tion-potential duration at the 90% repolarization level could be measured with a standard error of ±1%, as the mean of at least four (usually six to eight) cell samples. An increase of about 33% in APD was maximal for most cells, and hence estimates of the change in APD are reliable to ±3%. A single electrode voltage clamp[15] (using electrodes containing 0.5 M K_2SO_4) was used for the measurement of membrane currents. In some cases nisoldipine (2 μM) was present in the external solution to block currents carried by calcium, and in others cells were injected[15] with the calcium chelator BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid) to suppress currents activated by increases in cytosolic calcium.

Conduction velocity in isolated guinea pig atrial preparations was measured by placing stimulating and recording electrodes at a fixed distance of ca. 20 mm and measuring the time interval between the delivery of the stimulus pulse and the recording of the first action potential.

β-Blocking activity was determined from the extent of reduction of the rise in heart rate elicited in pithed rat preparations in response to doses of 0.25 μg/kg isoprenaline.

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Phosphonate-Containing Inhibitors of Tyrosine-Specific Protein Kinases†

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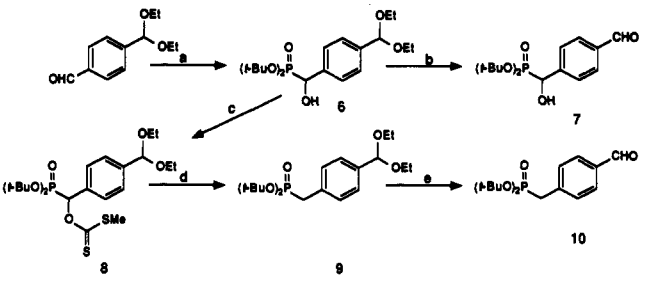
Tyrosine-specific protein kinases (TPK) are important signal transducing enzymes involved in normal cellular growth and differentiation and have been implicated in the etiology of a number of human neoplastic processes. Efforts to develop agents which inhibit the function of these enzymes by interfering with the binding of substrate have been limited by the lack of detailed three-dimensional structural data. Many inhibitors of substrate binding share a common styrene nucleus 1 which has been postulated to function as a conformationally constrained analogue of tyrosine. In an effort to develop high-affinity compounds based on this hypothesis, a number of derivatives were synthesized in which either methylphosphonate (**4a–c**) or (hydroxymethyl)phosphonate (**3a–c**) were appended to the aromatic 4-position of styrene-containing moieties. The intent of this approach was to prepare hydrolytically stable analogues which expressed additional enzyme recognition features present during the phosphorylation of tyrosine itself. None of the analogues showed inhibitory activity up to the maximum concentration tested (1000 μM) when assayed against autophosphorylation of A-431-derived epidermal growth factor receptor (EGFR) or $p56^{lck}$ (autophosphorylation and transphosphorylation of rabbit muscle enolase). Additionally, a series of naphthalene-based inhibitors including (1-naphthalenylhydroxymethyl)phosphonic acid (**14**), its known 2-positional isomer **16**, and sulfonate (**19, 20**) and phosphate derivatives (**17, 18**) were also tested under similar conditions. Only (2-naphthalenylhydroxymethyl)phosphonic acid (**16**) showed activity (IC_{50} = 250 μM in EGFR, in agreement with the reported literature value). These results suggest that the interaction of styrene-based inhibitors with the substrate binding domain of TPKS may not occur in a manner analogous to the interaction of tyrosine with this domain.

Tyrosine-specific protein kinases (TPK) constitute an important class of cyclic AMP-independent enzymes which transfer the γ-phosphate of either ATP or GTP to the 4-hydroxyl group of specific tyrosine residues within either peptide or protein substrates.[1] In normal cellular function TPKs are involved in the modulation of growth and differentiation[2] with ca. 50% of them functioning as the cytoplasmic signal-transducing domains of growth factor receptors.[3] A significant number of TPKs are the products of protooncogenes,[4] and their association with the etiology of a number of human cancers[5] makes them attractive targets for the development of new anticancer chemotherapeutics.[6] Since TPKs bind an appropriate nucleoside triphosphate along with a tyrosine-containing substrate, and catalyze the transfer of a phosphate group from one molecule to the other,[7] a possible approach in the design of agents which inhibit their function could involve (1) inhibition of nucleoside triphosphate binding or (2) inhibition of substrate binding to the enzyme.[8] While several potent inhibitors of nucleoside triphosphate binding are known[9] and active research continues in this area,[10] the homology of nucleoside triphosphate binding sites among a variety of different kinases[11] decreases the likelyhood of specificity for this class of inhibitors and makes them less desirable as therapeutic targets. Because

Scheme I[a]



[a] (a) $(t\text{-BuO})_2POH$, basic alumina; (b) HCl(aq)/$CHCl_3$; (c) NaH, CS_2, MeI; (d) $n\text{-Bu}_3SnH$, AIBN; (e) HCl(aq)/$CHCl_3$.

† A preliminary account of this work was presented at the 200th National Meeting of the American Chemical Society, Washington, DC, MEDI 138.

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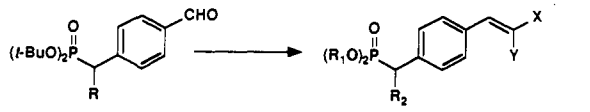
Scheme II. Synthesis of Phosphonate-Containing Styrenes

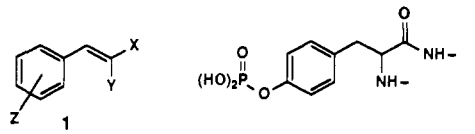


7 R = OH
10 R = H

11 R_1 = t-Bu, R_2 = OH
12 R_1 = t-Bu, R_2 = H
3 R_1 = H, R_2 = OH
4 R_1 = H, R_2 = H
a ; X = CN, Y = CN
b ; X = $CONH_2$, Y = CN
c ; X = $CONH_2$, Y = $CONH_2$

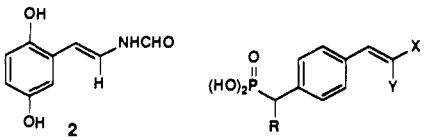
TPKs appear to be selective for tyrosine-containing substrates,[12] inhibitors that are competitive with respect to tyrosine binding sites have the potential for high levels of specificity both relative to serine/threonine-specific protein kinases and also among families of TPKs.[13] The development of this class of agents represents an area of current research interest.[14]

The absence of detailed three-dimensional information for any TPK enzyme has necessitated a trial and error approach in the design of substrate inhibitors involving modifications of structures which have previously been shown to be active. One structural motif common to a variety of competitive substrate inhibitors is the styrene nucleus (1). The importance of this structure was first



1
Styrene Nucleus

Phosphorylated Tyrosine Residue



2
Erbstatin

3 R = OH
4 R = H

demonstrated by Wong and Goldberg[15] who found that replacing Tyr[4] with ΔPhe in [Val[5]]angiotensin II transformed the peptide from a substrate to an inhibitor of pp60[src] TPK. Subsequently erbstatin, a natural product isolated from a strain of *Streptomyces*,[16] was shown to have a similar styrene-containing structure **2**.[17] Erbstatin is a potent inhibitor of epidermal growth factor receptor TPK (EGFR) which functions by competing with the peptide substrate.[18] Since the initial discovery of erbstatin, a variety of other small molecules which inhibit TPKs by competing with substrate have been reported,[13,14,19] and like erbstatin, these contain the styrene nucleus.

By analogy to Wong's work in which the substrate Tyr was replaced by ΔPhe, it can be speculated that these inhibitors function as nonphosphorylatable and conformationally constrained analogues of tyrosine which possess aromatic rings capable of occupying a pocket within the TPK catalytic site that would normally bind the tyrosine's phenolic ring in the substrate. This is further strengthened by the apparent enhancement in affinity of styrene-con-

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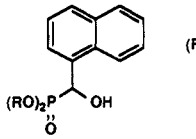
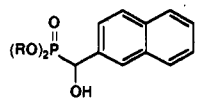
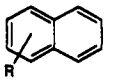
taining inhibitors which have aromatic hydroxyls.[14a] Additionally, the high activity of compounds having an amide at the vinyl position (1, X = amide) supports the hypothesis that these compounds bind to the TPK active site analogously to the tyrosine phosphate acceptor. In an effort to add structural features to this class of inhibitors which would enhance binding affinity, structural elements that mimic the transfer of the phosphate moiety were contrived. Thus the incorporation of a 4-phosphate ester onto the styrene nucleus offered the potential of additional enzyme–inhibitor interaction through bonding to active site groups constituently involved in stabilizing the transfer of phosphate. In light of the lability of the phosphate ester bond, alternate incorporation of the isosteric and hydrolytically stable phosphonic acid group (i.e., replacing the oxygen linkage of the ester with a methylene group) was considered and compounds of general structure 4 were postulated as targets. Similar considerations led Hangauer et al.[20] to prepare (2-naphthalenylhydroxymethyl)-phosphonic acid (16) in which the styrene moiety is con-



13 R = *t*-Bu
14 R = H



15 R = *t*-Bu
16 R = H



17 R = 1-Phosphate
18 R = 2-Phosphate
19 R = 1-Sulfonic acid
20 R = 2-Sulfonic acid

tained within the bicyclic naphthalene ring system. The hydroxyl group on the phosphonic acid methylene was designed in that study to add hydrogen-bonding interaction with elements at the catalytic site. The observed IC_{50} (200 μM) for 16 against autophosphorylation of the insulin receptor TPK, while modest, demonstrated the utility and potential of the concept. This paper describes the preparation and initial biological testing of a series of more elaborate phosphonate-containing styrene inhibitors of general structure 3 and 4 which are based on this approach. Additional naphthalene analogues were also examined to further explore structure–activity relationships of this nucleus.

Synthesis

Preparation of (hydroxymethyl)phosphonates by the Pudovik reaction proceedes readily through addition of phosphite to carbonyl compounds under basic catalysis.[21] In the present case however, reaction of (4-formylbenzylidene)malononitrile[14a] with di-*tert*-butyl phosphite[22] was unsuccessful and gave multiple products including that arising from conjugate 1,4-addition.[23] An alternate approach was therefore taken in which the phosphite was added prior to formation of the styrene structure. As shown in Scheme I, treatment of 4-(diethoxymethyl)-benzaldehyde with di-*tert*-butyl phosphite in the presence of basic alumina[24] gave the desired addition product 6 cleanly. Treatment of 6 with $CHCl_3$ saturated with aqueous HCl hydrolyzed the acetal selectively to provide aldehyde 7, which was subsequently used as a common intermediate in the synthesis of (hydroxymethyl)-phosphonate-containing styrenes. Radical deoxygenation of the secondary alcohol in 6 was achieved in a two-step process[25] through the intermediacy of xanthate 8, providing 9. Deprotection of 9 gave 10, which served as a common intermediate in the preparation of methylphosphonate-containing styrenes.

(Hydroxymethyl)phosphonates **11a–c** and methylphosphonates **12a–c** were prepared from the corresponding aldehydes 7 and 10, respectively, by reaction with the appropriate active methylene compounds (malononitrile, α-cyanoacetamide, or malonamide) in refluxing THF or THF/MeOH, containing a catalytic amount of pyridine (Scheme II). Malononitrile is roughly 1000 times more reactive toward aromatic aldehydes than α-cyanoacetamide,[26] and malonamide, which is the least reactive, necessitated longer reaction times and provides a lower yield of product compared with the previous reagents. Treatment of *tert*-butyl-protected phosphonates **11a–c** and **12a–c** with trifluoroacetic acid (TFA) yielded the corresponding free phosphonic acids **3a–c** and **4a–c**.

Both (1-naphthalenylhydroxymethyl)phosphonic acid (14) and (2-naphthalenylhydroxymethyl)phosphonic acid (16)[20] were prepared by TFA treatment of the corresponding *tert*-butyl-protected phosphonates 13 and 15, which in turn were obtained, respectively, from the reaction of 1-naphthaldehyde or 2-naphthaldehyde with di-*tert*-butyl phosphite in the presence of basic alumina.

Results and Discussion

The EGFR system is well-studied and has been implicated in a number of human neoplastic process.[4d] In addition the p56^{lck} enzyme has been shown to play an important role in CD4-mediated activation of T-lymphocytes,[27] making both EGFR and p56^{lck} important targets for therapeutic intervention and drug development. Most compounds to date which inhibit substrate interaction with TPKs possess the styrene nucleus.[13,14,19] Additionally, certain flavones and isoflavones, which interfere with ATP binding to TPKs, also contain within their structures a styrene-like moiety. In the absence of kinetic or molecular mechanisms for the enzymatic reaction and with no detailed structural information about the enzymes, the mechanism of action of this class of compounds can only be speculated. However by analogy to previous peptide work[15] the styrenes can be envisioned to function as conformationally restricted analogues of tyrosine. A large number of styrene-containing analogues have been prepared with only limited conclusions drawn regarding structure–activity requirements.[14a] On the basis of the hypothesized mode of action of these compounds the present study introduced the hydrolytically stable phosphate mimetics, methylphosphonate and (hydroxymethyl)phosphonate, into the skeleton of a select number of styrene-containing inhibitors in order to increase affinity and specificity through interactions expected to occur during the transfer of phosphate. Substitution at the 4-position was dictated by analogy to the 4-phosphate of tyrosine. The surprising inhibitory activity of (2-naphthalenylhydroxymethyl)phosphonic acid (IC_{50} = 200 μM against autophosphorylation in the insulin receptor and IC_{50} = 250 μM against transphosphorylation in the EGFR systems[20]) offered strong support for this approach. In the present case a number of acidic naphthalenes were prepared. In addition, two parallel series of phospho-

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nate-containing styrenes were synthesized; one with and one without an α-hydroxyl group. This parallel series of styrenes was intended to examine the importance of the α-hydroxyl group for binding to TPKs, since it has been rationalized that this group enhances receptor interaction.[20] Three styrene nuclei were employed. Two of these (**a** and **b**, Scheme II) are contained in known, potent TPK inhibitors, while the third (**c**, Scheme II) is new. All compounds were tested for their ability to inhibit autophosphorylation of the EGFR and both autophosphorylation and transphosphorylation of the exogenous substrate rabbit muscle enolase by $p56^{lck}$. None of the phosphonate-containing styrenes showed significant activity up to the maximum concentration tested (1000 μM). Additionally, while (2-naphthalenylhydroxymethyl)phosphonic acid (**16**) exhibited an IC_{50} = 250 μM in the EGFR system as reported,[20] it showed no activity in the $p56^{lck}$ system. The isomeric (1-naphthalenylhydroxymethyl)phosphonic acid (**14**) and the naphthalene analogues (phosphates **17** and **18** and sulfonates **19** and **20**) were also inactive in both EGFR and $p56^{lck}$ systems.

Taken together, these data may suggest that the styrene-containing TPK inhibitors interact with the enzyme in a manner other than simple mimicking of tyrosine itself, and that the 4-position of the styrene aromatic ring is not situated within the catalytic site analogously to the 4-position of tyrosine. In this light, the weak activity of (2-naphthalenylhydroxymethyl)phosphonic acid (**16**) may also result from interaction with the receptor in a manner other than mimicking phosphorylated tyrosine despite the fact that the inactivity of the isomeric **14** and other acidic naphthalene analogues suggests that a somewhat specific mode of receptor interaction for **16** would exist. Further studies will be required to attempt to rationally design analogues as probes to examine TPK active site geometries.

Experimental Section

Biological Procedures. Cells. HPB cells were grown in RPMI 1640 (Gibco) supplemented with glutamine and 10% fetal bovine serum (Gibco). A-431 cells were grown in Dulbecco's Modified Eagle Medium (D-MEM, Gibco).

Inhibition of $p56^{lck}$ Autophosphorylation and Transphosphorylation of Rabbit Muscle Enolase. Evaluation of the activity of the CD4-associated tyrosine kinase $p56^{lck}$ was performed as previously described.[28] HPB cells were lysed in 50 mM Tris HCl pH 8.0, 150 mM NaCl, 1% (v/v) NP40, 2 mM ethylenediaminetetraacetic acid, 100 μM orthovanadate, and 10 mg/mL each of aprotinin and leupeptin. Insoluble cellular debris was removed by centrifugation and 250 μg of cell lysate was incubated with 2 μL of the monoclonal antibody OKT4 (Becton Dickinson). Immune complexes were collected with formalin-fixed *Staphylococcus aureus* cells (Pansorbin, Calbiochem) precoated with affinity-purified rabbit anti-mouse immunoglobulin (Cappel). Following three washes in lysis buffer, immune complex kinase assays were performed by adding 20 mM morpholinepropanesulfonic acid, pH 7.0, 5 mM $MgCl_2$, 10 μM ATP, 20 μCi[γ-^{32}P]ATP (3000 Ci/mmol, New England Nuclear), 100 μg/L non acid denatured rabbit muscle enolase (Sigma) as an exogenous substrate, and inhibitors in various micromolar concentrations. The typical concentrations of the inhibitors in the assays were 0, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 250, 500, and 1000 μM. The enolase reactions were conducted at ambient temperature for 5 min and were terminated by the addition of SDS-PAGE sample buffer. Samples were analyzed on 8% SDS-polyacrylamide gels and radioactive bands were detected by autoradiography. Bands corresponding to autophosphorylated $p56^{lck}$ and transphosphorylated rabbit muscle enolase were removed and the ^{32}P-radioactivity was counted on a LS-1701 Liquid Scintillation System (Beckmann) using Econoflur-2 (Du Pont) as the scintillant. The percentage of the ^{32}P radioactivity was plotted against the concentration of the inhibitors.

Inhibition of Basal Autophosphorylation of EGFR. A-431 cells were lysed in the same lysis buffer as described above. Cell lysate (20 μg) was incubated with 5 μL (10 mg/mL) of monoclonal anti-human EGF receptor IgG (Upstate Biotechnology, Inc.). Immune complexes were collected with formalin-fixed *S. aureus* cells precoated with affinity-purified rabbit anti-mouse immunoglobulin. Following three washes with lysis buffer, one wash with lysis buffer containing 1 M NaCl, one wash with lysis buffer without NaCl, and one wash with kinase buffer (with $MnCl_2$), immune complex kinase assays were performed by adding 20 mM morpholinepropanesulfonic acid, pH 7.5, 5 mM $MnCl_2$, 10 μM ATP, 20 μCi[γ-^{32}P]ATP (3000 Ci/mmol, New England Nuclear), and inhibitors in various micromolar concentrations. The kinase reactions were conducted at ambient temperature for 1.5 min and were terminated by the addition of SDS-PAGE sample buffer. Samples were analyzed on 8% SDS-polyacrylamide gels and the radioactive bands were detected by autoradiography.

Chemical Procedures. Commercially available synthetic reagents and naphthalene derivatives (**17–20**) were purchased from either Aldrich or Fluka Chemical Co. Petroleum ether was of the boiling range 30–45 °C and removal of solvents was performed by rotary evaporation under reduced pressure. Silica gel chromatography was performed with a 4 cm diameter × 2 cm high column dry-packed with 10-μm silica gel run under negative vacuum pressure. Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from either Galbraith Laboratories, Knoxville, TN or Atlantic Microlab Inc., Norcross GA. Negative ion fast atom-bombardment (FAB) mass spectra were acquired with a VG Analytical 7070E mass spectrometer operated under the control of a VG 2035 data system. ^{1}H NMR data were obtained on a Varian XL-200 instrument.

Bis(1,1-dimethylethyl) [[4-(Diethoxymethyl)phenyl]-hydroxymethyl]phosphonate (6). A mixture of 4-(diethoxymethyl)benzaldehyde (20.8 g, 100 mmol), di-*tert*-butyl phosphite (19.4 g, 100 mmol), and Woelm basic alumina oxide (50 g; activity II) was set at ambient temperature overnight and then extracted by triturating with CH_2Cl_2/EtOH (90:10; 4 × 100 mL). Removal of solvent gave an oil which was crystallized from petroleum ether to yield **6** as white crystals: 33.24 g (83%); mp 98–100 °C; NMR (Me_2SO-d_6) δ 1.11 (t, 6 H, J = 7 Hz, 2 CH_3), 1.33 (s, 9 H, $C(CH_3)_3$), 1.39 (s, 9 H, $C(CH_3)_3$), 3.38–3.54 (m, 4 H, 2 CH_2), 4.63 (dd, 1 H, J = 5.6, 13.0 Hz, P-CH), 5.46 (s, 1 H, $(EtO)_2CH$), 5.86 (dd, 1 H, J = 5.6, 14.6 Hz, OH), 7.28–7.39 (m, 4 H, ArH). Anal. ($C_{20}H_{35}PO_6$) C, H.

Bis(1,1-dimethylethyl) [(4-Formylphenyl)hydroxymethyl]phosphonate (7). To a solution of **6** (10.05 g, 25 mmol) in $CHCl_3$ (150 mL) was added 1.0 mL of 1 N HCl along with 2 drops of 37% HCl and the mixture stirred at ambient temperature for 20 min. Removal of solvent gave an oil which crystallized to a solid mass. Trituration with petroleum ether gave product **7** as a white solid (8.35 g, 100%), mp 90–92 °C. A sample was recrystallized for analysis (CH_2Cl_2/petroleum ether): mp 91–94 °C; NMR ($CDCl_3$) δ 1.40 (s, 9 H, $C(CH_3)_3$), 1.44 (s, 9 H, $C(CH_3)_3$), 4.94 (d, 1 H, J = 11.6 Hz, PCH), 7.62 (dd, 2 H, J = 2, 8 Hz, ArH), 7.86 (d, 2 H, J = 8 Hz, ArH), 10.01 (s, 1 H, CHO). Anal. ($C_{16}H_{25}PO_5$) C, H.

Bis(1,1-dimethylethyl) [(4-Formylphenyl)methyl]-phosphonate (10). A suspension of NaH 80% in oil (938 mg, 31.2 mmol) was mixed well with petroleum ether, then the solvent was removed by decantation. To the free NaH was then added a solution of **6** (10.05 g, 25 mmol) in dry DMF (40 mL) along with CS_2 (7.1 mL, 9.0 mmol) and the resulting dark brown mixture stirred at ambient temperature for 15 min. Addition of iodomethane (2.3 mL, 37 mmol) gave a clear orange solution. After stirring for 15 min the floccular suspension was partitioned between aqueous NH_4Cl (300 mL) and CH_2Cl_2 (3 × 100 mL), washed with aqueous NH_4Cl (200 mL), and dried ($MgSO_4$) and solvent removed, yielding crude xanthate **8** as a yellow oil. Radical deoxygenation of **8** was achieved by refluxing for 3 h in toluene (100 mL) with n-Bu_3SnH (8.40 mL, 31 mmol) and azodiisobutyronitrile (AIBN) (64 mg, 0.4 mmol). Solvent was removed, yielding a light yellow oil which was dissolved in acetonitrile (200

(28) Veillette, A.; Horak, I. D.; Bolen, J. B. *Oncog. Res.* 1988, *2*, 385–401.

Table I. Analytical Data for Phosphonate-Containing Styrenes

no.	% yield	mp, °C	anal.	FABMS
11a	47	94	C, H, N	
11b	55	208–211	C, H, N	
11c	29	139 dec	C, H, N	
12a	38	117–119	C, H, N	
12b	77	227–229	C, H, N	
12c	29	137–139 dec	C, H, N	
3a·$^1/_2CH_3OH$·$^1/_3H_2O$	92	89 soften 270 dec	C, H, N	527 (M_2 - H), 263 (M - H)
3b	85	>220 dec	C, H, N	373 (M - H + glycerol), 281 (M - H)
3c·$^1/_2C_5H_{10}O$·$2H_2O$	100	109–110 dec	C, H, N	483 (M - H + 2 glycerol), 391 (M - H + glycerol), 299 (M - H)
4a·$^1/_3CH_3OH$	83	178–179	C, H, N	339 (M - H + glycerol), 247 (M - H)
4a·$^1/_3H_2O$	100	237–239	C, H, N	449 (M - H + 2 glycerol), 357 (M - H + glycerol), 265 (M - H)
4c	97	220–222 dec	C, H, N	467 (M_2 - H), 375 (M - H + glycerol), 283 (M - H)

mL), washed with petroleum ether (3 × 100 mL), and taken to dryness. The resulting oil was briefly treated with $CHCl_3$ saturated with 1 N HCl (200 mL; 10 min) then taken to dryness and purified by silica gel chromatography ($CHCl_3$). Crude product was obtained as a light yellow oil (3.74 g) which was crystallized from petroleum ether to yield **10** as white crystals (2.30 g, 29%): mp 61–65 °C; NMR ($CDCl_3$) δ 1.41 (s, 18 H, 2 $C(CH_3)_3$), 3.11 (d, 2 H, J = 22 Hz, PCH_2), 7.43 (dd, 2 H, J = 2, 8 Hz, ArH), 7.80 (d, 2 H, J = 8 Hz, ArH), 9.98 (s, 1 H, CHO). Anal. ($C_{16}H_{25}PO_4$) C, H.

Bis(2,2-dimethylethyl) [[4-(2,2-Dicyanoethenyl)phenyl]hydroxymethyl]phosphonate (11a). (General Procedure for the Preparation of Styrene-Containing Phosphonates 11a–c and 12a–c.) A solution of **7** (328 mg, 1.0 mmol) and malononitrile (73 mg, 1.1 mmol) in dry THF was stirred overnight at ambient temperature with 3 drops of piperidine. Solvent was removed and crude product purified by silica gel chromatography using $CHCl_3$, yielding **11a** as a white crystalline solid (177 mg, 47%). Recrystallization (CH_2Cl_2/petroleum ether) produced an analytical sample: mp 94 °C; NMR (Me_2SO-d_6) δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.45 (s, 9 H, $C(CH_3)_3$), 4.94 (d, 1 H, J = 12 Hz, PCH), 7.62 (dd, 2 H, J = 2, 8 Hz, ArH), 7.75 (s, 1 H, vinylic-H), 7.89 (d, 2 H, J = 8 Hz, ArH). Anal. ($C_{19}H_{25}N_2PO_4$) C, H, N. Analytical data for compounds **11a–c** and **12a–c** are listed in Table I.

[[4-(2,2-Dicyanoethenyl)phenyl]hydroxymethyl]phosphonic Acid (3a). (General Procedure for the Deprotection of *tert*-Butyl-Protected Phosphonates Yielding 3a–c and 4a–c.) A mixture of **11a** (338 mg, 0.90 mmol), TFA (10 mL), and anisole (486 mg, 4.5 mmol) in CH_2Cl_2 (10 mL) was stirred at ambient temperature (45 min). To this was then added H_2O (10 mL) and the mixture taken to dryness. The resulting oil was crystallized from MeOH/$CHCl_3$, yielding **3a** as light yellow needles 218 mg (92%): mp 89 °C soften, 270 °C dec; NMR (Me_2SO-d_6) δ 3.15 (s, 1.5 H, 0.5 mol CH_3OH), 4.80 (d, 1 H, J = 15.9 Hz, PCH), 6.1 (br s, 1 H, OH), 7.59 (d, 2 H, J = 8.3 Hz, ArH), 7.87 (d, 2 H, J = 8.3 Hz, ArH), 8.50 (s, 1 H, vinylic-H). Anal. ($C_{11}H_9N_2PO_4$·$^1/_3H_2O$·$^1/_2CH_3OH$) C, H, N. Analytical data for compounds **3a–c** and **4a–c** are listed in Table I.

Bis(2,2-dimethylethyl) (1-Naphthalenylhydroxymethyl)phosphonate (13). A mixture of 1-naphthaldehyde (1.56 g, 10 mmol), di-*tert*-butyl phosphite (1.94 g, 10 mmol), and basic Woelm aluminum oxide (5 g; activity II) was mixed well with CH_2Cl_2 (5 mL), then CH_2Cl_2 was removed by rotary evaporation. The reaction mixture was left at ambient temperature overnight, extracted (100 mL of CH_2Cl_2, 2 × 50 mL of EtOAc), and taken to dryness. The resulting white solid was recrystallized from CH_2Cl_2/petroleum ether, giving **13** as white crystals (2.0 g, 57%): mp 114.5–115.5 °C; NMR ($CDCl_3$) δ 1.25 (s, 9 H, $C(CH_3)_3$), 1.46 (s, 9 H, $C(CH_3)_3$), 5.71 (d, 1 H, J = 11.9 Hz, PCH), 7.49–7.57 (m, 3 H, ArH), 7.81–7.90 (m, 3 H, ArH), 8.11–8.16 (m, 1 H, ArH). Anal. ($C_{19}H_{27}PO_4$) C, H.

(1-Naphthalenylhydroxymethyl)phosphonic Acid (14). A solution of **13** (350 mg), anisole (540 mg, 5.0 mmol), and TFA (10 mL) was stirred at ambient temperature (1 h) then the mixture was taken to dryness to give a white solid which was triturated (petroleum ether) then washed ($CHCl_3$) and dried, yielding **14** as white crystals (148 mg, 62%): mp 126–128 °C soften, 152–156 °C dec; NMR (Me_2SO-d_6) δ 5.47 (d, 1 H, J = 14.3 Hz, PCH), 6.1 (br s, 3 H, exchangeable), 7.44–7.52 (m, 3 H, ArH), 7.72–7.90 (m, 3 H, ArH), 8.16–8.21 (m, 1 H, ArH); FABMS (negative ion) m/z 475 (M_2 - H), 329 (M - H + glycerol), 237 (M - H). Anal. ($C_{11}H_{11}PO_4$) C, H.

Bis(2,2-dimethylethyl) (2-Naphthalenylhydroxymethyl)phosphonate (15). Synthesis of **15** was accomplished by reacting 2-naphthaldehyde and di-*tert*-butyl phosphite by using the method described above to prepare **13**. It was necessary to first dissolve the reactants in CH_2Cl_2, mix with alumina, and then remove solvent by evaporation prior to setting overnight. The resulting reaction product was purified by silica gel chromatography eluting with CH_2Cl_2 then 10% EtOH in CH_2Cl_2, giving an oil which crystallized. Trituration with boiling petroleum ether produced product as white crystals (39%): mp 124–127 °C (lit.[20] no mp given); NMR ($CDCl_3$) δ 1.43 (s, 9 H, $C(CH_3)_3$), 1.47 (s, 9 H, $C(CH_3)_3$), 5.06 (d, 1 H, J = 10.3 Hz, PCH), 7.47–7.52 (m, 2 H, ArH), 7.58–7.64 (m, 1 H, ArH), 7.83–7.88 (m, 3 H, ArH), 7.92–7.98 (m, 1 H, ArH).

(2-Naphthalenylhydroxymethyl)phosphonic Acid (16). Synthesis of **16** was accomplished by deprotection of **15** as described for **14**, giving **16** as white crystals (84%): mp >250 °C (lit.[20] no mp given); NMR (Me_2SO-d_6) δ 5.47 (d, 1 H, J = 14.3 Hz, PCH), 7.44–7.48 (m, 2 H, ArH), 7.53–7.60 (m, 1 H, ArH), 7.78–7.90 (m, 1 H, ArH), 7.80–7.90 (m, 3 H, ArH); FABMS (negative ion) m/z 475 (M_2 - H), 329 (M - H + glycerol), 237 (M - H).

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Registry No. 3a, 132541-42-5; **3b**, 132541-57-2; **3c**, 132541-59-4; **4a**, 132541-43-6; **4a**·$^1/_3CH_3OH$, 132541-44-7; **4b**, 132541-60-7; **4c**, 132541-61-8; **6**, 132541-45-8; **7**, 132541-46-9; **10**, 132541-47-0; **11a**, 132541-48-1; **11b**, 132541-53-8; **11c**, 132541-54-9; **12a**, 132541-49-2; **12b**, 132541-55-0; **12c**, 132541-56-1; **13**, 132541-50-5; **14**, 115308-68-4; **15**, 132541-51-6; **16**, 132541-52-7; **17**, 1136-89-6; **18**, 13095-41-5; **19**, 85-47-2; **20**, 120-18-3; tyrosine-specific protein kinase, 80449-02-1; di-*tert*-butyl phosphite, 13086-84-5; 1-naphthaldehyde, 66-77-3; 2-naphthaldehyde, 66-99-9; 4-(diethoxymethyl)benzaldehyde, 81172-89-6.