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TRANSITION-STATE ANALOGUE INHIBITORS OF CHORISMATE MUTASE

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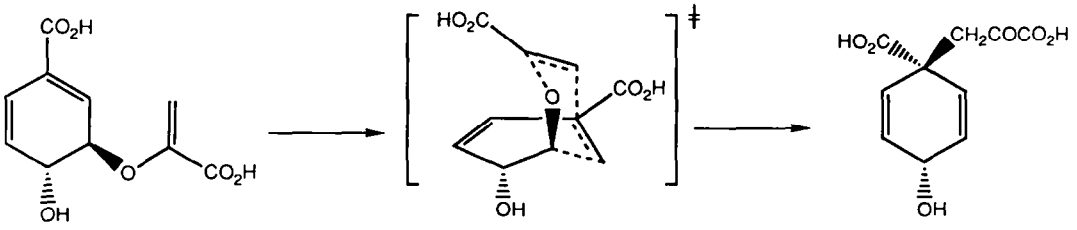
Abstract: The synthesis of seven bi- and tricyclic chorismate analogues 9-15 and their biological evaluation as mutase inhibitors is described. A tandem Cope rearrangement/Diels-Alder cycloaddition strategy was employed to prepare tricyclo[3.3.1.$0^{2,7}$]non-3-enes functionalized with carboxylic and/or phosphonic acid groups.

INTRODUCTION

The Claisen rearrangement of chorismic acid 1 to prephenic acid 2 (Scheme 1) represents the first committed step in the biosynthesis of phenylalanine and tyrosine along the shikimic acid pathway.[1] While the uncatalyzed reaction occurs readily, the rearrangement is accelerated more than a millionfold by the family of enzymes known as chorismate mutases.[2,3] To our knowledge, this transformation is the only example of an enzyme-catalyzed pericyclic reaction in primary metabolism.

SCHEME 1



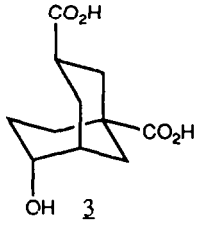
1 TS 2

Both the enzymic[4] and nonenzymic[5] processes occur via a chairlike geometry (TS, Scheme 1), and much effort has gone into the design and synthesis of potential enzyme inhibitors whose structure and overall shape closely resemble the rearrangement transition state. The earliest examples of this strategy included the bicyclic diacid 3[6] as well as adamantane derivatives 4[7] and 5[8] (Scheme 2). In 1985, the family of bicyclic diacids 6-8 was synthesized in Bartlett's laboratory.[9] Endo isomer 8 proved to be a considerably better inhibitor than 7 or 6 and was reported to be the most potent inhibitor of chorismate mutase ever prepared, with a K_i value of 0.12 μM against the chorismate mutase-prephenate dehydrogenase from *Escherichia coli*.[10]

Our own interest in the structure and mechanism of the chorismate mutases led us to explore a simple and highly convergent synthetic approach to transition state analogues which might be effective inhibitors of this key enzymatic step in plant and microbial metabolism. Our preliminary report in 1987 described an intramolecular Diels-Alder cycloaddition to prepare a functionalized tricyclo[3.3.1.$0^{2,7}$]non-3-ene whose smooth fragmentation led to the synthesis of exo and endo diacids 9 and 10.[11] Endo isomer 10, the carbocyclic analogue of 8, proved to be more potent than its exo counterpart 9, the carbocyclic analogue of 6. Together with Bartlett's findings, these

results confirm the importance of orientational effects in mutase inhibitors and suggest that in the chairlike transition state for the enzymic process, the enol pyruvate carboxyl group is markedly tilted towards the carbocyclic ring.

SCHEME 2



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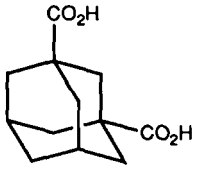

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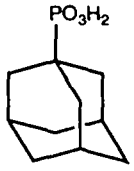

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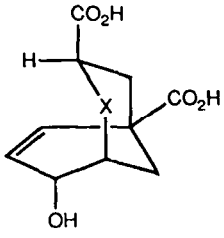
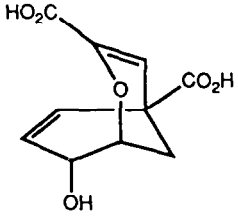


6 X= O

9 X= CH_2



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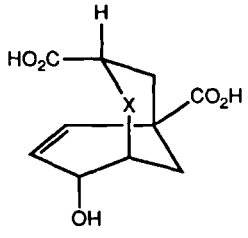
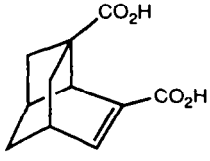


8 X= O

10 X= CH_2

In the course of this work, several other tricyclononene systems were also synthesized quite efficiently by a tandem Cope rearrangement-cycloaddition strategy. As a mimic of the mutase transition state, diacid 11 (Scheme 3) represented a new variation on the adamantane theme with a somewhat flattened ring framework. By replacing one or both of the carboxylic acids in 11 with phosphonic acids, structures 12, 13 and 14 probed the effect of modifying chorismate's dissociating residues. Previous studies[8] in the adamantane series had shown that phosphonate substituents led to more effective inhibitors, and that increasing the polar and hydrophilic character of the anionic groups resulted in tighter enzyme binding. This account describes in detail the synthesis of 9-15 and the biological evaluation of these substances as potential chorismate mutase inhibitors.

SCHEME 3



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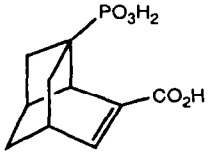

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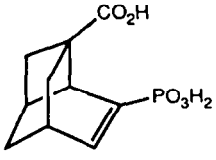

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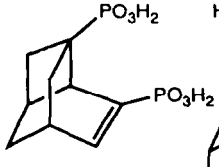

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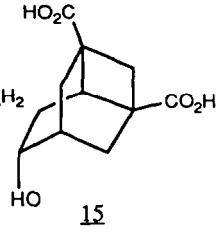

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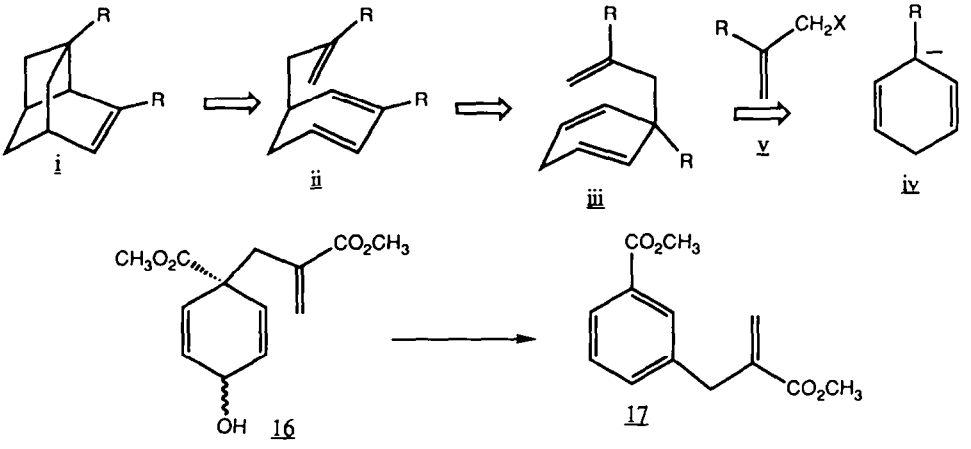
SYNTHETIC STUDIES

An initial plan of attack at the outset of this work was based on the retrosynthetic analysis presented in Scheme 4. The positioning of electron-withdrawing functional groups R in bicyclo[3.3.1.$0^{2,7}$]non-3-enes like i

seemed to make these structures ideal candidates for synthesis via the intramolecular Diels-Alder cycloaddition of trienes like ii. Several 5-alkenyl-cyclohexa-1,3-dienes were known to undergo thermal 4+2 cyclizations:[12] heating 5-allyl-cyclohexa-1,3-diene ii (R= H) at 225°C produced the parent tricyclic system i (R= H) along with benzene and two isomeric allylcyclopentadienes.[13] Construction of the appropriately cross-conjugated 1,3-cyclohexadienes ii (R= EWG) by Cope rearrangement of 1,4-cyclohexadienes iii (R= EWG) finds precedent in the facile isomerization of dimethyl carboprephenate 16 to the aromatic dehydration product 17 (Scheme 4).[14] A considerable simplification thus results since trienes like iii (R= carboxylate or phosphonate) are accessible by direct alkylation of the corresponding 1,4-dihydrobenzenes iv with allylic electrophiles v. The ready availability of all reactants, and the possibility of carrying out both the pericyclic rearrangement and 4+2 cycloaddition in one operation, were two of the tactical elements which strongly recommended the strategy outlined in Scheme 4.

SCHEME 4

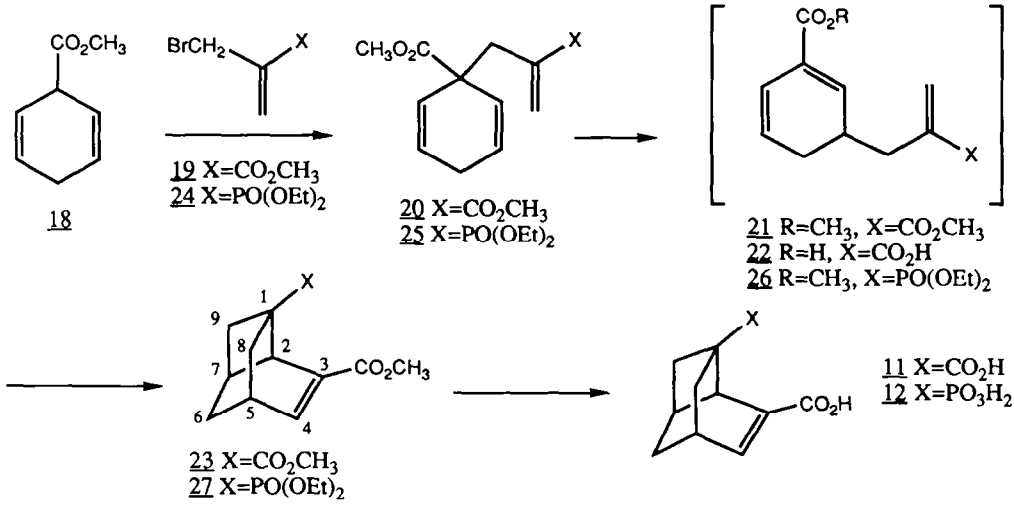


Structures 11 and 12. The synthesis of dicarboxylic acid 11 from methyl 1,4-dihydrobenzoate 18 is depicted in Scheme 5. Alkylation of the lithium enolate of 18 (THF, -60°C) with methyl 2-(bromomethyl)acrylate 19 furnished 20 in 83% yield. When heated in toluene (110°C, 24 h) this diester underwent both the hoped-for [3,3]-sigmatropic rearrangement and Diels-Alder cyclo-addition to afford tricyclic diester 23 virtually quantitatively. Saponification of 23 gave diacid 11 which was obtained as a crystalline white solid, mp 208°C.

Variable amounts of the Cope-rearranged intermediate 21 could be detected by NMR when 20 was boiled briefly in toluene (2-3 h, 19-35% yield), although obtaining a pure sample for purposes of characterization involved a difficult chromatographic separation from the starting material. Once in hand, pure diester 21 could be hydrolyzed in base to 22, an analogue of 4-deoxychorismic acid in which the enol pyruvate ether oxygen has been replaced with a methylene group. Diacid 22 thus produced (99% yield) was not sensitive to air oxidation and showed no tendency to decompose by aromatization or rearrangement at room temperature. Its behavior stands in sharp contrast to dimethyl 4-deoxychorismate which had independently been synthesized both in Berchtold's laboratory[15] and by us.[14] The half-lives for Claisen rearrangement (to 4-deoxyprephenate) and aromatization of disodium 4-deoxychorismate (30°C, D_2O, pD 7.2) were recently reported by the MIT group to be 3.5 and 8 min, respectively.[16] Clearly the different nature of the [3,3]-sigmatropic processes involved largely determines the

direction of each rearrangement. For 4-deoxychorismate, the direction of reaction is governed by the formation of a new carbonyl in deoxyprephenate, whereas the conversion of 20 to 21 results in a conjugated diene.

SCHEME 5



A parallel synthetic strategy was followed to prepare carboxyphosphonic acid 12. Alkylation of 18 with the known bromophosphonate 24[17] furnished 25 (79%) as a stable colorless oil. Cope rearrangement of this triester, unlike diester 20, gave only rearranged triene 26 (85% yield) with no indication of the intramolecular cycloaddition product 27. However the rearrangement was considerably slower, in accordance with expectations based on the Claisen rearrangement of dialkylphosphinyl-substituted allyl vinyl ethers.[18] To induce the desired Diels-Alder reaction, triene 26 was heated at 145°C for 72 h, after which time the tricyclic product 27 could be isolated in 77% yield. Cleavage of the phosphonic and carboxylic ester groups was achieved in a one-pot reaction by treating 27 first with TMSCl-NaI in CH_3CN, then with base to give the product triacid 12 in 66% yield. Structures 13 and 14. Scheme 6 outlines a parallel alkylation-rearrangement approach to phosphonates 13 and 14 by coupling bromides 19 and 24 with diethyl 2,5-cyclohexadienyl-1-phosphonate 28. This dihydroaromatic ester has been prepared by the Li/NH_3 reduction of diethyl benzenephosphonate,[8] however neither its anion nor the anion derived from the corresponding 1,5-cyclohexadienyl-1-phosphonate[19] has been successfully alkylated. Attempted conjugate addition of lithiated 28 (generated with n-butyllithium in THF at -78°C) to diethyl vinylphosphonate at rt led instead to the monoethyl ester of 1-ethyl-2,5-cyclohexadienyl-1-phosphonic acid.[8] This presumably occurred by rearrangement of lithiated 28 while undergoing a rapidly reversible Michael addition. A similar rearrangement of metallated 9,10-dihydro-acridine-9-phosphonates had earlier been noted by Redmore.[20]

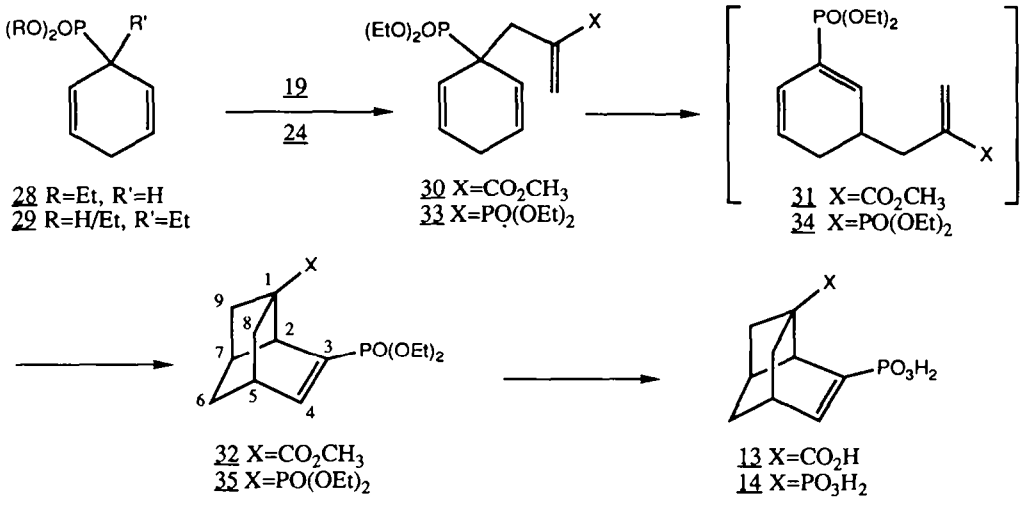
We hoped that the rapid and irreversible alkylations usually observed with reactive allylic bromides would meet with greater success. In the event, exposure of 28 to lithium isopropylcyclohexylamine (THF, -78°C) for 15 min generated a deep yellow solution of the corresponding anion which, when transferred by cannula into a slight excess of 19, immediately turned colorless. Workup afforded the α-alkylated phosphonate 30 in 72% yield with no trace of the rearranged starting material previously observed by Chao and Berchtold. However lithiated 28 exhibited unusual time-dependent alkylation behavior. When deprotonation was allowed to proceed for 30 min, alkylation gave 30 (50%) as well as 31, the apparent product of γ-substitution (22%). Further aging of the lithiated

species had little effect on the ratio of products [30 (46%) and 31 (22%) after 2 h]. In related work on the stereoselectivity of lithiated 1,4-dihydrobenzoate alkylations, Schultz et al. have reported solvent and temperature effects which also suggest the formation of a kinetic anion whose structure changes with time.[21]

Thermal isomerization of 30 could be monitored for the appearance of the Cope rearrangement product 31. However 30 rearranged and cyclized to tricyclic triester 32 (74%) without any observable steady-state concentration of 31. Thus the time-dependent alkylation of 28 provided the only source of 31, and its chemistry was not investigated further. Cycloadduct 32 was deprotected in the usual way to afford triacid 13 as a high-melting solid.

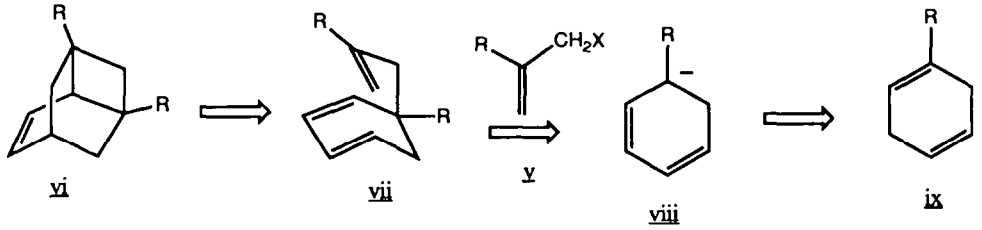
Another alkylation of lithiated 28, this time with bromophosphonate 24, was used for the synthesis of 14. The major α-substituted product 33 (60%) was obtained pure by flash chromatography and found to rearrange slowly to 34 (50% conversion at 125°C, 48 h) whereas prolonged heating at a higher temperature (145°C) was required to transform either 33 or 34 directly to the Diels-Alder cycloadduct 35. The best yield of 35 (64%) was obtained directly from 33 (72 h, 145°C). Cleavage of the phosphonate esters afforded tetraacid 14 (74%).

SCHEME 6



The efficiency with which the Cope/Diels-Alder strategy produced novel transition-state analogues of the chorismate-prephenate rearrangement led to a simple variation depicted retrosynthetically in Scheme 7. Here a different arrangement of electron-withdrawing functional groups R in the family of tricyclo[3.3.1.$0^{2,7}$]nonenes vi can be seen to arise by the 4+2 cycloaddition of triene vii. Anion viii might be produced by metallation of diene ix, itself a readily available Diels-Alder synthon. By introducing additional functionality R' in ix (from the reaction of substituted butadienes as in Scheme 7), the synthesis of heteroatom-containing systems like 15 (Scheme 3) might be achieved. It also seemed attractive to explore fragmentations of the functionalized tricyclic skeleton vi which might lead to bicyclic structures even more closely resembling the transition state geometry of interest.

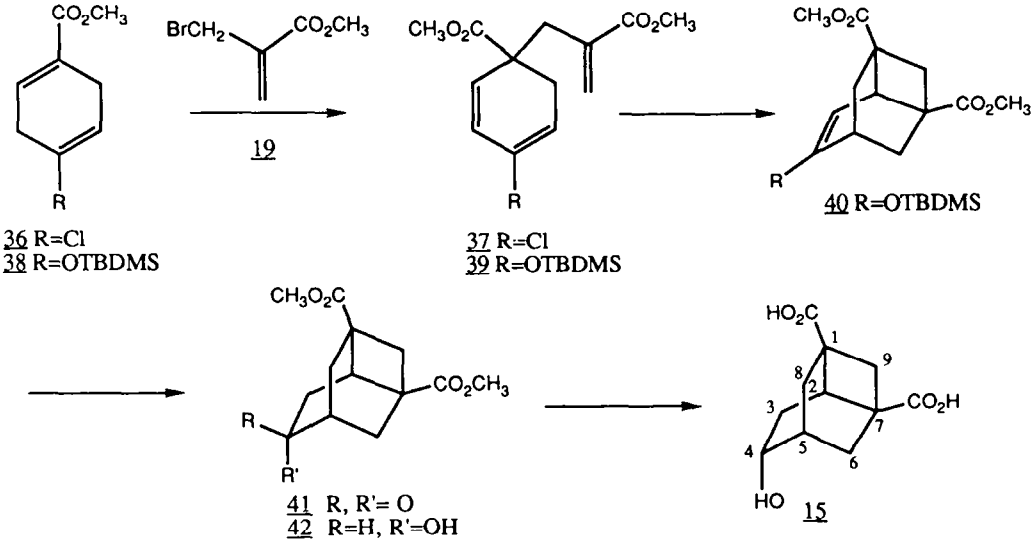
SCHEME 7



Structure 15. Preliminary studies focused on chlorodiene 36 (Scheme 8) since alkylation and intramolecular cycloaddition might furnish the tricyclic framework 40 (R=Cl). Based on a known route to the corresponding carboxylic acid,[22] chloroprene was heated with methyl propiolate and aluminum chloride[23] to form a 68:32 mixture favoring 36. A sample of 36 was deprotonated (LICA, -78°C) and added to bromoacrylate 19 to afford 37 as the major product. Not surprisingly, the undesired cycloaddition regioisomer also underwent alkylation, and separation of the two isomeric diesters could not be achieved.

By analogy with a known procedure,[24] methyl vinyl ketone was converted to 2-(tert-butyldimethylsilyl-oxy)-buta-1,3-diene. Heating this diene with methyl propiolate (benzene, reflux) furnished 38 in 81% yield after chromatography (Scheme 8). Alkylation of 38 as its lithium enolate (LICA, -78°C, inverse addition to 19) gave 39 in 95% yield. When a toluene solution of 39 was heated to reflux, the symmetrical tricyclo[3.3.1.$0^{2,7}$]non-3-ene 40 was obtained (90%). As expected, only twelve resonances were evident in the ^{13}C-NMR spectrum of 40. Hydrolysis of the enol silyl ether could be accomplished either in trifluoroacetic acid-CH_3OH or using silica gel impregnated with 5% aqueous H_2SO_4 to produce ketone 41. This symmetrical ketone was quantitatively reduced to a single alcohol 41 using $NaBH_4$ in CH_3OH. Saponification afforded 15 as a white crystalline solid.

SCHEME 8



Hydroxydiacid 15 incorporated all of chorismate's polar functional groups in a compact tricyclic system.

An interesting fragmentation of ketodiester 41 by cleavage of its C1-C2 bond in a base-catalyzed retro-Michael process would furnish a new family of bicyclic enones like 43 (Scheme 9). The endo configuration at C7 shown in 43 might be established by kinetic protonation of the intermediate ester enolate from the less-hindered (exo) face. Access to chorismate's allylic alcohol substructure might thus be gained in a few additional synthetic steps.

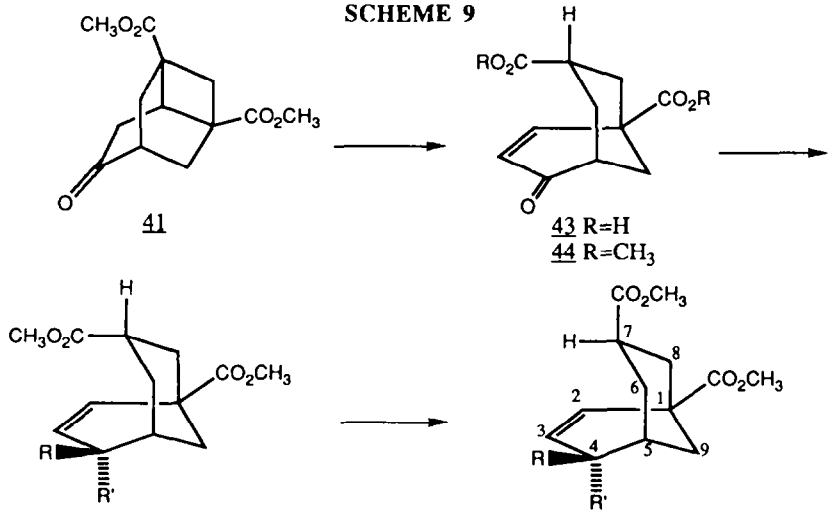
When ketone 41 was exposed to $NaOCH_3$ in CH_3OH at rt, a single diacid 43 was formed which could be converted to the corresponding dimethylester 44 using diazomethane. The ^{1}H-NMR spectrum of 44 showed C7 (t, 2.77, J=6.3 Hz) was coupled to only two protons. Examination of molecular models confirmed that an equatorial C7-H was indeed orthogonal to two neighboring spins at C6 and C8. Reduction of 44 with $NaBH_4$-$CeCl_3$ furnished hydroxydiester 45 (100%). The stereochemistry of alcohol 45 could be inverted using the Mitsunobu reaction with benzoic acid to furnish 46 possessing the desired pseudoaxial configuration at C4.

Careful control of conditions uncovered a rich tapestry of selective chemistry when triester 46 was exposed to base. Transesterification of triester 46 upon brief treatment with $NaOCH_3$ (1.5 equiv in CH_3OH, reflux) led to endo-hydroxydiester 47, while exhaustive saponification of 46 (4 equiv NaOH, rt) provided endo-diacid 10.

In stark contrast, when deprotonated at low temperature (KH, THF, -78°C) then worked up with trifluoroacetic acid, triester 46 epimerized to exo-triester 48. The stereochemical assignment relied on the multiplicity of the (now axial) C7-H (δ 2.60, tt, J=4.4, 12.8 Hz). Heating in the presence of $NaOCH_3$ both debenzoylated and epimerized 46, thus affording exo-hydroxydiester 49. Consistent with these observations, the same exo-diester 49 could be prepared from its endo-isomer 47 by prolonged exposure to methoxide in CH_3OH at reflux.

In the exo series, both triester 48 and diester 49 could be saponified using excess base to furnish the exo-diacid 9 which was also purified by reversed-phase HPLC and tested as an inhibitor of chorismate mutase *(vide infra)*. Thus, as might have been expected, thermodynamic equilibrium favored the exo series 48-49 in which the C7-carboxyl group was equatorial, while the more sterically congested endo-isomers 44-47 were formed by kinetic protonation of the intermediate ester enolate from its more accessible face.

SCHEME 9



ENZYME INHIBITION STUDIES

To evaluate the ability of racemic bicyclic and tricyclic structures 9-15 to block the enzyme-catalyzed rearrangement of 1 to 2 (Scheme 1), freshly purified samples of E. coli chorismate mutase-prephenate dehydrogenase were assayed under the conditions reported by SampathKumar and Morrison[25] by monitoring the disappearance of chorismate (decrease in UV absorbance at λ=274 nm) as it was converted to prephenate in the presence of NAD^+. IC_{50} measurements were readily obtained from plots of 1/V versus inhibitor concentration at a substrate concentration equal to the apparent K_M of chorismate (0.09mM).[26] These IC_{50} values represent the concentration of transition state analogue resulting in a 50% reduction in reaction rate and provide an initial measure of each inhibitor's effectiveness. Adamantane-1-phosphonic acid 5 was also prepared and assayed as a point of comparison with previous studies on synthetic inhibitors. Results are presented in Table 1.

Of all the synthetic structures tested, tricyclic diacid 11 was the least effective inhibitor of the mutase, with an I_{50} value more than 200-fold higher than the substrate's K_M (Entry 4). Prior incubation of 11 with the enzyme did not improve inhibition, thus ruling out slow binding of the inhibitor. Replacing either or both of the carboxyl groups in 11 with phosphonic acid substituents (12, 13 and 14, entries 5-7) resulted in somewhat enhanced binding, although even the best of these phosphonate analogues was only about one-fifth as effective as adamantane-1-phosphonate 5 (Entry 1). This might be due to steric effects or to repulsive interactions with the enzyme resulting from the second dissociation of each phosphonate function, which might be appreciable at the assay pH of 7.5.[27]

TABLE 1

INHIBITORS OF CHORISMATE MUTASE

ENTRY	INHIBITOR	I_{50} (mM)	I_{50}/K_M [a]
1	5	0.7	7.8
2	9	1.85	20.6
3	10	0.43	4.8
4	11	20	224
5	12	3.5	38.9
6	13	3.5	38.9
7	14	3.0	33.3
8	15	0.5	5.6

(a) K_M for chorismate was 89 μM.

The most potent of the compounds reported here was endo diacid 10 in the bicyclic series. Compound 10, first described in our preliminary communication in 1987, was a more active inhibitor than its exocyclic counterpart 9 and a better inhibitor of chorismate mutase than adamantane-1-phosphonate. Recently Bartlett et al. described an independent route to diacid 10 by a 14-step synthesis from dimethyl itaconate.[10] This achievement made possible a direct comparison (presumably under identical bioassay conditions) of the Berkeley oxabicyclic diacid 8 with the Cornell carbabicyclic diacid 10, both of which possess the endocyclic orientation of the "enolpyruvate"-derived carboxyl group. From this comparison it was concluded that substituting a CH_2 group for O reduced the binding affinity by a factor of 250.[10] However the fact that 10 is a fivefold better inhibitor than its exo isomer 9 follows the same trend observed by Bartlett et al. with structures 6, 7 and 8 (Scheme 2).[10] Overall it would appear that the specific orientation of the bridging carboxylate group, as well as dipolar or hydrogen bonding interactions with the enolpyruvate ether oxygen, play important roles in binding.

The unexpectedly high level of inhibition observed with hydroxydiacid 15 (Entry 8) deserves further comment. Diacid 15, which bears a general resemblance to 11-14 except for its additional hydroxyl group, proved to be the most potent tricyclic inhibitor made in this study. Despite the absence of chorismate's cyclohexene double bond (which is known to accelerate the in vitro Claisen rearrangement), compound 15 was nearly as effective an inhibitor as endo diacid 10. In view of the importance of the sidechain carboxyl group's orientation in the rearrangement transition state, molecular mechanics simulations[28] were used to calculate the lowest energy conformation of 15. The results are depicted in Figure 1. The four-membered ring in 15 can be seen to be more severely puckered than cyclobutane itself (41° versus ca. 25°), thus imparting a rather substantial downward "tilt" to the C1-carboxyl so that its orientation resembles that of endo stereoisomers 8 and 10. In fact a good fit was observed [root mean square (RMS) deviation of 0.380] when the calculated structure for 15 was superimposed on the energy-minimized form of carbocyclic endo diacid 10 so as to maximize overlap of the hydroxyl and two carboxyl groups. While such computer-generated molecular comparisons to an idealized mutase transition state have been attempted before with mixed success,[7] they involved synthetic inhibitors of limited potency. In this case, however, to determine whether more general structure-activity correlations might be constructed by disregarding the cyclohexene double bond and focusing only on the overlap of OH and CO_2H groups, several active transition state analogue inhibitors (6, 8, 9, 10 and 15) were energy-minimized and superimposed on endo-diacid 8, the most potent of the group.[10] Each structure's similarity to 8 was judged by RMS deviations, then compared with observed biological activity against chorismate mutase. While a qualitative correlation was evident from the data in Table 2, a rather good linear relationship was obtained in semilogarithmic plots of RMS deviations versus inhibitor activity as shown in Figure 2. Such structure-activity correlations may prove valuable in designing other synthetic targets as potential inhibitors of chorismate mutase.

FIGURE 1

MINIMIZED STRUCTURE OF CYCLOBUTANE 15

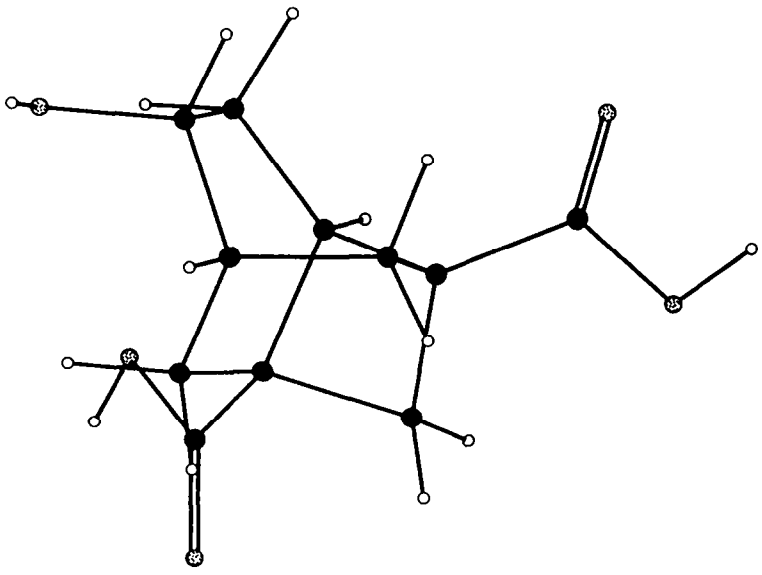


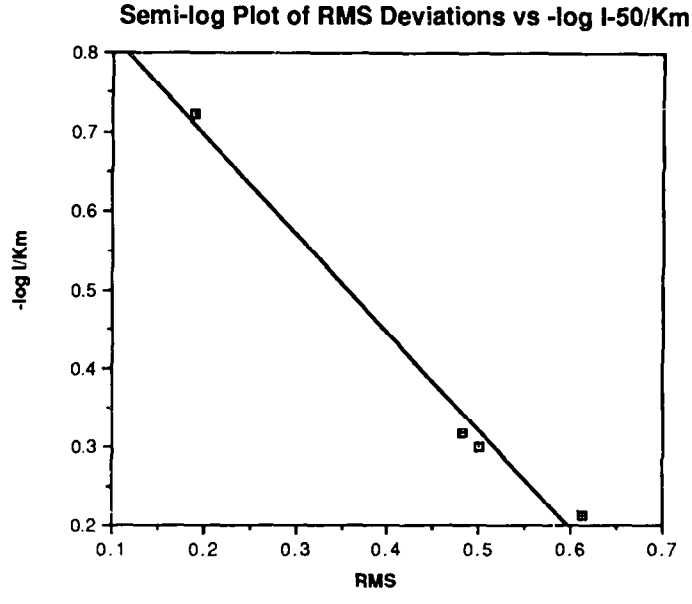
TABLE 2

CORRELATION OF ENERGY-MINIMIZED STRUCTURES WITH BIOLOGICAL ACTIVITY

STRUCTURE	INHIBITOR	RMS FIT (to 8) [a]	I_{50}/K_M
8	endo oxa diacid	0.000	0.008 [b]
10	endo carba diacid	0.190	4.8 [c]
6	exo oxa diacid	0.482	5.5 [b]
15	tricyclic cyclobutane	0.500	5.6 [c]
9	exo carba diacid	0.612	20.6 [c]

(a) Calculated using MacroModel, Version 2.0, by W.C. Still et al., 1988
(b) Ref. 10
(c) This work

FIGURE 2



In conclusion, a new, relatively short and efficient synthesis of bi- and tricyclic transition state analogues for a critical enzyme of the shikimate pathway has been developed. The greater activity noted in the endocyclic

versus exocyclic series (i.e. 10 > 9), the enhanced binding of carbobicyclic phosphonates 12-14 as compared with 11, and the unexpectedly potent inhibition for hydroxydiacid 15 all suggest new avenues for research in the design and preparation of chorismate mutase inhibitors.

EXPERIMENTAL SECTION

Proton NMR spectra were taken on a Bruker WM-300 (300 MHz) spectrometer. All chemical shifts were reported as δ scale in parts per million downfield from Me_4Si. Spectra taken in $CDCl_3$ were referenced to either Me_4Si (0.00, for compounds with aromatic protons) or residual $CHCl_3$ (7.25, for compounds without aromatic protons). Spectra taken in D_2O were referenced to HOD (4.75), and those in CD_3OD were referenced to CHD_2OD (3.35). Carbon-13 NMR spectra were taken on a Varian XL-400 (100 MHz), Bruker WM-300 (75 MHz) or JEOL FX-90Q (22.5 MHz) spectrometer. Infrared spectra were taken on a Perkin-Elmer Model 681 infrared spectometer and calibrated with polystyrene. Fourier Transform infrared spectra were taken on a Nicolet IR-44 infrared spectrometer. Ultraviolet absorption spectra were measured on a Hewlett-Packard HP 8451A Diode Array Spectrometer. Mass spectra were obtained from an AEI-MS 902 mass spectrometer. Chemical ionization spectra were obtained using isobutane as reagent gas; electron impact spectra were run at 70 eV ionizing voltage. Fast atom bombardment spectra were obtained in glycerol matrix on a Kratos MS-890 Spectrometer. Optical rotations were measured on a Perkin Elmer 241 Polarimeter. Sample concentrations were expressed in grams of sample per 100 cc of solvent. High performance liquid chromatography was performed with a Waters 6000A pump using a Model U6K injector with a 120 μM sample loop, μ-Bondapak C18 column (3.9 mm o.d.) and a Model 450 UV detector (202 nm). The phrase "the usual workup" refers to the following procedure: the combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo using a rotary evaporator.

Dimethyl 1-(2'-Carboxy-2'-propenyl)-1,4-dihydrobenzoate (20). To a solution of N-isopropylcyclohexylamine (339 mg, 2.40 mmol) in THF (8 mL) under Ar at -78°C was added dropwise n-BuLi (2.41 mmol, 1.5 M in hexane) and the mixture stirred 15 min at 0°C. After cooling (-78°C), methyl 1,4-dihydrobenzoate 18 (332 mg, 2.40 mmol) was added in THF (1 mL) and the deep-red anion solution stirred 20 min, during which time a precipitate formed. Warming to -60°C formed a homogeneous solution of the anion which was added dropwise by cannula to a solution of methyl α-bromomethacrylate 19 (461 mg, 2.58 mmol) in THF (2 mL). The resulting pale yellow solution was stirred 90 min at -78°C, poured into saturated aqueous NH_4Cl solution (25 mL) and extracted 3 x 25 mL with ethyl acetate. The usual workup followed by flash chromatography (1:9 EtOAc:hexane) gave 20 (470 mg, 83%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.17 (d, 1 H, J=1 Hz), 5.84 (dt, 2 H, J=9, 2 Hz), 5.72 (dt, 2 H, J=9, 1 Hz), 5.47 (d, 1 H, J=1 Hz), 3.69, 3.67 (2 s, each 3 H, CO_2CH_3), 2.73 (s, 2 H), 2.54 (m, 2 H); ^{13}C-NMR 174.3, 167.7, 135.9, 128.3, 126.3, 126.0, 52.9, 51.7, 48.0, 40.5, 25.9; IR ν_{max} (film) 3040, 2960, 1732, 1635, 1440, 1290, 1240, 1205, 1150, 1060, 945 cm^{-1}; HRMS calcd for $C_{13}H_{16}O_4$ $(M+H)^+$ 237.1127, found 237.1148.

Dimethyl 1-(2-Carboxy-2-propenyl)-3-carboxy-cyclohexa-2,4-diene [Dimethyl 4-Deoxycarbochorismate] (21). A solution of triene 20 (11 mg, 0.05 mmol) in toluene (2 mL) was heated at 105°C for 2.5 h under Ar. When the solution cooled, toluene was evaporated under reduced pressure and NMR analysis indicated that the rearrangement was approximately 40% complete. The reaction mixture was chromatographed (1:9 EtOAc:hexane) to furnish diester 21 (21%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.78 (d, 1 H, J=7 Hz), 6.35 (d, 1 H, J=10.6 Hz), 6.24 (d, 1 H, J=1.3 Hz), 5.85 (dt, 1 H, J=10.6, 4.6 Hz), 5.55 (d, 1 H, J=1.3 Hz), 3.74, 3.73 (2 s, each 3 H, CO_2CH_3), 2.68 (m, 1 H), 2.50-2.33 (m, 2 H), 2.30-2.19 (m, 1 H), 2.05-1.93 (m, 1 H); IR ν_{max} 1720, 1630, 1440, 1260, 1200, 1140 cm^{-1}; HRMS calcd for $C_{13}H_{16}O_4$ $(M+H)^+$ 237.1127, found 237.1135.

Tricyclo[3.3.1.0^{2,7}]non-3-ene-1,3-dicarboxylic acid Dimethyl Ester (23). A solution of triene 20 (163 mg, 0.69 mmol) in toluene (5 mL) was heated at 110°C for 24 h under Ar. When the solution cooled, toluene was evaporated under reduced pressure and the residue chromatographed (1:4 EtOAc:hexane) to furnish tricyclic diester 23 (159 mg, 98%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 7.62 (dd, H-4), 3.90 (d, H-2), 3.72, 3.58 (2 s, each 3 H, CO_2CH_3), 3.08 (br. s, H-5), 2.56 (dd, H-9), 2.11 (br. q, H-7), 1.86 (dd, H-8), 1.69 (dd, H-6), 1.37 (dd, H-8'), 1.23 (d, H-9'), 1.04 (m, H-6'): $J_{8,8'}$=12.4 Hz, $J_{6,6'}$= 12Hz, $J_{9,9'}$=9.3Hz, $J_{4,5}$= 7.3 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=5 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.7 Hz, $J_{2,4}$= 2.1 Hz, $J_{5,8'}$=1.7 Hz; ^{13}C-NMR 174.7, 165.6, 149.4, 130.9, 51.7, 51.5, 45.8, 39.5, 38.8, 34.1, 32.0, 30.8, 29.2; IR ν_{max} ($CHCl_3$) 3020, 2950, 1720, 1715, 1625, 1435, 1300, 1250, 1110, 1100 cm^{-1}; HRMS calcd for $C_{13}H_{17}O_4$ $(M+H)^+$ 237.1127, found 237.1146.

Tricyclo[3.3.1.0^{2,7}]non-3-ene-1,3-dicarboxylic Acid (11). To a solution of tricyclic diester 23 (18 mg, 0.076 mmol) in 1:1 THF:H_2O (1 mL) under Ar at 0°C was added aqueous NaOH (0.19 mmol) and the solution stirred at rt for 12 h. The bulk of THF was removed in vacuo, the aqueous residue acidified with 10% HCl and extracted 6 x 1 mL with EtOAc. After the usual workup, the crude product was chromatographed (EtOAc) to afford diacid 11 (15.8 mg, quantitative) which crystallized: mp 208°C; ^{1}H-NMR (300 MHz, CD_3OD) 7.39 (dd, H-4), 3.64 (d, H-2), 3.08 (br. s, H-5), 2.38 (dd, H-9), 2.02 (br. q, H-7), 1.87 (dd, H-8), 1.71 (dd, H-6), 1.24 (dd, H-8'), 1.23 (d, H-9'), 1.00 (m, H-6'); ^{13}C-NMR (22.5 MHz, Na_2 salt in D_2O) 186.3, 176.4, 146.7, 136.9, 49.4, 41.1, 41.0, 36.0, 32.8, 31.9, 28.7; IR ν_{max} (KBr) 2600-3000, 1690, 1625, 1425, 1290, 1120, 955, 732 cm^{-1}; HRMS calcd for $C_{11}H_{13}O_4$ $(M+H)^+$ 209.0814, found 209.0832.

Methyl 1-(2'-(Diethylphosphono)-2'-propenyl)-1,4-dihydrobenzoate (25). To a solution of N-isopropylcyclohexylamine (388 mg, 2.75 mmol) in THF (5 mL) under Ar at -78°C was added dropwise n-BuLi (2.76 mmol, 1.5 M in hexane) and the mixture stirred 5 min at 0°C. After cooling to -78°C, methyl 1,4-dihydrobenzoate 18 (380 mg, 2.75 mmol) was added in THF (5 mL). The deep-red anion solution was stirred 30 min, then added dropwise by cannula over 15 min to a solution of bromophosphonate 24 (718 mg, 2.79 mmol) in THF (6 mL). The resulting pale yellow solution was stirred 90 min at -78°C, poured into saturated aqueous NH_4Cl solution (40 mL) and extracted 4 x 30 mL with ether. The usual workup followed by flash chromatography (EtOAc) gave triester 25 (681 mg, 79%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.11 (d, 1 H, J=24.2 Hz), 5.80 (d, 1 H, J=30.2 Hz), 5.94-5.74 (m, 4 H), 4.05 (m, 4 H, -OCH_2-), 3.68 (s, 3 H, CO_2CH_3), 2.71-2.50 (m, 4 H, allylic), 1.30 (m, 6 H, OCH_3); IR ν_{max} 2950, 1735, 1440, 1390, 1240, 1050, 1030, 965 cm^{-1}; HRMS calcd for $C_{15}H_{24}O_5P$ $(M+H)^+$ 315.1361, found 315.1392.

3-Carbomethoxy-1-diethylphosphono-tricyclo[3.3.1.0^{2,7}]non-3-ene (27). A solution of triester 25 (108 mg, 0.34 mmol) in toluene (5 mL) was heated at 145°C for 72 h under Ar. When the solution cooled, it was poured into water (5 mL) and extracted 4 x 5 mL with EtOAc. The combined organic extracts were dried, concentrated under reduced pressure and the residue chromatographed (EtOAc) to afford tricyclic triester 27 (83 mg, 77%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 7.63 (dd, H-4), 4.08-3.94 (m, H-2 + OCH_2-), 3.72 (s, 3 H, OCH_3), 3.13 (br. s, H-5), 2.58 (dd, H-9), 2.22 (dq, H-7), 1.90 (dd, H-8), 1.76 (dd, H-6), 1.37 (dd, H-8'), 1.19 (d, H-9'), 1.27-1.15 (m, 6 H, -P-O-C-CH_3), 1.08 (m, H-6'): $J_{7,P}$=16 Hz (W-coupling), $J_{6,6'}$= 12.5Hz, $J_{8,8'}$=12 Hz, $J_{9,9'}$=9.4Hz, $J_{4,5}$= 7.3 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=6.5 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.8 Hz, $J_{2,4}$= 2.2 Hz, $J_{5,8'}$=1.5 Hz; IR ν_{max} ($CHCl_3$) 3000, 2965, 1720, 1640, 1445, 1265, 1060, 1035, 970 cm^{-1}; HRMS calcd for $C_{15}H_{24}O_5P$ $(M+H)^+$ 315.1361, found 315.1378.

3-Carboxy-tricyclo[3.3.1.0^{2,7}]non-3-ene-1-phosphonic Acid (12). To a solution of tricyclic triester 27 (71 mg, 0.23 mmol) and NaI (276 mg, 1.85 mmol) in CH_3CN (4 mL) at 0°C under Ar was added chlorotrimethylsilane (200 mg, 1.84 mmol) dropwise over 5 min. Sodium chloride precipitated immediately and the resulting suspension was stirred 15 min at 0°C and at rt for 10 h. The suspension was filtered using CH_3CN and the solvent evaporated under reduced pressure. The oily orange residue was taken up in 1:4 THF:H_2O (2 mL) and stirred overnight. The bulk of THF was removed in vacuo and the aqueous residue extracted 3 x 2 mL with ether to remove neutral impurities. The aqueous phase was cooled to 0°C under Ar and treated with NaOH (275 µL of an aqueous 1 mL solution containing 115 mg NaOH; 0.79 mmol) and the mixture stirred for 5 h. After extracting 3 x 1mL with ether, the aqueous layer was concentrated to give a white solid (74 mg) which was purified by redissolving in H_2O and passing through a prewashed ion-exchange resin (Dowex 50X8-200, 5 mL) eluting with H_2O (20 mL). The solvent was lyophilized to afford tricyclic triacid 12 (37 mg, 66%) as white crystals: mp >180°C (d); ^{1}H-NMR (300 MHz, D_2O) 7.79 (dd, H-4), 3.87 (ddd, H-2, J=2.0, 5.5, 9.9 Hz), 3.25 (br. s, H-5), 2.49 (m, H-9), 2.25 (ddd, H-7), 1.96 (dd, H-8), 1.85 (dd), 1.29, dd, H-8'), 1.25 (d, H-9'), 1.09 (m, H-6'): $J_{7,P}$=16 Hz (W-coupling), $J_{6,6'}$= 12.5Hz, $J_{8,8'}$=12 Hz, $J_{9,9'}$=9.4Hz, $J_{4,5}$= 7.3 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=6.5 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.8 Hz, $J_{2,4}$= 2.2 Hz, $J_{5,8'}$=1.5 Hz; IR ν_{max} (KBr) 3100-2500 (br), 2980, 1685, 1625, 1435, 1300, 1220, 1010, 980, 970, 925 cm^{-1}; HRMS calcd for $C_{10}H_{14}O_5P$ $(M+H)^+$ 245.0579, found 245.0589.

Diethyl 1-(2'-Carbomethoxy-2'-propenyl)cyclohexa-2,5-diene-1-phosphonate (30) and Diethyl 1-(2-Carbomethoxy-2-propenyl)cyclohexa-2,4-diene-3-phosphonate (31). To a solution of N-isopropylcyclohexylamine (30 mg, 0.22 mmol) in THF (1 mL) under Ar at -78°C was added dropwise n-BuLi (0.22 mmol, 1.6 M in hexane) and the mixture stirred 20 min at 0°C. After cooling to -78°C, diethyl 1,4-dihydrobenzene-1-phosphonate 28 (47 mg, 0.22 mmol) was added in THF (1 mL) and the deep-yellow anion solution stirred 15 min, then added dropwise by cannula to a solution of methyl α-bromomethacrylate 19 (49 mg, 0.27 mmol) in THF (1 mL). The resulting colorless solution was stirred 1 h at -78°C, poured into saturated aqueous NH_4Cl solution (4 mL) and extracted 3 x 5 mL with ethyl acetate. The combined organic extracts were washed

with water (6 mL) and brine (6 mL). The usual workup followed by flash chromatography (EtOAc) gave 30 (50 mg, 72%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.17 (d, 1 H, J=1.2 Hz), 5.87 (m, 2 H), 5.57 (m, 2 H), 5.45 (d, 1 H, J=1.2 Hz), 4.10 (m, 4 H), 3.69 (s, 3 H), 2.83 (d, 2 H, $J_{P,H}$ =7.5 Hz), 2.64 (m, 1 H), 2.57 (m, 1 H), 1.29 (m, 6 H); IR ν_{max} 2950, 1727, 1635, 1443, 1395, 1250, 1130, 1050, 1020, 960, 750 cm^{-1}; CIMS 315 (M+1, 44%); HRMS calcd for $C_{15}H_{24}O_5P$ $(M+H)^+$ 315.1361, found 315.1376.
When another sample of 28 (80 mg, 0.37 mmol) was lithiated in this fashion, then aged 30 min before reacting with 19 (0.48 mmol), a second, more polar spot on the thin-layer chromatogram also appeared (R_f=0.24 in EtOAc) corresponding to the γ-alkylated product 31. Careful flash chromatography afforded 30 (50%) and 31 (25 mg, 22%) as colorless oils. For 31: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.62 (dd, 1 H, J=24, 4 Hz), 6.23 (d, 1 H, J=1.2 Hz), 6.05 (m, 1 H), 5.87 (br. m, 1 H), 5.55 (d, 1 H, J=1.2 Hz), 4.10 (m, 4 H, -OCH_2-), 3.75 (s, 3 H, CO_2CH_3), 2.45-2.20 (m, 4 H), 1.29 (m, 6 H, P-O-C-CH_3); IR ν_{max} ($CHCl_3$) 2945, 1725, 1630, 1400, 1250, 1100, 1045 cm^{-1}; CIMS 315 (M+1, 38%).
1-Carbomethoxy-3-diethylphosphono-tricyclo[3.3.1.0^{2,7}]non-3-ene (32). A solution of triester 30 (48 mg, 0.15 mmol) in toluene (2 mL) was heated in a Kimble vial at 130°C for 48 h under Ar. When the solution cooled, it was poured into water (5 mL) and extracted 4 x 5 mL with EtOAc. The combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and the residue chromatographed (1:4 hexane:EtOAc) to afford the tricyclic triester 32 (35 mg, 74%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 7.57 (ddd, H-4), 4.00 (m, 4 H, -OCH_2-), 3.60 (s, CO_2CH_3), 3.59 (m, H-2), 3.08 (br. s, H-5), 2.57 (br. t, H-9), 2.11 (q, H-7), 1.87 (dd, H-8), 1.70 (dd, H-6), 1.43 (dd, H-8'), 1.27 (d, H-9'), 1.06 (m, H-6'): $J_{4,P}$=16.3 Hz, $J_{6,6'}$= 12.7 Hz, $J_{8,8'}$=12 Hz, $J_{9,9'}$=9.0 Hz, $J_{4,5}$= 7.1 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=6.5 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.8 Hz, $J_{2,4}$= 1.6 Hz, $J_{5,8'}$=1.5 Hz; IR ν_{max} (film) 2960, 1730, 1610, 1440, 1290, 1280, 1240, 1120, 1050, 1025, 965 cm^{-1}; HRMS calcd for $C_{15}H_{24}O_5P$ $(M+H)^+$ 315.1361, found 315.1381.
1-Carboxy-tricyclo[3.3.1.0^{2,7}]non-3-ene-3-phosphonic Acid (13). To a solution of tricyclic ester 32 (31 mg, 0.10 mmol) and NaI (119 mg, 0.79 mmol) in CH_3CN (2 mL) at 0°C under Ar was added chlorotrimethylsilane (86 mg, 0.79 mmol) dropwise. Immediate precipitation of NaCl was observed and the resulting suspension was stirred at rt for 8 h, filtered and washed with CH_3CN. The combined filtrates were concentrated in vacuo, the resulting oil was taken up in H_2O, stirred for 18 h, then extracted 3 x 1 mL with EtOAc to remove neutral impurities. The remaining aqueous phase was lyophilized to give the carbomethoxy phosphonic acid (25 mg) as a glassy oil. A fresh aqueous solution (0.5 mL) of the oil was treated with NaOH (150 μL of a 92 mg/mL aqueous solution. 0.35 mmol) at 5°C under Ar and stirred at rt for 5 h. The solvent was lyophilized and the residue repeatedly triturated with acetone, whereupon white crystals of the tris-sodium salt of 13 (31 mg) were deposited. The solid was dissolved in H_2O (1 mL) and passed through Dowex 50X8-200 ion exchange resin (4 mL) eluting with H_2O (10 mL). Lyophilization left tricyclic triacid 13 (17 mg, 69%) as a white, crystalline solid: m.p. >220°C (d); ^{1}H-NMR (300 MHz, $CDCl_3$) 7.60 (ddd, H-4), 3.61 (dd, H-2), 3.15 (m, H-5), 2.53 (br. t, H-9), 2.14 (dd, H-7), 1.98 (dd, H-8), 1.78 (dd, H-6), 1.34 (m, H-8', H-9'), 1.08 (m, H-6'): $J_{4,P}$=16 Hz, $J_{6,6'}$= 12.8 Hz, $J_{8,8'}$=12.4 Hz, $J_{9,9'}$=9.0 Hz, $J_{4,5}$= 7.1 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=6 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.8 Hz, $J_{2,4}$= 1.6 Hz, $J_{5,8'}$=1.4 Hz; IR ν_{max} (KBr) 3200-2700 (br.), 2960, 1720, 1620, 1315, 1220, 1130, 1010, 930, 670 cm^{-1}; HRMS calcd for $C_{10}H_{14}O_5P$ $(M+H)^+$ 245.0579, found 245.0590.
Tetraethyl 1-(2'-Propenyl)cyclohexa-2,5-diene-1, 2'-diphosphonate (33). To a solution of N-isopropyl-cyclohexylamine (35 mg, 0.25 mmol) in THF (1 mL) under Ar at -78°C was added dropwise n-BuLi (0.25 mmol, 1.6 M in hexane) and the mixture stirred 30 min at 0°C. After cooling to -78°C, diethyl 1,4-dihydro-benzene-1-phosphonate 28 (54 mg, 0.25 mmol) was added in THF (1 mL) and the deep-yellow anion solution stirred 12 min, then added dropwise by cannula to a solution of bromophosphonate 24 (66 mg, 0.26 mmol) in THF (1 mL) at -78°C. The reaction mixture was stirred 90 min at -78°C, poured into hexane and filtered. The solvent was evaporated under reduced pressure and the residue chromatographed (1:4 MeOH:EtOAc) to give triene 33 (58 mg, 60%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.11 (dd, 1 H, J=24.1, 1.3 Hz), 5.94 (dd, 1 H, J=49.5, 1.3 Hz), 5.90 (m, 2 H), 5.62 (m, 2 H), 4.06 (m, 8 H), 2.73 (dd, 2 H, $J_{P,H}$ =7.2 Hz, J_{gem}=12.6 Hz), 2.72 (m, 1 H), 2.63 (m, 1 H), 1.29 (m, 12 H); IR ν_{max} (neat) 3000, 1400, 1250, 1050, 1030, 968, 795 cm^{-1}; HRMS calcd for $C_{17}H_{31}O_6P$ $(M+H)^+$ 393.1596, found 393.1602.
Tetraethyl Tricyclo[3.3.1.0^{2,7}]non-3-ene-1,3-Diphosphonate (35). A solution of tetraester 33 (64 mg, 0.16 mmol) in toluene (3 mL) was heated in a Kimble vial at 145°C for 72 h under Ar. When the solution cooled, it was poured into water (5 mL) and extracted 5 x 5 mL with EtOAc. The combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and the residue chromatographed (1:19 EtOH:EtOAc) to afford the tricyclic tetraester 33 (41 mg, 64%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 7.55 (ddd, H-4), 4.05 (m, 8

H, -OCH_2-) 3.71 (m, H-2), 3.11 (br. s, H-5), 2.53 (dd, H-9), 2.20 (q, H-7), 1.90 (dd, H-8), 1.78 (dd, H-6), 1.42 (d, H-8'), 1.29 (m, 12 H), 1.19 (dd, H-9'), 1.07 (m, H-6'): $J_{4,P}$=$J_{7,P}$=16.4 Hz, $J_{6,6'}$= 12.5 Hz, $J_{8,8'}$=12 Hz, $J_{4,5}$= 7.1 Hz, $J_{6,7}$=$J_{2,7}$=$J_{9,7}$=6.5 Hz, $J_{5,6}$=3.8 Hz, $J_{5,8}$=3.8 Hz, $J_{2,4}$= 1.9 Hz, $J_{9',7}$= 1.3 Hz; IR ν_{max} (film) 2990, 2950, 1620, 1450, 1400, 1240, 1055, 1030, 970, 790 cm^{-1}; HRMS calcd for $C_{17}H_{31}O_6P$ $(M+H)^+$ 393.1596, found 393.1609.

Tricyclo[3.3.1.$0^{2,7}$]non-3-ene-1,3-Diphosphonic Acid (14). To a solution of tricyclic tetraester 35 (49 mg, 0.12 mmol) and NaI (297 mg, 0.1.98 mmol) in CH_3CN (3.5 mL) at 0°C under Ar was added chlorotrimethylsilane (215 mg, 1.98 mmol) dropwise. Immediate precipitation of NaCl was observed and the resulting suspension was stirred at rt for 10 h, filtered and washed with CH_3CN. The combined filtrates were concentrated in vacuo, the resulting orange oil was taken up in 2:1 H_2O:THF (2 mL), stirred for 12 h, then concentrated in vacuo to remove THF. The aqueous residue was extracted 3 x 2 mL with ether to remove neutral impurities, then treated with NaOH (215 µL of a 94 mg/mL aqueous solution. 0.51 mmol) at 5°C under Ar and stirred at rt for 1 h. The solvent was lyophilized and the residue repeatedly triturated with acetone, whereupon white crystals of the tetrasodium salt of 14 (46 mg) were deposited. The solid was dissolved in H_2O (1 mL) and passed through Dowex 50X8-200 ion exchange resin (4 mL) eluting with H_2O (10 mL). Lyophilization left tricyclic tetra-acid 14 (26 mg, 74%) as a white, crystalline solid: m.p. 165°C (d); ^{1}H-NMR (300 MHz, $CDCl_3$) 7.42 (ddd, H-4), 3.59 (dd, H-2), 3.13 (m, H-5), 2.43 (m, H-9), 2.20 (dd, H-7), 1.90 (dd, H-8), 1.79 (dd, H-6), 1.26 (m, H-8'), 1.21 (m, H-9'), 1.04 (m, H-6'): $J_{4,P}$=16.6 Hz, $J_{6,6'}$= 12.5 Hz, $J_{8,8'}$=12.4 Hz, $J_{4,5}$= 9.7 Hz, $J_{9,9'}$=9.0 Hz, $J_{5,6}$=3.6 Hz, $J_{5,8}$=3.5 Hz, $J_{2,4}$= 1.5 Hz; IR ν_{max} (KBr) 3100-2640 (br.), 2930, 1620, 1465, 1220, 1000 cm^{-1}.

4-(t-Butyldimethylsilyloxy)-1-carbomethoxy-cyclohexa-1,4-diene (38). A solution of 2-(t-butyldimethylsilyl-oxy)buta-1,3-diene (1.25 g, 6.78 mmol) and methyl propiolate (1.67 g, 19.86 mmol) in dry distilled benzene (6 mL) was heated at reflux under Ar for 16 h. After cooling, the solvent was evaporated under reduced pressure and the residue chromatographed (1:9 ether:hexane) to give 38 (1.47 g, 81%) as a colorless oil; ^{1}H-NMR (300 MHz, $CDCl_3$) 6.87 (m, H-2), 4.86 (m, H-5), 3.72 (s, CO_2CH_3), 2.97 (m, 2 H), 2.82 (m, 2 H), 0.89 (s, 9 H, t-Bu), 0.13 (s, 6 H, Si-CH_3); ^{13}C-NMR (22.5 MHz, $CDCl_3$) 166.8, 146.2, 135.5, 127.7, 100.5, 51.3, 31.2, 25.8, 25.4, 17.8, -4.6; IR ν_{max} (film) 2950, 2925, 2850, 1720, 1690, 1470, 1435, 1375, 1255, 1200, 1085, 1050, 1000, 870, 835 cm^{-1}; CIMS 269 (M+1, 67%).

Dimethyl 1-(2'-Carboxy-2'-propenyl)-4-(t-butyldimethylsilyloxy)-2,4-dihydrobenzoate (39). To a solution of N-isopropylcyclohexylamine (214 mg, 1.52 mmol) in THF (3 mL) under Ar at -0°C was added dropwise n-BuLi (1.52 mmol, 1.52 M in hexane) and the mixture stirred 15 min. After cooling to -78°C, cyclohexadiene 38 (370 mg, 1.38 mmol) was added in THF (2 mL) and the deep-yellow anion solution stirred 20 min. The anion was then added dropwise by cannula to a solution of methyl α-bromomethacrylate 19 (306 mg, 1.79 mmol) in THF (3 mL) and the pale yellow solution was stirred 30 min at -78°C, at which time tlc indicated complete consumption of starting material. Hexane (20 mL) was added, forming a precipitate, and the resulting suspension was washed with NH_4Cl solution (2 mL) and brine (2 mL). The usual workup of the organic phase, followed by flash chromatography (1:9 ether:hexane), gave triene 39 (478 mg, 95%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 6.20 (d, 1 H, J=1.4 Hz), 5.94 (d, 1 H, J=10 Hz), 5.74 (dd, 1 H, J=10, 2.1 Hz), 5.48 (br. s, 1 H), 4.81 (m, 1 H), 3.70, 3.62 (2 s, each 3 H, CO_2CH_3), 2.76 (d, 1 H, J=13.3 Hz), 2.62, (d, 1 H, J=17.3 Hz), 2.61 (d, 1 H, J=13.3 Hz), 2.32 (dd, 1 H, J=5.5, 17.3 Hz), 0.90 (s, 9 H), 0.11 (s, 6 H); IR ν_{max} (film) 2957, 2930, 2860, 1730, 1655, 1975, 1440, 1400, 1250, 1200, 1150, 1055, 900, 840 cm^{-1}; CIMS 307 (M+1, 69%); HRMS calcd for $C_{19}H_{31}O_5Si$ $(M+H)^+$ 367.1940, found 367.1953.

1,7-Bis-carbomethoxy-4-(t-butyldimethylsilyloxy)-tricyclo[3.3.1.$0^{2,7}$]non-3-ene (40). A solution of triene 39 (1.01 g, 2.76 mmol) in toluene (20 mL) was heated at reflux under Ar for 68 h. After cooling, the pale yellow solution was concentrated in vacuo and the residue chromatographed (1:4 ether:hexane) to afford the symmetrical tricyclic adduct 40 (905 mg, 90%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 4.88 (dd, J=2.1, 6.9 Hz, H-3), 3.63 (s, 6 H, CO_2CH_3), 3.37 (d, J=6.9 Hz, H-2), 2.75 (br. d, J=9.4 Hz, H-9), 2.69 (m, H-5), 1.68, 1.79 (ABX, J=3.4, 12.5 Hz, H-6, H-6', H-8, H-8'), 1.33 (d, J=9.4 Hz, H-9'), 0.92 (s, 9 H, t-Bu), 0.14 (s, 6 H, Si-CH_3); ^{13}C-NMR (22.5 MHz, $CDCl_3$); 174.7, 163.4, 97.6, 51.8, 44.9, 49.5, 40.8, 37.6, 34.9, 25.7, 18.1, -4.5; IR ν_{max} (film) 2945, 2850, 1730, 1640, 1470, 1431, 1365, 1280, 1215, 1170, 1115, 900, 850, 835 cm^{-1}; HRMS calcd for $C_{19}H_{31}O_5Si$ $(M+H)^+$ 367.1940, found 367.1961.

1,7-Bis-carbomethoxytricyclo[3.3.1.$0^{2,7}$]nonan-4-one (41). To a solution of silyl enol ether 40 (271 mg, 0.74 mmol) in dry CH_3OH (2 mL) at 0°C under Ar was added freshly distilled trifluoroacetic acid (444 mg, 3.89 mmol) and the resulting solution stirred at rt for 9 h. The solvents were evaporated under reduced pressure and the residue chromatographed (3:2 ether:hexane) to afford the symmetrical ketone 41 (179 mg, 96%) as a colorless

oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 3.69 (s, 6 H, CO_2CH_3), 3.04 (m, H-2), 2.79 (br. d, J=9.7 Hz, H-9), 2.66 (m, H-5), 2.45 (d, 2 H, H-3, H-3'), 2.23 (br. s, 4 H, H-6, H-6', H-8, H-8'), 1.75 (d, J=9.7 Hz, H-9'); ^{13}C-NMR (22.5 MHz, $CDCl_3$); 211.6, 173.8, 52.1, 43.2, 42.0, 41.8, 34.8, 31.4; IR ν_{max} (film) 2950, 1730, 1430, 1290, 1105, 1029 cm^{-1}; HRMS calcd for $C_{13}H_{17}O_5$ (M+H)$^+$ 253.1076, found 253.1087.

1,7-Bis-carbomethoxytricyclo[3.3.1.0^{2,7}]nonan-4-ol (42). To a solution of 41 (27 mg, 0.10 mmol) in CH_3OH (1.5 mL) was added $NaBH_4$ (5 mg, 0.21 mmol) in one portion. After stirring 20 min at rt, the solution was poured into saturated aqueous NH_4Cl (1 mL) and extracted 3 x 3 mL with ether. The combined organic extracts were dried ($MgSO_4$), concentrated in vacuo and the residue chromatographed (3:2 ether:hexane) to furnish the desired hydroxydiester 42 (20 mg, 72%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 3.96 (m, H-4), 3.65, 3.66 (2 s, each 3 H, CO_2CH_3), 2.62 (m, 2 H), 2.44 (br. d, J=13.3 Hz), 2.14 (ddd, 1 H, J=3.3, 9.9, 15.8 Hz), 2.07-1.92 (m, 2 H), 1.86-1.73 (m, 2 H, including OH), 1.68 (dd, 1 H, J=4.3, 13.3 Hz), 1.61 (d, 1 H, J=9.3 Hz, H-7'), 1.48 (ddd, 1 H, J=3.2, 3.2, 15.8 Hz); ν_{max} ($CHCl_3$) 3600, 3470, 3020, 2950, 1725, 1435, 1290, 1215, 1130, 1095, 1040, 905 cm^{-1}; HRMS calcd for $C_{13}H_{18}O_5$ (M$^+$) 254.1154, found 254.1169.

Tricyclo[3.3.1.0^{2,7}]nonan-4-ol-1,7-dicarboxylic Acid (15). To a solution of hydroxydiester 42 (15 mg, 0.05 mmol) in 1:1 THF:H_2O (1 mL) at 0°C was added NaOH (89 µL of a 2.31 M aqueous solution, 0.21 mmol) and the resulting homogeneous solution stirred 4 h at rt. The bulk of THF was evaporated under reduced pressure and the residual aqueous solution washed 3 x 1 mL with ether. The aqueous phase was loaded onto an ion exchange column (Dowex 50X8-200 resin, 1 mL) and eluted with H_2O (20 mL) to afford, after lyophilization, tricyclic hydroxydiacid 15 (12 mg, 88%): mp 227°C; ^{1}H-NMR (300 MHz, CD_3OD) 3.95 (m, H-4), 2.60 (m, 2 H), 2.53 (d, 1 H, J=13.5 Hz), 2.16 (ddd, 1 H, J=3.4, 9.9, 15.6 Hz), 2.10-1.99 (m, 2 H), 1.88 (d, 1 H, J=12.1 Hz), 1.72 (dd, 1 H, J=3.9, 13.1 Hz), 1.64 (d, 1 H, J=9 Hz), 1.56 (m, 1 H); ν_{max} (KBr) 3410, 3200-2400 (br.), 1695, 1430, 1305, 1280, 1200, 1140, 1095, 1045, 975 cm^{-1}; HRMS calcd for $C_{11}H_{15}O_5$ (M+H)$^+$ 227.0919, found 227.0940.

Bicyclo[3.3.1]non-2-en-4-one-1-endo,7-dicarboxylic Acid (43). To a solution of ketodiester 41 (169 mg, 0.67 mmol) in CH_3OH (6 mL) at 0°C under Ar was added $NaOCH_3$ (670 µL of a 3.5 M CH_3OH solution, 2.35 mmol) and the pale yellow solution was stirred 19 h at rt. The solvent was evaporated under reduced pressure and the solid residue was taken up in H_2O (1.5 mL), then washed 3 x 2 mL with ether. The aqueous phase was loaded onto an ion-exchange column (Dowex 50X8-200 resin, 4 mL) and eluted with H_2O (80 mL) to afford, after lyophilization, endo-ketodiacid 43 (146 mg, 97%) as a waxy solid: ^{1}H-NMR (300 MHz, CD_3OD) 7.18 (dd, J=2.2, 10 Hz, H-2), 5.93 (dd, J=0.8, 10 Hz, H-3), 2.85 (t, J=6.8 Hz, H-7), 2.69-2.58 (m, H-5, H-8), 2.53 (d, J=14.2 Hz, H-6), 2.40 (d, J=13.2 Hz, H-9), 2.18 (dd, J=6.7, 14 Hz, H-8'), 2.14 (ddd, J=2.4, 2.4, 13.2 Hz, H-9'), 1.92 (ddd, J=5, 7.1, 14.2 Hz, H-6'), ^{13}C-NMR (22.5 MHz, dioxane) 200.5, 180, 179.3, 178.4, 154.1, 131.3, 44.9, 41.7, 36.5, 35.9, 30.4, 26.8; IR ν_{max} (KBr) 3300-2600 (br.), 1715, 1682, 1450, 1290, 1190, 1070, 1060, 880 cm^{-1}; HRMS calcd for $C_{11}H_{13}O_5$ (M+H)$^+$ 225.0763, found 225.0811.

Dimethyl Bicyclo[3.3.1]non-2-en-4-one-1-endo,7-dicarboxylate (44). Ethereal diazomethane was added carefully dropwise to a solution of ketodiacid 43 (120 mg, 0.54 mmol) in ether (6 mL) at -78°C until starting material was no longer detectable by tlc. Argon was bubbled through the reaction mixture for 10 min to remove any excess CH_2N_2, then the solvent was removed in vacuo and the residue chromatographed (4:1 ether:hexane) to afford enone diester 44 (118 mg, 87%) as a colorless oil: ^{1}H-NMR (300 MHz, CD_3OD) 6.99 (dd, J=2.1, 10.2 Hz, H-2), 5.91 (d, J=10.2 Hz, H-3), 3.75, 3.55 (2 s, each 3 H, CO_2CH_3), 2.77 (t, J=6.7 Hz, H-7), 2.69-2.54 (m, 3 H), 2.36 (d, J=12.8 Hz, H-9), 2.08 (dd, J=6.5, 13.9 Hz, H-8'), 2.04 (ddd, J=2.4, 2.4, 12.8 Hz, H-9'), 1.80 (ddd, J=4.6, 6.9, 14 Hz, H-6'); ^{13}C-NMR (22.5 MHz, $CDCl_3$) 200.5, 174.3, 174.1, 149.8, 130.3, 52.6, 51.6, 44.1, 41.2, 36.3, 35.5, 30.8, 26.6; IR ν_{max} ($CHCl_3$) 3020, 2960, 1735, 1685, 1460, 1437, 1292, 1232, 1192, 1070, 1060, 817 cm^{-1}; HRMS calcd for $C_{13}H_{17}O_5$ (M+H)$^+$ 253.1076, found 253.1098.

Dimethyl 4-(Endo-hydroxy)bicyclo[3.3.1]non-2-en-1-endo,7-dicarboxylate (45). Sodium borohydride (25 mg, 0.66 mmol) was added to a solution of diester 44 (115 mg, 0.46 mmol) and $CeCl_3$ (137 mg, 0.55 mmol) in CH_3OH (3 mL) at 0°C and the reaction stirred 5 min, by which time starting material had been consumed. Hydrochloric acid (10%, 60µL) was added, the solvent evaporated under reduced pressure, the residue taken up in H_2O (1 mL) and extracted 3 x 2 mL with ether. The combined organic extracts were worked up in the usual way and chromatographed (3:2 ether:hexane) to afford allylic alcohol 45 (115 mg, 99%) as a low-melting solid: mp 0-10°C; ^{1}H-NMR (300 MHz, $CDCl_3$) 5.66 (br. d, J=10.3 Hz, H-2/3), 5.60 (br. d, J=10.3 Hz, H-3/2), 4.28 (dd, J=6.1, 12.3 Hz, H-4), 3.98 (d, J=12.3 Hz, OH), 3.64, 3.69 (2 s, each 3 H, CO_2CH_3), 2.69-2.57 (m, H-6, H-7), 2.45 (dd, J=1.8, 13.9 Hz, H-8), 2.29 (m, H-5), 2.04 (m, H-9), 1.98 (dd, J=7.5, 13.9 Hz, H-8'), 1.86 (ddd, J=1.8, 4.0, 12.8 Hz, H-9'), 1.62 (m, H-6'); ^{13}C-NMR (22.5 MHz, $CDCl_3$) 177.6, 175.8, 134.7, 128.1,

69.2, 52.0, 51.9, 42.3, 36.2, 34.8, 33.0, 32.6, 23.5; IR ν_{max} ($CHCl_3$) 3960, 3010, 2960, 1730, 1465, 1438, 1365, 1255, 1080, 1060, 930, 830 cm^{-1}; HRMS calcd for $C_{13}H_{18}O_5$ (M^+) 254.1154, found 254.1159.

Dimethyl 4-(Exo-benzoyloxy)bicyclo[3.3.1]non-2-en--1-endo,7-dicarboxylate (46). Diisopropyl azodicarboxylate (183 mg, 0.91 mmol) was added dropwise over 10 min to a solution of alcohol 45 (115 mg, 0.45 mmol), triphenylphosphine (239 mg, 0.91 mmol) and benzoic acid (115 mg, 0.95 mmol) in THF (5 mL) at 0°C under Ar. The pale yellow solution was stirred 15 min at 0°C then 18 h at rt. The solvent was removed in vacuo and the residue chromatographed (3:7 ether:hexane) to furnish allylic benzoate 46 (149 mg, 92%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 8.00 (m, 2 H), 7.50 (m, 1 H), 7.40 (m, 2 H), 6.09 (d, J=10 Hz, H-3), 5.85 (ddd, J=1.1, 4.5, 10 Hz, H-2), 5.39 (d, J=4.5 Hz, H-4), 3.72, 3.63 (2 s, each 3 H, CO_2CH_3), 2.70-2.50 (m, H-6, H-7, H-8), 2.34 (m, H-5), 2.18 (d, J=12.6 Hz, H-9), 1.91 (dd, J=6.3, 13.6 Hz, H-8'), 1.80-1.70 (m, H-9', H-6'); ^{13}C-NMR (22.5 MHz, $CDCl_3$) 175.6, 174.7, 165.7, 134.5, 132.8, 130.4, 129.6, 128.2, 126.4, 70.0, 52.0, 51.5, 43.0, 35.7, 32.5, 31.8, 30.2, 27.0; IR ν_{max} (film) 2960, 1730, 1605, 1455, 1440, 1270, 1110, 1040, 1028, 970, 855 cm^{-1}; HRMS calcd for $C_{20}H_{22}O_6$ $(M+H)^+$ 359.1495, found 359.1519.

Dimethyl 4-(Exo-hydroxy)bicyclo[3.3.1]non-2-en--1-endo,7-dicarboxylate (47). A solution of triester 46 (91 mg, 0.11 mmol) in CH_3OH (2 mL) in a Kimble vial was treated with $NaOCH_3$-CH_3OH (61 µL of a 2.43 M solution, 0.15 mmol). The vial was heated in a 70°C oil bath for 70 min, then cooled and poured into a mixture of ice-cold saturated aqueous $NaHCO_3$ (2.5 mL) and ether (15 mL). More H_2O (4 mL) was added to dissolve the white precipitate which formed, then the aqueous phase was extracted 4 x 15 mL with ether. The combined ether layers were worked up in the usual way and the crude product chromatographed (2:1 EtOAc:hexane) to afford 47 (31 mg, 85%) as a colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) 5.92 (d, J=10 Hz, H-2), 5.80 (dd, J=4.3, 10 Hz, H-3), 4.11 (t, J=5 Hz, H-4), 3.72, 3.60 (2 s, each 3 H, CO_2CH_3), 2.60 (t, J=6.6 Hz, H-7), 2.56-2.42 (m, H-6, H-8), 2.19 (s, 1 H, H-5), 2.09 (d, J=12.8 Hz, H-9), 1.91 (dd, J=6.5, 13.8 Hz, H-8'), 1.79-1.62 (m, H-6', H-9'); IR ν_{max} (film) 3480, 2950, 1730, 1435, 1150-1300, 1062, 1015, 940, 880, 760 cm^{-1}; CIMS 255 (M+1, 19%).

4-(Exo-hydroxy)bicyclo[3.3.1]non-2-en--1-endo,7-dicarboxylic Acid (10). To a solution of triester 46 (16 mg, 0.04 mmol) in CH_3OH (750 µL) was added NaOH (71 µL of a 2.44 M aqueous solution, 4 equiv) and the colorless solution stirred 8 h at rt. The solvent was removed in vacuo and the residual white solid taken up in H_2O (15 mL) and washed 4 x 10 mL with ether. The aqueous layer was then lyophilized and the white solid remaining (24 mg) was purified on an ion-exchange column (Dowex 50X8-200 resin, 4 mL) eluting with H_2O (10 mL). Lyophilization thus afforded diacid 10 as a white solid (7.7 mg, 78%). Further purification by reversed-phase HPLC (H_2O, 2 mL/min) gave a sample of 10 for bioassay: ^{1}H-NMR (300 MHz, CD_3OD) 6.00 (d, J=9.8 Hz, H-2), 5.75 (dd, J=4.1, 9.8 Hz, H-3), 4.12 (d, J=4.1 Hz, H-4), 2.60 (t, J=6.3 Hz, H-7), 2.50 (m, H-8), 2.45 (m, H-6), 2.14 (m, H-5, H-9), 1.94 (dd, J=6.4, 13.3 Hz, H-8'), 1.79 (m, H-6'), 1.70 (m, H-9'); IR ν_{max} (KBr, di-Na^+ salt) 3400, 2940, 1650, 1555, 1455, 1400, 1310, 1000 cm^{-1}; HRMS calcd for $C_{11}H_{15}O_5$ $(M+H)^+$ 227.0919, found 227.0934.

Dimethyl 4-(Exo-benzoyloxy)bicyclo[3.3.1]non-2-en--1-exo,7-dicarboxylate (48). A slurry of KH/mineral oil (20 mg, 6.8 mg pure KH, 0.17 mmol) in a 5 mL round-bottomed flask was washed with dry hexanes under Ar. The KH was then suspended in THF (1 mL) containing a trace of I_2 and the slightly yellow suspension was stirred 20 min at rt (note: color was discharged after 5 min). The flask was then cooled to -78°C and triester 46 (10 mg, 0.03 mmol) in THF (0.5 mL) was added over 4 min. The cloudy mixture was stirred 5 min at -78°C, 10 min at 0°C then at rt for 60 min. After recooling to -78°C, a solution of CF_3CO_2H in THF (120 mg/mL, 0.5 mL) was added rapidly by syringe and gas evolution was noted. Another 0.5 mL portion of acid was added and more gas was evolved. After warming to rt, a final portion of acid was added (0.5 mL) but no further gas was observed. The reaction mixture was poured into H_2O (5 mL) and extracted 4 x 5 mL with ether. The combined ether extracts were worked up in the usual way and the crude product chromatographed (1:3 EtOAc:hexane) to give the desired exo-triester 48 (9.2 mg, 89%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) 8.02 (m, 2 H), 7.55 (m, 1 H), 7.42 (m, 2 H), 6.22 (d, J=10 Hz, H-2), 6.13 (dd, J=4, 10 Hz, H-3), 5.20 (d, J=3.5 Hz, H-4), (3.75, 3.67 (2 s, each 3 H, C)$_2$$CH_3$, 2.60 (tt, J=4.4, 12.8 Hz, H-7), 2.41 (br. s, H-5), 2.15 (m, H-6, H-9), 1.99 (br. d, J=12.8 Hz, H-8), 1.82 (m, H-8', H-9'), 1.70 (dt, J=4.8, 4.8, 13.5 Hz, H-6'); IR ν_{max} (film) 2950, 1730, 1430, 1320, 1260, 1190, 1110, 1065, 1020, 950, 710 cm^{-1}; CIMS 359 (M+1, 3%).

Dimethyl 4-(Exo-hydroxy)bicyclo[3.3.1]non-2-en--1-exo,7-dicarboxylate (49). To a solution of exo-triester 48 (1 mg) in CH_3OH (200 µL) in a 5 mm NMR tube was added $NaOCH_3$-CH_3OH (50 µL of a 4 mg/mL solution, 1.5 equiv) and the tube was heated at 70°C for 24 h, by which time tlc indicated complete disappearance of 48. The reaction was cooled to rt and acidified (10% HCl, 2 drops), then poured into H_2O (1.5 mL) and extracted 4 x 2 mL with ether. The combined organic extracts were worked up in the usual way and the crude product

chromatographed (2:1 EtOAc:hexane) to furnish triester 49 (0.3 mg, 43%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) 6.07 (d, J=10.4 Hz, H-3), 6.03 (d, J=10.4 Hz, H-2), 3.87 (br. s, H-4), 3.73, 3.66 (2 s, each 3 H, CO_2CH_3), 2.51 (tt, J=4.7, 12.8 Hz, H-7), 2.26 (br. s, H-5), 2.04 (br. d, J=12 Hz, H-6), 1.91 (m, H-8, H-9), 1.79 (d, J=12.6 Hz, H-8'), 1.71 (br. d, J=11.5 Hz, H-6'), 1.63 (dd, J=4.3, 13.8 Hz, H-9'); ^{13}C-NMR ($CDCl_3$) 175.7, 175.0, 132.0, 131.0, 68.1, 52.3, 51.8, 43.7, 36.3, 35.5, 33.0, 31.2, 29.1; IR ν_{max} (film) 3450, 2955, 1735, 1440, 1260, 1070, 1020 cm^{-1}; HRMS calcd for $C_{13}H_{19}O_5$ $(M+H)^+$ 255.1233, found 255.1250.

4-(Exo-hydroxy)bicyclo[3.3.1]non-2-en--1-exo,7-dicarboxylic Acid (9). Exo-triester 48 (5.4 mg, 0.015 mmol) dissolved in CH_3OH (260 µL) was treated with NaOH (24 µL of a 2.44 M aqueous solution, 4 equiv) and the resulting colorless solution stirred 9 h at rt. After removing the solvent in vacuo, the residual white solid (11.5 mg) was taken up in H_2O and washed 3 x 2 mL with ether. The aqueous phase was then lyophilized and the residue dissolved in H_2O (1 mL) and acidified by passage through prewashed Dowex 50X8-200 resin (1.5 mL) eluting with H_2O (4 mL). Lyophilization afforded 3.6 mg of 9 contaminated with traces of benzoic acid. Final purification was achieved by reversed-phase HPLC: ^{1}H-NMR ($CDCl_3$) 6.03-5.87 (m, H-2, H-3), 3.84 (d, J=3.5 Hz, H-4), 2.95-2.77 (m, H-6, H-8, H-9), 2.45 (tt, J=4.3, 13.1 Hz, H-7), 2.12 (br. s, H-5), 1.75-1.58 (m, H-8', H-9'), 1.52 (dt, J=4.7, 13.5 Hz, H-6'); 1.91 (m, H-8, H-9), 1.79 (d, J=12.6 Hz, H-8'), 1.71 (br. d, J=11.5 Hz, H-6'), 1.63 (dd, J=4.3, 13.8 Hz, H-9'); IR ν_{max} (nujol) 3720-2320 (br), 1710 cm^{-1}; CIMS 227 (M+1, 10%).

Enzyme Assays. Chorismic acid was isolated from *K. pneumoniae* strain 62-1 according to Gibson.[29] Chorismate mutase-prephenate dehydrogenase was isolated and purified from *E. coli* strain JFM-30 with minor modifications of the procedure described by SampathKumar and Morrison.[25] One unit of activity corresponds to that amount of enzyme which catalyzes the conversion of 1 µmole of chorismate per minute. Thus 16 g of wet cells were French pressed and subjected to ammonium acetate precipitation, then DEAE Sephacel fractionation using a gradient of 0 to 0.3 M KCl in a stabilizing buffer containing 100 mM N-ethylmorpholine, 1 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM citrate and 0.1 mM phenylmethyl sulfonyl fluoride (PMSF). Combining fractions of highest activity (60 mL) resulted in a total activity of 960 units. The sample was dialyzed overnight against buffer (4 x 1 L) and applied to a column of Blue Sepharose (1.5 x 6.5 cm, 11 mL) and eluted at a flow rate of 18 mL/h using 20 mL buffer. The mutase was eluted with buffer containing 0.5 M KCl and 5 mM NAD^+ (20 mL total) and dialyzed against buffer containing 20 mM citrate and 20 mM DTT to remove KCl and NAD^+. The dialysate, which contained 34 mg of protein with a specific activity of 48 units/mg, was quick-frozen in liquid N_2 and stored in 0.5 mL fractions at -78°C.

Assays were performed at 30°C in a buffer consisting of 50 mM N-ethylmorpholine, 0.5 mM DTT, 0.5 mM EDTA, 1 mM sodium citrate, 10% v/v glycerol and 0.1 mg/mL bovine serum albumin. The buffer was adjusted to pH 7.5 with 2-(N-morpholino)ethanesulfonic acid. Assays (1 mL volume) were initiated by adding substrate to a solution of enzyme preincubated at 30°C and progress of the reaction was monitored by following the decrease in absorbance at λ=274 nm.

The I_{50} values for all inhibitors were obtained by plotting the reciprocal of reaction velocity against inhibitor concentration with chorismate concentration held at 89µM, the observed K_M at the time of assays. The data were fit by linear regression analysis and I_{50} values obtained as follows: 9: 0-200 µM, I_{50}=1.85±.15 mM; 10: 0-100 µM, I_{50}=0.43±.10 mM; 11: 0-500 µM, I_{50}=20±3 mM; 12: 0-500 µM, I_{50}=3.5±.2 mM; 13: 0-100 µM, I_{50}=3.5±.15 mM; 14: 0-100 µM, I_{50}=3.0±.3 mM; 15: 0-100 µM, I_{50}=0.5±.16 mM.

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