Synthesis of Disodium 3-[(1-Carboxylatoethenyl)oxy]cyclohepta-1,6-diene-1-carboxylate: A Seven-Membered Ring Analogue of Chorismate

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The synthesis of a seven-membered ring analogue of chorismate, disodium 3-[(1-carboxylatoethenyl)oxy]-cyclohepta-1,6-diene-1-carboxylate (**4c**), was accomplished in 3% overall yield from cycloheptanone. Compound **4c** was not processed by chorismate mutase, but it was a moderate inhibitor of the normal enzyme-catalyzed conversion of chorismate to prephenate. Prephenate analogue **5c** did not inhibit the prephenate dehydrogenase catalyzed decarboxylation of prephenate. Thermolysis of the dimethyl ester of **4c** led to a complex mixture of products via 1,5-hydrogen migrations, [3,3] Claisen rearrangement, and intramolecular Michael and Diels–Alder reactions. The products were separated and identified on the basis of spectral and analytical data.

The shikimate pathway is utilized by plants, bacteria, and fungi for the biosynthesis of aromatic substances.[1] Chorismate (**1**) resides at the branch-point of this pathway and is converted by separate enzymes to prephenate, anthranilate, *p*-aminobenzoate, isochorismate, and *p*-hydroxybenzoate (Scheme I). These intermediates then are converted to phenylalanine and tyrosine, tryptophan, folic acid, substituted benzoic acids, and the ubiquinones.

The Claisen rearrangement of chorismate to prephenate has attracted considerable attention.[2,3] The enzyme chorismate mutase (CM) accelerates the rate of [3,3] rearrangment by more than a million times in relation to the already facile thermal rearrangement, which has a half-life of ~16 h at 30 °C in pH 7.5 buffered water.[4] Experiments using specifically labeled chorismate definitively showed that both the enzymic[5] and thermal[6] reactions proceed through chair-like transition states. For the thermal reaction, secondary tritium isotope effects[7] and analogue studies[3,8] indicate an unsymmetrical transition state where C–O bond breakage precedes C–C bond formation. A number of proposals have been put forth to explain the observed enzymic rate enhancement,[2] and recent studies have focused on what role the C-4 hydroxyl moiety might play.[9]

Additional work has focused on the enzyme-catalyzed aminations of chorismate. Although no intermediates have been isolated, synthetic **2** and **3** have been shown to be viable substrates for the biosynthesis of anthranilate[10] and *p*-aminobenzoate[11] by the enzymes anthranilate synthase (AS) and *p*-aminobenzoate synthase (PABS). Recent studies by Walsh and Berchtold have begun to probe these apparently mechanistically similar but poorly understood enzymic reactions.[12]

In view of our interest in the design of substances that act as pseudosubstrates or inhibitors of the enzymes that process chorismate, we have synthesized a seven-membered ring analogue, disodium 3-[(1-carboxylatoethenyl)oxy]-cyclohepta-1,6-diene-1-carboxylate (**4c**, Scheme II). It was hoped that **4c** would be conformationally and electronically similar to chorismate and could be used to probe the mechanisms of the enzyme-catalyzed conversions of **1**. For example, if **4c** were turned over by chorismate mutase to **5c** (Scheme II), it would provide evidence that the C-4 hydroxyl group of chorismate is not essential for catalysis. As previously reported, analogue **4c** was found to be the most potent inhibitor tested for both anthranilate synthase and *p*-aminobenzoate synthase.[12] In the present paper, we describe the total synthesis of **4c**, the thermolysis of **4a–c**, and the enzymic studies of **4c** with chorismate mutase and **5c** with prephenate dehydrogenase.

The route used for the synthesis of the cycloheptadienyl analogue of chorismate is depicted in Scheme III. Cycloheptanone was converted to the cyanohydrin by reaction with excess HCN,[13] which eliminated water under the action of thionyl chloride in refluxing benzene[14] to give α,β-unsaturated nitrile **6** in 61% yield from cycloheptanone. Acid-catalyzed methanolysis of **6** followed by an aqueous workup gave methyl ester **7** in 88% yield after distillation.[13] Bis allylic bromination with *N*-bromosuccinimide in refluxing CCl_4 gave a mixture of epimeric dibromides, which were dehalogenated with sodium iodide in acetone to give diene **8** in 40% yield from **7** after distillation.

Selective epoxidation of **8** with MCPBA in CH_2Cl_2 proceeded smoothly to give sensitive epoxide **9**, which was opened with catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene

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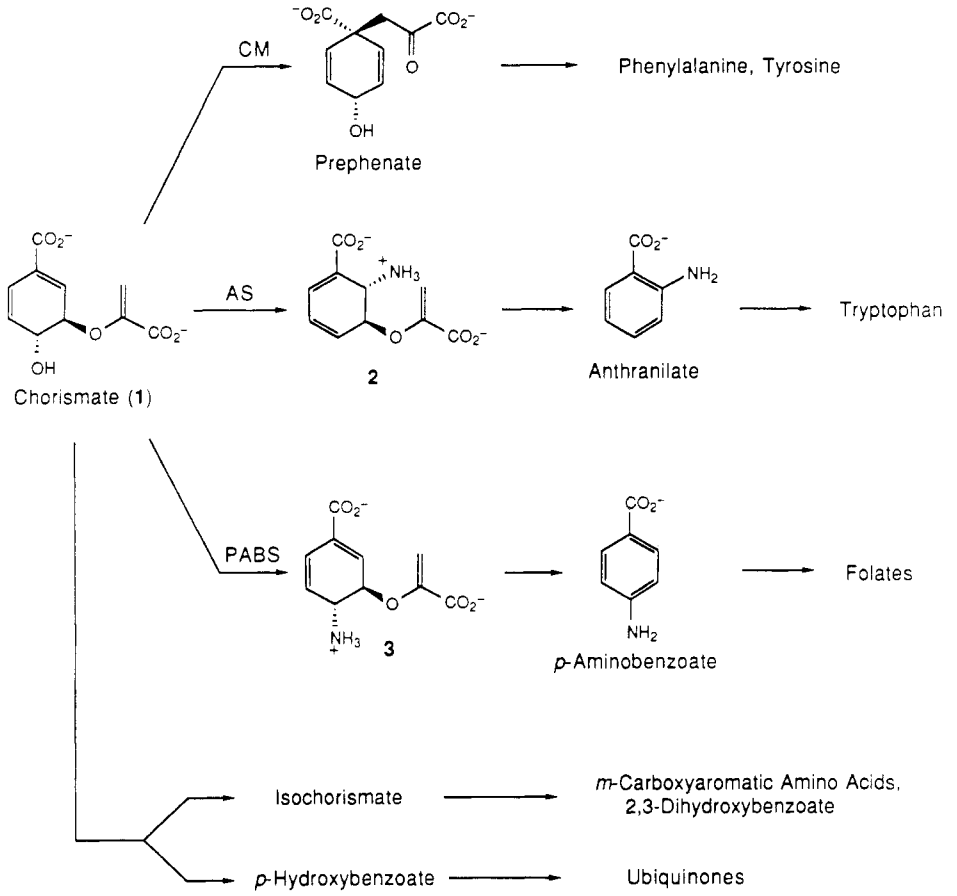
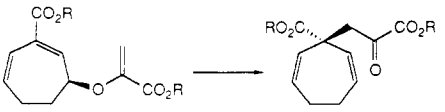
Scheme I



Scheme II



4a, R = CH_3
4b, R = H
4c, R = Na

5a, R = CH_3
5b, R = H
5c, R = Na

(DBU) in THF to give dienol **10**. Coupling of **10** and dimethyl diazomalonate with rhodium acetate catalysis[15] in benzene at 65 °C gave malonate **11** in 28% yield from **8** after flash chromatography. Malonate **11** was somewhat unstable; therefore, a crystalline adduct was made by reaction of **11** with 4-methyl-1,2,4-triazoline-3,5-dione in CH_2Cl_2 to give **12**, which was then fully characterized. Reaction of **11** with Eschenmoser's salt and triethylamine in CH_2Cl_2 gave Mannich base **13**, which was quaternized with excess methyl iodide in CH_2Cl_2 to give **14**. Fragmentation of salt **14** was initiated with NaOH in THF/H_2O to give enolpyruvate **4a** in 74% yield from **11**. Saponification of **4a** with NaOH in THF/H_2O, followed by acidification wth Amberlite IR-120 (plus) resin, gave diacid **4b** in 87% yield. Treatment of diacid **4b** with cation exchange resin gave disodium salt **4c** in 71% yield.

Dimethyl ester **4a** was quite stable and showed no tendency to rearrange in $CDCl_3$. This was in stark contrast to the behavior of dimethyl 4-*deshydroxy*chorismate (Scheme IV), which had a half-life for disappearance of starting material of 3.6 h at 30 °C in $CDCl_3$.[9] Dimethyl chorismate was stable under these conditions; however, complete rearrangement of dimethyl chorismate was observed after heating for 36 h at 55 °C in DMSO-d_6.[15b] In comparison, the half-life for disappearance of **4a** in DMSO-d_6 at 80 °C was found to be ~12.4 h.

Perhaps most interesting was that thermolysis of **4a** led to a complex mixture of products (Scheme V). Thus, diester **4a** was heated at 80 °C in DMSO-d_6 while the course of the reaction was monitored by ^1H NMR spectroscopy. After a total of 67 h, the mixture was concentrated and purified by MPLC on silica gel. The ^1H NMR spectrum of the least polar compound (~10% of the mixture) showed one less olefinic proton than **4a** and no carbinol proton. In addition, coupled protons at 5.77 and 5.17 ppm (J = 1.9 Hz) were indicative of the presence of an enolpyruvate moiety. This suggested isomerization via a 1,5-hydrogen migration to **15a**. This structure was confirmed by reaction with 4-methyl-1,2,4-triazoline-3,5-dione to give crystalline adduct **19**. The spectral and analytical data for **19** were fully consistent with the proposed structure.

The next compound to elute from the column was prephenate analogue **5a** (~12% of the mixture), the expected [3,3] rearrangement product. The ^1H NMR spectrum of this compound had olefinic resonances at δ 5.93 (2 H, dm, J = 11 Hz) and 5.59 (2 H, d, J = 11 Hz), which were characteristic of a prephenate-type structure. The rest of the spectral and analytical data confirmed the structure of **5a**.

The third compound obtained (~4% of the mixture) was assigned structure **16a**. Although **16a** had the same number of protons as starting material, there were only two remaining olefinic resonances. The proposed structure,

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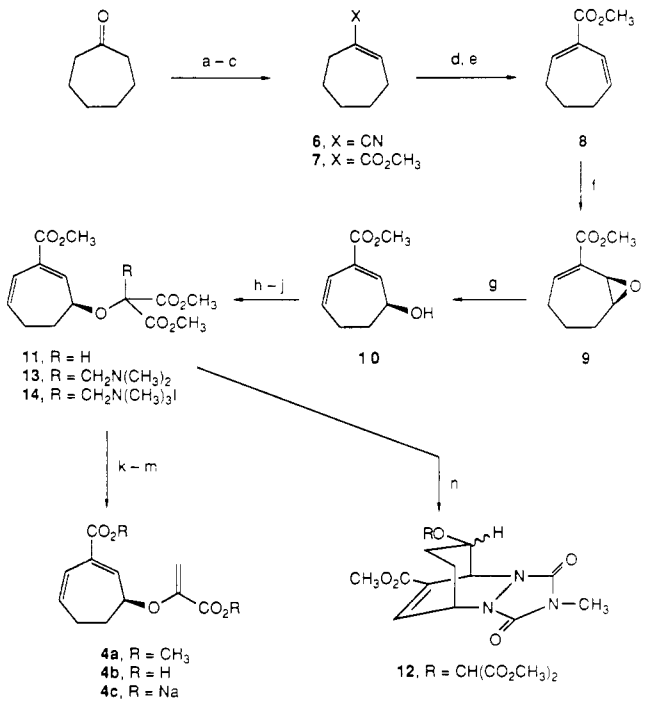
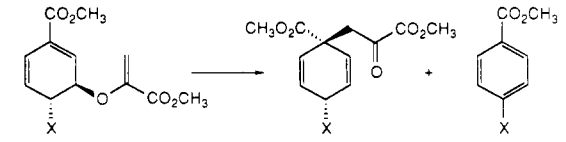
Scheme III[a]



[a] Reagents: (a) NaCN, $NaHSO_3$, H_2O; (b) $SOCl_2$, benzene, Δ; (c) CH_3OH, H_2SO_4, Δ; (d) NBS, CCl_4, AIBN, Δ; (e) NaI, acetone; (f) MCPBA, K_2CO_3, CH_2Cl_2; (g) DBU, THF; (h) $CH_3O_2CC(N_2)CO_2CH_3$, rhodium acetate, benzene, Δ; (i) $CH_2N(CH_3)_2I$, Et_3N, CH_2Cl_2; (j) CH_3I, CH_2Cl_2; (k) NaOH, THF/H_2O; (l) NaOH, THF/H_2O; (m) Na^+ exchange resin; (n) 4-methyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2.

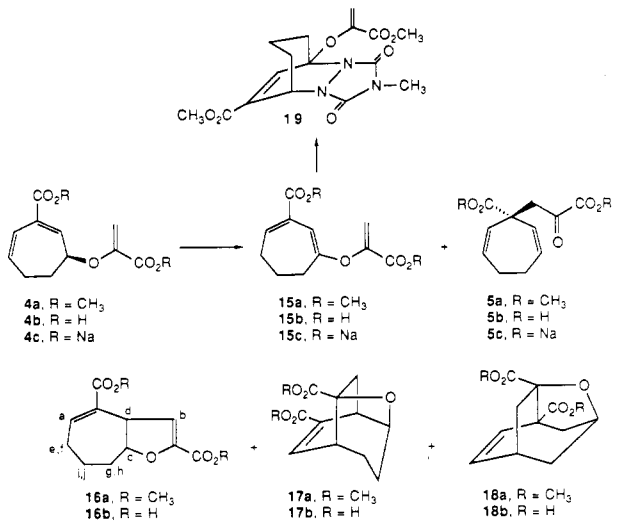
Scheme IV



Dimethyl 4-*deshydroxy*-Chorismate, X = H

Dimethyl Chorismate, X = OH

Scheme V



the result of an intramolecular Michael reaction, accommodates this requirement and was corroborated by complete ^1H–^1H decoupling experiments (see Experimental Section). The observed coupling patterns and chemical shifts were consistent with structure **16a**.

Chart I

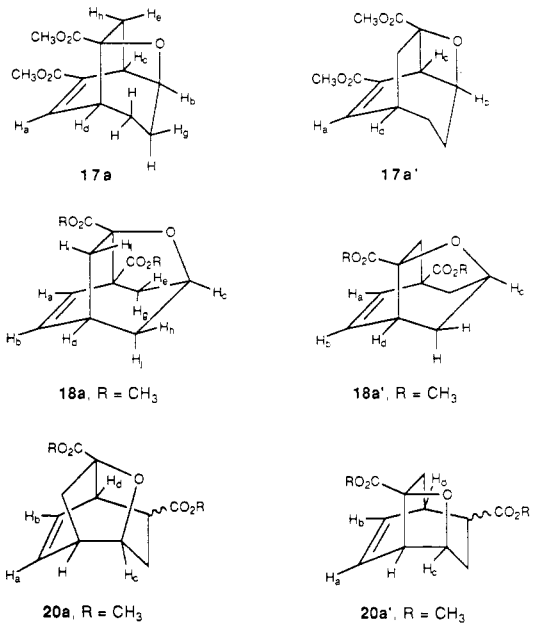


Table I. ^1H NMR Data for Compound 17a

proton (δ)[a]	coupling partner (J)[b,c]	mult
a (6.81)	c (1.8), d (6.1)	dd
b (4.48)	c (6.8), g (6.8)	t
c (3.59)	a (1.8), b (6.8), e (5.9)	ddd
d (3.19)	f, d or i	m
e (2.11)	c (5.9), h (9.8)	dd
f, g (2.07–1.91)	d	m
h (1.68)	e (9.8)	d
i, j (1.47–1.26)	d	m

[a] Chemical shifts are in ppm downfield of tetramethylsilane. [b] Coupling constants are in hertz. [c] This includes only unambiguously coupled protons.

The final two compounds were also structural isomers of **4a** that had a reduced number of olefinic protons. It was clear from examination of models that compound **4a** could adopt the conformations necessary for intramolecular [4 + 2] reactions,[16] which would be consistent with the observed loss of olefinic protons. The Diels–Alder products possible from intramolecular rearrangement of **4a** are depicted in Chart I. Examples of cycloheptadienes that undergo intramolecular Diels–Alder reactions to give carbocyclic analogues of structures **17a**, **17a'**, **20a**, **20a'**, and **18a**, **18a'** are known.[17,18]

The presence of only one olefinic proton in the second most polar compound (~42% of the mixture) suggested that it was the result of a direct Diels–Alder reaction of **4a**, which could lead to either anti-bridged **17a** or anti-fused **17a'**. FMO theory would predict the formation of **17a**.[19] Additionally, the presence of a four-membered ring in **17a'** would make formation of this product more difficult. Complete ^1H–^1H decoupling experiments unambiguously demonstrated that the correct structure was **17a**

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Table II. NMR Data for Compound 18a

proton (δ, mult)[a]	coupling partner (J)[b,c]	correlated ^{13}C, δ (mult, J)
a (6.76, d)	b (9.3)	134.5 (d, 176)
b (6.51, dd)	a (9.3), d (7.6)	137.0 (d, 168)
c (4.75, t)	e (5.4), h (5.4), j	79.3 (d, 156)
d (2.64, m)	b (7.6), f (3.6), h (5.4), i	29.3 (d, 147)
e (2.55, dd)	c (5.4), g (11)	47.9 (t, 144)
f (2.23, dd)	d (3.6), i (12)	41.1 (t, 138)
g (2.06, d)	e (11)	47.9 (t, 144)
h (1.98, dt)	c (5.4), d (5.4), j (14)	29.7 (t, 132)
i (1.80, dm)	d, f (12), j	41.1 (t, 138)
j (1.54, dm)	c, h (14), i	29.7 (t, 132)

[a] Chemical shifts are in ppm downfield of tetramethylsilane. [b] Coupling constants are in hertz. [c] This includes only unambiguously coupled protons.

(Chart I, Table I). The two structures can be differentiated by looking at the coupling pattern of proton H_c. In structure **17a**, H_c could show coupling to as many as four other protons, while **17a'** should exhibit appreciable coupling constants with only two other protons. Thus, since H_c shows appreciable coupling to three other protons, structure **17a'** is effectively ruled out. In structure **17a**, protons H_c and H_h are nearly orthogonal, which explains why H_c shows coupling to only three partners.

The most polar compound (~32% of the mixture) exhibited two olefinic protons; therefore, Diels–Alder adducts **18a**, **18a'**, **20a**, and **20a'** were possible structures. Structures **18a** and **18a'** would be the result of cyclization of the 1,3-diene (from **4a** via a 1,5-hydrogen shift), while **20a** and **20a'** would come from the 2,4-diene (from **4a** after two consecutive hydrogen migrations). Of these four possibilities, FMO considerations would favor the formation of isomer **18a**.[19] In fact, ^1H–^1H decoupling experiments, in combination with various 2D NMR techniques, unambiguously demonstrated that the correct structure was **18a** and also led to the complete assignment of all protons (Chart I, Table II). The fact that H_a appears as a sharp doublet militates against structures **20a** and **20a'**; models of these structures clearly indicate that both H_a and H_b should show similar coupling patterns, and thus both should appear as a doublet of doublets. In addition, various ^{13}C NMR techniques showed the presence of three methylene carbons, while only two are present in **20a** and **20a'**. The position of H_d is firmly established by its coupling to H_b. Proton H_d is a broad multiplet that is coupled to at least four other protons: H_b, H_f, H_h and H_i. Structure **18a'** is eliminated since it cannot accommodate this requirement.

The thermal interconversion of cycloheptadienyl species via 1,5-hydrogen migrations is well documented.[16a] The species that undergoes cyclization usually has an interconnecting chain length of three atoms. In the more general case of an intramolecular Diels–Alder reaction with an acyclic diene, the vast majority involve substrates with connecting chains of three or four atoms that usually cyclize to a fused regiochemistry, regardless of the presence of strong directing groups. The formation of adducts **17a** and **18a** are both examples of regiospecific (with respect to the reactive dienyl species) intramolecular Diels–Alder reactions with inverse electron demand. The regiochemical outcome found for these relatively facile reactions are in accord with FMO predictions.[19] Conversion of **4a** to **17a** is somewhat unusual since it involves reaction of the 1,6-diene with a connecting chain of only two atoms. In addition, the product of cyclization is the less common anti-bridged isomer. The preference for reaction of **4a** to give more **17a** than **18a** probably indicates that cyclization is somewhat faster than 1,5-hydrogen migration. The lack of any detectable amounts of adducts **20a** and **20a'** is consistent with this observation and is in keeping with the expectation that the nonconjugated 2,4-diene would be less reactive.

Thermolysis of **4b** in DMSO-d_6 at 80 °C led to a similarly complex mixture of products (Scheme V). It was possible to estimate the half-life for disappearance of **4b** to be between 11 and 16 h under these conditions. Even though separation of the product mixture proved to be impossible, ^1H NMR analysis of the crude mixture indicated that the products formed were analogous to those formed from the diester. Attempted rearrangement of **4b** at 50 °C in pH 7 phosphate-buffered D_2O led only to hydrolysis of the side chain to give a mixture of alcohol **10** and pyruvic acid. When disodium salt **4c** was thermolyzed at 70 °C in DMSO-d_6, the only product detected by ^1H NMR was double-bond isomer **15c**.

Dimethyl ester **5a** was converted into the disodium salt by saponification with 1.0 M NaOH in THF/H_2O (Scheme V). After acidification to pH 6.8 with Amberlite IR-120 (plus) resin, compound **5c** was obtained in quantitative yield.

Prephenate analogue **5c** was tested as an inhibitor of the dehydrogenase activity of chorismate mutase–prephenate dehydrogenase (CM–PD) from *E. coli*[20] by modification of the procedure of Heyde and Morrison.[21] It showed no inhibition at a concentration of 0.2 mM. Prephenate was found to have a V_{max} = 3.8 μmol min^{-1} mg^{-1} (enzyme) and K_m = 0.099 mM ([NAD] = 0.1 mM) under the conditions of the assay.

Chorismate analogue **4c** was not metabolized by the mutase portion of CM-PD;[20] however, it was shown to be a moderate competitive inhibitor with a K_i = 0.43 mM ([**4c**] = 0.31 mM).[21] Chorismate had a K_m = 0.14 mM under the conditions of the assay. Additionally, **4c** was not metabolized by the enzymes anthranilate synthase (AS) and *p*-aminobenzoate synthase (PABS).[12] However, it was shown to be the best inhibitor tested so far with these enzymes. It was a competitive inhibitor of AS with a K_i = 30 μM, and it displayed an approximate K_i = 230 μM for PABS. Chorismate had a K_m = 5.6 μM for AS and K_m = 42 μM for PABS.

In summary, the synthesis of chorismate analogue **4c** was achieved in 3% overall yield from cycloheptanone. Thermolysis of dimethyl chorismate analogue **4a** led to a complex mixture of products, which were identified on the basis of spectral and analytical data. Prephenate analogue **5c** did not inhibit the dehydrogenase activity of CM–PD. Finally, chorismate analogue **4c** was not a pseudosubstrate for CM–PD, AS, or PABS; however, it was a good inhibitor of the reactions catalyzed by these enzymes.

Experimental Section[22]

Cyclohept-1-enecarbonitrile (6). A solution of NaCN (43.8 g, 0.893 mol, 2.0 equiv) in H_2O (180 mL) was added to cycloheptanone (50.0 g, 0.446 mol), and the resulting two-phase mixture was cooled to ~0 °C (ice/H_2O). A solution of $NaHSO_3$ (92.8 g, 0.892 mol, 2.0 equiv) in H_2O (250 mL) was added via dropping funnel over 45 min. After 8 h, the mixture was gravity filtered, the precipitate was rinsed with Et_2O (50 mL), and the combined filtrates were extracted with Et_2O (3 × 100 mL). The organic

(20) CM–PD from *E. coli* JFM30 was obtained from Professor J. F. Morrison: (a) SampathKumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 204–211. (b) Bhosale, S. B.; Rood, J. I.; Sneddon, M. K.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *717*, 6–11.

(21) Heyde, E.; Morrison, J. F. *Biochemistry* **1978**, *17*, 1573–1580.

(22) ^1H NMR spectra were obtained at 250, 270, or 300 MHz. ^{13}C NMR spectra were obtained at 67.9 or 75.4 MHz. Flash chromatography refers to the procedure developed by Still and co-workers: Still, W. C.; Kahn, M.; Mitra, A. *J. Orga. Chem.* **1978**, *43*, 2923–2925.

extracts were dried over $MgSO_4$, filtered, and concentrated to a liquid. The majority of unreacted cycloheptanone was removed by short-path distillation (30–35 °C, 0.3 Torr) to give 58.0 g of the cyanohydrin of cycloheptanone, which ^1H NMR analysis indicated was ~70% pure.[13] The crude material was used without further purification in the next experiment: ^1H NMR ($CDCl_3$, 60 MHz) δ 3.80 (1 H, s), 2.00 (4 H, m), 1.60 (8 H, br s).

The crude cyanohydrin (58.0 g, ~0.29 mol) was dissolved in benzene (100 mL) and cooled to ~0 °C (ice/H_2O). Thionyl chloride (76 mL, 1.04 mol, 3.6 equiv) was added dropwise to the stirred solution over 45 min. The cooling bath was removed, and the mixture was heated to a gentle reflux for 13 h. The solution was cooled to room temperature, poured into ice/H_2O (~300 mL), and stirred vigorously to destroy excess thionyl chloride. The layers were separated, and the aqueous portion was extracted with benzene (2 × 50 mL). The combined organic portions were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated to give 56.0 g of crude **6**. The dark liquid was short-path-distilled under reduced pressure to give 33.0 g (61% from cycloheptanone) of **6** as a clear, nearly colorless liquid:[14] bp 47–51 °C (0.25 Torr); ^1H NMR ($CDCl_3$) δ 6.78 (1 H, t, J = 7.5 Hz), 2.40 (2 H, m), 2.30 (2 H, dd, J = 11, 7.5 Hz), 1.77 (2 H, m), 1.61 (4 H, m).

Methyl Cyclohept-1-ene-1-carboxylate (7). To a stirred solution of nitrile **6** (96.6 g, 0.80 mol) in CH_3OH (800 mL) at ~0 °C (ice/H_2O) was added concentrated H_2SO_4 (160 mL), dropwise. The cooling bath was removed, and the mixture was refluxed for ~3 days; CH_3OH lost through the reflux condenser was replaced as necessary. When the ^1H NMR spectrum of an aliquot showed complete reaction, the mixture was cooled, poured into ice/H_2O (~1000 mL), and stirred vigorously until all the ice had melted. The solution was extracted with CH_2Cl_2 (300 mL, 3 × 150 mL) and the combined extracts were washed with 5% $NaHCO_3$ (200 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 115.5 g of crude **7**. Short-path distillation under reduced pressure gave 108.0 g (88%) of **7** as a colorless liquid:[14] bp 42–46 °C (0.15 Torr); ^1H NMR ($CDCl_3$) δ 7.18 (1 H, t, J = 7.5 Hz), 3.72 (3 H, s), 2.53 (2 H, m), 2.30 (2 H, dd, J = 11, 7.5 Hz), 1.78 (2 H, m), 1.54 (4 H, m).

Methyl Cyclohepta-1,6-diene-1-carboxylate (8). To a solution of **7** (25.0 g, 0.162 mol) in carbon tetrachloride (400 mL) were added N-bromosuccinimide (61.0 g, 0.343 mol, 2.1 equiv) and a catalytic amount of α,α′-azobis(isobutyronitrile) (AIBN, 0.107 g, 0.653 mmol, 0.4 mol %). The system was flushed with N_2 and brought to a gentle reflux. The mixture was cooled after 2.5 h, and the floating solid was removed by suction filtration. The filtrate was washed with 5% Na_2SO_3 (100 mL) and H_2O (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 49.8 g of the dibromide as a yellow oil.

To the dibromide (49.8 g, 0.16 mol) in acetone (400 mL) was added all at once a solution of NaI (59.8 g, 0.40 mol, 2.5 equiv) in acetone (600 mL). The initially light yellow solution became dark, and a copious amount of precipitate formed. The mixture was stirred for 2 h and suction filtered to remove the solid, and the filtrate was concentrated. The dark residue was dissolved in Et_2O (100 mL) and suction filtered, and the filter cake was rinsed with additional Et_2O (2 × 100 mL). The combined filtrates were cooled to ~0 °C (ice/H_2O) and washed with 5% Na_2SO_3 (300 mL, 100 mL). The aqueous layers were combined and extracted with Et_2O (100 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to give 28.2 g of crude **8**. The colored liquid was decolorized with n-Bu_3SnH (0.6 mL)[23] and short-path-distilled under vacuum to give 9.91 g (40% from **7**) of diene **8**[24] as a colorless liquid: bp 30–35 °C (0.35 Torr); ^1H NMR ($CDCl_3$) δ 7.16 (1 H, t, J = 5.9 Hz), 6.36 (1 H, dm, J = 12 Hz), 5.99 (1 H, dt, J = 12, 5.2 Hz), 3.76 (3 H, s), 2.45 (2 H, q, J = 5.9 Hz), 2.35 (2 H, q, J = 5.9 Hz), 1.88 (2 H, quintet, J = 5.9 Hz).

Methyl 1,7-Epoxybicyclo[5.1.0]cyclohept-2-ene-2-carboxylate (9). To **8** (14.93 g, 98.1 mmol) in CH_2Cl_2 (25 mL) under N_2 at ~0 °C (ice/H_2O) was added K_2CO_3 (17.61 g, 127.4 mmol, 1.3 equiv). As a solution of MCPBA (27.50 g, 127.5 mmol, 1.3 equiv) in CH_2Cl_2 (500 mL) was added via addition funnel, a copious precipitate formed. The mixture was warmed to room temperature and stirred overnight. The mixture was suction filtered after 25 h, and the filtrate was washed with 5% Na_2SO_3 (100 mL) and 5% $NaHCO_3$ (2 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 15.67 g of crude epoxide **9** (~95%) that was used directly in the next reaction. In a separate experiment, pure **9** (liquid) was obtained by double elution chromatography on a silica gel plate [acetone/petroleum ether (1:5), 0.1 × 20 × 20 cm plate]: IR (neat) 2960, 1710, 1440, 1260, 1230, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.26 (1 H, dd, J = 7.0, 3.9 Hz), 3.81 (1 H, m), 3.80 (3 H, s), 3.46 (1 H, q, J = 4.1 Hz), 2.46 (1 H, m), 2.27 (1 H, m), 2.10 (2 H, m), 1.66 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 167.8 (s), 147.8 (d), 128.5 (s), 58.7 (d), 52.6 (d), 52.1 (q), 30.3 (t), 29.2 (t), 21.3 (t); mass spectrum, m/e (rel intensity) 168 (M^+, 1.6), 152 (6.3), 140 (18.5), 139 (57.7), 137 (26.1), 136 (35.8), 135 (12.3), 109 (68.1), 108 (77.3), 107 (41.3), 95 (25.9), 93 (20.5), 91 (33.8), 81 (62.3), 80 (47.0), 79 (100), 77 (45.7); high-resolution mass spectrum, calcd for $C_8H_9O_2$ ($M^+ - CH_3O$) 137.0603, found 137.0604.

Methyl 3-Hydroxycyclohepta-1,6-diene-1-carboxylate (10). To a solution of crude epoxide **9** (15.67 g, ~93 mmol) in dry THF (250 mL, from Na) under N_2 was added 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 1.40 mL, 9.4 mmol, 0.1 equiv) via syringe. The nearly colorless solution became brown as the solution was stirred at room temperature. After 20 h, TLC analysis indicated remaining **9**, so additional DBU (0.2 mL, 1.3 mmol, 0.01 equiv) was added. The mixture was concentrated after a total of 23 h, and the residue was taken up in Et_2O (300 mL) and filtered. The filtrate was washed with 2% HCl (3 × 100 mL) and 5% $NaHCO_3$ (2 × 100 mL), dried over $MgSO_4$, filterd, and concentrated to give 10.76 g (~65%)[25] of crude alcohol **10**. This material was carried on without any additional purification. An analytical sample of **10** (liquid) was prepared by chromatography on silica gel [Et_2O/CH_2Cl_2 (3:1), 0.1 × 20 × 20 cm plate]: IR (neat) 3600–3200, 3040, 2960, 1720, 1440, 1265, 1220, 1060, 1025, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.10 (1 H, d, J = 3.4 Hz), 6.35 (1 H, d, J = 12 Hz), 6.06 (1 H, dt, J = 12, 5.2 Hz), 4.51 (1 H, br s), 3.77 (3 H, s), 2.36 (2 H, m), 2.08 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 167.7 (s), 145.4 (d), 135.7 (d), 126.9 (s), 122.5 (d), 70.2 (d), 52.1 (q), 35.5 (t), 26.3 (t); mass spectrum, m/e (rel intensity) 168 (M^+, 6.8), 166 (3.5), 150 (1.2), 137 (19.3), 136 (59.3), 108 (38.3), 107 (28.9), 91 (27.4), 81 (29.6), 80 (34.1), 79 (100), 77 (55.6); high-resolution mass spectrum, calcd for $C_9H_{12}O_3$ 168.0787, found 168.0781.

Methyl 3-[Bis(methoxycarbonyl)methoxy]cyclohepta-1,6-diene-1-carboxylate (11). A solution of dimethyl diazomalonate (5.309 g, 33.6 mmol, 1.1 equiv)[26] in benzene (50 mL) was added to a mixture of alcohol **10** (5.129 g, ~30.5 mmol)[27] and $Rh_2(OAc)_4$ (0.238 g, 0.538 mmol, 2 mol %) in benzene (50 mL) under a N_2 atmosphere. Additional benzene (20 mL) was used to complete the transfer. The green solution was heated at 65 °C until N_2 evolution had ceased (2 h). The mixture was cooled to room temperature and suction filtered. The filtrate was washed with 5% $NaHCO_3$ (50 mL), saturated aqueous NaCl (50 mL), and H_2O (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 9.20 g of a brown oil. The oil was purified by flash chromatography on silica gel [ethyl acetate/petroleum ether (1:2), 7 × 15 cm column] to give 3.90 g (28% from **8**) of pure **11**: IR (neat) 3040, 3010, 2960, 1750, 1720, 1440, 1250, 1125, 1025 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.15 (1 H, d, J = 3.7 Hz), 6.34 (1 H, d, J = 12 Hz), 6.07 (1 H, dt, J = 12, 5.1 Hz), 4.68 (1 H, s), 4.27 (1 H, dt, J = 8.1, 3.7 Hz), 3.83 (3 H, s), 3.82 (3 H, s), 3.77 (3 H, s), 2.35 (2 H, m), 2.19 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 167.0 (s), 166.9 (s), 166.8 (s), 142.1 (d), 135.8 (d), 128.3 (s), 122.9 (d), 78.7 (d), 77.3 (d), 52.9 (q), 52.1 (q), 33.4 (t), 26.5 (t); mass spectrum, m/e (rel intensity) 298 (M^+, 0.7), 266 (4.6), 250 (5.6), 229 (9.0), 218 (4.4), 167 (21.6), 151 (13.3), 150 (14.0), 149 (10.7), 135 (41.8), 132 (22.0), 119 (18.0), 107 (18.0), 91 (100). This compound was characterized further as the methyltriazolinedione adduct.

(23) This was added to reduce halogen-containing species that interfered with the distillation.

(24) (a) Pikulik, I.; Childs, R. F. *Can. J. Chem.* **1977**, *55*, 251–258. (b) Paquette, L. A.; Zon, G. *J. Am. Chem. Soc.* **1974**, *96*, 224–233.

(25) This yield was calculated from **8**, with the assumption that **10** is pure.

(26) Anod, W.; Yagihara, T.; Tozune, S.; Imai, I.; Suzuki, J.; Toyama, T.; Nakaido, S.; Migita, T. *J. Org. Chem.* **1972**, *37*, 1721–1727.

(27) The calculation of the number of millimoles is uncorrected for any impurities that were present with **10**.

To a solution of diene **11** (25 mg, 0.084 mmol) in CH_2Cl_2 (1 mL) under N_2 at ~0 °C was added 4-methyl-1,2,4-triazoline-3,5-dione (12 mg, 0.106 mmol, 1.3 equiv) until a faint pink color persisted. The mixture was stirred for 2 h and concentrated to give 35 mg (100%) of **12**. Preparative TLC on silica gel [acetone/petroleum ether (1:1), 0.025 × 20 × 20 cm plate] gave 13 mg (38%) of adduct **12** as a white solid: mp 130–135 °C; IR (neat) 2960, 1760, 1715, 1450, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (1 H, dd, J = 6.9, 1.1 Hz), 5.58 (1 H, dd, J = 3.9, 1.1 Hz), 5.04 (1 H, dt, J = 6.9, 3.9 Hz), 4.82 (1 H, s), 3.91 (1 H, m), 3.83 (3 H, s), 3.82 (3 H, s), 3.77 (3 H, s), 3.06 (3 H, s), 2.21 (1 H, m), 2.05 (1 H, m), 1.95–1.70 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 167.0 (s), 165.8 (s), 163.5 (s), 153.6 (s), 153.3 (s), 137.3 (d), 130.9 (s), 77.2 (d), 52.9 (q), 52.4 (q), 52.2 (d), 50.0 (d), 27.4 (t), 25.7 (q), 24.5 (t); mass spectrum, m/e (rel intensity) 411 (M^+, 7.0), 380 (2.3), 292 (4.3), 264 (32.2), 263 (100), 224 (36.2), 206 (12.0), 167 (9.1), 149 (11.9); high-resolution mass spectrum, calcd for $C_{17}H_{21}N_3O_9$ 411.1278, found 411.1254.

Methyl 3-[[1-(Methoxycarbonyl)ethenyl]oxy]cyclohepta-1,6-diene-1-carboxylate (4a). Eschenmoser's salt (2.606 g, 14.08 mmol, 1.3 equiv) was added to a solution of malonate **11** (3.199 g, 10.72 mmol) in CH_2Cl_2 (70 mL) under a N_2 atmosphere. Et_3N (1.60 mL, 11.5 mmol, 1.1 equiv) was added to the two-phase mixture to give a clear solution that was stirred overnight. After 14.5 h, the reaction mixture was washed with H_2O (2 × 50 mL) and saturated aqueous NaCl (35 mL). The organic portion was dried over $MgSO_4$, filtered, and concentrated to give 3.590 g (~94%) of crude **13**: IR (neat) 2960, 1745, 1720, 1435, 1255, 1100, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.10 (1 H, d, J = 3.7 Hz), 6.32 (1 H, d, J = 12 Hz), 6.02 (1 H, dt, J = 12, 5.6 Hz), 4.58 (1 H, m), 3.78, 3.75 (9 H, s), 2.94 (2 H, AB q), 2.31 (6 H, s), 2.40–2.10 (4 H, m).

To Mannich base **13** (3.590 g, ~10.1 mmol) in CH_2Cl_2 (65 mL) under N_2 was added CH_3I (3.20 mL, 51.4 mmol, 5 equiv) via syringe. The cloudy solution was stirred for 18 h, and the solvent was removed in vacuo. The solid residue was taken up in CH_2Cl_2 (500 mL) and washed with H_2O (50 mL). The aqueous layer was extracted with CH_2Cl_2 (50 mL), and the combined organic portions were dried over $MgSO_4$, filtered, and concentrated to give 4.35 g (~82% from **11**) of **14** as a yellow solid: IR ($CHCl_3$) 3020, 2960, 1745, 1715, 1440, 1250, 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.97 (1 H, d, J = 3.7 Hz), 6.33 (1 H, d, J = 12 Hz), 6.08 (1 H, dt, J = 12, 5.6 Hz), 4.83 (1 H, m), 4.32 (2 H, AB q), 3.97 (3 H, s), 3.87 (3 H, s), 3.80 (3 H, s), 3.65 (9 H, s), 2.35–2.10 (4 H, m).

To a solution of **14** (4.35 g, ~8.7 mmol) in THF/H_2O (55:45, 100 mL) at ~0 °C (ice/H_2O) was added 1.0 M NaOH (13.2 mL, 13.2 mmol, 1.5 equiv) via syringe over several minutes. The mixture was stirred for 20 min, CH_2Cl_2 (500 mL) was added, and the aqueous portion was saturated with NaCl. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic portions were washed with H_2O (100 mL), dried over $MgSO_4$, filtered, and concentrated to give 2.46 g of crude **4a**. The ^1H NMR spectrum of the crude material showed a small amount of unreacted starting material that was removed by addition of ethyl acetate (150 mL) and petroleum ether (150 mL), followed by suction filtration of the cloudy solution. The filtrate was concentrated to give 2.007 g (74% from **11**) of diester **4a** as a pale yellow oil that was pure by ^1H NMR: IR (neat) 2960, 1720, 1620, 1440, 1250, 1200, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06 (1 H, m), 6.39 (1 H, d, J = 12 Hz), 6.06 (1 H, dt, J = 12, 4.9 Hz), 5.48 (1 H, d, J = 2.9 Hz), 4.66 (1 H, dm, J = 9.3 Hz), 4.54 (1 H, d, J = 2.9 Hz), 3.83 (3 H, s), 3.77 (3 H, s), 2.44 (2 H, m), 2.37–2.18 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 173.8 (s), 170.3 (s), 155.0 (s), 147.6 (d), 139.7 (d), 131.2 (s), 126.2 (d), 97.5 (dd), 76.0 (d), 50.2 (q), 49.8 (q), 28.9 (t), 23.2 (t); mass spectrum, m/e (rel intensity) 252 (M^+, 1.4), 221 (1.7), 220 (8.5), 192 (2.6), 151 (14.7), 133 (13.0), 119 (16.0), 118 (17.5), 105 (13.1), 92 (12.9), 91 (100); high-resolution mass spectrum, calcd for $C_{13}H_{16}O_5$ 252.0998, found 252.0995.

3-[(1-Carboxyethenyl)oxy]cyclohepta-1,6-diene-1-carboxylic Acid (4b). Cold 1.0 M NaOH (4.5 mL, 4.5 mmol, 2.2 equiv) was added dropwise to a solution of diester **4a** (0.510 g, 2.02 mmol) in THF/H_2O (2:1, 21 mL) at ~0 °C. The mixture was stirred for 5.5 h, and the pH was adjusted to ~4 with Amberlite IR-120 (plus) acidic resin. The resin was removed by suction filtration. The filtrate was saturated with NaCl and extracted with ethyl acetate (4 × 20 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to give 0.276 g (61%) of **4b** as an oil. The aqueous layer was re-acidified with Amberlite resin and filtered, and the filtrate was extracted with ethyl acetate (50 mL, 3 × 15 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to give another 0.116 g (26%) of **4b**. An analytically pure sample was prepared by trituration of the oil with CH_2Cl_2 (3 × 10 mL) to give 0.174 g (38%) of **4b** as a white powder: mp 136–138 °C; UV (pH 7.5 Tris-HCl buffer) λ_{max} 255 (ϵ 5050); IR (KBr) 3300–2700, 1690, 1615, 1430, 1290, 1205 cm^{-1}; ^1H NMR (CD_3OD) δ 7.02 (1 H, s), 6.37 (1 H, d, J = 12 Hz), 6.05 (1 H, dt, J = 12, 4.8 Hz), 5.46 (1 H, d, J = 2.6 Hz), 4.94 (acid protons + H_2O, br), 4.75 (1 H, dm, J = 9.3 Hz), 4.64 (1 H, d, J = 2.6 Hz), 2.45 (2 H, m), 2.31 (1 H, m), 2.14 (1 H, m); ^{13}C NMR (CD_3OD) δ 169.8 (s), 166.3 (s), 150.9 (s), 143.5 (d), 136.3 (d), 129.1 (s), 123.7 (d), 96.7 (t), 77.5 (d), 33.5 (t), 28.3 (t); mass spectrum, m/e (rel intensity) 224 (M^+, 2.4), 206 (4.3), 171 (10.6), 152 (3.3), 149 (9.4), 137 (14.4), 119 (13.2), 105 (9.4), 93 (19.3), 92 (15.5), 77 (45.4); high-resolution mass spectrum, calcd for $C_{11}H_{12}O_5$ 224.0685, found 224.0689.

Disodium 3-[(1-Carboxylatoethenyl)oxy]cyclohepta-1,6-diene-1-carboxylate (4c). Diacid **4b** (18.5 mg, 0.083 mmol) was dissolved in CH_3OH/H_2O (1:1, 4 mL) and passed through a Bio-Rex 70 cation exchange column (Na^+ form, 300 mg), which had been pre-wetted with CH_3OH/H_2O. The eluant was passed through the column twice, and the column was washed with H_2O (15 mL). The combined eluants were concentrated to give 15.7 mg (71%) of **4c** as a solid: ^1H NMR (DMSO-d_6) δ 6.91 (1 H, br s), 6.60 (1 H, d, J = 12 Hz), 5.80 (1 H, dm, J = 12 Hz), 5.13 (1 H, s), 4.55 (1 H, m), 4.12 (1 H, s), 2.36 (2 H, m), 2.16 (1 H, m), 1.98 (1 H, m).

Thermolysis of 4a. Dimethyl ester **4a** (215 mg, 0.852 mmol) was dissolved in DMSO-d_6 (1 mL) under N_2 and heated at 80 °C. The reaction was followed by ^1H NMR spectroscopy, and the half-life for disappearance of **4a** was determined to be ~12.4 h. After 67 h, the ^1H NMR spectrum showed a complete disappearance of starting material and the formation of a complex mixture of products. The solvent was removed in vacuo, and the residual oil was purified by MPLC on silica gel [toluene/ethyl acetate (16:1), 25 × 310 mm column] to give five major products.

The least polar compound was shown to be double-bond isomer **15a** (see Results and Discussion section). It was obtained as 24 mg (~11%) of a somewhat impure oil: ^1H NMR ($CDCl_3$) δ 7.09 (1 H, t, J = 5.6 Hz), 5.90 (1 H, s), 5.77 (1 H, d, J = 1.9 Hz), 5.17 (1 H, d, J = 1.9 Hz), 3.82 (3 H, s), 3.74 (3 H, s), 2.56 (2 H, t, J = 5.6 Hz), 2.45 (2 H, q, J = 5.6 Hz), 1.94 (2 H, quintet, J = 5.6 Hz). It was characterized further as the methyltriazolinedione adduct.

Crude **15a** (24 mg, 0.095 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to ~0 °C. To the colorless solution was added 4-methyl-1,2,4-triazoline-3,5-dione (5.5 mg, 0.05 mmol, 0.5 equiv) until a faint pink color persisted. The mixture was concentrated and the residual oil was purified by flash chromatography on silica gel [ethyl acetate/petroleum ether (1:1)] to give 12 mg (34%) of crystalline adduct **19**: mp 100–110 °C; IR (KBr) 3027, 2956, 1774, 1720, 1628, 1460, 1333, 1217, 1169 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06 (1 H, d, J = 1.5 Hz), 5.68 (1 H, d, J = 3.0 Hz), 5.36 (1 H, d, J = 3.4 Hz), 4.92 (1 H, d, J = 3.0 Hz), 3.86 (3 H, s), 3.83 (3 H, s), 3.10 (3 H, s), 2.45 (1 H, m), 2.29 (1 H, m), 1.93 (1 H, m), 1.72–1.40 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 162.9 (s), 162.8 (s), 153.6 (s), 149.9 (s), 146.1 (s), 138.3 (d), 132.3 (s), 102.6 (t), 90.4 (s), 52.7 (q), 52.6 (q), 52.1 (d), 34.2 (t), 26.3 (t), 25.5 (q), 18.6 (t); mass spectrum, m/e (rel intensity) 365 (M^+, 9.1), 334 (13.9), 333 (43.9), 264 (16.0), 251 (23.0), 250 (70.8), 236 (12.9), 235 (22.4), 220 (22.6), 219 (92.7), 218 (100), 191 (91.4), 190 (38.4), 159 (34.6), 149 (42.7), 119 (31.6), 105 (19.0), 91 (63.6); high-resolution mass spectrum, calcd for $C_{16}H_{19}N_3O_7$ 365.1223, found 365.1246.

The next compound to elute off the column was prephenate analogue **5a**. It was obtained as 20 mg (9%) of an oil (pure by ^1H NMR): IR (neat) 3025, 2960, 1740, 1730, 1435, 1280, 1260, 1230, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.93 (2 H, dm, J = 11 Hz), 5.59 (2 H, d, J = 11 Hz), 3.86 (3 H, s), 3.72 (3 H, s), 3.45 (2 H, s), 2.27 (4 H, br s); ^{13}C NMR ($CDCl_3$) δ 191.0 (s), 174.2 (s), 161.0 (s), 133.0 (d), 129.6 (d), 53.0 (q), 52.8 (q), 52.1 (s), 49.0 (t), 26.4 (t); mass spectrum, m/e (rel intensity) 252 (M^+, 2.3), 221 (1.4), 220 (4.6), 193 (29.7), 165 (54.4), 150 (46.7), 133 (74.4), 105 (92.2), 91 (100); high-resolution mass spectrum, calcd for $C_{13}H_{16}O_5$

252.0998, found 252.100 ± 0.001.

The third compound to elute was compound **16a**. It was obtained as 6 mg (3%) of fairly pure oil: ^{1}H NMR ($CDCl_3$) δ 7.20 (H_a, t, J_{ae} = 7.5 Hz, J_{af} = 7.5 Hz), 5.86 (H_b, d, J_{bd} = 3.8 Hz), 5.06 (H_c, dd, J_{cd} = 12 Hz, J_{cg} = 5.6 Hz), 4.52 (H_d, dd, J_{cd} = 12 Hz, J_{bd} = 3.8 Hz), 3.82 (3 H, s, carbomethoxy), 3.75 (3 H, s, carbomethoxy), 2.47 (H_e, m, J_{ae} = 7.5 Hz, also coupled to H_f), 2.20 (H_f and H_g, m, J_{af} = 7.5 Hz, J_{cg} = 5.6 Hz, also coupled to $H_{e,i,j}$), 2.02 (H_h, m), 1.76 (H_i, m, coupling was observed to $H_{e,f,g}$), 1.57 (H_j, m, coupling was observed to $H_{e,f,g}$).

Diels–Alder adduct **17a** was next to elute and was obtained as 74 mg (34%) of oil: IR (neat) 2960, 1740, 1715, 1635, 1440, 1285, 1270, 1250, 1200, 1105, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.86 (1 H, dd, J = 6.1, 1.8 Hz), 4.48 (1 H, t, J = 6.8 Hz), 3.83 (3 H, s), 3.78 (3 H, s), 3.59 (1 H, ddd, J = 6.8, 5.9, 1.8 Hz), 3.19 (1 H, m), 2.11 (1 H, dd, J = 9.8, 5.9 Hz), 2.07–1.91 (2 H, m), 1.68 (1 H, d, J = 9.8 Hz), 1.47–1.26 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 172.2 (s), 165.5 (s), 140.0 (d), 139.4 (s), 81.3 (s), 74.1 (d), 52.6 (q), 52.0 (q), 45.6 (t), 39.8 (d), 39.1 (d), 24.0 (t), 18.6 (t); mass spectrum, m/e (rel intensity) 252 (M^+, 26.4), 221 (16.1), 220, (36.7), 213 (19.8), 195 (43.6) , 193 (34.8), 163 (26.5), 161 (66.2), 149 (47.4), 133 (55.6), 119 (27.2), 118 (30.8), 105 (96.6), 91 (100), 84 (88.3), 77 (46.0). Anal. Calcd for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39. Found: C, 61.90; H, 6.50.

The final compound to elute was Diels–Alder adduct **18a**, and it was obtained as 53 mg (25%) of a white powder. An analytically pure sample was prepared by sublimation (50–60 °C, 0.1 Torr): mp 109–110 °C; IR ($CHCl_3$) 3010, 2955, 1740, 1440, 1290, 1270, 1205 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.76 (1 H, d, J = 9.3 Hz), 6.51 (1 H, dd, J = 9.3, 7.6 Hz), 4.75 (1 H, t, J = 5.4 Hz), 3.76 (3 H, s), 3.73 (3 H, s), 2.64 (1 H, m), 2.55 (1 H, dd, J = 11, 5.4 Hz), 2.23 (1 H, dd, J = 12, 3.6 Hz), 2.06 (1 H, d, J = 11 Hz), 1.98 (1 H, dt, J = 14, 5.4 Hz), 1.80 (1 H, dm, J = 12 Hz), 1.54 (1 H, dm, J = 14 Hz); ^{13}C NMR ($CDCl_3$) δ 173.4 (s), 172.1 (s), 137.0 (d), 134.5 (d), 88.1 (s), 79.3 (d), 56.5 (s), 52.4 (q), 52.3 (q), 47.9 (t), 41.1 (t), 29.7 (t), 29.3 (d); mass spectrum, m/e (rel intensity) 252 (M^+, 11.8), 221 (10.8), 220 (27.0), 193 (9.3), 151 (13.3), 150 (41.0), 133 (21.5), 119 (25.7), 118 (26.1), 105 (53.9), 91 (100). Anal. Calcd for $C_{13}H_{16}O_5$: C, 61.90; H, 6.39. Found: C, 62.11; H, 6.49.

Thermolysis of 4b. Diacid **4b** was heated at 80 °C in DMSO-d_6 under a N_2 atmosphere. This gave a complex mixture of products that could not be separated. The ^{1}H NMR spectrum of the crude reaction mixture suggested the formation of products analogous to those formed from the thermolysis of **4a**. The half-life for disappearance of **4b** under these conditions was crudely estimated between 11 and 16 h.

Thermolysis of 4c. Disodium salt **4c** (15.7 mg, 0.059 mmol) was dissolved in DMSO-d_6 (1 mL) and placed in a NMR tube under a N_2 atmosphere. Complete conversion to double-bond isomer **15c** was observed after heating the sample at 70 °C for 11 h: ^{1}H NMR (DMSO-d_6) δ 6.73 (1 H, t, J = 5.5 Hz), 6.04 (1 H, s), 5.16 (1 H, s), 4.50 (1 H,s), 2.44 (2 H, t, J = 5.5 Hz), 2.30 (2 H, q, J = 5.5 Hz), 1.82 (2 H, quintet, J = 5.5 Hz).

Disodium 1-(2-Carboxylato-2-oxoethyl)cyclohepta-2,6-diene-1-carboxylate (5c). Cold 1.0 M NaOH (0.064 mL, 0.064 mmol, 3.0 equiv) was added to a solution of diester **5a** (5.4 mg, 0.021 mmol) in THF/H_2O (2:1, 1.5 mL) at ~0 °C (ice/H_2O). The yellow solution was stirred for 5 h, and the pH was adjusted to 6.8 with Amberlite IR-120 (plus) acidic resin. The resin was removed by suction filtration, and the filtrate was concentrated to give 7.0 mg (>100%) of hygroscopic disodium salt **5c** as an off-white solid: ^{1}H NMR (CD_3OD) δ 5.74 (4 H, m), 3.18 (2 H, br s), 2.25 (4 H, br m).

A sample of diacid **5b** (pale yellow solid) was prepared as indicated above, except that the pH was adjusted to ~4 before filtration: IR (neat) 3400–3000, 3027, 2926, 1773, 1719, 1636, 1437, 1186 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 5.90 (2 H dt, J = 11, 5.4 Hz), 5.63 (2 H, d, J = 11 Hz), 3.33 (4 H, br s, acid + methylene protons), 2.46 (2 H, m), 2.19 (2 H, m); mass spectrum, m/e (rel intensity) 224 (M^+, 0.6), 180 (8.9), 151 (17.4), 150 (10.6), 149 (10.2), 136 (29.9), 135 (25.1), 133 (25.3), 105 (64.2), 91 (100).

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Synthesis of α- and γ-Alkyl-Substituted Phosphinothricins: Potent New Inhibitors of Glutamine Synthetase

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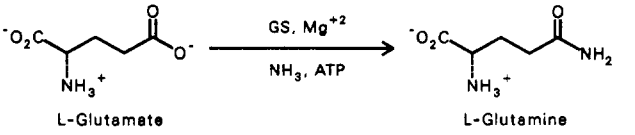
Considerations of substrate structural variability for the enzyme glutamine synthetase (GS) have led to the design of α- and γ-substituted analogues of the naturally occurring GS inhibitor phosphinothricin (PPT). The novel cyclic inhibitor D,L-cyclohexanephosphinothricin (CHPPT) was prepared via conjugate addition of diethyl methylphosphonite to 2-cyclohexenone, followed by stereospecific Bucherer–Bergs amino acid synthesis. CHPPT stereochemistry was determined by COSY and NOESY 2D NMR techniques. The substituted phosphinothricins function as active site probes useful for elucidating the mechanism of GS inhibition by PPT.

Introduction

The enzyme glutamine synthetase (GS, EC 6.3.1.2) catalyzes a reaction of central importance in nitrogen metabolism, the conversion of L-glutamate to L-glutamine.[1] The amide functionality of glutamine is the ultimate source of nitrogen introduced into α-amino acids via transaminase-catalyzed reactions and also provides nitrogen for the urea cycle and pyrimidine biosynthesis.[2]



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