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Synthesis of Vinyl Pyrophosphonate Analogues of Farnesyl Pyrophosphate: New Potential Inhibitors of Farnesyl Protein Transferase

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ABSTRACT: *The synthesis of new bioisosteric analogues of farnesyl pyrophosphate where a vinyl pyrophosphonate replaced the pyrophosphate moiety is described. These compounds have been prepared using a Horner–Wadsworth–Emmons procedure and a modified Michelson reaction. They have been evaluated for the inhibition of farnesyl protein transferase.* © 2002 Wiley Periodicals, Inc. Heteroatom Chem 13:654–661, 2002; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10081

INTRODUCTION

Protein prenylation is an important posttranslational protein modification, which appears to play a major role in protein association with membranes as well as in protein–protein interactions [1]. This process involves covalent addition of either farnesyl or geranyl–geranyl groups to cystein residues localized at or near protein's C- terminus.

Ras proteins play an important role in a signaling the pathway that controls cell proliferation, e.g. mutations in Ras proteins have been estimated to be intimately associated with 30% of all human cancers [2]. Ras gene mutations produce mutated Ras that remains locked in an activated GTP-bound state thereby relaying an uncontrolled proliferative signal resulting in malignant transformations [3,4].

To function in the signal transduction, Ras protein must be bound to the cell membrane. This binding is facilitated by a sequence of postranslational modifications [5]. It has been shown, in particular, that farnesylation of Ras is necessary for proper functioning in cell signaling; this prenylation is catalyzed by an enzyme, protein farnesyl transferase, and this discovery provides the rational of the development of FTase inhibitors as a new type of antiproliferative agents.

Several types of farnesyl transferase inhibitors, especially peptides or peptidomimetics, have been designed for use as anticancer drugs depending on their mechanism of activity [6]. Farnesyl pyrophosphate analogues as potential chemotherapeutic agents have also been extensively studied. Among them, several mono-, bi-, or triphosphonate compounds, such as compound **1a**, have been synthesized for an examination of their ability to inhibit FTase [6,7]. Furthermore, to gain information about the interaction between proteins and prenylated pyrophosphate, compounds that incorporate a benzophenone crosslinking group have been synthesized to exploit their photochemical properties. In this respect, the synthesis of compounds **1b** and **1c** that contain stable ether linkages between the photoactive benzophenone and the isoprenic moieties has been described. Both compounds were found to

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be competitive inhibitors of yeast protein farnesyl transferase, with respect to farnesyl pyrophosphate (FPP), the presence of benzoyl benzyl group inducing a higher affinity for FTase when compared to FPP [8,9].

Here, we report the synthesis of bioisosteric analogues of isoprenoid pyrophosphates: vinylpyrophosphonate derivatives, which are known to be stable towards enzymatic hydrolysis.

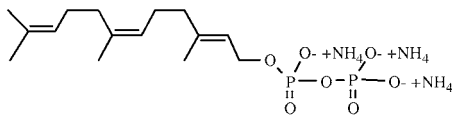
The first compound targeted was the vinyl pyrophosphonate **2a** corresponding to phosphonate **1a** whose inhibitory activity on FTase had already been observed [6]. Compared to **1a**, pyrophosphonate **2a**, which contains one more phosphorus atom, is more bioisosteric with respect to farnesyl pyrophosphate, and therefore an increased activity on FTase may be expected.

Other components are vinylpyrophosphonate analogues of **1b** and **1c**, which were synthesized to test the effect of the replacement of the pyrophosphate moiety on their inhibitory activity on FTase (Scheme 1).

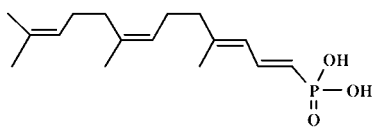
SYNTHESIS

Compounds **2b,c** and the triphosphorylated compounds **3b,c** were synthesized in a nine steps sequence as illustrated in Schemes 2 and 3. Dimethylallyl alcohol (**4b**) [8] and geraniol (**4c**) were first protected as THP derivatives by reaction with 3,4-dihydropyran and pyridinium *p*-toluenesulfonate (PPTS) [10]. The resulting ethers **5b** and **5c** were oxidized with *t*-butylhydroperoxide and catalytic H_2SeO_3 in dichloromethane affording **6b** and **6c** (38 and 47% yields respectively) [11], which were then treated with NaH and 4-(bromomethyl)benzophenone to give **7b** and **7c** (36 and 49% yields) [8]. Deprotection of compounds **7b** and **7c** was accomplished with catalytic PPTS in methanol and afforded **8b** and **8c** in quantitative yield [10]. The alcohols **8b** and **8c** were oxidized with pyridinium dichromate in DMF and yielded the corresponding aldehydes **9b** and **9c** in 68 and 70% yield, respectively [12]. This reaction is very clean, and the isolation of products is simple. There is no overoxidation of the aldehydes and no E to Z isomerization of the aldehyde α–β double bond such that we observed with manganese dioxide [13] or the Swern reagent [14]. Farnesal (**4a**) was obtained according to the same procedure (91%) from farnesol.

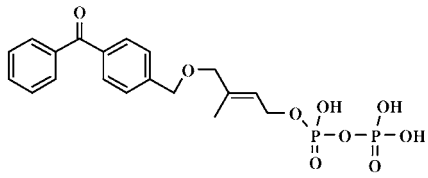
Treatment of the aldehydes **4a, 9b**, and **9c** with tetraethylmethylene diphosphonate and NaH in benzene gave via a Horner–Wadsworth–Emmons condensation the expected vinyl phosphonates **5a, 10b**, and **10c** (60, 95, and 75% yields) [15]. The resulting



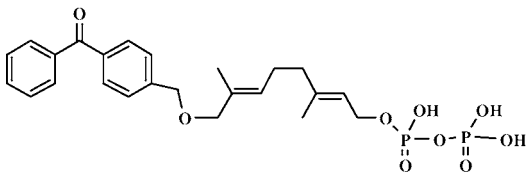
Farnesyl pyrophosphate



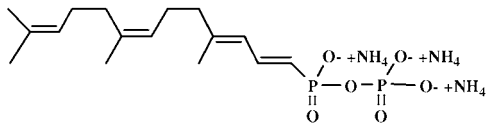
1a



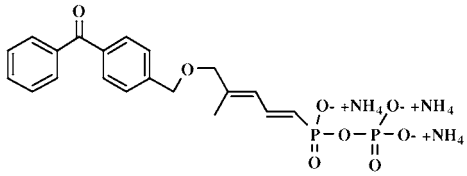
1b



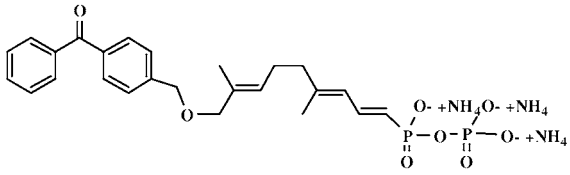
1c



2a

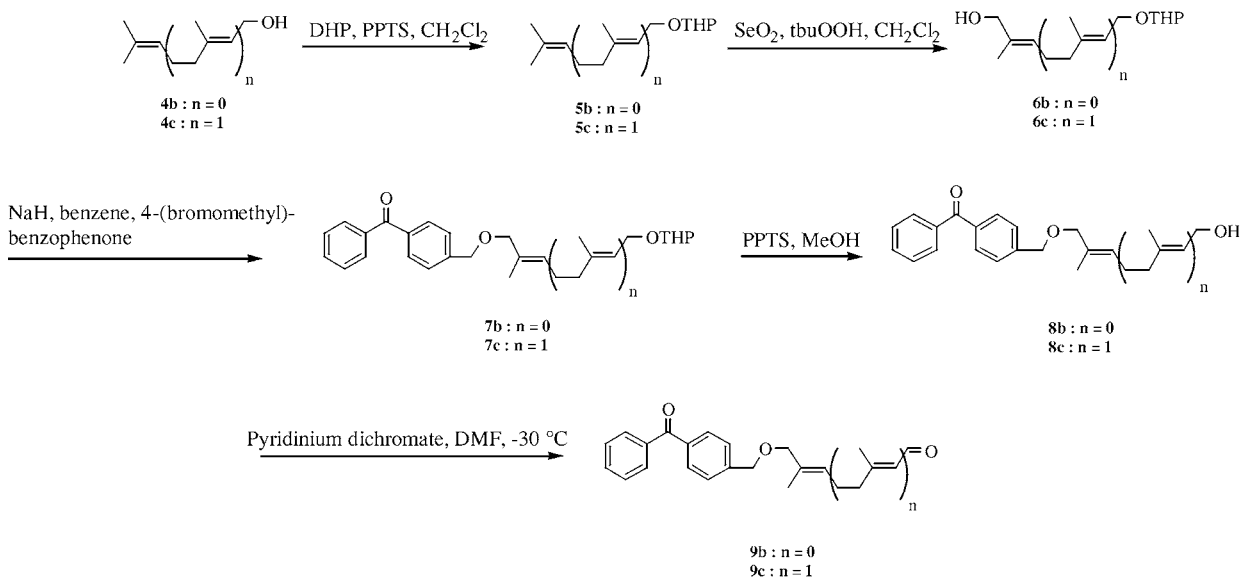


2b



2c

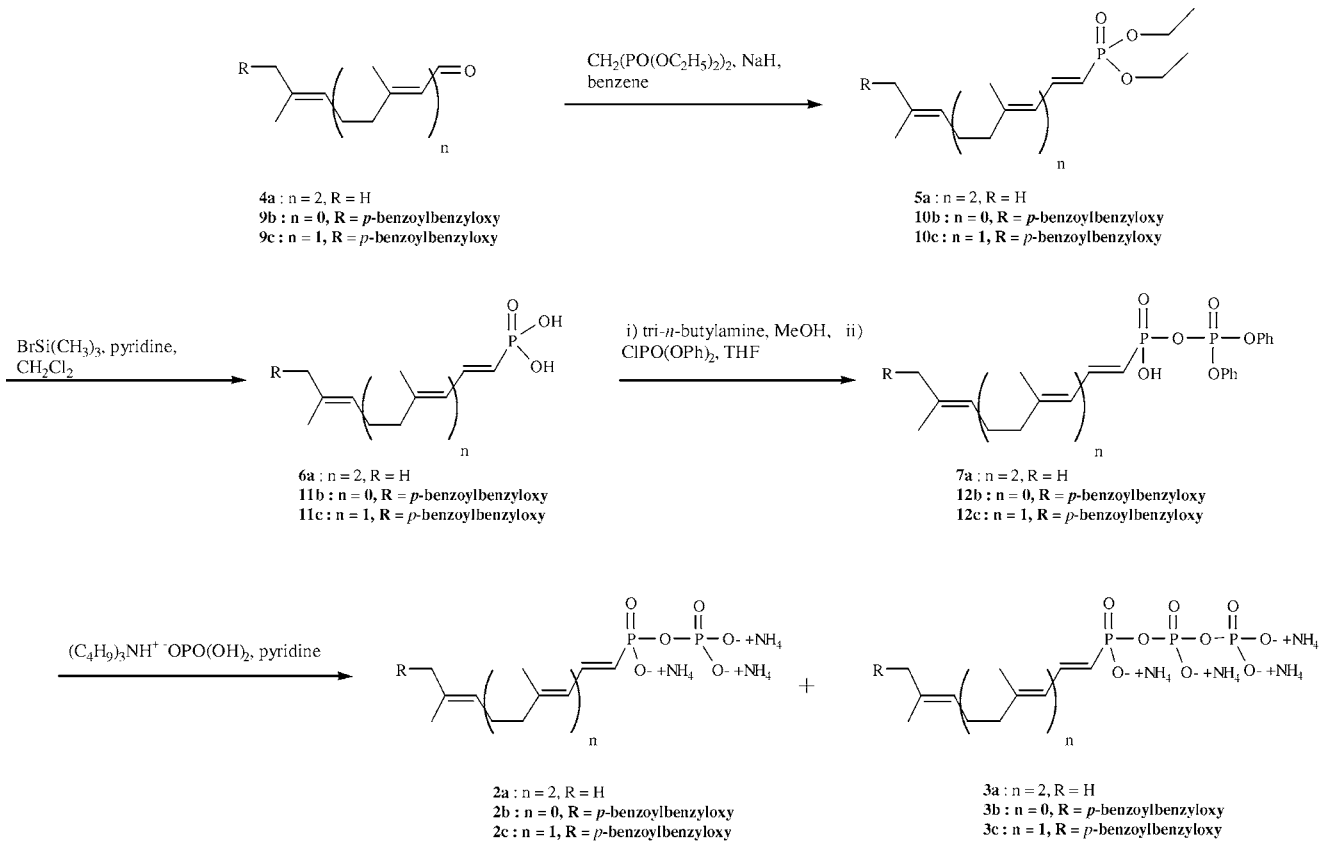
SCHEME 1 Inhibitors of FTase (**1a–c**) and their pyrophosphonate analogues (**2a–c**).



SCHEME 2 Synthesis procedure of aldehyde precursors.

phosphonates were converted to the corresponding phosphonic acids **6a, 11b**, and **11c** by standard treatment with bromotrimethylsilane and pyridine in CH_2Cl_2 (84, 70, and 85% yields, respectively) [16].

The synthesis of vinyl pyrophosphonates was performed using modified Michelson's conditions (detailed in the experimental section) [17]. Compounds **6a, 11b**, and **11c** were first activated by



SCHEME 3 Synthesis procedure of pyrophosphonates from aldehydes.

the reaction of their tri-*n*-butylammonium salt in dry THF with diphenyl phosphorochloridate and tri-*n*-butylamine. Compounds **7a, 12b**, and **12c** obtained by this reaction were then combined without any purification in dry pyridine with the tri-*n*-butylammonium orthophosphate to give compounds **2a, 2b**, and **2c** in 49, 48, and 57% yields, respectively, after purification. Besides, formation of the corresponding triphosphorylated derivatives **3a, 3b**, and **3c** was observed and these compounds were obtained in 18, 6, and 17% yields, respectively. The formation of compounds **3a, 3b**, and **3c** is due to the fact that the activation is not complete, so that the remaining diphenyl phosphochloridate can react with the tri-*n*-butylammonium orthophosphate producing the ^1P-diphenyl pyrophosphate. The subsequent displacement of the diphenyl phosphate group by the tri-*n*-butylammonium salts of compound **2a, 2b**, or **2c** conduced finally to compound **3a, 3b**, or **3c**.

BIOLOGICAL EVALUATION

Compounds **2a–c** and **3a–c** were tested for their ability to inhibit farnesyl transferase. None of the compounds showed inhibitory activity at a concentration up to 1 μM as shown by fluorimetry testing on an isolated enzyme from bovine brain [18–20].

CONCLUSION

The incorporation of a second phosphorus in the farnesyl phosphonic acid **1a** did not ameliorate significantly its activity. To make a comparison with the benzophenone containing molecules (**1b** and **1c**) that were more efficiently recognized by FTase than the farnesyl pyrophosphate, compounds **2b** and **2c** have been prepared. Their biological evaluation indicates that both compounds do not inhibit the FTase at a micromolar concentration. These results may probably be explained by a too large difference in the polarity or/and steric properties with the substrate.

EXPERIMENTAL

Reactions were monitored by TLC, using aluminum-coated plates with silica gel 60 F_{254} (MERCK). Columns chromatography were carried with Carlo Erba silica gel 60 A (35–70 μm). All solvents were dried and distilled before use [21]. ^{1}H and ^{31}P NMR spectra were recorded with an AC 50 Bruker 200 MHz spectrometer and ^{13}C NMR spectra with a WP-200-SY Bruker spectrometer by using $CDCl_3$ or D_2O as solvent. Chemical shifts are given using residual solvent peaks as a reference relative to TMS. Mass spectra were measured with a Jeol DX-300 spectrometer in the FAB^+ (NBA) or FAB^- (GT) ion mode.

General Procedure for the Preparation of the Aldehydes from Corresponding Alcohols

To a solution of pyridinium dichromate (4.45 mmol, 1.2 eqiv.) in DMF (30 ml), which was cooled to −30°C and maintained under nitrogen, the alcohol **8b, 8c**, or farnesol (3.56 mmol, 1 eqiv.) was added and the mixture was stirred for 3 h, then allowed to warm to room temperature; finally water (100 ml) was added. The aqueous solution was extracted with ether (3 × 50 ml) and the combined ether extracts were washed with aqueous HCl (1 N, 80 ml) then with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The oily residue was chromatographed on a silica gel column with ethyl ether – petroleum ether (3:7) as eluent.

*(E)-3-Methyl-4-(4′-benzoylbenzyloxy)-2-butenal (**9b**).* (712 mg, 68%).^{1}H NMR: δ 2.15 (d, $J_{5\text{-}2} = 1.4$ Hz, 3H, H_5), 4.1 (s, 2H, H_4), 4.62 (s, 2H, $OC\underline{H}_2C_6H_4$), 6.21 (qd, $J_{2\text{-}1} = 8.0$ Hz, 1H, H_2), 7.42–7.55 (m, 4H, H_{ar}), 7.55–7.66 (m, 1H, H_{ar}), 7.78–7.86 (m, 4H, H_{ar}), 10.08 (d, 1H, H_1). ^{13}C NMR: δ 14.88 (1C, C_5), 72.56 (1C, C_4), 74.23 (1C, $OC\underline{H}_2C_6H_4$), 126.15 (1C, C_2), 127.52 (2C, C_{ar}), 128.69 (2C, C_{ar}), 130.4 (2C, C_{ar}), 130.72 (2C, C_{ar}), 132.75 (1C, C_{ar}), 137.47 (1C, $C_{1''}$), 137.92 (1C, $C_{4'}$), 142.67 (1C, $C_{1'}$), 152.42 (1C, C_3), 191.3 (1C, C_1), 196.69 (1C, C_{ketone}). FAB^+ (NOBA): *m/z* 295 $(M + H)^+$; 317 $(M + Na)^+$. Anal calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16; O, 16.31. Found: C, 77.55; H, 6.14; O, 16.30.

*(E,E)-3,7-Dimethyl-8-(4′-benzoylbenzyloxy)-2,6-octadienal (**9c**).* (900 mg, 70%). ^{1}H NMR: δ 1.60 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 2.28–2.38 (m, 4H, H_4 and H_5), 3.97 (s, 2H, H_8), 4.56 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.45 (m, 1H, H_6), 5.92 (d, $J_{2\text{-}1} = 7.9$ Hz, 1H, H_2), 7.45–7.56 (m, 4H, H_{ar}), 7.57–7.68 (m, 1H, H_{ar}), 7.78–7.88 (m, 4H, H_{ar}), 10.02 (d, 1H, H_1). ^{13}C NMR: δ 14.41 (1C, C_9), 18.10(1C, C_{10}), 25.73 (1C, C_5), 40.54 (1C, C_4), 71.45 (1C, C_8), 76.71 (1C, $O\underline{C}H_2C_6H_4$), 126.78 (1C, C_2), 127.20 (2C, C_{ar}), 127.55 (1C, C_6), 128.69 (2C, C_{ar}), 130.71 (2C, C_{ar}), 130.84 (2C, C_{ar}), 132.83 (1C, $C_{4''}$), 133.82 (1C, C_7), 137.16 (1C, $C_{1''}$), 138.08 (1C, $C_{4'}$), 143.85 (1C, $C_{1'}$), 163.78 (1C, C_3), 191.62 (1C, C_1), 196.96 (1C, C_{ketone}). FAB^+ (NOBA): *m/z* 363 $(M + H)^+$; 385 $(M + Na)^+$. Anal calcd for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23; O, 13.24. Found: C, 79.56; H, 7.20; O, 13.25.

General Procedure for the Horner–Wadsworth–Emmons Reaction

To a suspension of sodium hydride (2.98 mmol, 1.2 eqiv.) in dry benzene (20 ml) was added under a nitrogen atmosphere tetraethylmethylene diphosphonate (2.98 mmol, 1.2 eqiv.) at room temperature. After 15 min, a solution of the aldehyde **4a, 9b**, or **9c** (2.48 mmol, 1 eqiv.) in dry benzene (10 ml) was added and the mixture was stirred for 2 h. After dilution with CH_2Cl_2 (100 ml), the mixture was washed with water (100 ml), aqueous NaOH (0.1 N, 100 ml), and water (100 ml).The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Silica gel chromatography with ethyl acetate gave pure diethylphosphonate.

*(E,E,E)-1-Diethylphosphono-4,8,12-trimethyl-1,3,7,11-tridecatetraene (***5a***).* (526 mg, 60%; ^{1}H NMR: δ 1.2 (t, 6H, H_{18}, H_{20}), 1.53 (s, 6H, H_{13} and H_{16}), 1.62 (s, 3H, H_{15}), 1.82 (s, 3H, H_{14}), 1.93 (m, 4H, H_9 and H_{10}), 2.1 (s, 4H, H_5 and H_6), 4.02 (m, 4H, H_{17} and H_{19}), 4.05 (m, 2H, H_7 and H_{11}), 5.48 (dd, $J_{1\text{-}2} = 16.6$ Hz, $J_{1\text{-P}} = 20.2$ Hz, 1H, H_1), 5.9 (d, $J_{3\text{-}2} = 11.2$ Hz, 1H, H_3), 7.3 (ddd, $J_{2\text{-P}} = 21.0$ Hz, 1H, H_2); ^{31}P NMR: δ 21.1.^{13}C NMR: 14.78–15.10 (2C, C-18); 15.17–16.13–16.45 (3C, C-14, C-15 and C-16); 24.47 (1C, C-13); 24.93–25.44 (1C, C-6 and C-10); 38.44–38.89 (2C, C-5 and C-9); 60.32–60.37 (2C, C-17); 112 (d, $J_{1\text{-P}} = 192.2$ Hz, 1C, C-1); 121.98–122.97 (2C, C-7 and C-11); 123.07 (d, $J_{3\text{-P}} = 26.4$ Hz, 1C, C-3); 130.15–134.62 (2C, C-8 and C-12); 144.21 (d, $J_{2\text{-P}} = 6.8$ Hz, 1C, C-2); 147.73 (1C, C-4). FAB$^+$ (NOBA): *m/z* 355 $(M + H)^+$; 377 $(M + Na)^+$. Anal calcd for $C_{20}H_{35}O_3P$: C, 67.78; H, 9.89; O, 13.56; P, 8.75. Found: C, 67.75; H, 9.90; O, 13.59; P, 8.76.

*(E,E)-[4-(5′-Diethylphosphono-2′-methyl-2′,4′-pentadienyloxymethyl)-phenyl]-phenyl-methanone (***10b***).* (1010 mg, 95%). ^{1}H NMR: δ 1.34 (t, $J_{8'\text{-}7'} = J_{10'\text{-}9'} = 6.9$ Hz, 6H, $H_{8'}$ and $H_{10'}$), 1.92 (s, 3H, CH_3), 4.06 (s, 2H, $H_{1'}$), 4.08–4.28 (m, 4H, $2H_{7'}$ and $2H_{9'}$), 4.60 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.70 (dd, $J_{5'\text{-P}} = 19.5$ Hz, $J_{5'\text{-}4'} = 16.9$ Hz, 1H, $H_{5'}$), 6.26 (d, $J_{3'\text{-}4'} = 11.6$ Hz, 1H, $H_{3'}$), 7.30–7.65 (m, 6H, $H_{4'}$ and $5H_{ar}$), 7.60–7.88 (m, 4H, H_{ar}). ^{31}P NMR: δ 20.9. ^{13}C NMR: δ 16.70 (1C, $C_{6'}$), 16.81 (2C, $C_{8'}$ and $C_{10'}$), 63.15 (2C, $C_{7'}$ et $C_{9'}$), 72.15 (1C, $C_{1'}$), 75.32 (1C, $O\underline{C}H_2C_6H_4$), 117.18 (d, $J_{5'\text{-P}} = 191.4$ Hz, 1C, $C_{5'}$), 125.13 (d, $J_{3'\text{-P}}$ 26.9 Hz, 1C, $C_{3'}$), 127.54 (2C, C_{ar}), 128.72 (2C, C_{ar}), 130.50 (2C, C_{ar}), 130.74 (2C, C_{ar}), 132.88 (1C, $C_{4''}$), 137.37 (1C, $C_{1''}$), 138.02 (1C, C_1), 143.20 (1C, C_4), 143.99 (1C, $C_{2'}$), 144.53 (d, $J_{4'\text{-P}} = 6.7$ Hz, 1C, $C_{4'}$), 196.82 (1C, C_{ketone}). FAB$^+$ (NOBA): *m/z* 429 $(M + H)^+$; 451 $(M + Na)^+$. Anal calcd for $C_{24}H_{29}O_5P$: C, 67.28; H, 6.82; O, 18.67; P, 7.23. Found: C, 67.29; H, 6.82; O, 18.69; P, 7.24.

*(E,E,E)-[4-(9′-Diethylphosphono-2′,6′-dimethyl-2′,6′,8′-nonatrienyloxymethyl)-phenyl]-phenyl-methanone (***10c***).* (870 mg, 75%). ^{1}H NMR: δ 1.24 (t, $J_{13'\text{-}12'} = J_{15'\text{-}14'} = 7.0$ Hz, 6H, $H_{13'}$ and $H_{15'}$), 1.73 (s, 3H, CH_3), 1.91 (s, 3H, CH_3), 2.20–2.30 (m, 4H, $2H_{4'}$ and $2H_{5'}$), 3.97 (s, 2H, $H_{1'}$), 4.09 (m, 4H, $H_{12'}$ and $H_{14'}$), 4.55 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.45 (m, 1H, $H_{3'}$), 5.59 (dd, $J_{9'\text{-P}} = 20.2$ Hz, $J_{9'\text{-}8'} = 16.2$ Hz, 1H, $H_{9'}$), 5.99 (d, $J_{7'\text{-}8'} = 12.1$ Hz, 1H, $H_{7'}$), 7.32–7.65 (m, 1H, $H_{8'}$), 7.46–7.65 (m, 5H, H_{ar}), 7.76–7.90 (m, 4H, H_{ar}). ^{31}P NMR: δ 21.7. ^{13}C NMR: δ 14.41 (1C, $C_{10'}$), 16.78 (2C, $C_{13'}$ and $C_{15'}$), 17.75 (1C, $C_{11'}$), 26.32 (1C, $C_{4'}$), 40.11 (1C, $C_{5'}$), 62 (2C, $C_{12'}$ and $C_{14'}$), 71.31 (1C, $C_{1'}$), 76.91 (1C,$O\underline{C}H_2C_6H_4$), 114.94 (d, $J_{9'\text{-P}} = 192.2$ Hz, 1C, $C_{9'}$), 124.94 (d, $J_{7'\text{-P}} = 26.56$ Hz, 1C, $C_{7'}$), 127.61 (2C, C_{ar}), 127.83 (1C, $C_{3'}$), 128.69 (2C, C_{ar}), 130.44 (2C, C_{ar}), 130.69 (2C, C_{ar}), 132.80 (1C, $C_{4''}$), 133.12 (1C, C_2), 137.16 (1C, $C_{1''}$), 138.11 (1C, C_1), 143.84 (1C, C_4), 145.52 (d, $J_{8'\text{-P}} = 6.6$ Hz, 1C, $C_{8'}$), 148.66 (1C, $C_{6'}$), 196.89 (1C, C_{ketone}). FAB$^+$ (NOBA): *m/z* 497 $(M + H)^+$; 519 $(M + Na)^+$. Anal calcd for $C_{29}H_{37}O_5P$: C, 70.14; H, 7.50; O, 16.11; P, 6.23. Found: C, 70.16; H, 7.49; O, 16.22; P, 6.25.

General Procedure for the Conversion of Vinyl Phosphonates to Vinyl Phosphonic Acids

To a solution of compound **5a, 10b**, or **10c** (1.61 mmol, 1 eqiv.) in CH_2Cl_2 (30 ml) were added successively pyridine (16.1 mmol, 10 eqiv.) and bromotrimethylsilane (8.1 mmol, 5 eqiv.). The mixture was stirred for 6 h at room temperature and under nitrogen. The solvent was then removed and aqueous NaOH (1 N, 30 ml) was added. The aqueous solution was stirred for 20 min then extracted with ether to remove the pyridine excess. After acidification to pH 2, the mixture was extracted with ether (3 × 100 ml) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give pure vinyl phosphonic acid **6a, 11b**, or **11c**.

*(E,E,E)-1-Phosphono-4,8,12-trimethyl-1,3,7,11-tridecatetraene (***6a***).* (334 mg, 84%). ^{1}H NMR: δ 1.61 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.94–2.17 (m, 8H), 5.02–5.21 (m, 2H), 5.58–5.76 (m, 1H), 5.91 (m, 1H), 7.12–7.5 (m, 1H), 10.6 (br s, 2H); ^{31}P NMR: δ 21.7; ^{13}C NMR: δ 15.66–16.43–17.70–18.11 (4C, C_{13}, C_{14}, C_{15} and C_{16}), 26.12–27.12 (2C, C_5 and C_9), 40.10–40.54 (2C, C_6 and C_{10}), 119.53 (d, $J_{1\text{-P}} = 184.5$ Hz, 1C, C_1), 123.71–124.67 (2C, C_7 and C_{11}), 124.79 (d, $J_{3\text{-P}} = 24.6$ Hz, 1C, C_3), 131.79–136.23

(2C, C_8 and C_{12}), 144.33 (d, $J_{2\text{-P}} = 5.9$ Hz, 1C, C_2), 149.04 (1C, C_4). FAB$^+$ (NOBA): *m/z* 293 $(M + H)^+$; 321 $(M + Na)^+$.

*(E,E)-[4-(5′-Phosphono-2′-methyl-2′-4′-pentadienyloxymethyl)-phenyl]-phenyl-methanone (***11b***).* (460 mg, 70%). ^{1}H NMR: δ 1.91 (s, 3H, CH_3), 4.06 (s, 2H, $H_{1'}$), 4.60 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.80 (dd, $J_{5'\text{-P}} = 21.1$ Hz, $J_{5'\text{-}4'} = 16.6$ Hz, 1H, $H_{5'}$), 6.25 (d, $J_{3'\text{-}4'} = 11.4$ Hz, 1H, $H_{3'}$), 7.35–7.60 (m, 6H, $H_{4'}$ and $5H_{ar}$), 7.72–7.88 (m, 4H, H_{ar}), 10.25 (s, 2H, $PO\underline{H}$). ^{31}P NMR: δ 23.6. ^{13}C NMR: δ 15.23 (1C, $C_{6'}$), 72.06 (1C, $C_{1'}$), 75.35 (1C, $OC\underline{H}_2C_6H_4$), 118.59 (d, $J_{5'\text{-P}} = 188.5$ Hz, 1C, $C_{5'}$), 126.65 (d, $J_{3'\text{-P}} = 26.6$ Hz, 1C, $C_{3'}$), 127.55 (2C, C_{ar}), 128.71 (2C, C_{ar}), 130.44 (2C, C_{ar}), 130.74 (2C, C_{ar}), 132.84 (1C, $C_{4''}$), 134.50 (1C, $C_{1''}$), 135.0 (1C, C_1), 137.36 (d, $J_{4'\text{-P}} = 6.1$ Hz, 1C, $C_{4'}$), 143.19 (1C, C_4), 144.0 (1C, $C_{2'}$), 198.0 (1C, C_{ketone}). FAB$^+$ (NOBA): *m/z* 373 $(M + H)^+$; 395 $(M + Na)^+$.

*(E,E,E)-[4-(9′-Phosphono-2′,6′-dimethyl-2′,6′,8′-nonatrienyloxymethyl)-phenyl]-phenyl-methanone (***11c***).* (600 mg, 85%). ^{1}H NMR: δ 1.72 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 2.12–2.30 (m, 4H, $2H_{4'}$ and $2H_{5'}$), 3.96 (s, 2H, $H_{1'}$), 4.54 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.45 (m, 1H, $H_{3'}$), 5.66 (dd, $J_{9'\text{-P}} = 20.6$ Hz, $J_{9'\text{-}8'} = 16.8$ Hz, 1H, $H_{9'}$), 5.95 (d, $J_{7'\text{-}8'} = 10.9$ Hz, 1H, $H_{7'}$), 7.35–7.60 (m, 1H, $H_{8'}$), 7.40–7.68 (m, 5H, H_{ar}), 7.75–7.88 (m, 4H, H_{ar}). ^{31}P NMR: δ 24.4. ^{13}C NMR: δ 14.39 (1C, $C_{10'}$), 16.80 (1C, $C_{11'}$), 26.21 (1C, $C_{4'}$), 40.10 (1C, $C_{5'}$), 71.20 (1C, $C_{9'}$), 76.88 (1C, $O\underline{C}H_2C_6H_4$), 114.89 (d, $J_{9'\text{-P}} = 190$ Hz, 1C, $C_{9'}$), 124.93 (d, $J_{7'\text{-P}} = 26.3$ Hz, 1C, $C_{7'}$), 127.58 (2C, C_{ar}), 127.80 (1C, $C_{3'}$), 128.67 (2C, C_{ar}), 130.47 (2C, C_{ar}), 130.71 (2C, C_{ar}), 132.81 (1C, $C_{4''}$), 133.06 (1C, $C_{2'}$), 137.04 (1C, $C_{1''}$), 138.0 (1C, $C_{1'}$), 143.80 (1C, C_4), 145.46 (d, $J_{8'\text{-P}} = 6.6$ Hz, 1C, $C_{8'}$), 148.69 (1C, $C_{6'}$), 196.77 (1C, C_{ketone}). FAB$^+$ (NOBA): *m/z* 441 $(M + H)^+$; 463 $(M + Na)^+$.

General Method for the Phosphorylation of Vinyl Phosphonic Acids

- *Preparation of the activated phosphonoanhydride*: To a solution of compound **6a, 11b**, or **11c** (1.36 mmol, 1 eqiv.) in methanol (20 ml) was added tri-*n*-butylamine (1.36 mmol, 1 eqiv.). The mixture was stirred at room temperature for 30 min, then methanol was evaporated under reduced pressure and the residue was dried by repeated coevaporation with dry pyridine (3 × 10 ml). The tri-*n*-butylammonium salt of the vinyl phosphonic acid was dissolved in dry THF (20 ml), then diphenylphosphorochloridate (1.36 mmol, 1 eqiv.) and tri-*n*-butylamine (4.1 mmol, 3 eqiv.) were added successively. The mixture was stirred at room temperature under nitrogen for 2 h.
- *Preparation of the tri-n-butylammonium orthophosphate*: To a solution of orthophosphoric acid (4.1 mmol, 3 eqiv.) in methanol (20 ml) was added tri-*n*-butylamine (4.1 mmol, 3 eqiv.). The mixture was stirred for 30 min at room temperature. Methanol was evaporated under reduced pressure and the residue was dried by repeated coevaporation with dry pyridine (3 × 10 ml).
- *Coupling procedure*: To a stirred solution of the tri-*n*-butylammonium orthophosphate (4.1 mmol, 3 eqiv.) in dry pyridine (20 ml) was added the activated phosphonoanhydride prepared in situ in THF within a period of 10 h at room temperature and under nitrogen. The mixture was stirred for two additional hours and then the solvent was removed. Column chromatography with isopropanol-aqueous ammonia (27%) (6:4) gave **2a–c** and **3a–c**.

*(E,E,E)-1-Pyrophosphono-4,8,12-trimethyl-1,3,7,11-tridecatetraene (***2a***).* (286 mg, 49%). ^{1}H NMR: δ 1.49 (s, 6H, $2CH_3$), 1.56 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 1.73–2.10 (m, 8H, H_5, H_6, H_9 and H_{10}), 4.93–5.12 (m, 2H, H_7 and H_9), 5.5 (m, 1H, H_1), 5.98 (d, $J_{3\text{-}2} = 11$ Hz, 1H, H_3), 6.88–7.13 (m, 1H, H_2). ^{31}P NMR: δ −9.92 (d, $J_{\beta-\alpha} = 22.2$ Hz, 1P, P_β), 8.42 (d, 1P, P_α). ^{13}C NMR: δ 15.87–16.75–16.89–17.48 (4C, C_{13}, C_{14}, C_{15} and C_{16}), 25.52–26.58 (2C, C_5 and C_9), 39.59–40.03 (2C, C_6 and C_{10}), 120.91 (d, $J_{1\text{-P}} = 188.9$ Hz, 1C, C_1), 124.36–124.80 (2C, C_7 and C_{11}), 125.04 (d, $J_{3\text{-P}} = 21.2$ Hz, 1C, C_3), 132.56–136.38 (2C, C8 and C_{12}), 140.52 (1C, C_2), 146.90 (1C, C_4).

*(E,E,E)-1-Triphosphono-4,8,12-trimethyl-1,3,7,11-tridecatetraene (***3a***).* (128 mg, 18%) ^{1}H NMR: δ 1.47 (s, 6H, $2CH_3$), 1.54 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.73–2.08 (m, 8H, H_5, H_6, H_9 and H_{10}), 4.91–5.10 (m, 2H, H_7 and H_9), 5.48–5.76 (m, 1H, H_1), 5.97 (d, $J_{3\text{-}2} = 11.0$ Hz, 1H, H_3), 6.86–7.12 (m, 1H, H_2). ^{31}P NMR: δ −21.3 (dd, $J_{\beta-\alpha} = 23.1$ Hz, $J_{\beta-\gamma} = 20$ Hz, P_β), −6.3 (d, P_γ), 8.6 (d, P_α). ^{13}C NMR: δ 15.83–16.72–16.91–17.52 (4C, C_{13}, C_{14}, C_{15} and C_{16}), 25.53–26.62 (2C, C_5 and C_9), 39.59–40.09 (2C, C_6 and C_{10}), 120.99 (d, $J_{1\text{-P}} = 189.2$ Hz, 1C, C_1), 124.34–124.81 (2C, C_7 and C_{11}), 125.14 (d, $J_{3\text{-P}} = 22.33$ Hz, 1C, C_3), 132.40–136.43 (2C, C_8 and C_{12}), 140.58 (1C, C_2), 146.92 (1C, C_4).

*(E,E)-[4-(5′-Pyrophosphono-2′-methyl-2′,4′-pentadienyloxymethyl)-phenyl]-phenyl-methanone (***2b***).* (294 mg, 48%). ^{1}H NMR: δ 1.64 (s, 3H, CH_3), 4.0 (s, 2H, $H_{1'}$), 4.51 (s, 2H, $OC\underline{H}_2C_6H_4$), 5.90 (dd,

$J_{5'\text{-P}} = 19.2$ Hz, $J_{5'\text{-}4'} = 16.8$ Hz, 1H, $H_{5'}$), 6.13 (d, $J_{3'\text{-}4'} = 10.9$ Hz, 1H, $H_{3'}$), 7.10 (ddd, $J_{4'\text{-P}} = 20.4$ Hz, 1H, $H_{4'}$), 7.35–7.50 (m, 4H, H_{ar}), 7.54–7.62 (m, 1H, H_{ar}), 7.62–7.74 (m, 4H, H_{ar}). ^{31}P NMR: δ −7.16 (d, $J_{\beta-\alpha} = 23.0$ Hz, 1P, P_β), 6.54 (d, 1P, P_α). ^{13}C NMR: δ 14.33 (1C, $C_{6'}$), 71.22 (1C, $C_{1'}$), 75.62 (1C, O<u>C</u>$H_2C_6H_4$), 124.53 (d, $J_{5'\text{-P}} = 185.0$ Hz, 1C, $C_{5'}$), 127.48 (d, $J_{3'\text{-P}} = 26.2$ Hz, 1C, $C_{3'}$), 128.19 (2C, C_{ar}), 128.84 (2C, C_{ar}), 130.74 (2C, C_{ar}), 130.88 (2C, C_{ar}), 133.82 (1C, $C_{4''}$), 136.40 (1C, $C_{1''}$), 136.88 (1C, C_1), 138.83 (d, $J_{4'\text{-P}} = 5.7$ Hz, 1C, $C_{4'}$), 140.92 (1C, C_4), 143.40 (1C, $C_{2'}$), 200.37 (1C, C_{ketone}).

*(E,E)-[4-(5′-Triphosphono-2′-methyl-2′,4′-pentadienyloxymethyl)-phenyl]-phenyl-methanone (***3b***).* (43 mg, 6%). ^{1}H NMR: δ 1.79 (s, 3H, CH_3), 4.03 (s, 2H, $H_{1'}$), 4.54 (s, 2H, OC<u>H</u>$_2C_6H_4$), 5.92 (dd, $J_{5'\text{-P}} = 19.9$ Hz, $J_{5'\text{-}4'} = 16.6$ Hz, 1H, $H_{5'}$), 6.14 (d, $J_{3'\text{-}4'} = 11.1$ Hz, 1H, $H_{3'}$), 7.12 (ddd, $J_{4'\text{-P}} = 20.0$ Hz, 1H, $H_{4'}$), 7.30–7.50 (m, 4H, H_{ar}), 7.52–7.68 (m, 5H, H_{ar}). ^{31}P NMR: δ −22.0 (dd, $J_{\beta-\alpha} = 23.1$ Hz, $J_{\beta-\gamma} = 19.9$ Hz, 1P, P_β), −7.6 (d, 1P, P_γ), 9.5 (d, 1P, P_α). ^{13}C NMR: δ 14.34 (1C, $C_{6'}$), 71.28 (1C, $C_{1'}$), 75.65 (1C, O<u>C</u>$H_2C_6H_4$), 123.58 (d, $J_{5'\text{-P}} = 186.0$ Hz, 1C, $C_{5'}$), 127.44 (d, $J_{3'\text{-P}} = 26.8$ Hz, 1C, $C_{3'}$), 128.41 (2C, C_{ar}), 128.87 (2C, C_{ar}), 130.62 (2C, C_{ar}), 131.01 (2C, C_{ar}), 133.84 (1C, $C_{4''}$), 136.69 (1C, $C_{1''}$), 137.14 (1C, C_1), 139.69 (d, $J_{4'\text{-P}} = 5.8$ Hz, 1C, $C_{4'}$), 140.82 (1C, C_4), 143.43 (1C, $C_{2'}$), 201.04 (1C, C_{ketone}).

*(E,E,E)-[4-(9′-Pyrophosphono-2′,6′-dimethyl-2′,6′,8′-nonatrienyloxymethyl)-phenyl]-phenyl-methanone (***2c***).* (410 mg, 57%). ^{1}H NMR: δ 1.51 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 1.95–2.15 (m, 4H, $H_{4'}$ and $H_{5'}$), 3.80 (s, 2H, $H_{1'}$), 4.40 (s, 2H, OC<u>H</u>$_2C_6H_4$), 5.23–5.35 (m, 1H, $H_{3'}$), 5.78 (dd, $J_{9'\text{-P}} = 19.7$ Hz, $J_{9'\text{-}8'} = 16.8$ Hz, 1H, $H_{9'}$), 5.90 (d, $J_{7'\text{-}8'} = 11.1$ Hz, 1H, $H_{7'}$), 7.04 (ddd, $J_{8'\text{-P}} = 20.5$ Hz, 1H, $H_{8'}$), 7.28–7.46 (m, 4H, H_{ar}), 7.51–7.63 (m, 5H, H_{ar}). ^{31}P NMR: δ −6.45 (d, $J_{\beta-\alpha} = 23.2$ Hz, 1P, P_β), 7.14 (d, 1P, P_α). ^{13}C NMR: δ 13.67 (1C, $C_{10'}$), 16.75 (1C, $C_{11'}$), 25.97 (1C, $C_{4'}$), 39.27 (1C, $C_{5'}$), 70.46 (1C, $C_{1'}$), 76.47 (1C, O<u>C</u>$H_2C_6H_4$), 121.43 (d, $J_{9'\text{-P}} = 187.1$ Hz, 1C, $C_{9'}$), 124.91 (d, $J_{7'\text{-P}} = 26.1$ Hz, 1C, $C_{7'}$), 128.0 (2C, C_{ar}), 128.84 (2C, C_{ar}), 130.34 (2C, C_{ar}), 130.73 (2C, C_{ar}), 129.53 (1C, $C_{3'}$), 132.20 (1C, $C_{4'}$), 133.65 (1C, $C_{2'}$), 136.35 (1C, $C_{1''}$), 137.04 (1C, C_1), 140.13 (1C, C_4), 143.85 (d, $J_{8'\text{-P}} = 5.6$ Hz, 1C, $C_{8'}$), 146.41 (1C, $C_{6'}$), 199.35 (1C, C_{ketone}).

*(E,E,E)-[4-(9′-Triphosphono-2′,6′-dimethyl-2′,6′,8′-nonatrienyloxymethyl)-phenyl]-phenyl-methanone (***3c***).* (150 mg, 17%). ^{1}H NMR: δ 1.54 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 2.0–2.20 (m, 4H, $H_{4'}$ and $H_{5'}$), 3.69 (s, 2H, $H_{1'}$), 4.44 (s, 2H, OC<u>H</u>$_2C_6H_4$), 5.36 (m, 1H, $H_{3'}$), 5.74 (dd, $J_{9'\text{-P}} = 19.6$ Hz, $J_{9'\text{-}8'} = 16.9$ Hz, 1H, $H_{9'}$), 5.92 (d, $J_{7'\text{-}8'} = 11.3$ Hz, 1H, $H_{7'}$), 7.06 (ddd, $J_{8'\text{-P}} = 20.7$ Hz, 1H, $H_{8'}$), 7.30–75.1 (m, 4H, H_{ar}), 7.51–7.71 (m, 5H, H_{ar}). ^{31}P NMR: δ −21.04 (dd, $J_{\beta-\alpha} = 23.1$ Hz, $J_{\beta-\gamma} = 19.4$ Hz, 1P, P_β), −7.45 (d, 1P, P_γ), 9.01 (d, 1P, P_α). ^{13}C NMR: δ 13.62 (1C, $C_{10'}$), 16.76 (1C, $C_{11'}$), 25.91 (1C, $C_{4'}$), 39.24 (1C, $C_{5'}$), 70.41 (1C, $C_{1'}$), 76.46 (1C, O<u>C</u>$H_2C_6H_4$), 120.53 (d, $J_{9'\text{-P}} = 192.5$ Hz, 1C, $C_{9'}$), 124.74 (d, $J_{7'\text{-P}} = 26.3$ Hz, 1C, $C_{7'}$), 128.08 (2C, C_{ar}), 128.82 (2C, C_{ar}), 130.34 (2C, C_{ar}), 130.74 (2C, C_{ar}), 129.61 (1C, $C_{3'}$), 132.14 (1C, $C_{4''}$), 133.66 (1C, $C_{2'}$), 136.32 (1C, $C_{1''}$), 136.99 (1C, $C_{5'}$), 140.88 (d, $J_{8'\text{-P}} = 5.8$ Hz, 1C, $C_{8'}$), 143.79 (1C, C_4), 147.03 (1C, $C_{6'}$), 199.56 (1C, C_{ketone}).

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