Articles

Synthesis and Evaluation of 3-Dehydroquinate Synthase Transition State Analogues

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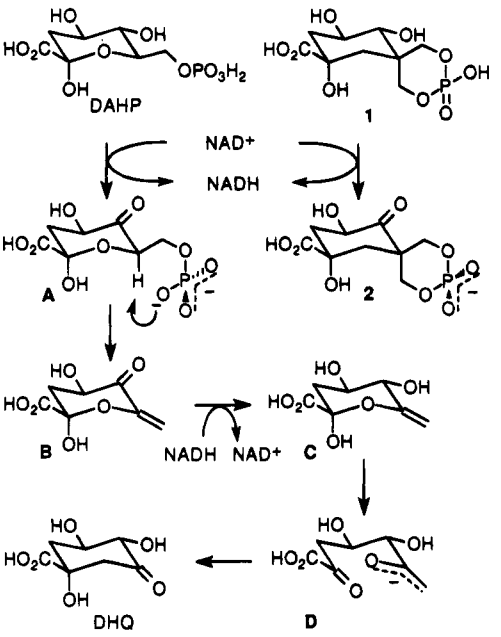
Analogues have been synthesized of a six-membered, cyclic transition state likely involved in elimination of inorganic phosphate from an enzyme-bound, reactive intermediate formed during 3-dehydroquinate (DHQ) synthase catalysis. A spirocyclic carbaphosphodiester and ketocarbaphosphodiester analogue were both synthesized from quinic acid via a route where the essential spiro center was introduced by a ytterbium triflate-catalyzed aldol condensation. Spirocyclic carbaphosphodiester was a modest competitive inhibitor of DHQ synthase with an inhibition constant (K_i) of 6.7×10^{-5} M. Enzyme-bound NADH formed during inhibition of enzyme by the spirocyclic carbaphosphodiester. Spirocyclic ketocarbaphosphodiester, even at concentrations of 0.5 mM, failed to inhibit DHQ synthase. These observations are discussed from the perspective of possible conformational constraints imposed by DHQ synthase on the reactive intermediate which undergoes elimination of inorganic phosphate relative to the ionization state of this reactive intermediate.

Synthesis of structural mimics of both putative reactive intermediates and transition states found along enzyme reaction coordinates has proven to be a highly effective strategy for discovery of potent enzyme inhibitors. 3-Dehydroquinate synthase provides an ideal test case for such strategies[1] given the numerous chemical steps (Scheme 1) that are required for enzymatic conversion of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) into product 3-dehydroquinate (DHQ). Each turnover of DAHP into DHQ requires an oxidation, elimination, reduction, and aldol condensation. A substantive body of evidence[2] has established that the elimination of inorganic phosphate from reactive intermediate A (Scheme 1) is intramolecularly mediated by the ionized phosphate monoester. To assist in defining as well as exploiting the role of DHQ synthase during this elimination, spirocyclic carbaphosphodiester **1** (Scheme 1) and spirocyclic ketocarbaphosphodiester **2** (Scheme 1) have been synthesized.

The cyclic phosphodiesters of spirocyclic **1** and **2** were designed to be conformationally restrained mimics of the six-membered transition state wherein the phosphate monoester of reactive intermediate A (Scheme 1) removes the methine proton at C-5. If DHQ synthase accelerates the elimination of inorganic phosphate from reactive intermediate A by restraining the degrees of freedom available to the ionized phosphomethyl group, spirocyclic **1** and **2** might be potent inhibitors of the enzyme. Significant charge reduction (Scheme 2) would also be likely in the immediate vicinity of the phosphate monoester during its removal of the C-5 methine proton of reactive intermediate A. Stabilization by DHQ synthase

Scheme 1



of the monobasic, ionized state of the phosphate monoester during the E1cb-like transition state (A′, Scheme 2) would further stabilize the transition state separating reactive intermediate A from reactive intermediate B. The cyclic phosphodiesters of spirocyclic **1** and **2** could take advantage of this stabilization as a consequence of their monobasic ionization state.

By examining the extent spirocyclic **1** and **2** inhibit DHQ synthase and comparing this inhibition with that determined for other structurally related inhibitors (Scheme 2) of the enzyme, insights are provided into the role DHQ synthase plays during the elimination of inorganic phosphate from reactive intermediate A. An opportunity is also provided for identifying new inhibitors of DHQ synthase more amenable to penetration into

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(1) For a recent overview, see: Haslam, E. *Shikimic Acid Metabolism and Metabolites*; Wiley: New York, 1993; Chapters 2 and 3.

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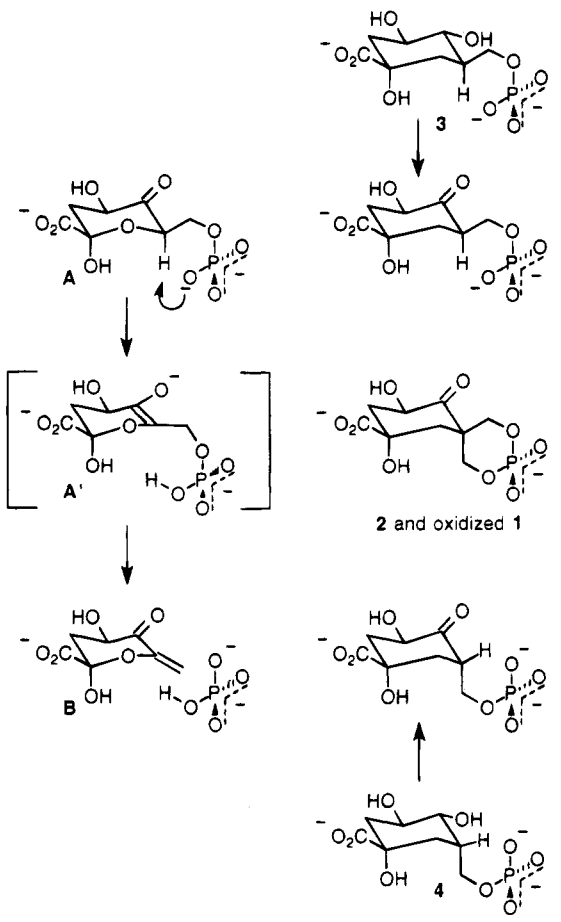
Scheme 2

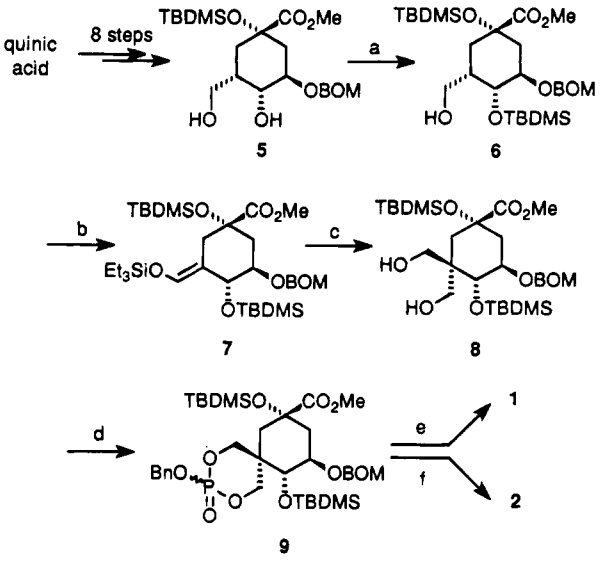


bacterial and plant cytosols by virtue of ionization states that are significantly reduced relative to previously reported inhibitors of this enzyme.[3]

Results and Discussion

Spirocyclic carbaphosphodiester **1** is best viewed as a precursor to a transition state analogue requiring oxidation of its C-4 hydroxyl group by DHQ synthase (Scheme 1) before the active site is presented with a transition state analogue. This expected oxidation follows from a wide variety of micromolar to submicromolar inhibitors of DHQ synthase whose binding to the enzyme results in oxidation of the inhibitor and formation of enzyme-bound NADH.[2bc,4] DHQ synthase-catalyzed oxidation of **1** and the accompanying oxidation of enzyme-bound NAD to enzyme-bound NADH would generate an active site configuration similar to that encountered by the actual transition state separating reactive intermediate A from reactive intermediate B. Spirocyclic ketocarbaphosphodiester **2** with its C-4 carbonyl group (Scheme 1) directly challenges DHQ synthase with an analogue of one of its transition states. Binding of spirocyclic ketocarbaphosphodiester **2** would lead to a nonnative configuration involving the transition state analogue being bound at

Scheme 3[a]



[a] Key: (a) (i) *t*BuMe$_2$SiOTf, Et$_3$N, CH$_2$Cl$_2$, rt, (ii) *p*TsOH, MeOH, rt, 96%; (b) (i) (COCl)$_2$, DMSO, Et$_3$N, CH$_2$Cl$_2$, −78 °C, (ii) Et$_3$SiOTf, Et$_3$N, C$_6$H$_6$, reflux, 81%; (c) (i) CH$_2$O, Yb(OTf)$_3$, H$_2$O, THF, reflux, (ii) NaBH$_4$, MeOH, 0 °C, 65%; (d) (i) BnOP(NEt$_2$)$_2$, tetrazole, CH$_3$CN, rt, (ii) *m*-CPBA, CH$_2$Cl$_2$, −40 °C, 68%; (e) (i) Bu$_4$NF, THF, 0 °C to rt, (ii) H$_2$, Pd/C, THF/H$_2$O (2:1), (iii) NaOH, H$_2$O, rt, 43%; (f) (i) Bu$_4$NF, THF, 0 °C to rt, (ii) (COCl)$_2$, DMSO, Et$_3$N, CH$_2$Cl$_2$, −78 °C, 45%, (iii) NaOH, THF/H$_2$O (10:1), rt, (iv) H$_2$, Pd/C, THF/H$_2$O (4:1), 40%.

an active site possessing NAD instead of NADH. A carbocyclic analogue of reactive intermediate A possessing a carbonyl at C-4 has been reported to lead to potent inhibition of DHQ synthase.[5]

Transition State Analogue Synthesis. The synthesis employed for assembly of 5-(phosphomethyl)-5-deoxyquinate **4** (Scheme 2) provided the foundation for synthesis (Scheme 3) of spirocyclic analogues **1** and **2**.[6] 5-Phosphomethyl-5-deoxyquinate **4** and a series of phosphoryl and phosphonyl derivatives were inhibitors of DHQ synthase that were generally oxidized by the enzyme to their C-4 carbonyl forms.[6] These interactions were used to establish a region of diastereomeric promiscuity in the C-5 region of substrate analogues of DHQ synthase that was supportive of a phosphate monoester-mediated elimination. Diol **5** (Scheme 3), which was a key intermediate in the synthesis of 5-phosphomethyl-5-deoxyquinate **4**, was prepared in eight steps in 20–30% overall yield from quinic acid. The key step in this sequence is the use of the C-4 hydroxyl group to set the stereochemistry of the C-5 hydroxymethyl group by use of the Nishiyama–Stork reaction.[7]

Conversion of diol **5** into spirocyclic analogues **1** and **2** required that a spiro center be created at C-5 by addition of another hydroxymethyl group at this position. In route to assembly of the spiro center, diol **5** was disilylated followed by selective hydrolysis of the primary silyl ether to afford alcohol **6**. Swern oxidation of the primary alcohol yielded an aldehyde. All attempts to alkylate the enolate derived from this aldehyde resulted in elimination of the C-4 substituent and formation of an unsatur-

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ated aldehyde. The aldehyde derived from alcohol **6** was therefore reacted in refluxing benzene with Et_3N and triethylsilyl trifluoromethanesulfonate to form triethylsilyl enol ether **7**. Under these conditions, little or no elimination occurred. Triethylsilyl enol ether **7** was stable enough to be purified by silica gel chromatography.

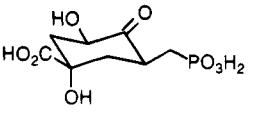
Reaction of **7** with activated hydroxymethyl equivalents under conditions requiring Lewis acid catalysts such as $TiCl_4$ and $ZnCl_2$ produced the aforementioned α,β-unsaturated aldehyde product. However, use of Kobayashi methodology[8] employing water-soluble lanthanide Lewis acids avoided unwanted elimination side reactions. Ytterbium triflate catalyzed the reaction of triethyl silyl enol ether **6** with aqueous formaldehyde in THF at reflux to give the desired aldol product which was immediately reduced with $NaBH_4$ to the C-5 bis(hydroxymethyl derivative **8**. Reaction[9] of benzyl N,N,N',N'-tetraethylphosphordiamidite with bishydroxymethyl **8** in the presence of tetrazole produced the corresponding cyclic phosphite which was oxidized in situ with *m*-CPBA to form triester **9** as an approximately equimolar mixture of diastereomers. This mixture of diastereomers was then submitted to stepwise deprotection. Treatment with $Bu_4N^+F^-$ removed both silyl protecting groups. The resulting diol was hydrogenolyzed to remove the benzyl protection group of the phosphate diester and the BOM protecting group of the C-3 hydroxy group. Subsequent hydrolysis of the methyl ester yielded spirocyclic carbaphosphodiester **1**.

Synthesis of spirocyclic ketocarbaphosphodiester **2** also employed the diol product resulting from $Bu_4N^+F^-$ deprotection of triester **9.** Swern oxidation of this intermediate provided the requisite carbonyl at C-4. The order of the subsequent deprotection steps required to obtain transition state analogue **2** was important. Hydrogenolysis to remove the benzyl ester from the cyclic phosphate followed by hydrolysis of the methyl ester under basic conditions led to substantial decomposition. Switching the order of deprotection such that methyl ester hydrolysis preceded benzyl ester hydrogenolysis circumvented the decomposition problems and afforded the desired spirocyclic ketocarbaphosphodiester **2**.

Enzymology. Spirocyclic carbaphosphodiester **1** displayed classical competitive inhibition of DHQ synthase with an inhibition constant (K_i) of 6.7×10^{-5} M. Incubation of DHQ synthase with spirocyclic **1** resulted in an increase in absorbance at 340 nm indicative of accumulation of NADH at the enzyme active site. This suggests that spirocyclic **1** fits in the enzyme active site similarly to a number of other inhibitors. Incubation of DHQ synthase with concentrations of spirocyclic ketocarbaphosphodiester **2** as high as 0.5 mM failed to reveal any inhibition of the enzyme.

One reason for the modest inhibition of DHQ synthase by spirocyclic **1** may be adverse steric interactions between the enzyme active site and the cyclic phosphodiester portion of **1**. However, this is not consistent with the tolerance by the active site of DHQ synthase of both carbaDAHP[2d,10] **3** (Scheme 2) and 5-phosphomethyl-5-deoxyquinate[6] **4** (Scheme 2). CarbaDAHP **3**, which differs from substrate DAHP only in the substitution of a methylene group for a pyranosyl ring oxygen, is a classical, competitive inhibitor of DHQ synthase ($K_i = 1.2 \times 10^{-7}$ M).[2d,10] 5-Phosphomethyl-5-deoxyquinate **4**, a C-5 epimer of carbaDAHP **3**, is a potent, slowly-reversible inhibitor ($K_i = 3.0 \times 10^{-8}$ M)[6] even with its epimerized C-5 phosphomethyl group. Accumulation of enzyme-bound NADH is indicated by an increase in absorbance at 340 nm when both **3** and **4** are incubated with DHQ synthase which suggests a similar fit in the active site of DHQ synthase. The potent inhibition observed for 5-(phosphomethyl)-5-deoxyquinate **4** and the similar fit for both **3** and **4** in the active site indicates that DHQ synthase is quite tolerant of the type of steric/stereochemical challenge presented to the active site by the phosphodiester portion of spirocyclic **1**.

The lack of inhibition of DHQ synthase by spirocyclic ketocarbaphosphodiester **2** is consistent with some of the kinetic behavior observed for ketocarbaphosphonate **10**.[5]



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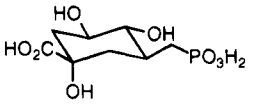

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Incubation of DHQ synthase with ketocarbaphosphonate **10** is characterized by initial reversible inhibition ($K_i = 1.5 \times 10^{-7}$ M).[5] Apparent irreversible inhibition is observed only after prolonged incubation of ketocarbaphosphonate **10** with enzyme. The initial, reversible inhibition constant is substantially weaker than the inhibition constant ($K_i = 5.1 \times 10^{-9}$ M) determined for carbaphosphonate **11**.[3b,4b] This reduction in inhibitor potency over short time periods suggests that active site interactions are lost when the C-4 hydroxyl group of carbaphosphonate **11** is converted into the carbonyl group of ketocarbaphosphonate **10**. A similar loss in active site interactions could explain the loss of enzyme inhibition observed when the C-4 hydroxyl group of spirocyclic carbaphosphodiester **1** is changed to the C-4 carbonyl group of spirocyclic ketocarbaphosphodiester **2**.

Extent of ionization is the remaining factor to consider as an explanation for the modest inhibition of DHQ synthase by spirocyclic carbaphosphodiester **1**. Spirocyclic carbaphosphodiester **1**, carbaDAHP **3**, and 5-(phosphomethyl)-5-deoxyquinate **4** can all undergo ionization to either monobasic or dibasic forms. However, the tribasic ionization state available for carbaDAHP **3** and 5-phosphomethyl-5-deoxyquinate **4** is not accessible to spirocyclic **1**. Previous research has shown that V_{max} increases to an optimum at pH 7.4 and then declines as pH increases further.[11] The inhibition of DHQ synthase by several inhibitors has also been discovered to improve as the solution pH is increased.[2c] While use of these parameters to infer the ionization state of substrate, reactive intermediates, and their respective analogues at the active site of DHQ synthase is problematic,[12] insights can readily be gleaned from the respective inhibition of DHQ synthase by spirocyclic carbaphosphodiester **1**, carbaDAHP **3**, and 5-(phosphomethyl)-5-deoxyquinate **4**. The extent of interaction between these molecules and DHQ synthase is consistent with the enzyme either selectively binding the tribasic ionization states of substrate or initially binding the dibasic form of substrate

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followed by enzyme-mediated deprotonation to the tribasic form. In this view of DHQ synthase catalysis, the enzyme would not play a direct role in catalyzing the elimination of inorganic phosphate from reactive intermediate A. DHQ synthase merely ensures that fully ionized reactive intermediate A is present in the active site. After elimination, the monobasic reactive intermediate B would be present in the active site with dibasic inorganic phosphate. Tribasic carbaDAHP **3** can then be viewed (Scheme 2) as a substrate analogue oxidized by DHQ synthase to generate an analogue of fully ionized reactive intermediate A. Fully ionized 5-phosphomethyl-5-deoxyquinate **4** can then be proposed (Scheme 2) after oxidation by DHQ synthase, to be an analogue of monobasic enzyme-bound reactive intermediate B and enzyme-bound dibasic inorganic phosphate.

Conclusion. The modest inhibition of DHQ synthase by spirocyclic carbaphosphodiester **1** and the complete absence of inhibition by spirocyclic ketocarbaphosphodiester **2** are not consistent with DHQ synthase aggressively stabilizing the transition state (A', Scheme 2) separating reactive intermediate A and reactive intermediate B. Comparison of phosphate diesters **1** and **2** with structurally related phosphate monoesters **3** and **4** suggests that ionization states and not steric or stereochemical factors likely explain the differences in observed inhibition of DHQ synthase. These comparisons also point towards a rather limited catalytic role for DHQ synthase during the elimination of inorganic phosphate from reactive intermediate A. DHQ synthase could play some role in restricting the degrees of freedom available to the phosphate monoester of reactive intermediate A during removal of the C-5 methine proton. However, the importance of this conformational restriction appears to be less of a factor in the design of inhibitors than presenting the enzyme with reactive intermediate or transition state analogues capable of existing in suitable, tribasic ionization states.

Experimental Section

General Chemistry. ^{1}H NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts for ^{1}H NMR spectra are reported (in parts per million) relative to internal tetramethylsilane (Me_4Si, $\delta = 0.0$ ppm) with $CDCl_3$ as solvent, and to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP, $\delta = 0.0$ ppm) when D_2O was the solvent. ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts for ^{13}C NMR spectra are reported (in parts per million) relative to $CDCl_3$ ($\delta = 77.0$ ppm) or internal acetonitrile (CH_3CN, $\delta = 3.69$ ppm) in D_2O. ^{31}P NMR spectra were recorded on a 121 MHz spectrometer and chemical shifts reported (in parts per million) relative to external 85% phosphoric acid (0.0 ppm). FAB and CI mass spectra were obtained on a double-focusing mass spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Phosphorus and phosphate were determined by the method of Ames.[13] Hydrogenations were carried out with a Parr hydrogenation apparatus (Parr Instrument Co.). Radial chromatography was carried out with a Harrison Associates Chromatotron using 1, 2 or 4 mm layers of silica gel 60 PF_{254} containing gypsum (E. Merck). Silica gel 60 (40–63 μm, E. Merck) was used for flash chromatography.[14] Analytical thin-layer chromatography (TLC) utilized precoated plates of silica gel 60 F-254 (0.25 mm, E. Merck or Whatman). TLC plates were visualized by immersion in anisaldehyde stain (by volume: 93% ethanol, 3.5% sulfuric acid, 1% acetic acid and 2.5% anisaldehyde) followed by heating. Dimethylformamide was dried and stored over activated Linde 4A molecular sieves under nitrogen. Pyridine, Et_3N and CH_2Cl_2 were distilled from calcium hydride under nitrogen. Benzene was distilled from sodium under nitrogen. Tetrahydrofuran was distilled under nitrogen from sodium benzophenone ketyl. Methanol was dried over activated 3A molecular sieves. Enzymological manipulations followed established literature procedures.[4b] Organic solutions of products were dried over $MgSO_4$. Yb_2O_3 was purchased from Adrich.

Methyl [1(*S*)-(1α,3β,4α,5α)]-3-[(Benzyloxymethyl)oxy]-1,4-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-(hydroxymethyl)cyclohexane-1-carboxylate (6). Diol **5** (5.60 g, 12.3 mmol) was dissolved in CH_2Cl_2 (100 mL) and anhydrous Et_3N (4.30 mL, 30.8 mmol) added. The resulting solution was cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (5.90 mL, 25.8 mmol) was added dropwise under Ar. After the reaction mixture was stirred an additional 1 h at 0 °C, water and ether were added. The resulting organic layer was washed with water (1×), aqueous $CuSO_4$ (2×) and brine (1×). Drying and concentration afforded a yellow oil. CH_3OH (100 mL) was added followed by *p*-TsOH monohydrate (0.27 g). After 30 min at rt, pyridine (1 mL) was added and the solution was concentrated to an oil. Ether was added and the solution was washed with aqueous $CuSO_4$ (1×) and brine (1×). The organic layer was dried and concentrated to yield alcohol **6** (6.73 g, 96%) as a light yellow oil which did not require further purification: ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 5 H), 4.73 (d, $J = 7$ Hz, 1 H), 4.60 (d, $J = 12$ Hz, 1 H), 4.59 (d, $J = 7$ Hz, 1 H), 4.50 (d, $J = 12$ Hz, 1 H), 3.80–3.90 (m, 2 H), 3.67 (s, 3 H), 3.50–3.70 (m, 2 H), 2.46 (d, $J = 14$ Hz, 1 H), 2.15–2.25 (m, 1 H), 2.07 (ddd, $J = 12$, 3, 3 Hz, 1 H), 1.92 (dd, $J = 14$, 2 Hz, 1 H), 1.60 (dd, $J = 12$, 12 Hz, 1 H), 0.90 (s, 9 H), 0.84 (s, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H), 0.05 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 173.4, 137.6, 128.4, 127.8, 127.7, 92.5, 75.3, 74.3, 69.5, 68.7, 64.3, 51.6, 37.9, 35.1, 32.8, 25.7, 25.5, 18.0, 17.9, −2.9, −3.1, −4.7, −5.2; MS *m*/*z* (rel inten) EI 91 (100); CI 569 ($M + H^+$, 17), 434 (14), 433 (57), 432 (29), 431 (100); HRMS (CI) calcd. for $C_{29}H_{52}O_7Si_2$ ($M + H^+$) 569.3330, found 569.3347. Anal. Calcd for $C_{29}H_{52}O_7Si_2$: C, 61.22; H, 9.21. Found: C, 61.23; H, 9.24.

Methyl [1(*S*)-(1α,3β,4α,5α)]-3-[(Benzyloxymethyl)oxy]-1,4-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-[[(triethylsilyl)oxy]ylidene]cyclohexane-1-carboxylate (7). Oxalyl chloride in CH_2Cl_2 (2.0 M, 5.70 mL, 11.4 mmol) was added to anhydrous CH_2Cl_2 (30 mL) at −78 °C, and anhydrous DMSO (1.10 mL, 15.2 mmol) was added slowly under Ar. After 5 min, alcohol **6** (4.33 g, 7.60 mmol) in CH_2Cl_2 (30 mL) was cannulated into the reaction mixture. Triethylamine (5.3 mL, 38.1 mmol) was added via syringe 45 min later. After an additional 30 min at −78 °C, the reaction was warmed to rt and quenched with water. Ether was added and the organic layer was washed successively with aqueous HCl (1 N, 1×), water (1×) and brine (1×). Drying and concentration afforded the aldehyde as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.67 (s, 1 H), 7.25–7.40 (m, 5 H), 4.77 (d, $J = 7$ Hz, 1 H), 4.64 (d, $J = 7$ Hz, 1 H), 4.62 (d, $J = 12$ Hz, 1 H), 4.55 (d, $J = 12$ Hz, 1 H), 4.19 (dd, $J = 3$, 3 Hz, 1 H), 3.91 (d, $J = 3$ Hz, 1 H), 3.68 (s, 3 H), 2.85–3.00 (m, 1 H), 2.41 (dd, $J = 12$, 12 Hz, 2 H), 1.93 (dd, $J = 12$, 12 Hz, 2 H), 0.86 (s, 9 H), 0.85 (s, 9 H), 0.08 (s, 6 H), 0.04 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 202.7, 173.1, 137.5, 128.4, 127.8, 92.9, 75.0, 74.3, 69.7, 67.8, 51.7, 49.6, 35.3, 30.5, 25.5, 18.0, 17.8, −2.9, −3.1, −4.5, −5.1.

Without purification, the aldehyde was dissolved in anhydrous benzene (50 mL) and Et_3N (2.10 mL, 15.2 mmol) was added. Triethylsilyl trifluoromethanesulfonate (2.6 mL, 11.4 mmol) was added neat at rt, under Ar. The resulting solution darkened upon subsequent heating to reflux. After 4 h at reflux, the reaction mixture was allowed to cool to rt and ice-water and ether were added. The resulting organic layer was successively washed with ice-water (1×), aqueous $CuSO_4$ (1×) and brine (1×). Drying and concentration afforded a brown oil which was purified by flash chromatography (hexane, 1:5 EtOAc/hexane, v/v). The silyl enol ether **7** was obtained as a yellow oil (4.22 g, 81%): ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 5 H), 6.51 (s, 1 H), 4.87 (d, $J = 7$ Hz, 1 H), 4.81 (d, $J = 7$ Hz, 1

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H), 4.69 (d, J = 12 Hz, 1 H), 4.54 (d, J = 12 Hz, 1 H), 3.96 (d, J = 8 Hz, 1 H), 3.65–3.80 (m, 1 H), 3.72 (s, 3 H), 2.96 (d, J = 14 Hz, 1 H), 2.20–2.40 (m, 2 H), 2.00 (dd, J = 13, 10 Hz, 1 H), 0.80–1.05 (m, 27 H), 0.66 (q, J = 8 Hz, 6 H), 0.11 (s, 3 H), 0.10 (s, 6 H), 0.08 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.4, 138.1, 136.7, 128.3, 128.2, 127.7, 127.4, 126.2, 115.0, 95.0, 78.3, 76.7, 75.0, 69.1, 51.8, 40.5, 33.7, 25.9, 25.8, 18.6, 18.1, 6.5, 4.5, −3.4, −3.6, −4.7, −5.0; MS m/z (rel inten) EI 91 (100); CI 681 (M + H^+, 3), 519 (100); HRMS (CI) calcd for $C_{35}H_{64}O_7Si_3$ (M + H^+) 681.4038, found 681.4052. Anal. Calcd for $C_{35}H_{64}O_7Si_3$: C, 61.71; H, 9.47. Found: C, 61.79; H, 9.44.

Methyl [1(*S*)-(1α,3β,4α)]-3-[(Benzyloxymethyl)oxy]-1,4-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5,5-bis(hydroxymethyl)cyclohexane-1-carboxylate (8). Silyl enol ether **7** (4.06 g, 5.96 mmol) was dissolved in THF (45 mL) and aqueous formaldehyde (37 wt.%, 15 mL) was added. Ytterbium triflate (0.6 g), prepared[8a] from Yb_2O_3 and dried at rt, was added and the resulting solution was heated to reflux. Heating was stopped when the starting material had completely disappeared by TLC (typically 24–48 h). The reaction mixture was partitioned between ether and water, and the resulting organic layer was washed with brine (1×). The organic phase was dried and concentrated to a yellow oil which was dissolved in CH_3OH (100 mL). The solution was cooled to 0 °C and $NaBH_4$ (5 g) was added in portions. After 1 h at 0 °C, acetone (10 mL) was added and the resulting mixture was concentrated to a thick cloudy syrup. The residue was partitioned between water and EtOAc. The aqueous layer was saturated with NaCl and extracted again (3×) with EtOAc. The combined organic layers were dried and concentrated to a slightly yellow oil. Purification by flash chromatography (1:5 EtOAc/hexane, 1:1 EtOAc/hexane, v/v) afforded the diol **8** as a clear oil (2.33 g, 65%): ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 5 H), 4.84 (d, J = 7 Hz, 1 H), 4.78 (d, J = 7 Hz, 1 H), 4.72 (d, J = 12 Hz, 1 H), 4.50 (d, J = 12 Hz, 1 H), 4.01 (dd, J = 12, 4 Hz, 1 H), 3.92 (ddd, J = 11, 9, 4 Hz, 1 H), 3.65–3.80 (m, 3 H), 3.68 (s, 3 H), 3.58 (d, J = 11 Hz, 1 H), 2.94 (br, 1 H), 2.59 (br, 1 H), 2.45 (ddd, J = 14, 4, 3 Hz, 1 H), 2.14 (dd, J = 14, 11 Hz, 1 H), 1.98 (dd, J = 14, 3 Hz, 1 H), 1.71 (d, J = 14 Hz, 1 H), 0.91 (s, 9 H), 0.90 (s, 9 H), 0.13 (s, 3 H), 0.12 (s, 3 H), 0.09 (s, 3 H), −0.05 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.2, 137.5, 128.3, 127.8, 127.6, 95.6, 77.4, 77.0, 76.4, 69.3, 66.4, 65.6, 52.0, 45.7, 39.6, 38.7, 26.0, 25.8, 18.2, −3.2, −3.6, −4.4, −5.0. Anal. Calcd for $C_{30}H_{54}O_8Si_2$: C, 60.16; H, 9.09. Found: C, 60.19; H, 9.05.

[8(*S*)-(8α,10β,11α)]-3-(Benzyloxy)-10-[(benzyloxymethyl)oxy]-8-(methoxycarbonyl)-8,11-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2,4-dioxa-3-phosphaspiro[5.5]undecane 3-Oxide (9). To a solution of benzyl N,N,N′,N′-tetraethylphosphordiamidite (2.35 g, 8.33 mmol) and diol **8** (4.79 g, 8.00 mmol) in dry CH_3CN (30 mL) was added in one portion 1H-tetrazole (1.15 g, 16.0 mmol) under Ar. A mild exothermic reaction immediately took place. After 5 h at rt, the reaction mixture was cooled to −40 °C (CH_3CN/dry ice) and a solution of *m*-CPBA (100%, 1.69 g, 9.59 mmol) in CH_2Cl_2 (40 mL) was slowly added via cannula. The cold bath was then removed and the reaction mixture stirred at rt. Water and ether were added 3 h later. The organic layer was washed with aqueous $Na_2S_2O_3$ (2×), saturated aqueous $NaHCO_3$ (2×), water (1×), aqueous $CuSO_4$ (1×) and brine (1×). The organic layer was dried and concentrated to a yellow residue which was dissolved in CH_2Cl_2. Filtration through silica gel under reduced pressure and concentration afforded a light yellow oil. Hexane was added and white crystals (0.69 g, 12%) corresponding to one diastereomer formed. The filtrate was concentrated to an oil which was purified by radial chromatography (4 mm thickness, hexane, 1:5 EtOAc/hexane, 1:1 EtOAc/hexane, v/v, EtOAc) to afford a diastereomeric mixture of fully protected spirocyclic carbaphosphodiester **9** as a colorless oil (3.34 g, 56%). The crystalline diastereomer corresponded to a slower eluting radial chromatographic fraction: ^{1}H NMR ($CDCl_3$) δ 7.25–7.45 (m, 10 H), 5.10 (dd, J = 8, 2 Hz, 1 H), 4.80 (d, J = 7 Hz, 1 H), 4.69 (d, J = 7 Hz, 1 H), 4.68 (d, J = 12 Hz, 1 H), 4.59 (dd, J = 10, 2 Hz, 1 H), 4.49 (d, J = 12 Hz, 1 H), 3.60–3.80 (m, 3 H), 3.72 (s, 3 H), 3.34 (d, J = 9 Hz, 1 H), 2.76 (dd, J = 15, 3 Hz, 1 H), 2.46 (ddd, J = 14, 4, 3 Hz, 1 H), 2.07 (dd, J = 14, 11 Hz, 1 H), 1.41 (d, J = 15 Hz, 1 H), 0.88 (s, 9 H), 0.81 (s, 9 H), 0.07 (s, 3 H), 0.04 (s, 3 H), 0.00 (s, 3 H), −0.02 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.3, 137.3, 135.4 (J_{POCC} = 7 Hz), 128.5, 128.4, 128.3, 128.2, 127.7, 127.6, 95.2, 77.1 (J_{POC} = 7 Hz), 76.4, 75.7, 74.9, 70.9 (J_{POC} = 7 Hz), 69.4, 68.8 (J_{POC} = 5 Hz), 52.0, 41.1 (J_{POCC} = 5 Hz), 39.6, 37.3, 25.9, 25.6, 18.2, 18.0, −3.3, −3.4, −4.5, −5.1; MS m/z (rel inten) FAB 751 (M + H^+, 15), 693 (100); HRMS (FAB) calc for $C_{37}H_{59}O_{10}PSi_2$ (M + H^+) 751.3465, found 751.3459. A small amount of the second, pure diastereomer was isolated as an oil from the faster eluting radial chromatographic fraction: ^{1}H NMR ($CDCl_3$) δ 7.25–7.50 (m, 10 H), 5.13 (d, J = 7 Hz, 2 H), 4.30–4.80 (m, 6 H), 3.90–4.20 (m, 4 H), 3.68 (s, 3 H), 2.53 (dd, J = 14, 3 Hz, 1 H), 2.01 (dd, J = 14, 3 Hz, 1 H), 1.81 (d, J = 13 Hz, 1 H), 1.50 (d, J = 13 Hz, 1 H), 0.91 (s, 9 H), 0.82 (s, 9 H), 0.15 (s, 3 H), 0.08 (s, 3 H), 0.06 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 172.5, 137.1, 135.6 (J_{POCC} = 7 Hz), 128.4 (2), 128.3 (2), 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 92.3, 74.9 (J_{POC} = 7 Hz), 74.5, 74.1, 73.7, 69.5, 68.7 (J_{POC} = 5 Hz), 65.7, 51.8, 40.2 (J_{POCC} = 5 Hz), 34.7, 34.0, 25.6, 25.3, 17.8 (2), −3.2, −3.4, −4.9, −5.3; MS m/z (rel inten) CI 751 (M + H^+, 15), 693 (100); HRMS (CI) calcd for $C_{37}H_{59}O_{10}PSi_2$ (M + H^+) 751.3465, found 751.3461.

Anal. (Mixture of diastereomers) Calcd for $C_{37}H_{59}O_{10}PSi_2$: C, 59.17; H, 7.92. Found: C, 59.01; H, 7.99.

[8(*S*)-(8α,10β,11α)]-8-Carboxy-3,8,10,11-tetrahydroxy-2,4-dioxa-3-phosphaspiro[5.5]undecane 3-Oxide (1). The fully protected spirocyclic carbaphosphodiester **9** (0.27 g, 0.36 mmol) was dissolved in THF (10 mL) and a solution of $Bu_4N^+F^-$ in THF (1.0 M, 1.80 mL, 1.80 mmol) was added at 0 °C. After 10 min the ice-bath was removed and the solution was stirred at rt for 10 min. Water and EtOAc were added and the aqueous layer was reextracted with EtOAc (2×). The combined organic layers were dried and concentrated to a yellow oil. Purification by radial chromatography (2 mm thickness, 1:1 EtOAc/hexane, v/v, EtOAc) afforded the corresponding diol as a colorless oil (0.12 g, 62%, combined yield) of a faster eluting diastereomer: ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 10 H), 5.08 (d, J = 8 Hz, 2 H), 4.50–5.00 (m, 6 H), 4.00 (s, 1 H), 3.65–3.90 (m, 2 H), 3.74 (s, 3 H), 3.27 (d, J = 9 Hz, 1 H), 2.57 (d, J = 15 Hz, 1 H), 2.10–2.25 (m, 1 H), 1.85 (dd, J = 12, 12 Hz, 1 H), 1.62 (d, J = 15 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 175.1, 136.8, 135.5 (J_{POCC} = 6 Hz), 128.4, 128.3, 127.8, 127.7, 94.9, 77.6 (J_{POC} = 7 Hz), 76.6, 73.9, 73.7, 70.6 (J_{POC} = 7 Hz), 69.8, 68.5 (J_{POC} = 6 Hz), 53.0, 40.3 (J_{POCC} = 6 Hz), 39.0, 35.4 and a slower eluting diastereomer: ^{1}H NMR ($CDCl_3$) δ 7.25–7.40 (m, 10 H), 5.12 (d, J = 12 Hz, 2 H), 4.55–5.00 (m, 6 H), 3.60–3.95 (m, 2 H), 3.77 (s, 3 H), 3.55 (br, 1 H), 3.35 (d, J = 14 Hz, 1 H), 2.10–2.25 (m, 2 H), 1.83 (dd, J = 18, 18 Hz, 1 H), 1.62 (d, J = 18 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 175.3, 137.0, 135.5 (J_{POCC} = 6 Hz), 128.5, 127.8, 94.9, 77.6, 77.0, 76.4, 74.5, 73.9, 70.3 (J_{POC} = 7 Hz), 69.8 (J_{POC} = 7 Hz), 53.2, 40.8 (J_{POCC} = 6 Hz), 39.3, 36.7.

The mixture of diol diastereomers was dissolved in THF/water (2:1 v/v, 8 mL) and hydrogenolyzed over 10% Pd on C (0.057 g) at 50 psi H_2 pressure for 1 h. The suspension was filtered through Celite which was subsequently washed with 50% aqueous THF followed by water. The aqueous layer was washed with CH_2Cl_2 (2×) and EtOAc (1×). Concentration *in vacuo* afforded a colorless crystalline solid: ^{1}H NMR (D_2O) δ 4.45–4.75 (m, 3 H), 3.70–3.95 (m, 2 H), 3.77 (s, 3 H), 3.34 (d, J = 9 Hz, 1 H), 2.44 (d, J = 15 Hz, 1 H), 2.16 (d, J = 14 Hz, 1 H), 1.89 (dd, J = 14, 14 Hz, 1 H), 1.76 (d, J = 15 Hz, 1 H). Aqueous NaOH (1 N, 5 mL) was added and the resulting solution was stirred at rt for 11 h. Dowex 50 (H^+) was added and the suspension was loaded onto a short column of Dowex 50 (H^+). The colorless filtrate was concentrated to dryness to afford spirocyclic carbaphosphodiester **1** as a colorless film (43%): ^{1}H NMR (D_2O) δ 4.42–4.70 (m, 3 H), 3.74–3.98 (m, 2 H), 3.36 (d, J = 9 Hz, 1 H), 2.47 (dd, J = 15, 3 Hz, 1 H), 2.18 (dd, J = 14, 3 Hz, 1 H), 1.91 (dd, J = 14, 14 Hz, 1 H), 1.78 (d, J = 15 Hz, 1 H); ^{13}C NMR (D_2O) δ 180.9, 78.5 (J_{POC} = 6 Hz), 77.9, 77.2, 71.8 (J_{POC} = 6 Hz), 69.3, 43.7 (J_{POCC} = 6 Hz), 42.7, 38.0; MS m/z (rel inten) FAB 321 (M + Na^+, 100), 299 (M + H^+, 50); HRMS (FAB) calcd for $C_9H_{15}O_9P$ (M + H^+) 299.0532, found 299.0525.

[8(*S*)-(8α,10β,11α)]-8-carboxy-3,8,10-trihydroxy-2,4-di-

oxa-3-phosphaspiro[5.5]undecan-11-one 3-Oxide (2). To oxalyl chloride (2.0 M in CH_2Cl_2, 1.20 mL, 2.40 mmol) in CH_2Cl_2 (10 mL) was added DMSO (0.20 mL, 2.7 mmol) at −78 °C under Ar. After 5 min, a CH_2Cl_2 (10 mL) solution of intermediate diol (0.779 g, 1.04 mmol) obtained from the aforementioned treatment of spirocyclic carbaphosphodiester **9** with $Bu_4N^+F^-$ in THF was cannulated into the reaction mixture. After 30 min at −78 °C, Et_3N (0.50 mL, 3.75 mmol) was added, and after an additional 30 min, the cold bath was removed. The reaction was quenched with water. Ether was then added and the organic layer washed successively with aqueous HCl (1 N, 1×), water (1×) and brine (1×). Drying and concentration afforded a yellow oil. Purification by radial chromatography (4 mm thickness, hexane, 1:5 EtOAc/hexane, 1:1 EtOAc/hexane, v/v, EtOAc) yielded a light yellow oil (0.25 g, 45%). This intermediate was dissolved in THF (7 mL) and aqueous NaOH (1 N, 0.7 mL) was added at 0 °C. The resulting homogeneous solution was stirred for 40 min. Acidification with aqueous HCl (1 N, 2 mL) and addition of saturated aqueous NaCl resulted in separation of the phases. Saturation with NaCl and extraction with EtOAc (3×) followed by drying and concentration afforded a yellow oil. This was dissolved in THF/H_2O (4:1, v/v, 10 mL) and was hydrogenolyzed over 10% Pd on C (0.120 g) at 50 psi H_2 pressure. After 3 h, the suspension was filtered through Celite. The aqueous layer was washed with EtOAc (3×) and was then concentrated *in vacuo* to yield the spirocyclic ketocarbaphosphodiester **2** as a colorless film (0.055 g, 40%): ^1H NMR (D_2O) δ 4.96 (dd, J = 13, 6 Hz, 1 H), 4.40−4.60 (m, 3 H), 3.91 (dd, J = 12, 7 Hz, 1 H), 2.48 (ddd, J = 13, 6, 3 Hz, 1 H), 2.21 (dd, J = 13, 13 Hz, 1 H), 2.16 (dd, J = 15, 3 Hz, 1 H), 2.12 (d, J = 15 Hz, 1 H); ^{13}C NMR (D_2O) δ 216.9, 182.7, 79.7, 78.1 (J_{POC} = 6 Hz), 76.8 (J_{POC} = 7 Hz), 74.8, 55.0, 49.2, 44.7. MS m/z (rel inten) FAB 319 (M + Na^+, 40), 297 (M + H^+, 59); HRMS (FAB) calcd for $C_9H_{14}O_6P$ (M + H^+) 297.0376, found 297.0371.

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