Synthesis and Biological Evaluation of a Phosphonate Analog of the Natural Acetyl Cholinesterase Inhibitor Cyclophostin

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · OCTOBER 2008

Impact Factor: 4.72 · DOI: 10.1021/jo801453v · Source: PubMed

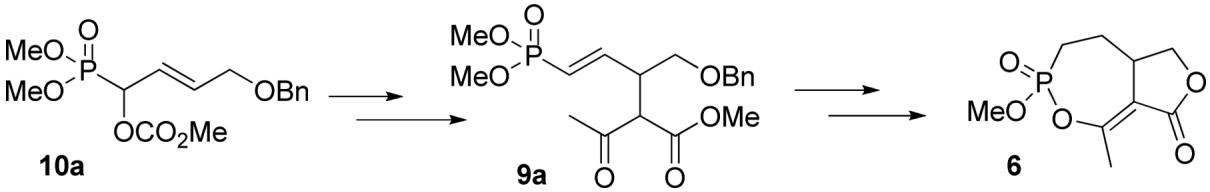
Published in final edited form as:
J Org Chem. 2008 November 7; 73(21): 8386–8391. doi:10.1021/jo801453v.

Synthesis and Biological Evaluation of a Phosphonate Analog of the Natural Acetyl Cholinesterase Inhibitor Cyclophostin

Saibal Bandyopadhyay, **Supratik Dutta**[†], **Christopher D. Spilling**, **Cynthia M. Dupureur**[†], and **Nigam P. Rath**[†]

[†]Department of Chemistry and Biochemistry and Center for Nanoscience, University of Missouri-St. Louis, One University Boulevard, St. Louis, MO 63121, USA

Abstract



Two diastereomers of a phosphonate analog **6** of the AChE inhibitor cyclophostin were synthesized. The substitution reaction of phosphono allylic carbonate **10a** with methyl acetoacetate gave the vinyl phosphonate **9a**. Attempted hydrogenation/debenzylation gave an unexpected enolether lactone. Alternatively, selective hydrogenation, demethylation, cyclization and debenzylation gave the phosphonate analog of cyclophostin as a separable mixture of diastereomers **6**. The *trans* phosphonate isomer was more active than *cis* isomer against AChE from two sources.

Introduction

Cyclophostin **1**, a novel bicyclic organophosphate, was isolated from a fermentation solution of *Streptomyces lavendulae* (strain NK901093) during a search for natural insecticides.[1] The natural product **1** showed potent inhibition of acetyl cholinesterase (AChE) from housefly (CSMA strain) and the brown plant hopper with reported IC_{50} of 7.6×10^{-10} M. The structure of cyclophostin was first assigned by spectroscopic methods and then confirmed by single crystal X-ray diffraction studies as a bicyclic structure with a seven-membered cyclic enol-phosphate triester fused to a butyrolactone ring. There are chiral centers at both C3a and the phosphorus atom. The absolute configurations of the chiral centers were determined to be 3a*R*, 6*S* by the anomalous scattering method.

The unusual bicylic enolphosphate is found in some related natural compounds **2** and **3** and the enolphosphate moiety adjacent to a carbonyl is also found in the synthetic insecticides monocrotphos **4** and phosphamidan **5**.[2-4] The unnamed tetrahydrofuran fused enolphosphates **2a** and **2b** were isolated during an earlier search for insecticides and were shown to be AChE inhibitors.[3] The cyclipostins **3** posses a core structure similar to that of cyclophostin, but differ in the phosphate ester.[4] The cyclipostins **3** are phosphate esters of long chain lypophilic alcohols of various lengths and structures and all are potent inhibitors of hormone sensitive lipase.[4]

cspill@umsl.edu.

Supporting Information **Available**. General experimental, ^{1}H and ^{13}C NMR spectra for compounds 10a, 10b, 9a, 9b, 9c, 9d, 14a, 14b, 15, 16, 8, 8a, 6b, and **6a.** X-Ray data for compounds **6b**. This material is available free of charge via the internet at http://pubs.acs.org.

AChE has been identified as a therapeutic target for myasthenia gravis,[5] glaucoma[6] and Alzheimer's disease[7] and is well known as the target for insecticides and “nerve gas” chemical warfare agents. The exact mode of inhibition of AChE by cyclophostin has not been reported. Since other phosphate inhibitors of AChE are known to form a covalent bond between the phosphorus and the serine residue of enzyme active site, it is likely that the mode of inhibition by cyclophostin involves similar kind of interaction (Figure 2). It is also probable that the enolphosphate acts as a leaving group on reaction with the active site serine.

Phosphonate analogs of biologically active phosphates have been shown to be an extremely useful tool in investigating mechanistic detail of various enzymatic systems.[8] This success is usually attributed to the non-hydrolyzability of a P-C bond (phosphonate analog) when compared to the P-O bond of the corresponding phosphate leading to enhanced compound lifetime in vivo. It should be possible to replace the non-critical oxygen at position 5 in cyclophostin (and the cyclipostins) with a methylene and still retain the AChE inhibitory activity (Figure 2), whereas loss of the enol oxygen (position 7) should eliminate activity. Herein, we report the synthesis of the first phosphonate analog **6** of cyclophostin.

Results and Discussion

A retrosynthetic analysis of the bicyclic phosphonate **6** suggested that either the lactone or the enol phosphonate bond could be formed first giving rise to intermediates **7** and **8**, respectively. The common intermediate **9** could be formed *via* a palladium catalyzed substitution reaction of the carbonate derivative **10** of an allylic hydroxy phosphonate with an acetoacetate ester. We reported the use of a similar strategy for the synthesis of the lignan enterolactone.[9]

It is now well established that allylic hydroxy phosphonate derivatives can be used as intermediates in the synthesis of γ-substituted vinyl phosphonates by 1,3-transposition of functionality.[10-12] Following the original work of Zhu and Lu,[11] we reported that the facile addition of soft nucleophiles to optically pure carbonate derivatives proceeded with complete transfer of chirality.[9,12]

4-Benzyloxy- and 4-(*tert*-butyldimethyl)silyloxy- *cis*-2-buten-1-ol **11a** and **11b** were oxidized using PCC in CH_2Cl_2 to give the known aldehydes **12a** and **12b**, respectively.[13] The Et_3N catalyzed Pudovik reaction of the α,β-unsaturated aldehydes with dimethyl phosphite gave the racemic hydroxy phosphonates **13a** and **13b**, which were converted into the corresponding carbonate derivatives **10a** and **10b** by reaction with methyl chloroformate in pyridine.

The palladium catalyzed substitution reaction of phosphono allylic carbonates **10a** and **10b** with acetoacetates and malonates was investigated. The reaction of methyl acetoacetate with phosphonate **10a** gave the vinyl phosphonate **9a** in 85% yield (Table 1). The formation of the vinyl phosphonate **9a** was always accompanied by diene formation, especially after prolonged reaction times. However, the use of freshly distilled methyl acetoacetate and careful monitoring of the reaction (^{31}P NMR spectroscopy) minimized diene formation. The phosphonate **10b** reacted with methyl acetoacetate similarly to give the vinyl phosphonate **9c** in 64% yield. The reaction of *tert*-butyl acetoacetate with phosphonate **10a** was slower than with the methyl acetoacetate, and a moderate amount of diene formation was always observed. The palladium (0) catalyzed malonate susbstitution reaction of phosphono allylic carbonate **10a** was again comparatively slow and required the presence of a weak base like BSA. In each case, the products **9a-d** were formed as a mixture of diastereomers.

The attempted concomitant hydrogenation and debenzylation of vinyl phosphonate **9a** with hydrogen over palladium on carbon unexpectedly gave the lactone methylenolether **14a** independent of the solvent used. More surprisingly, a similar product was also observed with *t*-butyl acetoacetate substituted vinyl phosphonate **9b** giving the *t*-butyl enolether substituted

butyrolactone **14b** in quantitative yield. The TBS protected phosphonate **9c** was uneventfully hydrogenated to give the saturated phosphonate **15**. Attempted deprotection and lactonization of phosphonate **15** withHF/Py also produced the lactone methylenolether **14a**. All attempts to cleave either the methyl or *t*-butyl enolether failed to produce desired 2-acetyl butyrolactone system.

An alternate strategy (Scheme 4) involving the construction of the seven membered enolphosphonate ring prior to the butyrolactone was pursued. Selective hydrogenation of vinyl phosphonate **9a** using hydrogen over palladium on carbon poisoned with pyridine cleanly generated methyl acetoacetate substituted saturated phosphonate **16**. The phosphonate was selectively mono-demethylated using one equivalent of sodium iodide in refluxing acetonitrile. The sodium salt was protonated with Amberlite® resin (IR 120) to generate the corresponding phosphonic acid **17**. Enol phosphonate ring formation was successfully achieved by reaction of the phosphonic acid **17** with EDC, HOBt and Hunig's base in CH_2Cl_2 giving monocyclic enolphosphonate **8** as a 1:1.4 mixture of diastereomers. Finally, selective debenzylation of **8** with hydrogen over palladium on carbon resulted in clean hydrogenolysis to give the primary alcohol which cyclized to the butyrolactone without over reduction of enolphosphonate. The phosphonate analogue of cyclophostin **6** was obtained as a mixture of two diastereoisomers having characteristic peaks in the ^{31}P NMR spectrum at 21 (6a) and 25 (6b) ppm. Mixture of diastereomers was separated using silica gel chromatography. The diastereoisomer **6b** is a crystalline solid and was further purified by crystallization from EtOAc and hexane. An X-ray crystal structure (Figure 3) showed the relative stereochemistry of the C3a and the phosphorus atom to be the same as the natural product (H and methoxy are *cis*).

In order to examine the effect of the bicylic ring structure on the AChE activity, a simple enolphosphate analog **19** was prepared for comparison (Scheme 5). Phosphorylation of 2-acetyl butyrolactone **18** was accomplished by reaction with dimethyl chlorophosphate using a reported procedure.[14] Alternatively, the enol phosphate **19** was formed as a mixture of geometrical isomer by reaction of 2-acetyl butyrolactone **18** with dimethyl phosphoric acid and DCC.

The new compounds were examined for inhibitory activity against AChE from human and electric eel using an Elman assay.[15] Surprisingly, the phosphonate analog **6a** with the “unnatural” relative stereochemistry (Table 2, Entry 1) showed the most potent activity against both human and electric eel acetylcholinesterase (AChE). Interestingly, compound **6a** is more effective against AChE from human than eel. This type of species dependent difference in inhibition has been observed with phosphate inhibitors like soman, tabun, and VX.[16] The phosphonate analog **6b** with natural relative stereochemistry was 10 fold less potent against AChE from both sources when compared to the diastereomer **6a** (Table 2, Entry 2). However, the phosphonate analogs were significantly less potent than the natural product cyclophostin **1** (reported for insect AchE only). The fused butyrolactone did not appear to be necessary for activity since the monocyclic diastereomers **8a** and **8b** (Table 2, Entries 3 and 4) showed IC_{50} similar to those of the bicycles **6a** and **6b**. However, the more active monocycle **8b** had the opposite relative stereochemistry to the more active bicycle **6a**. Furthermore, the simple enolphosphate derivative **19** of acetyl butyrolactone was inactive (Table 2, Entry 5). Not surprisingly the methyl enolether **14a** was also inactive (Table 2, Entry 6).

In summary, two diastereomers of a phosphonate analog of cyclophostin were synthesized. The *trans* isomer was more active than *cis* isomer against AChE from two sources. Since the natural product has the *cis* configuration, the unnatural isomer may well prove more potent. We are currently pursuing a synthesis of both isomers of the natural product.

Experimental section

Dimethyl [1-(methoxycarbonyloxy)-4-(benzyloxy)-2-butenyl]phosphonate 10a

To a mixture of dimethyl phosphite (8.2 mL, 89 mmol) and aldehyde **12a**[13a] (9.2 g, 52 mmol) was added Et_3N (3.1 mL, 22 mmol). The reaction mixture was stirred overnight and then the volatiles were evaporated in *vacuo* to give crude hydroxy phosphonate **13a** (16.9 g). The crude hydroxy phosphonate **13a** (16.9 g, 59.1 mmol) was dissolved in anhydrous CH_2Cl_2 (60 mL) and the solution was cooled to 0 °C. Pyridine (7.6 mL, 88 mmol) and DMAP (0.10 g, 0.8 mmol) were added to the solution, followed by the slow addition of methyl chloroformate (9.1 mL, 120 mmol). After the complete addition of methyl chloroformate, the reaction mixture was slowly allowed to warm up to room temperature and then it was stirred until the reaction was complete (TLC, 24 h). The reaction mixture was washed with H_2O (2x) and saturated $CuSO_4$ (2x), and then the organic layer was dried over $MgSO_4$. The solvent was evaporated in *vacuo* and the crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 60 : 40) to give **10a** as a colorless oil (10.4 g, 61% in two steps). IR (neat) 1758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.31−7.36 (5H, m), 6.09 (1H, m), 5.89 (1H, m), 5.55 (1H, ddd, J_{HH} = 1.1, 6.6 Hz, J_{HP} = 14 Hz), 4.52 (2H, s), 4.08 (2H, m), 3.84 (3H, s), 3.83 (6H, d, J_{HP} = 10.7 Hz); ^{13}C NMR ($CDCl_3$) δ 154.9 (d, J_{CP} = 9.3 Hz), 138.2, 133.1 (d, J_{CP} = 12 Hz), 128.6, 127.95, 127.92, 122.8 (d, J_{CP} = 3.9 Hz), 72.5 (d, J_{CP} = 169 Hz), 72.6, 69.5 (d, J_{CP} = 1.5 Hz), 55.7, 54.2 (d, J_{CP} = 7.0 Hz), 54.0 (d, J_{CP} = 6.5 Hz); ^{31}P NMR ($CDCl_3$) δ 19.7; HRMS (FAB, *NBA*, MH^+) calcd. for $C_{15}H_{22}O_7P$: 345.1103. Found. 345.1108; Anal. Calcd for $C_{15}H_{21}O_7P$: C, 52.33; H, 6.15. Found: C, 52.35; H, 6.02.

Dimethyl [1-(methoxycarbonyloxy)-4-(t-butyldimethylsilyloxy)-2-butenyl] phosphonate 10b

To a mixture of dimethyl phosphite (3.8 mL, 41 mmol) and aldehyde **12b**[13b] (4.73 g, 23.6 mmol) was added Et_3N (1.5 mL, 11 mmol). The reaction mixture was stirred overnight then the volatiles were evaporated in *vacuo* to give the crude hydroxy phosphonate **13b** (6.6 g). The crude hydroxyphosphonate **13b** (6.6 g, 21 mmol) was dissolved in anhydrous CH_2Cl_2 (60 mL) and the solution was cooled to 0 °C. Pyridine (2.9 mL, 36 mmol) and DMAP (0.41 g, 3.4 mmol) were added to the solution, followed by the slow addition of methyl chloroformate (3.74 mL, 48.4 mmol). After the addition of methyl chloroformate was complete, the reaction mixture was slowly allowed to warm up to room temperature then it was stirred until the reaction was complete (TLC, 24 h). The reaction mixture was washed with H_2O (2x) and saturated $CuSO_4$ (2x), and then the organic layer was dried over $MgSO_4$. The solvent was evaporated in *vacuo* and the crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 60 : 40) to give **10b** as a colorless oil (4.09 g, 46 % in two steps). IR (neat) 1757 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.04 (1H, m), 5.85 (1H, m), 5.54 (1H, ddd, J_{HH} = 1.2, 7.1 Hz, J_{HP} = 13.4 Hz), 4.23 (2H, m), 3.83 (3H, s), 3.82 (6H, d, J_{HP} = 10.7 Hz), 0.91 (9H, s), 0.07 (6H, s); ^{13}C NMR ($CDCl_3$) δ 154.7 (d, J_{CP} = 9.3 Hz), 136.0 (d, J_{CP} = 10 Hz), 119.8 (d, J_{CP} = 3.4 Hz), 71.0 (d, J_{CP} = 169 Hz), 62.6, 55.3, 53.8 (d, J_{CP} = 6.8 Hz), 25.9, 18.3, −5.4; ^{31}P NMR ($CDCl_3$) δ 20.0; HRMS (FAB, *NBA*, MNa^+) calcd. for $C_{14}H_{29}O_7PSiNa$: 391.1319. Found. 391.1321.

(*E*)-methyl 2-acetyl-3-(benzyloxymethyl)-5-(dimethoxyphosphoryl)pent-4-enoate 9a

$Pd_2(dba)_3$ (0.049 g, 0.053 mmol) and dppe (0.063 g, 0.16 mmol) were dissolved in anhydrous THF (15 mL). The reaction mixture was stirred at room temperature for 3−4 minutes under argon. Freshly distilled methyl acetoacetate (0.58 mL, 5.4 mmol) was added followed by a solution of phosphonate **10a** (0.93 g, 2.7 mmol) in anhydrous THF (5 mL). The resulting reaction mixture was heated at 70 °C in a preheated oil bath for 2.5 h. The reaction mixture was allowed to cool to room temperature and was then partitioned between brine and Et_2O. After separation, the aqueous layer was re-extracted with Et_2O and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo* and the crude product

was purified by chromatography (SiO_2, EtOAc : Hexane, 60 : 40) to give of **9a** as a colorless oil (1.2:1 mixture of diastereomers, 0.89 g, 86%). IR (neat) 1742, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (5H, m), 6.73 (1H, m), 5.75 (1H, m), 4.43 (2H, m), 3.87 (1H, dd, J_{HH} = 3.5, 8.9 Hz), 3.67 (9H, m), 3.56 (1H, m), 3.48 (1H, m), 3.31 (1H, m), 2.22 (1.5H, s), 2.21 (1.5H, s); ^{13}C NMR ($CDCl_3$) δ 201.3, 201.1, 168.4, 168.3, 150.8 (d, J_{CP} = 3.9 Hz), 150.7 (d, J_{CP} = 4.8 Hz), 137.7, 137.6, 128.5, 128.4, 127.9, 127.81, 127.78, 119.1 (d, J_{CP} = 185 Hz), 118.9 (d, J_{CP} = 185 Hz), 73.7, 70.0, 69.8, 59.9, 59.8, 52.6, 52.5 (d, J_{CP} = 6.9 Hz), 52.4 (d, J_{CP} = 5.6 Hz), 43.6 (d, J_{CP} = 22 Hz), 43.5 (d, J_{CP} = 22 Hz), 30.7, 30.6; ^{31}P NMR ($CDCl_3$) δ 20.6, 20.5 ppm; HRMS (EI, MH^+) calcd. for $C_{18}H_{26}O_7P$: 385.1416. Found. 385.1418.

(*E*)-*tert*-butyl 2-acetyl-3-(benzyloxymethyl)-5-(dimethoxyphosphoryl)pent-4-enoate 9b

$Pd_2(dba)_3$ (0.13 g, 0.14 mmol) and dppe (0.17 g, 0.43 mmol) were dissolved in anhydrous THF (15 mL). The reaction mixture was stirred at room temperature for 3−4 minutes under argon. *tert*-Butyl acetoacetate (0.96 mL, 5.8 mmol) was added followed by a solution of phosphonate **10a** (0.50 g, 1.5 mmol) in anhydrous THF (5 mL). The resulting reaction mixture was heated at 70 °C in a preheated oil bath for 2 h. The reaction mixture was allowed to cool to room temperature and was then partitioned between brine and Et_2O. After separation, the aqueous layer was re-extracted with Et_2O and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated in *vacuo* and the crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 60 : 40) to give **9b** as a colorless oil (1.4:1 mixture of diastereomers, 0.38 g, 62%). IR (neat) 1736, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.29 (5H, m), 6.74 (1H, m) 5.74 (1H, m), 4.45 (2H, m), 3.78 (2H, m), 3.69 (6H, d, J_{HP} = 12 Hz), 3.48 (1H, m), 3.26 (1H, m), 2.20 (3H, s), 1.43 (9H, s); ^{13}C NMR ($CDCl_3$) δ 201.81, 201.76, 167.2, 167.0, 151.5 (d, J_{CP} = 4.9 Hz), 151.3 (d, J_{CP} = 5.0 Hz), 138.0, 137.8, 128.6, 128.0, 127.94, 127.87, 118.8 (d, J_{CP} = 185 Hz), 118.7 (d, J_{CP} = 186 Hz), 82.8, 73.5, 70.2, 61.2, 61.0, 52.5 (d, J_{CP} = 5.7 Hz), 43.9 (d, J_{CP} = 22 Hz), 43.6 (d, J_{CP} = 22 Hz) 30.3, 30.1, 28.03, 28.01; ^{31}P NMR ($CDCl_3$) δ 20.80, 20.76; HRMS (FAB, NBA, MH^+ calcd. for $C_{21}H_{31}O_7P$ 426.1876. Found. 371.1323 (M-*tert*-Bu); Anal. Calcd for $C_{21}H_{31}O_7P{\cdot}H_2O$: C, 58.32; H, 3.38. Found: C, 58.32; H, 3.37.

(*E*)-methyl 2-acetyl-3-((*tert*-butyldimethylsilyloxy)methyl)-5-(dimethoxyphosphoryl)pent-4-enoate 9c

$Pd_2(dba)_3$ (0.09 g, 0.09 mmol) and dppe (0.11 g, 0.28 mmol) were dissolved in anhydrous THF (20 mL). The reaction mixture was stirred at room temperature for 3−4 minutes under argon. Freshly distilled methyl acetoacetate (0.82 mL, 7.6 mmol) was added followed by a solution of phosphonate **10b** (1.4 g, 3.8 mmol) in anhydrous THF (5 mL). The resulting reaction mixture was heated at 70 °C in a preheated oil bath for 2 h. The reaction mixture was allowed to cool to room temperature and was then partitioned between brine and Et_2O. After separation, the aqueous layer was re-extracted with Et_2O and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated in *vacuo* and the crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 60 : 40) to give **9c** as a colorless oil (1.2:1 mixture of diastereomers, 0.99 g, 64%). IR (neat) 1740, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.71 (1H, m), 5.65 (1H, m), 3.88 (1H, dd, J_{HH} = 4.2, 9.0 Hz), 3.70 (10H, m), 3.60 (1H, m), 3.17 (1H, m), 2.27 (1.5H, s), 2.22 (1.5H, s), 0.87 (9H, s), 0.02 (6H, s); ^{13}C NMR ($CDCl_3$) δ 201.7, 201.5, 168.7, 168.6, 151.0 (d, J_{CP} = 4.8 Hz), 119.3 (d,J_{CP} = 185 Hz), 119.0 (d, J_{CP} = 185 Hz), 63.4, 63.3, 59.6, 59.5, 52.7 (d, J_{CP} = 5.4 Hz), 52.6 (d, J_{CP} = 5.1 Hz), 52.5, 45.9 (d, J_{CP}= 21 Hz), 45.7 (d, J_{CP}= 21 Hz), 30.6, 30.4, 26.0, 18.5, −5.4; ^{31}P NMR ($CDCl_3$) δ 20.6, 20.5; HRMS (FAB, NBA, MH^+) calcd. for $C_{17}H_{34}O_7PSi$: 409.1812. Found. 409.1817.

(*E*)-1-*tert*-butyl 3-methyl 2-(1-(benzyloxy)-4-(dimethoxyphosphoryl)but-3-en-2-yl)malonate 9d

$Pd_2(dba)_3$ (0.21 g, 0.23 mmol) and dppe (0.27 g, 0.68 mmol) were dissolved in anhydrous THF (30 mL). The reaction mixture was stirred at room temperature for 3−4 minutes under argon. A mixture of *tert*-Butyl methyl malonate (3.4 mL, 20 mmol) and BSA (4.85 mL, 19.8 mmol) in THF (5 mL) was added followed by a solution of phosphonate **10a** (3.08 g, 8.91 mmol) in anhydrous THF (5 mL). The resulting reaction mixture was heated at 70 °C in a preheated oil bath for 6 h. The reaction mixture was allowed to cool to room temperature and was then partitioned between brine and Et_2O. After separation, the aqueous layer was re-extracted with Et_2O and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated in *vacuo* and the crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 70 : 30) to give **9d** as a colorless oil (unresolved mixture of diastereomers, 3.21 g, 81%). IR (neat) 1748, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (5H, m), 6.79 (1H, m), 5.76 (1H, m), 4.46 (2H, m), 3.79 (1H, m), 3.67 (6H, d, J_{HP} = 10.9 Hz), 3.66 (3H, s), 3.58 (2H, m), 3.22 (1H, m), 1.42 (9H, s); ^{13}C NMR ($CDCl_3$) δ 168.9, 168.7, 167.0, 166.8, 150.8 (d, J_{CP} = 5.3 Hz), 138.0, 128.60, 128.59, 127.96, 127.91, 119.0 (d, J_{CP} = 187 Hz), 82.8, 73.5, 70.2, 70.0, 53.6, 53.5, 52.59 (d, J_{CP} = 5.7 Hz), 52.57 (d, J_{CP} = 9 Hz), 44.2 (d, J_{CP} = 22 Hz) , 44.0 (d, J_{CP} = 22 Hz), 28.1; ^{31}P NMR (CDCl3) δ 20.7; HRMS (FAB, NBA, MNa^+) calcd. for $C_{21}H_{31}O_8PNa$: 465.1654. Found. 465.1653.

Dimethyl 2-(4-(1-methoxyethylidene)-5-oxotetrahydrofuran-3-yl)ethylphosphonate 14a

Vinyl phosphonate **9a** (0.4 g, 1.0 mmol) was dissolved in CH_2Cl_2 (3 mL). The reaction flask was flushed with argon for 10 minutes and then 5% Pd on C (0.10 g) was added. The resulting reaction mixture was flushed with argon for another 5 minutes and then with hydrogen. The reaction mixture was stirred under hydrogen (balloon) for 6 h and then filtered through Celite®. The Celite® was washed with CH_2Cl_2 and the solvent was evaporated in *vacuo* to give **14a** (0.28 g, 100%) as an oil. IR (neat) 1693, 1638 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.42 (1H, dd, J_{HH} = 9.6, 9.6 Hz), 4.14 (1H, dd, J_{HH} = 4.5, 9.5 Hz), 3.75 (3H, d, J_{HP} = 10.8 Hz), 3.74 (3H, d, J_{HP} = 10.8 Hz), 3.72 (3H, s), 3.26 (1H, m), 2.20 (3H, d, J_{HH} = 1.1 Hz), 1.68 (4H, m); ^{13}C NMR ($CDCl_3$) δ 170.1, 166.4, 105.2, 75.2, 52.6 (d, J_{CP} = 6.4 Hz), 51.0, 42.4 (d, J_{CP} = 18 Hz), 26.1 (d, J_{CP} = 4.5 Hz), 21.5 (d, J_{CP} = 141 Hz), 14.5; ^{31}P NMR ($CDCl_3$) δ 35.2; HRMS (EI, M^+) calcd. for $C_{11}H_{19}O_6P$: 278.0919. Found. 278.0923; Anal. Calcd for $C_{11}H_{19}O_6P{\cdot}3H_2O$: C, 46.2; H, 7.00 Found: C, 46.13; H, 7.00.

Dimethyl 2-(4-(1-*tert*-butoxyethylidene)-5-oxotetrahydrofuran-3-yl)ethylphosphonate 14b

Vinyl phosphonate **9b** (0.10 g, 0.23 mmol) was dissolved in MeOH (3 mL). The reaction flask was flushed with argon for 10 minutes. Then 5% Pd on C (0.05 g) was added and the reaction mixture was flushed with argon for another 5 minutes and then with hydrogen. The reaction mixture was stirred under hydrogen (balloon) for 6 h and then filtered through Celite®. The Celite® was washed with CH_2Cl_2 and the solvent was evaporated in *vacuo* to give **14b** (0.07 g, quantitative) as an oil. IR (neat) 1689, 1638 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.37 (1H, dd, J_{HH} = 9.7, 9.7 Hz), 4.07 (1H, dd, J_{HH} = 4.6, 9.5 Hz), 3.72 (3H, d, J_{HP} = 10.8 Hz), 3.72 (3H, d, J_{HP} = 10.8 Hz), 3.20 (1H, m), 2.15 (3H, d, J_{HH} = 1.0 Hz) 1.75 (4H, m), 1.47 (9H, s); ^{13}C NMR ($CDCl_3$) δ 168.8, 165.5, 106.5, 79.9, 74.9, 52.6 (d, J_{CP} = 6.5 Hz), 42.7 (d, J_{CP} = 18 Hz), 28.6, 26.0 (d, J_{CP} = 4.4 Hz), 21.4 (d, J_{CP} = 141 Hz), 14.5; ^{31}P NMR ($CDCl_3$) δ 35.4; HRMS (EI, M^+) calcd. for $C_{14}H_{25}O_6P$: 320.1389. Found. 320.1389.

Methyl 2-acetyl-3-(*tert*-butyldimethylsilyloxymethyl)-5-(dimethoxyphosphoryl)pentanoate 15

The vinyl phosphonate **9c** (0.3 g, 0.7 mmol)was dissolved in MeOH (3 mL) and the reaction flask was flashed with argon for 5 minutes. 10% Pd on C (0.08 g) was added and the reaction

flask was flushed with argon for another 5 minutes and then with hydrogen. The reaction mixture was stirred under hydrogen (balloon) for 3 h, and then through Celite®. The Celite® was washed with CH_2Cl_2 and the solvent was evaporated in *vacuo* to give the saturated phosphonate **15** (1:1 mixture of diastereoisomers, 0.3 g, 100%) as a colorless oil. IR (neat) 1743, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.72 (9H, m), 3.49 (3H, m), 2.34 (1H, m), 2.27 (1.5H, s), 2.26 (1.5H, s), 1.74 (4H, m), 0.88 (9H, s), 0.02 (6H, s); ^{13}C NMR ($CDCl_3$) δ 202.9, 202.8, 169.50, 169.48, 62.0, 60.9, 60.8, 52.6, 52.5 (d, J_{CP} = 6.5 Hz), 40.93 (d, J_{CP} = 17 Hz), 40.90 (d, J_{CP} = 17 Hz), 30.5, 30.1, 26.0, 22.7 (d, J_{CP} = 140 Hz), 22.6 (d, J_{CP} = 140 Hz), 22.0 (d, J_{CP} = 6.5 Hz), 21.9 (d, J_{CP} = 6.6 Hz), 18.4, −5.5 (m); ^{31}P NMR ($CDCl_3$) δ 34.6; HRMS (FAB, *NBA*, MH^+) calcd. for $C_{17}H_{36}O_7PSi$: 411.1968. Found 411.1969.

Methyl 2-acetyl-3-(benzyloxymethyl)-5-(dimethoxyphosphoryl)pentanoate 16

The vinyl phosphonate **9a** (1.5 g, 3.9 mmol) was dissolved in MeOH (3 mL) and the reaction flask was flushed with argon for 5 minutes. 5% Pd on C (0.68 g) and pyridine (0.05 mL) were added. The resulting reaction mixture was flushed with argon for another 5 minutes and then hydrogen. The mixture was stirred under hydrogen (balloon) for 6 h, and then filtered through Celite®. The Celite® was washed with CH_2Cl_2 (100 mL) and the solvent was evaporated in *vacuo* to give the saturated phosphonate **16** (1:1 mixture of diastereomers, 1.52 g, 100%). IR (neat) 1740, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (5H, m), 4.43 (1H, s), 4.42 (1H, s), 3.72 (10H, m), 3.45 (2H, m), 2.49 (1H, m), 2.25 (1.5H, s), 2.22 (1.5H, s), 1.70 (4H, m); ^{13}C NMR ($CDCl_3$) δ 202.5, 202.2, 169.11, 169.07, 137.8, 137.7, 128.1, 128.2, 127.6, 127.5, 73.0, 69.2, 69.1, 60.7, 52.2, 52.1 (d, J_{CP} = 6.6 Hz), 38.8 (d, J_{CP} = 17 Hz), 29.9, 29.7, 22.20 (d, J_{CP} = 140 Hz), 22.24 (d, J_{CP} = 4.4 Hz), 22.1 (d, J_{CP} = 140 Hz); ^{31}P NMR ($CDCl_3$) δ 34.6, 34.5; HRMS (FAB, *NBA*, MH^+) calcd. for $C_{15}H_{28}O_7P$: 387.1573. Found 387.1555.

Synthesis of monocyclic phosphonate analog 8

To a solution of phosphonate **16** (1.13 g, 2.91 mmol) in CH_3CN (1.5 mL) was added NaI (0.44 g, 2.9 mmol). The resulting mixture was heated at reflux overnight. The solvent was removed in *vacuo* to obtain the mono-sodium salt as a white solid (1.43 g, crude). To a suspension of the sodium salt in acetone (5 mL) was added Amberlite® IR 120 resin. The resulting mixture was shaken on an orbital shaker until the sodium salt completely dissolved and the color of the solution became amber. The resin was removed by filtration and washed with acetone to give the phosphonic acid **17** as an amber colored solution in acetone. The acetone was evaporated in *vacuo* to obtain the crude mono-phosphonic acid as a red viscous liquid (1.03 g). To a solution of the monophosphonic acid (1.03 g, 2.75 mmol) in freshly distilled CH_2Cl_2 (14 mL) was added EDC (0.67 g, 3.5 mmol), HOBt (0.54 g, 4.0 mmol) and Hunig's base (0.66 mL, 3.9 mmol). After stirring the resulting solution for 24 h the solvent was evaporated in *vacuo*. The resulting crude product was dissolved in EtOAc (150 mL) and washed with 0.5 M HCl (2x) and saturated $NaHCO_3$ (2x). The organic layer was dried over $MgSO_4$ and the solvent was evaporated in *vacuo*. The crude product was purified by column chromatography (SiO_2, EtOAc : Hexane, 70 : 30) to give a 1:1.4 diastereomeric mixture of **8** as a colorless oil (0.73 g, 73 % in 3 steps). Further chromatographic separation (SiO_2, EtOAc : Hexane, 50 : 50) gave the pure diastereomer **8a**. IR (neat) 1717, 1643 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (5H, m), 4.50 (2H, m), 3.80 (3H, d, J_{HP} = 11 Hz), 3.73 (3H, s), 3.65 (1H, d, J_{HH} = 9.2 Hz), 3.53 (1H, dd, J_{HH} = 6.5, 9.3 Hz), 3.38 (1H, m), 2.24 (3H, s), 2.07 (4H, m); ^{13}C NMR ($CDCl_3$) δ 168.6, 157.4 (d, J_{CP} = 7.5 Hz), 138.3, 128.6, 127.9, 127.8, 120.2 (J_{CP} = 5.0 Hz), 73.1, 68.9, 52.3 (J_{CP} = 7.0 Hz), 52.2, 37.7, 22.12 (J_{CP} = 134 Hz), 22.5 (J_{HP} = 7.1 Hz), 21.6; ^{31}P NMR ($CDCl_3$) δ 26.6. HRMS (FAB, NBA, MH^+) calcd. for $C_{17}H_{23}O_6P$: 354.1232. Found. 355.1315.

Phosphonate analog of cyclophostin 6

The monocyclic enolphosphonate **8** (1.06 g, 2.99 mmol) was dissolved in MeOH (6 mL). The reaction flask was flushed with argon for 10 minutes. 10% Pd on C (0.50 g) was added and the reaction mixture was flushed with argon for another 5 minutes followed by hydrogen. The resulting mixture was stirred under hydrogen (balloon) for 3 h. The reaction mixture was filtered through Celite®. The Celite® was washed with CH_2Cl_2 and the solvent was evaporated in *vacuo* to give the bicyclic phosphonate **6** (1.4:1mixture of diastereoisomers, 0.70 g, 100%) as a colorless oil. Chromatographic separation (SiO_2, EtOAc : Hexane, 50 : 50) gave *cis*-diastereomer **6b** was white crystalline solid which was further purified by crystallizing from EtOAc/hexane and the *trans*-diastereomer **6a** as a low melting solid.

cis-phosphonate analog of cyclophostin 6b

(mp 119 °C) IR (neat) 1747, 1673 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.5 (1H, dd, J_{HH} = 9.5, 9.0 Hz), 3.87 (3H, d, J_{HP} = 11 Hz), 3.83 (1H, m), 3.34 (1H, m), 2.46 (3H, s), 2.34 (1H, m), 2.04 (3H, m); ^{13}C NMR ($CDCl_3$) δ 170.2, 161.1 (d, J_{CP} = 6.6 Hz), 114.8 (d, J_{CP} = 3.8 Hz), 70.1, 52.8 (d, J_{CP} = 7.2 Hz), 39.1, 26.46 (d, J_{CP} = 136 Hz), 26.51 (d, J_{CP} = 6.9 Hz),18.92, 18.90; ^{31}P NMR ($CDCl_3$) δ 25.4.

trans-phosphonate analog of cyclophostin 6a

IR (neat) 1749, 1672 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.5 (1H, dd, , J_{HH} = 9.0, 6.0 Hz), 3.83 (3H, d, , J_{HP} =11 Hz), 3.81 (1H, app m), 3.40 (1H, m), 2.42 (3H, s), 2.05 (4H, m); ^{13}C NMR ($CDCl_3$) δ 170.2, 160.3 (d, J_{CP} = 9.8 Hz), 113.9, 69.9, 52.9 (d, J_{CP} = 6.8 Hz), 38.5, 26.1 (d, J_{CP} = 134 Hz), 26.8 (d, J_{CP} = 7.7 Hz), 18.6; ^{31}P NMR ($CDCl_3$) δ 21.9 ppm; HRMS (EI, M^+) calcd. for $C_9H_{13}O_5P$: 232.0501. Found. 232.0503.

Dimethyl 1-(2-oxodihydrofuran-3(*2H*)-ylidene)ethyl phosphate 19

A solution of 2-acetylbutyrolactone (0.37 g, 2.9 mmol) and Hunig's base in anhydrous CH_2Cl_2 (5 mL) was added to a solution of dimethylphosphoric acid (0.30 g, 2.4 mmol) and DCC (0.74 g, 3.6 mmol) in anhydrous CH_2Cl_2 (10 mL). Then DMAP (0.12 g, 0.98 mmol) was added and the reaction mixture was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted with CH_2Cl_2 (2x) and the combined organic layers were dried over $MgSO_4$. The solvent was evaporated in *vacuo* and the residue was purified by column chromatography (SiO_2, EtOAc:Hexane, 1:1) to yield the enolphosphate **19** (0.36 g, 64%) as a colorless oil. IR (neat) 1751, 1689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.31 (2H, t, J_{HH} = 7.5 Hz), 3.87 (6H, d, J_{HP} = 11 Hz), 3.04 (2H, m), 2.53 (3H, d, J_{HP} = 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 170.7, 157.3 (d, J_{CP} = 7.1 Hz), 111.7 (d, J_{CP} = 9.1 Hz), 64.7, 55.2 (d, J_{CP} = 6.2 Hz), 26.1, 16.7; ^{31}P NMR ($CDCl_3$) δ −4.7; HRMS (EI, M^+) calcd. for $C_8H_{13}O_6P$: 236.0450 Found 236.0447.

Quantitation of Anti-acetylcholinesterase Activity

The action of the new compounds against recombinant human and electric eel acetylcholinesterase (AChE) were determined by using Ellman's assay.[15] The lyophilized AChE was solubilized in 20 mM Tris HCl buffer, pH 7.5 and 1% BSA. The compounds were solubilized in isopropanol and preincubated with the enzyme for 30 min at room temperature and the residual activity of the enzyme was determined with 0.5 mM acetylthiocholine iodide and 0.3 mM 5,5'-dithiobis-2-nitrobenzoic acid in 100 mM sodium phosphate, pH 8.0 at 37°C. The absorbance of thionitrobenzoate anion at 412 nm was monitored every 2 sec for 50 sec. Reaction rates (average of triplicate) in absorbance/sec were converted to umol/min by using thionitrobenzoate anion extinction co-efficient 14,150 $M^{-1}cm^{-1}$. In the assay, the concentration of isopropanol was always maintained below 10%. The activity of the enzyme was not altered by the presence of 10% of isopropanol. The enzyme activity at each

concentration (0 − 320 μM) of test compound was expressed as a percent of the activity in the absence of compound to get % residual activity. Residual activity (%) was plotted against the compound concentration (uM) and the inhibitory action was calculated as an IC_{50}, defined as the concentration of compound (μM) required to inhibit 50% of enzymatic activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

The project described was supported by grant number R01-GM076192 from the National Institute of General Medicine. We thank National Science Foundation (CHE-0313736) for funding the early studies on the reactions of allylic hydroxy phosphonates employed in this synthesis, and for grants to purchase the NMR spectrometer (CHE-9974801), the mass spectrometer (CHE-9708640) and the x-ray diffractometer (CHE-0420497) used in this work. We thank Mr. Joe Kramer and Prof. R.E.K. Winter for mass spectra.

References

1. Kurokawa T, Suzuki K, Hayaoka T, Nagakawa T, Izawa T, Kobayashi M, Harada M. J. Antibiot 1993;46:1315–1318. [PubMed: 8407597]
2. He WH. Phosphorus, Sulfur Silicon Relat. Elem 2008;183:266.
3. Neumann R, Peter HH. Experientia 1987;43:1235.
4. Wink J, Schmidt FR, Seibert G, Aretz W. J. Antibiot 2002;55:472. [PubMed: 12139016] d Věrtesy L, Beck B, Brönstrup M, Ehrlich K, Kurz M, Műller G, Schummer D, Seibert G. J. Antibiot 2002;55:480. [PubMed: 12139017]
5. Garica-Carrasco M, Escárcega RO, Fuentes-Alexandro S, Riebeling C, Cervera R. Autoimmunity Reviews 2007;6:373. [PubMed: 17537383]
6. Kaur J, Zhang M-Q. Curr. Med. Chem 2000;3:273. [PubMed: 10637365]
7. a Li WM, Kan KK, Carlier PR, Pang YP, Han YF. Curr. Alzheimer Res 2007;4:386. [PubMed: 17908041] b Lleó A, Greenberg SM, Growdon JH. Annu. Rev. Med 2006;57:513. [PubMed: 16409164]
8. For examples see: aHilderbrand RL. The Role of Phosphonates in Living Systems. 1983CRC PressBoca Raton, FLbBlackburn GM, Taylor GE, Tattershall RH, Thatcher GRJ, McLennan A. Bruzik KS, Stec WJ. Phosphonate Analogues of Biological Phosphates. Biophosphates and their Analogues - Synthesis, Structure, Metabolism and Activity 1987:451.Elsevier Science Publishers B. V.Amsterdam, NetherlandscEngel R. Phosphonates as Analogues of Natural Phosphates. Chem. Rev 1977;77:349.dPompliano DL, Rands E, Schaber MD, Mosser SD, Anthony NJ, Gibbs JB. Biochemistry 1992;31:3800. [PubMed: 1567835]eMagnin DR, Biller SA, Chen Y, Dickson JK Jr. Fryszman OM, Lawrence RM, Logan JVH, Sieber-McMaster ES, Sulsky RB, Traeger SC, Hsieh DC, Lan S-J, Rinehart JK, Harrity TW, Jolibois KG, Kunselman LK, Rich LC, Slusarchyk DA, Ciosek CP Jr. J. Med. Chem 1996;39:657. [PubMed: 8576905]fBerkowitz DB, Bose M, Pfannenstiel TJ, Doukov T. J. Org. Chem 2000;65:4498. [PubMed: 10959850]gHakogi T, Monden Y, Iwama S, Katsumura S. Org. Lett 2000;2:2627. [PubMed: 10990413]
9. Yan B, Spilling CD. J. Org. Chem 2004;69:2859. [PubMed: 15074940]
10. Öhler E, Kanzler S. Liebigs Ann. Recueil 1997:1437. d Öhler E, Kanzler S. Phosphorus, Sulfur Silicon Relat. Elem 1996;112:71.
11. a Zhu J, Lu X. Chem. Commun 1987:1318. b Zhu J, Lu X. Tetrahedron Lett 1987;28:1897.
12. He A, Yan B, Thanavaro A, Spilling CD, Rath NP. J. Org. Chem 2004;69:8643. [PubMed: 15575740] Rowe BJ, Spilling CD. J. Org. Chem 2003;68:9502. [PubMed: 14629182]
13. a Giner J-L. Tetrahedron Lett 1998;39:2479. b Haruki E, Arakawa M, Matsumura N, Otsuji Y, Imoto E. Chem. Lett 1974;1:427.
14. Buechel KH, Roechling H, Korte F. Leibigs Ann 1965;685:10.
15. Ellman GJ, Courtney KD, Andres V Jr. Featherstone RM. Biochem Pharm 1961;7:88. [PubMed: 13726518]

16. Luo C, Tong M, Chilukuri N, Brecht K, Maxwell DM, Saxena A. Biochemistry 2007;46:11771. [PubMed: 17900152]

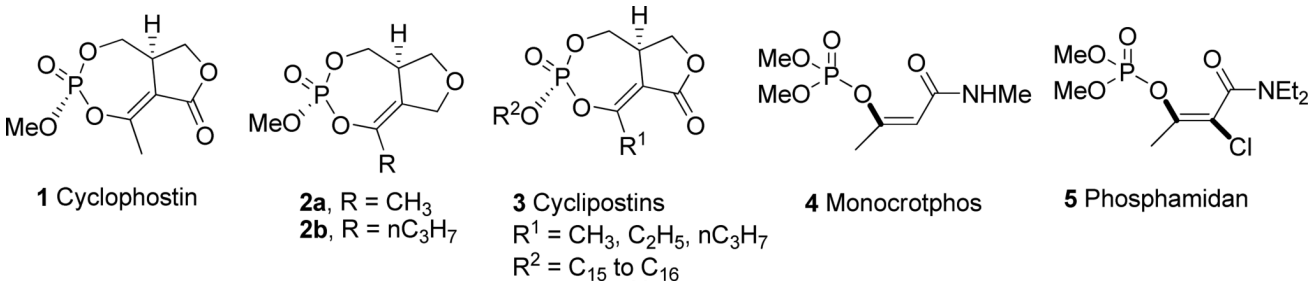


Figure 1.
Cyclophostin and Related Structures

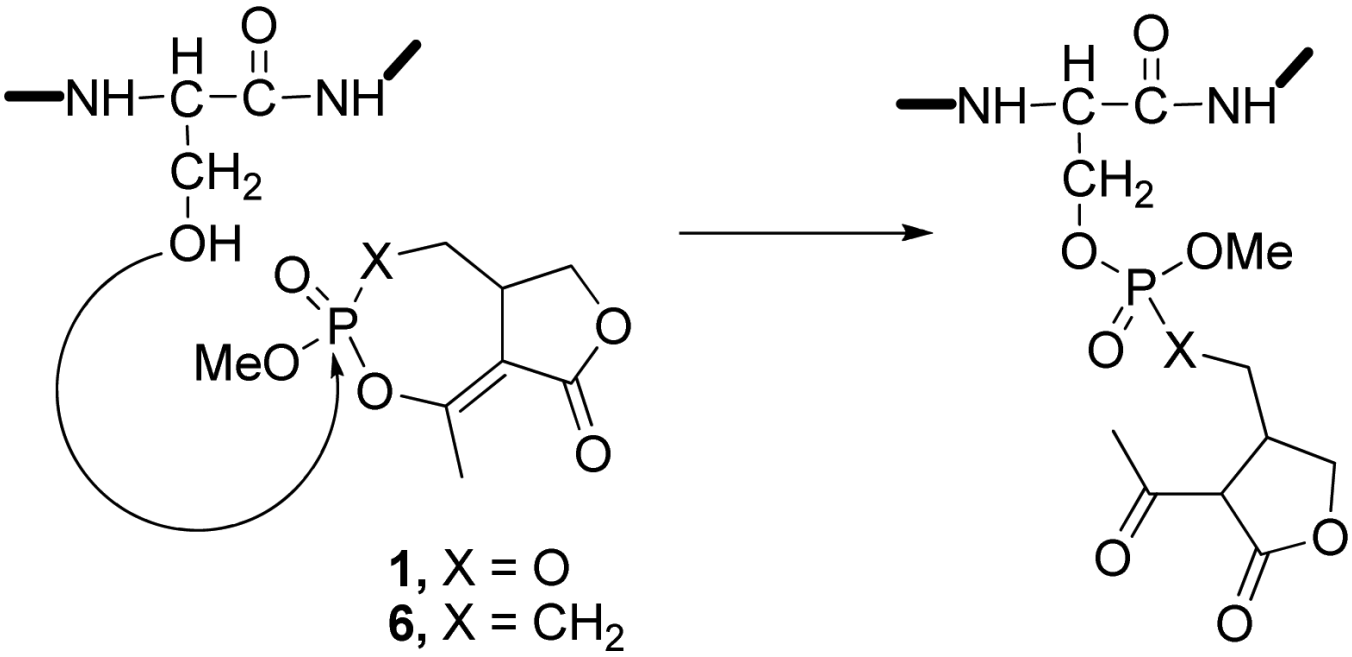
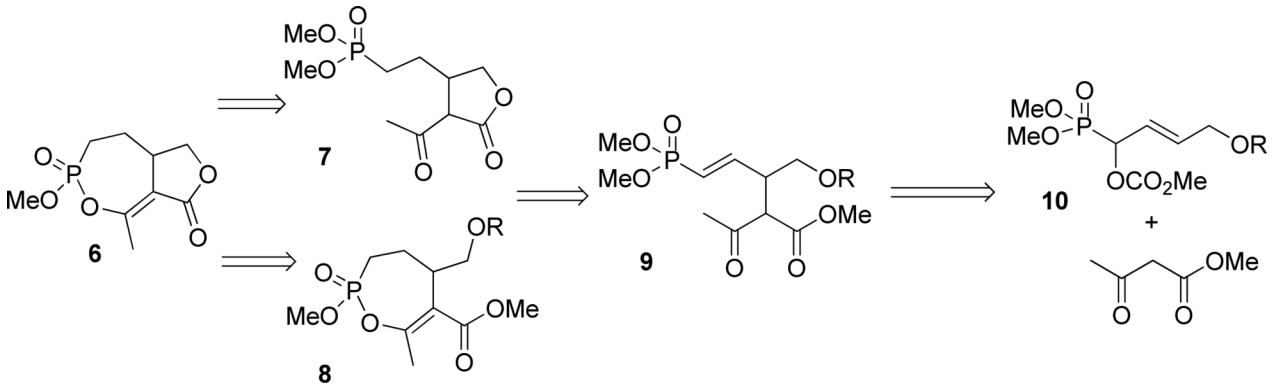
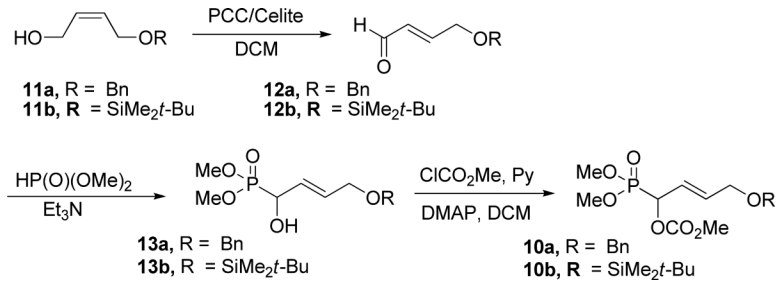


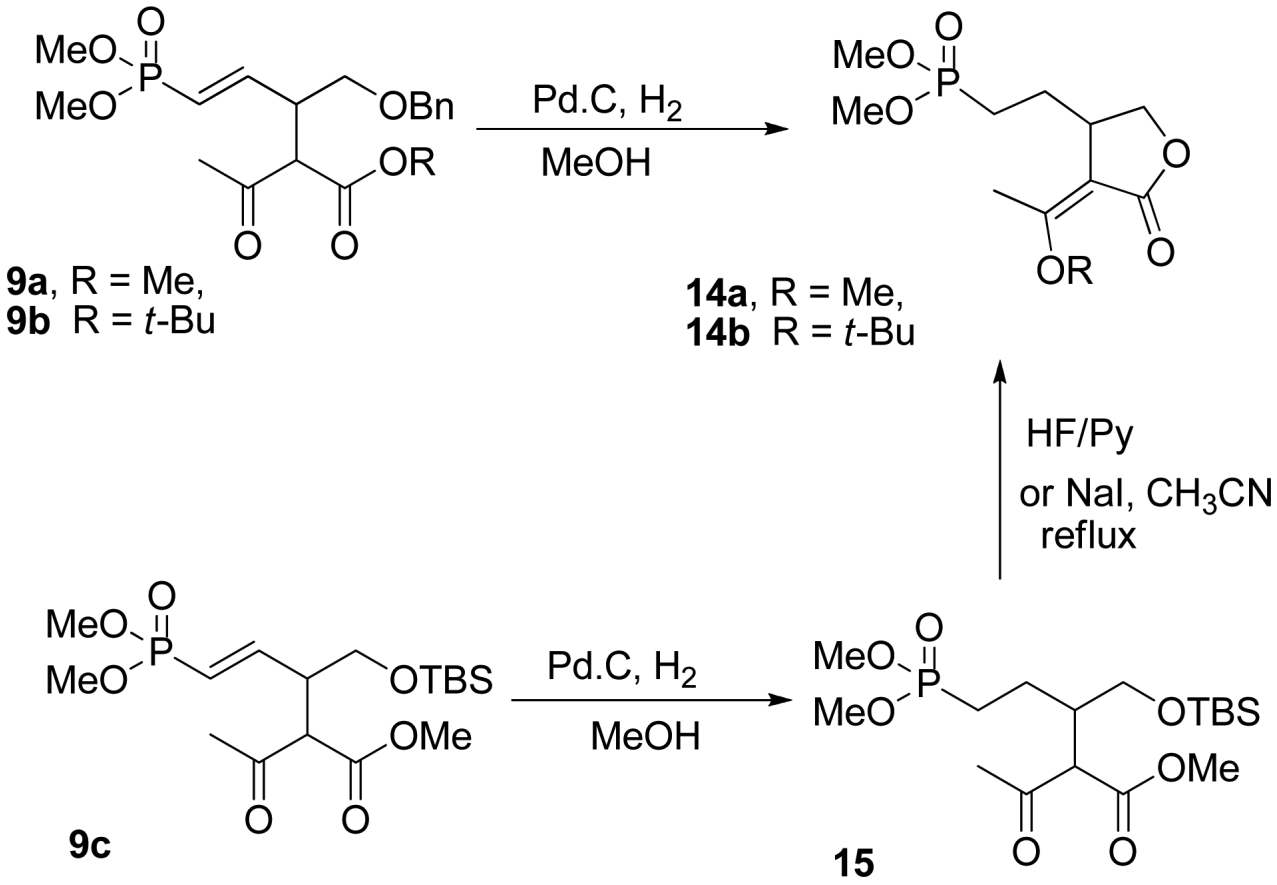
Figure 2.
Proposed Reaction with the Active Site Serine and a Phosphonate Analog of Cyclophostin



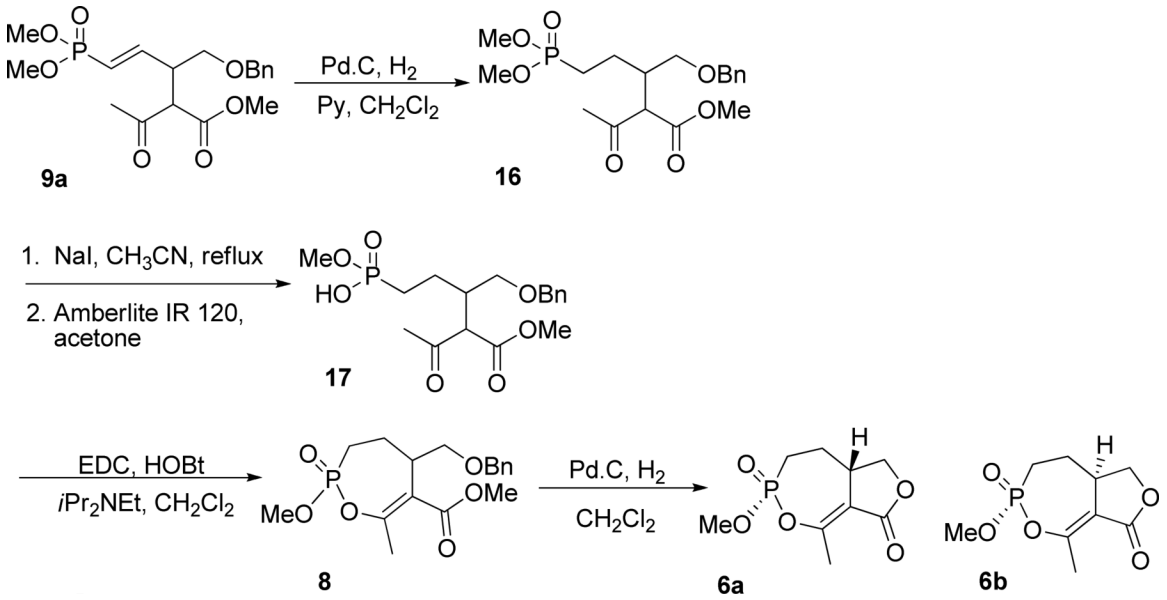
Scheme 1.
Retrosynthetic Analysis for the Phosphonate Analog of Cyclophostin.



Scheme 2.
Preparation of the Allylic Hydroxy Phosphonates



Scheme 3.
Hydrogenation and Debenzylation of the Vinyl Phosphonates



Scheme 4.
Synthesis of the Phosphonate Analog

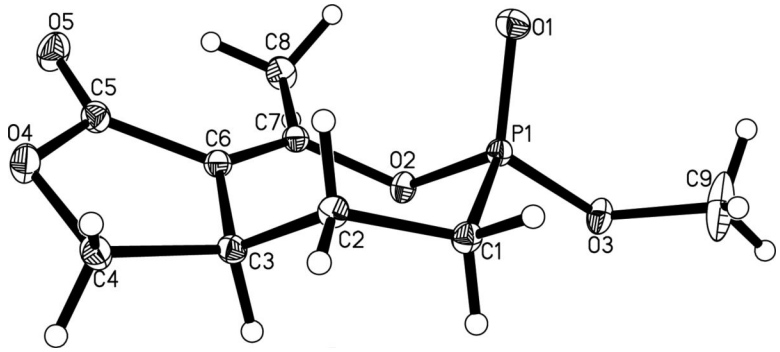
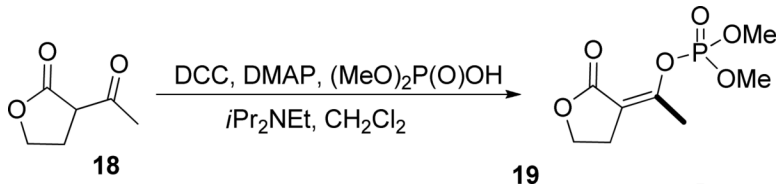


Figure 3.
Projection view of the Phosphonate 6b with 50% thermal ellipsoids

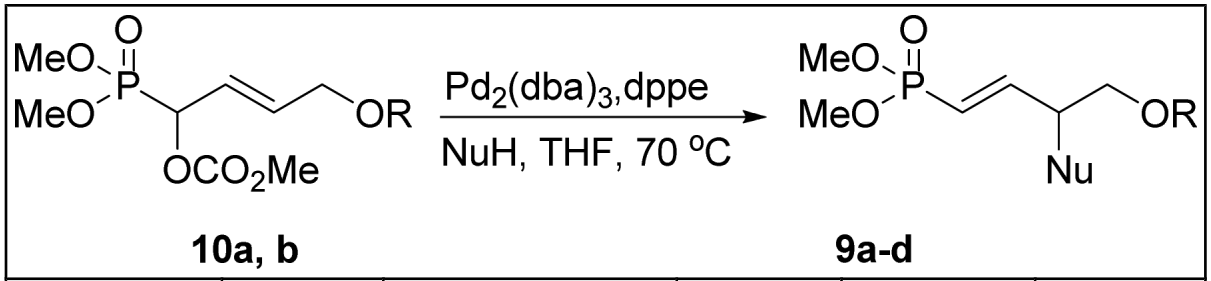


18 19

Scheme 5.
Synthesis of an Enol Phosphate Analog of Cyclophostin

Table 1

Palladium Catalyzed Allylic Substitution Reactions with Acetoacetates and Malonates

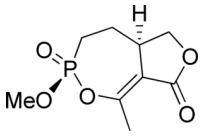
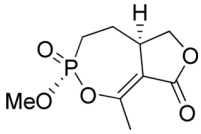
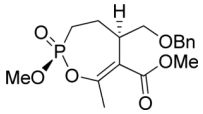
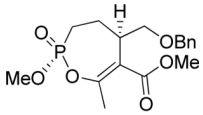
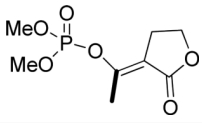
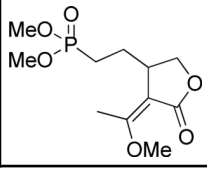


10a, b **9a-d**

Carbonate	R	NuH	Base	Product	Yield
10a	Bn	$CH_3COCH_2CO_2Me$	none	**9a**	85%
10a	Bn	$CH_3COCH_2CO_2$*t*-Bu	none	**9b**	56%
10b	TBS	$CH_3COCH_2CO_2Me$	none	**9c**	64%
10a	Bn	*t*-$BuO_2CCH_2CO_2Me$	BSA	**9d**	81%

Table 2

The Inhibition Data for AChE from Human and Eel.

Entry	Compound	Structure	IC_{50}(uM) Eel AChE	IC_{50}(uM) Human AChE
1	6a		~70	~3
2	6b		>400	~30
3	8a		~150	~35
4	8b	 4:1 mixture	~110	~6
5	18		>1000	>1000
6	14a		>1000	>1000