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PREPARATION OF (±)-(ERYTHRO)-AND (±)-(THREO)-2-VINYL CITRIC ACIDS AS POTENTIAL MECHANISM-BASED INHIBITORS OF ATP-CITRATE LYASE

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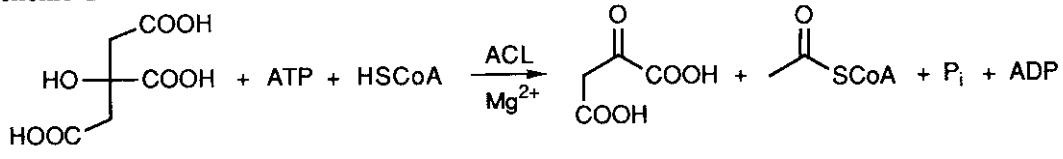
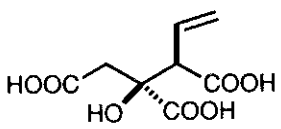
Summary: The dianion of diethyl-1,3-acetone dicarboxylate **6** was reacted with a vinyl cation equivalent, 2-bromoethyl phenyl selenide, to give the mono alkylated 3-oxoglutarate **7** in 80% yield. Subsequent four-step elaboration gave the title citric acid analogues. These agents were designed as potential mechanism-based inhibitors of ATP-citrate lyase, an enzyme involved in cholesterol and lipid biosynthesis.

ATP-citrate lyase (ACL; E.C. 4.1.3.8.) catalyzes a retro-aldol reaction of citrate **1** to oxaloacetate **2**[2] (Scheme I). The reaction is coupled both to the hydrolysis of ATP to ADP and inorganic phosphate (P_i) and the formation of one mole of acetyl CoA **3**. Cytosolic **3** is utilized in mammalian cholesterol and fatty acid biosynthesis, and inhibition of ACL may represent a useful drug strategy for treating hyperlipidaemia.[2b] For this reason we were interested in preparing and evaluating (±)-(erythro)- and (±)-(threo)-2-vinyl citrates **4** and **5** as potential mechanism-based inhibitors of ACL.[3,4]

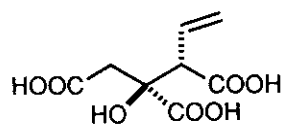
Scheme I



1 **2** **3**

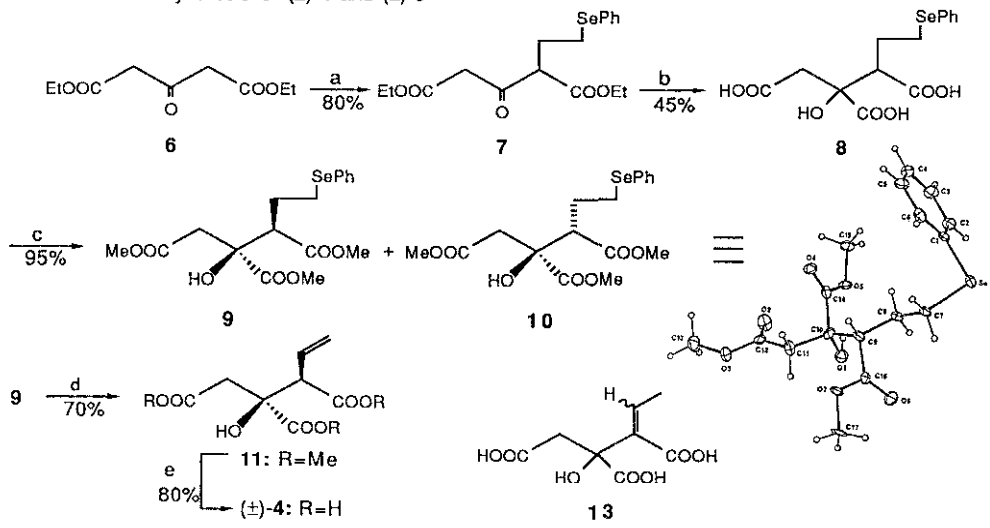


(±)-**4**



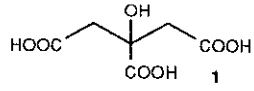
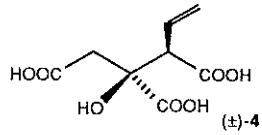
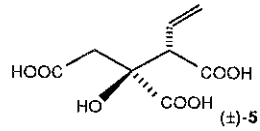
(±)-**5**

Scheme II. Synthesis of (±)-**4** and (±)-**5**



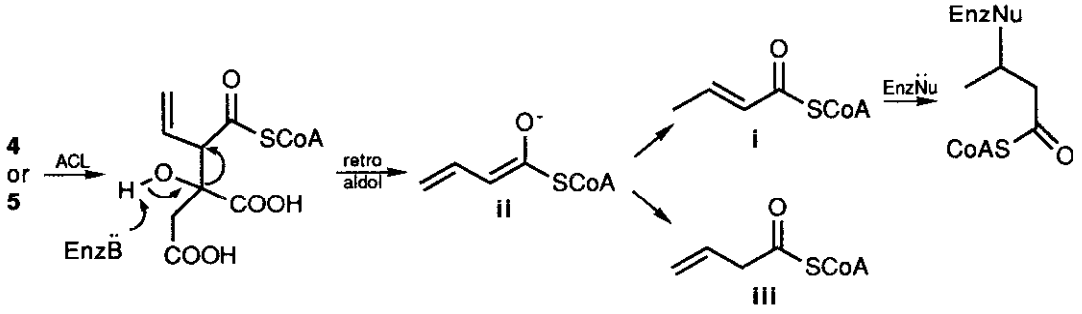
Reagents and conditions: a) 2 equiv. LDA, 4:1 THF--HMPA, -78 °C, then 1.5 equiv. $PhSeCH_2CH_2Br$ (ref 6), -78→40 °C, 2 h; b) NaCN, 12 M HCl, Et_2O, 12 h, then 3 M aqueous HCl, reflux, 4 h; c) MeOH, TsOH, reflux, 2 h; then reverse phase HPLC; d) 1.1 equiv. O_3, CCl_4, -10 °C, then 60 °C, 10 min; e) 3 M aqueous HCl, reflux, 3 h.

Table I. Results of ATP-Citrate Lyase Assay of Vinyl Citrates

Compound	Reversible Binding[a] K_i (μM)	C-C Skeletal Cleavage[b] V_{max} (rel.)	K_m (μM)	Irreversible Inactivation $k_i \times 10^{-3} h^{-1}$
 **1**	—	1	106	$5.0 \times 10^{-3} h^{-1}$[c] ($t_{1/2}$ = 138 h)
 (±)-**4**	480	.0077	68	nd[d]
 (±)-**5**	530	.003	140	nd[d]

a) Assayed against 100 μM citrate (=K_m) under saturating ATP (5 mM) and CoA (200 μM) concentrations using malate dehydrogenase (MDH) coupled assay monitoring oxaloacetate production at 25 °C, pH 8.0. See ref 10. b) MDH coupled assay monitoring oxaloacetate production using vinyl citrate as substrate. c) Enzyme decay rate under assay conditions. d) Not detectable at 2 mM inhibitor concentration above enzyme decay rate of 5.0×10^{-3} h^{-1}.

Vinyl citrates **4** and **5** are latent electrophiles designed to profit from the incipient anion formed by the enzyme to generate a reactive Michael acceptor **i** directly in the active site.[5] Subsequent alkylation of an active site nucleophile would lead to irreversible enzyme inactivation.



The dianion of diethyl 1,3-acetonedicarboxylate **6**[6a] was reacted with a previously unrecognized vinyl cation equivalent, 2-bromoethyl phenyl selenide[6b,c] to give C-alkylated 3-oxoglutarate **7** in 80% isolated yield[7,8a] (Scheme II). Elaboration of **7** to an inseparable mixture of diastereomeric triacids **8** was accomplished using previously described cyanohydrin/hydrolysis conditions.[8b] Esterification of **8** in methanol afforded (±)-(erythro)-triester **9** and (±)-(threo)-triester **10** after reverse phase HPLC purification (30% THF--water, C18 bonded silica). X-ray crystallographic analysis[9] of the less polar crystalline **10** (Et_2O, mp 61-63 °C) established the relative threo-configuration found in **10** (ORTEP drawing, Scheme II). Conversion of **9** (**10**) to the chromatographically stable vinyl citrate triester **11** (**12**) was realized via phenyl selenoxide elimination in the absence of base (1.1 equiv. O_3, CCl_4, -10 °C then 60 °C, 10 min; 70%). Vinyl citrate (±)-**4** ((±)-**5**) was obtained from **11** (**12**) following acid catalyzed ester hydrolysis (3 M aqueous HCl, reflux, 3 h; 85%). These hydrolysis conditions also produced ca. 10% of the α,β-unsaturated citrate **13** as a 2:1 mixture of geometric isomers.

Evaluation of **4** and **5** using purified rat liver ACL[10] revealed reversible binding constants (K_i) of similar magnitude (530 μM and 480 μM, compared to K_m citrate = 106 μM, Table I). The vinyl citrates were substrates for enzyme (K_m's within 1.5x that of natural substrate **1**) submitting to retro-aldol skeletal cleavage at a substantially lower rate (V_{max}) relative to **1**. Time dependent inactivation of ACL was not observed at 2 mM concentrations of **4** or **5** as evidenced by changes in the decay rate for enzyme under the assay conditions. Assuming the formation of **i**, this result is surprising in light of an active site nucleophile believed to play a critical role in covalently binding citrate during catalysis.[2] Alternatively, regioselective protonation of **ii** may have occurred in the enzyme active site yielding **iii** which is incapable of irreversibly inactivating ACL.

REFERENCES AND NOTES

1. a) Department of Medicinal Chemistry, Sterling Drug Inc., 25 Great Valley Parkway, Malvern, PA 19355. b) Department of Biochemistry, University of Edinburgh, Edinburgh EH8 9XD, Scotland. c) Glaxo Institute for Molecular Biology, 14 chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland. d) Department of Physical Organic Chemistry. e) Department of Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals

2. a) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman and Company: San Francisco, **1979**; pp 765-768 and references therein. b) Srere, P. A. In *Advances in Enzymology;* Meister, A., Ed.; John Wiley & Sons: New York, 1975; vol. 43, p 85.

3. (2S,3S)-2-Hydroxycitrate, a naturally occurring reversible inhibitor (K_i = 0.15 μM) of ACL, has been extensively used in studies on lipogenesis in vitro and in vivo. Lowenstein, J. M.; Brunengraber, H. In *Methods in Enzymology,* Lowenstein, J. M., Ed.; Academic Press: New York, 1981; p 486

4. For recent treatise on mechanism-based enzyme inhibition strategies see: a) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology;* CRC Press, Inc: Boca Raton, Florida 1988; vols. 1 and 2. b) Sandler, M.; Smith, H. J. *Design of Enzyme Inhibitors as Drugs;* Oxford University Press: Oxford, 1989.

5. ACL mediated cleavage of citrate **1** is highly regiospecific in that the pro S arm of prochiral **1** is activated by enzyme and then liberated as acetyl CoA (see ref 2a and Marletta, M. A.; Srere, P. A.; Walsh, C. *Biochemistry*, **1981**, *20*, 2719). Thus turnover of **4** and **5** will generate the reactive Michael species **i** only in substrate stereoisomers where the vinyl group is positioned in the pro S arm of **1**.

6. a) Lambert, J. B.; Wharry, S. M. J. Am. Chem. Soc. **1982**, *104*, 5857. b) Lindgren, B. *Acta Chem. Scan., Ser. B* **1977**, *B31*, 1. c) Kataev, E. G.; Mannafov, T. G.; Bendnikov. E. G.; Komarovskaya, O. A. *Zh. Org. Khim.* **1973**, *9*, 1983.

7. a) All new compounds exhibited physical and spectroscopic properties consistent with their structure. b) Vinyl citric acids **4** and **5** were converted to their di-sodium salts using a Bio Rad AG50W ion exchanger in the Na^+ form. The hygroscopic di-Na salts were stable to storage at 0 °C in a dessicator. For **4** (di-Na): ^{1}H NMR δ (D_2O) 5.91 (m, 1H, C<u>H</u>=CH_2), 5.22 and 5.17 (m, 2H, CH=C<u>H</u>$_2$), 3.25 (d, 1H, C<u>H</u>CH=CH_2, J = 5.0 Hz), 3.05 and 2.75 (doublets, 1 H each, $NaOOCCH_2$, J = 10.8 Hz). For **5** (di-Na): ^{1}H NMR δ (D_2O) 5.95 (m, 1H, C<u>H</u>=CH_2), 5.35 and 5.25 (m, 2H, CH=C<u>H</u>$_2$), 3.45 (d, 1H, C<u>H</u>CH=CH_2, J = 4.5 Hz), 2.78 (s, 2H, $NaOOCCH_2$).

8. a) Diethyl 3-oxoglutarate **6** has been alkylated with MeI using NaOEt/EtOH to give diethyl 2-methyl-3-oxoglutarate in 85% yield (Beach, R. L.; Aogaichi, T.; Plaut, G. W. E. *J. Biol. Chem.* **1977**, *252*, 2702). Alkylation of **6** with 2-bromoethyl phenyl selenide did not occur under these conditions. b) Hydrocyanation/hydrolysis conditions described for the conversion of 2-methyl-3-oxoglutarate to 2-methylcitric acid.[8a]

9. Triester **10** (1:1:1 MeOH--EtOH--iPrOH), monoclinic, $P2_1/n$ with **a** = 9.749(6), **b** = 5.846(2), **c** = 31.069(12)Å, β = 97.85(2)° at 188K, V = 1754.2(13)Å^3, Z = 4, ρ(calc) = 1.58 g cm^{-3}. A quadrant of intensity data were collected on an Enraf Nonius CAD-4 diffractometer using variable speed ω–θ scans and graphite monochromated MoKα radiation (λ = 0.71073Å). A total of 4210 unique reflections (2°<2θ<56°) were collected and 2587 reflections with (F_o<3σ(F_o)) were considered observed after correction for Lorentz polarization and absorption effects. The structure was solved by direct methods and refined by full matrix least squares to R = 0.064; Rw = 0.064; S = 1.955, (w = 4F_o^2/δ^2(I)). Archival data have been deposited with the Cambridge Crystallographic Data Center (University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.).

10. Enzyme isolation: Wraight, C.; Day, A.; Hoogenraad, N.; Scoopes, R. *Anal. Biochem.* **1985**, *144*, 604. Assay: Houston, B.; Nimmo, H. G. *Biochem. J.* **1984**, *224*, 437 except that buffer was Tris-HCl pH 8.0 (50 mM). Data analysis was carried out using Enzfitter (Elsevier Biosoft).

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