8.1 Hz, $CHH'CHCO$), 0.99 (s, 9 H, $SiCMe_3$), 0.21 and 0.20 (singlets, 3 H each, $SiMe_2$); mass spectrum m/z (rel intensity) 294 (70), 250 (20), 180 (100), 131 (50).

[*S*-(α*R,3*R**)]-1-(*tert*-Butyldimethylsilyl)-2-oxo-α-2-propenyl-3-hydroxy-3-azetidinepropanoic Acid Benzyl Ester (35a) and the [*S*-(α*S**,3*R**)]-Diastereomer (35b).** To a solution of the 1:1.5 mixture of **33a:33b** (1.08 g, 2.26 mmol) in methanol (18 mL) was added glacial acetic acid (2 mL), and the reaction mixture was stirred for 18 h. Toluene (10 mL) was added and the solution was concentrated under reduced pressure (<30 °C). The residue was purified by chromatography (50% Et_2O–hexane) to give a 1:1.5 mixture of **35a:35b** (855 mg; 94%) as an oil which was used directly in the subsequent oxidation. For **35b**: IR (thin film) 3350, 2950, 2910, 2855, 1730, 1460, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.79 (ddt, 1 H, J = 17.0, 12.0, 7.0 Hz, $CH{=}CH_2$), 5.04 (d, 1 H, J = 17.0 Hz, $CH{=}CH_2$), 5.14 (s, 2 H, CH_2Ar), 5.03 (d, 1 H, J = 10.0 Hz, $CH{=}CH_2$), 3.8 (br s, 1 H, OH), 3.13 (m, 2 H, CH_2N), 3.05 (m, 1 H, $COCHCH_2$), 2.44 and 2.30 (doublet of triplets, 1 H each, J = 14.0, 7.0 Hz, $COCHCH_2$), 2.25 (dd, 1 H, J = 14.0, 10.0 Hz, $CHH'CH{=}CH_2$), 1.97 (dd, 1 H, J = 14.0, 3.0 Hz, $CHH'CH{=}CH_2$), 0.92 (s, 9 H, $SiCMe_3$), 0.21 and 0.18 (singlets, 3 H each, $SiMe_2$); high-resolution mass spectrum calcd for $C_{18}H_{24}NO_4Si$ (M − 57) 346.1475, found 346.1464.

[*R*-(4*S-*trans*)]-2-(*tert*-Butyldimethylsilyl)-6-hydroxy-5-oxa-1-oxo-2-azaspiro[3.5]nonane-8-carboxylic Acid Benzyl Ester (36a) and the [*R*-(4*R**-*cis*)-Diastereomer (36b).** Ozone was passed through a stirred solution of a 1:1.5 mixture of **35a**: **35b** (1.32 g, 3.3 mmol) in CH_2Cl_2 (20 mL) at −78 °C until a persistent blue color was observed. The excess ozone was removed by passing oxygen through the solution until the exhaust failed to react with starch iodide. Triphenylphosphine (1.3 g, 5 mmol) was then added and the reaction mixture was brought to 25 °C. The solvent was evaporated under reduced pressure and the residue purified by chromatography (30% Et_2O–hexane) to give spiro-lactols **36a** (423 mg; 32%) and **36b** (658 mg; 50%). For **36a** (1:1 mixture of anomers): ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.53 (ddd, 0.5 H, J = 13.0, 12.0, 2.0 Hz, OCHO), 5.3 (d, 0.5 H, J = 13.0 Hz, OCHO), 5.14 (s, 2 H, CH_2Ar), 5.07 (d, 0.5 H, J = 12.0 Hz, CH_2CHO), 3.62 (d, 0.5 H, J = 5.0 Hz, CH_2CHO), 3.33 and 3.19 (doublets, 0.5 H each, J = 6.4 Hz, CH_2N), 3.32 and 3.17 (doublets, 0.5 H each, J = 6.4 Hz, CH_2N), 3.25 (m, 1 H, CH), 2.01 (m, 3.5 H, CH_2CHCH_2CHO), 1.52 (dt, 0.5 H, J = 13.0, 9.0 Hz, CH_2CHCH_2CHO), 0.95 and 0.94 (singlets, 9 H total, $SiCMe_3$), 0.26, 0.23, and 0.21 (singlets, 6 H total, $SiMe_2$).

For **36b** (3:1 mixture of anomers): ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H), 5.48 (m, 1 H, OCHO), 5.14 (s, 2 H, CH_2Ar), 3.51, 3.31 and 3.17 (doublets, 2 H total, J = 6.2 Hz, CH_2N), 3.10 (m, 2 H, CH_2CHO), 2.65 (m, 0.25 H, CH_2CHO), 1.85 (m, 4 H, $HCCH_2$), 0.94 (s, 9 H, $SiCMe_3$), 0.24 and 0.21 (singlets, 3 H each, $SiMe_2$).

[*R*-(4*S-*trans*)]-2-(*tert*-Butyldimethylsilyl)-1,6-dioxo-5-oxa-2-azaspiro[3.5]nonane-8-carboxylic Acid Benzyl Ester (37a) and the [*R*-(4*S**-*cis*)]-Diastereomer (37b).** Pyridinium chlorochromate (4 × 290 mg, 5.2 mmol total) was added at 30-min intervals to a vigorously stirred solution of **36a** (450 mg, 1.1 mmol) in CH_2Cl_2 (20 mL) containing NaOAc (1.1 g, 14.4 mmol). The reaction mixture was stirred for 30 min after the final addition of oxidant. The mixture was filtered through a bed of silica (eluted with diethyl ether) and concentrated under reduced pressure. Recrystallization of the residue (Et_2O–hexane) gave spiro-lactone **37a** as white needles (260 mg; 58%). The mother liquor was concentrated to afford an additional 80 mg of **37a** (76% combined yield): mp 98–99 °C; IR ($CHCl_3$ solution) 2955, 2930, 2860, 1750, 1465, 1255 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.16 (s, 2 H, CH_2Ar), 3.51 (dddd, 1 H, J = 10.9, 9.1, 7.0, 5.1 Hz, CH_2CH), 3.49 and 3.31 (doublets, 1 H each, J = 6.8 Hz, CH_2N), 3.08 (ddd, 1 H, J = 17.9, 7.1, 1.0 Hz, CH_2CO), 2.71 (dd,

1 H, J = 17.9, 9.1 Hz, CH_2CO), 2.54 (ddd, 1 H, J = 14.3, 5.0, 1.0 Hz, CH_2CH), 2.20 (dd, 1 H, J = 14.3, 10.8 Hz, CH_2CH), 0.94 (s, 9 H, $SiCMe_3$), 0.25 (s, 6 H, $SiMe_3$); mass spectrum (FAB +ve ion) m/z (rel intensity) 404 (15), 314 (10), 185 (95), 144 (15), 93 (100). Anal. ($C_{21}H_{29}NO_5Si$) C, H, N.

Spiro-lactone **37b** was prepared from **36b** (630 mg, 1.6 mmol) in an identical manner as described for the preparation of **37a**. For **37b** (360 mg; 62%): white needles (Et_2O–hexane); mp 97–99 °C; IR ($CHCl_3$ soln) 2960, 2930, 2820, 1760, 1465, 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (m, 5 H, Ar), 5.18 (s, 2 H, CH_2Ar), 3.51 and 3.33 (doublets, 1 H each, 6.6 Hz, CH_2N), 3.14 (dd, 1 H, J = 16.3, 10.9 Hz, $CH_2C{=}O$), 3.01 (m, 1 H, $CHCH_2$), 2.87 (ddd, 1 H, J = 16.4, 6.0, 0.8 Hz, $CH_2C{=}O$), 2.58 (dd, 1 H, J = 14.6, 7.7 Hz, $CHCH_2$), 2.37 (ddd, 1 H, J = 14.6, 7.0, 0.8 Hz, $HCCH_2$), 0.95 (s, 9 H, $SiC(Me_3)_3$), 0.25 and 0.24 (singlets, 3 H each, $SiMe_2$); mass spectrum (FAB +ve ion) m/z (rel intensity) 404 (30), 314 (10), 185 (45), 93 (100). Anal. ($C_{21}H_{29}NO_5Si$) C, H, N.

[*R*-(4*S-*trans*)]-1,6-Dioxo-5-oxa-2-azaspiro[3.5]nonane-8-carboxylic Acid Benzyl Ester (38a) and the [*R*-(4*S**-*cis*)]-Diastereomer (38b).** To a stirred solution of **37a** (201 mg, 0.5 mmol) in THF (1 mL) were added glacial acetic acid (60 mL, 1 mmol) and tetrabutylammonium fluoride (0.5 mL of a 1 M solution in THF, 0.5 mmol). The reaction mixture was stirred for 20 min and poured onto silica gel (2 g). The volatiles were removed under reduced pressure to give a white powder which was purified by chromatography (Et_2O and then EtOAc). The EtOAc fractions were combined and concentrated to give spiro-lactam **38a** (127 mg; 88%): white needles (EtOAc); mp 133–135 °C; IR ($CHCl_3$ soln) 3420, 3020, 1780, 1735, 1255, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 5.95 (br s, 1 H, NH), 5.15 (s, 2 H, CH_2Ar), 3.60 and 3.46 (doublets, 1 H each, J = 6.2 Hz, CH_2N), 3.49 (m, 1 H, CH_2CH), 3.07 (ddd, 1 H, J = 17.9, 6.0, 1.0 Hz, CH_2COO), 2.74 (dd, 1 H, J = 17.9, 9.4 Hz, CH_2COO), 2.61 (ddd, 1 H, J = 14.5, 5.0, 1.0 Hz, CH_2CH), 2.25 (dd, 1 H, J = 14.4, 10.9 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/z (rel intensity) 288 (8), 275 (15), 183 (100), 151 (9), 91 (75). Anal. ($C_{15}H_{15}NO_5$) C, H, N.

Spiro-lactam **38b** was prepared from **37b** (201 mg, 0.5 mmol) in an identical manner as described for the preparation of **38a**. For **38b** (135 mg; 93%): white solid (EtOAc); mp 124–125 °C; IR ($CHCl_3$ soln) 3420, 3010, 1780, 1740, 1205 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ar), 6.0 (br s, 1 H, NH), 5.18 (s, 2 H, CH_2Ar), 3.62 and 3.47 (doublets, 1 H each, J = 6 Hz, CH_2N), 3.15 (dd, 1 H, J = 15.0, 10.5 Hz, CH_2COO), 3.05 (m, 1 H, CH_2CH), 2.88 (dd, 1 H, J = 15.0, 5.0 Hz, CH_2COO), 2.62 (dd, J = 14.5, 7.0 Hz, CH_2CH), 2.4 (dd, 1 H, J = 14.5, 6.0 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/z (rel intensity) 288 (35), 275 (100), 256 (10). Anal. ($C_{15}H_{15}NO_5$) C, H, N.

[R-(4*S-*trans*)]-1,6-Dioxo-5-oxa-2-azaspiro[3.5]nonane-8-carboxylic Acid (39a) and the [*R*-(4*S**-*cis*)]-Diastereomer (39b).** A solution of **38a** (166 mg, 0.6 mmol) in THF (5 mL) was shaken over 10% Pd/C (10 mg) under H_2 at atmospheric pressure for 3 h. The reaction mixture was diluted with methanol (30 mL), filtered through Celite, and concentrated under reduced pressure. The residue was recrystallized (CH_3CN) to give acid **39a** (107 mg; 93%): white needles (CH_3CN); mp >250 °C dec; IR (KBr disc) 3360 (Br), 3180 (Br), 1760, 1740, 1730, 1260, 1185, 1110 cm^{-1}; ^1H NMR (DMSO) δ 8.46 (s, 1 H, NH), 3.42 and 3.33 (doublets, 1 H each, J = 6.5 Hz, CH_2N), 3.20 (m, 1 H, CH_2CH), 2.81 (dd, 1 H, J = 17.0, 6.3 Hz, CH_2COO), 2.63 (dd, 1 H, J = 17.0, 8.5 Hz, CH_2COO), 2.52 (dd, 1 H, J = 14.5, 6.0 Hz, CH_2CH), 2.24 (dd, 1 H, J = 14.5, 9.3 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/z (rel intensity) 200 (8), 185 (100). Anal. ($C_8H_9NO_5$) C, H, N.

Acid **39b** was prepared from **38b** (145 mg, 0.5 mmol) in an identical manner as described for the preparation of **39a**. For **39b** (83 mg; 82%): mp >250 °C dec; IR (KBr disc) 3420, 3240, 1765, 1745, 1725, 1275, 1140 cm^{-1}; ^1H NMR (DMSO) δ 8.43 (s, 1 H, NH), 3.45 and 3.34 (doublets, 1 H each, J = 6.4 Hz), 3.15 (m, 1 H, CH_2CH), 2.76 (dd, 1 H, J = 14.2, 8.9 Hz, CH_2COO), 2.65 (dd, 1 H, J = 14.5, 6.5 Hz, CH_2CH), 2.35 (dd, 1 H, J = 4.2, 6.4 Hz, CH_2COO), 2.11 (dd, 1 H, J = 14.2, 9.6 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/e (rel intensity) 200 (20), 185 (30), 93 (100). Anal. ($C_8H_9NO_5$) C, H, N.

[*S*-(*R,3*R**)]-[(3-Hydroxy-2-oxo-3-azetidinyl)methyl]butanedioic Acid (17a) and the [*S*-(*S**,3*R**)]-Diastereomer (17b).** To a stirred suspension of **39a** (40 mg; 0.2 mmol) in water (1 mL) was added aqueous sodium hydroxide (0.8 mL of a 1 M aqueous solution; 0.8 mmol). The reaction mixture was stirred for 5 min and passed through a mixed bed of carboxylate ion exchange resin (Amberlite IRC50), eluting with water. Concentration under reduced pressure and azeotroping with ethanol gave diacid **17a** (56 mg; >100%), containing $NaHCO_3$ (<10%) which coeluted from the ion-exchange column: amorphous solid; IR (KBr disc) 3430, 1744, 1573, 1403, 1206 cm^{-1}; ^1H NMR (D_2O, buffered at pH 8 with $NaHCO_3$) δ 3.54 and 3.33 (doublets, 1 H each, J = 6.3 Hz, CH_2N), 2.75 (m, 1 H, CH_2CH), 2.58 (dd, 1 H, J = 14.9, 5.0 Hz, CH_2COO), 2.25 (dd, 1 H, J = 14.8, 9.9 Hz, CH_2COO), 2.21 (dd, 1 H, J = 14.6, 9.0 Hz, CH_2CH), 1.81 (dd, 1 H, J = 14.6, 3.7 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/z (rel intensity) 216 (20), 183 (100).[32]

Diacid **17b** was prepared from **39b** (40 mg, 0.2 mmol) in an identical manner described for the preparation of **17a**. For **17b** (64 mg): amorphous solid; IR (KBr disc) 3405, 1743, 1575, 1411, 1272, 930 cm^{-1}; ^1H NMR (D_2O, buffered at pH 8 with $NaHCO_3$) δ 3.53 and 3.30 (doublets, 1 H each, J = 6.4 Hz, CH_2N), 2.78 (m, 1 H, CH_2CH), 2.54 (dd, 1 H, J = 14.9, 5.4 Hz, CH_2COO), 2.26 (dd, 1 H, J = 14.9, 9.7 Hz, CH_2COO), 2.11 (dd, 1 H, J = 14.5, 10.0 Hz, CH_2CH), 1.92 (dd, 1 H, J = 14.5, 3.3 Hz, CH_2CH); mass spectrum (FAB –ve ion) m/e (rel intensity) 216 (20), 183 (100).[32]

Rat ATP-Citrate Lyase Assay. ATP-citrate lyase was purified from rat liver as previously described.[11] Reversible binding K_i were measured by inhibition of the carbon–carbon cleavage activity,[12b] using 1.0 mM citrate ($S = K_m$) as previously described.[13] Data were fitted to the equation $V = V_m/(1 + [I]/K_i)$ using the program Grafit.[33] ATPase activity was measured using a pyruvate kinase coupled assay[10a] under previously described conditions.[13]

Supplementary Material Available: Experimental details of the X-ray structure determination of **24**, including tables of fractional atomic coordinates, thermal parameters, interatomic distances, and angles (8 pages). Ordering information is given on any current masthead page.

Registry No. (±)-**12a**, 144373-40-0; (±)-**12b**, 144373-41-1; (±)-**17a**, 144373-60-4; (±)-**17b**, 144373-61-5; **18**, 32807-28-6; **19**, 64127-51-1; (±)-**20**, 144373-33-1; (±)-**21**, 144373-34-2; (±)-**22a**, 144373-35-3; (±)-**22b**, 144373-36-4; (±)-**23a**, 144373-37-5; (±)-**23b**, 144373-38-6; (±)-**24**, 144373-39-7; **25**, 117505-49-4; (±)-**26**, 144373-42-2; (±)-**27**, 137627-17-9; **28**, 126830-73-7; **28** acid, 18187-17-2; (±)-**29** (isomer 1), 144373-43-3; (±)-**29** (isomer 2), 144373-44-4; (±)-**30**, 144373-47-7; (±)-**31**, 144373-45-5; (±)-**32**, 144373-46-6; (±)-**33a**, 144373-48-8; (±)-**33b**, 144373-49-9; (±)-**34**, 144373-50-2; (±)-**35a**, 144373-51-3; (±)-**35b**, 144373-52-4; (±)-α-**36a**, 144373-53-5; (±)-β-**36a**, 144409-36-9; (±)-α-**36b**, 144409-37-0; (±)-β-**36b**, 144409-38-1; (±)-**37a**, 144373-54-6; (±)-**37b**, 144373-55-7; (±)-**38a**, 144373-56-8; (±)-**38b**, 144373-57-9; (±)-**39a**, 144373-58-0; (±)-**39b**, 144373-59-1; ATP-citrate lyase, 9027-95-6.

(32) Diacids **17a** and **17b** were contaminated with $NaHCO_3$ (<10%) which coeluted with the diacids from the ion-exchange system. As a result, accurate CHN data could not be obtained.

(33) Leatherbarrow, R. J. Grafit Version 2.0. Erithacus Software Ltd., 1990; Staines, England.