Synthesis of Prenyl Pyrophosphonates as New Potent Phosphoantigens Inducing Selective Activation of Human Vγ9Vδ2 T Lymphocytes

Ibrahim Zgani,[§] Chantal Menut,*[,§] Michel Seman,[#] Valerie Gallois,[#] Virginie Laffont,[†] Jeanine Liautard,[†] Jean-Pierre Liautard,[†] Marc Criton,[§] and Jean-Louis Montero*[,§]

LCBM, UMR 5032, Université Montpellier II, CNRS, MAYOLY-SPINDLER, 8 Rue de l'Ecole Normale, 34296 Montpellier, France, Laboratoire d'Immunodifférenciation, Université Denis Diderot, 2 Place Jussieu, 75251 Paris, Cedex 05, France, and Microbiologie et Pathologie Cellulaire Infectieuse, INSERM U 65, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier, France

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γ9δ2T cells represent the most abundant population of human blood $\gamma\delta$T lymphocytes. They produce and promote strong cytotoxic activity against many pathogens that are implicated in several human infectious diseases. Their activation requires their exposure to small phosphorus-containing antigens in the family of prenyl pyrophosphates and their related biosynthetic precursors such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are naturally occurring metabolites in mycobacteria and several other microbial pathogens. The broad specificity in the recognition of these molecules by the T-lymphocyte population expressing a Vγ9Vδ2 cell receptor might facilitate their manipulation by designing small potent synthetic agonist ligands. In this paper, we describe the synthesis and the biological evaluation of new pyrophosphonate compounds as new isosteric analogues of natural prenyl pyrophosphates. Several prenyl and alkenyl pyrophosphonate with different chain lengths and degrees of insaturation (**24**−**28**, **48**−**50**, and **64**−**66**) were tested as well as the alkoxymethylpyrophosphonic analogue of IPP (compound **76**) as its closest isostere. Several of them appeared to be better activators of Vγ9Vδ2 T cell proliferation than IPP. These results open the perspective of a potential use of isoprenoides pyrophosphonates as specific immunoregulatory molecules.

Introduction

T cells bearing the $\gamma\delta$ T-cell antigen receptor (TCR) represent a distinct but minor lymphocyte subset in all mammalian species. In human blood, however, a predominant $\gamma\delta$ T-lymphocyte subset is found, with limited diversity harboring a Vγ9Vδ2 TCR.[1] This γ9δ2 T cell population expand during infections by various intracellular pathogens such as mycobacteria.[2] Vγ9Vδ2 T cells also exhibit cytotoxic activities against several tumor cell types.[3] Hence, this T cell subset may represent a selective target for immunotherapy of cancer and infectious diseases. This concept is also supported by the fact that unlike other T lymphocytes that recognize antigenic peptides associated with major histocompatibility complex molecules, Vγ9Vδ2 T cells respond to nonconventional antigens corresponding to non-peptide phosphoesters, collectively referred to as phosphoantigens.[4] Among these, isoprenoid-PP (PP = pyrophosphate) such as isopentenyl-PP (IPP), dimethylallyl-PP (DMAPP), farnesyl-PP, and geranyl-PP have been identified as natural phosphoantigens produced by mycobacteria.[2]

However, pyrophosphomonoesters such as IPP, DMAPP, and 3-formyl-1-butyl pyrophosphate,[5] which can be considered as natural activators of γ9δ2 T cells, are likely to be rapidly dephosphorylated under physiological conditions by several enzymes such as apyrases and phosphatases present in body fluids or on the surface of many cell types. The metabolic stability of these compounds is probably crucial for putative clinical application in immunotherapy. To prevent this degradation, we intended to replace the P−O bond in the pyrophosphomonoester moiety by a P−C bond that may be more resistant to enzymatic attacks. The use of phosphonic acids in place of the naturally occurring phosphate might be expected to enhance the lifetime and thereby the integrated activity of the metabolite, on condition that its geometry and binding capabilities are preserved.[6]

To allow a systematic examination of our hypotheses, we designed several prenyl and alkenyl pyrophosphonates with different chain lengths and degrees of unsaturation including alkyl, vinyl, or oxymethyl pyrophosphonic groups. In the present report, we decribe the preparation and biological evaluation of new pyrophosphonic derivatives as analogues of natural phosphoantigens. Several of these new phosphonate analogues appear to be better activators of γ9δ2T cell proliferation than natural compounds and open the exciting perspective of a potential use in clinics as new and selective immunomodulators.

Chemistry

The first compounds synthesized were vinyl pyrophosphonates. They were derived from their alcohol precursors **1**−**3** or from commercially available alde-

* To whom correspondence should be addressed. For C.M.: phone and fax, 33 (0)46714434; e-mail, cmenut@univmontp2.fr. For J.-L.M.: phone and fax, 33 (0)467144343; e-mail, montero@univ-montp2.fr.
[§] LCBM, Université Montpellier II.
[#] Université Denis Diderot.
[†] INSERM U65, Université Montpellier II.

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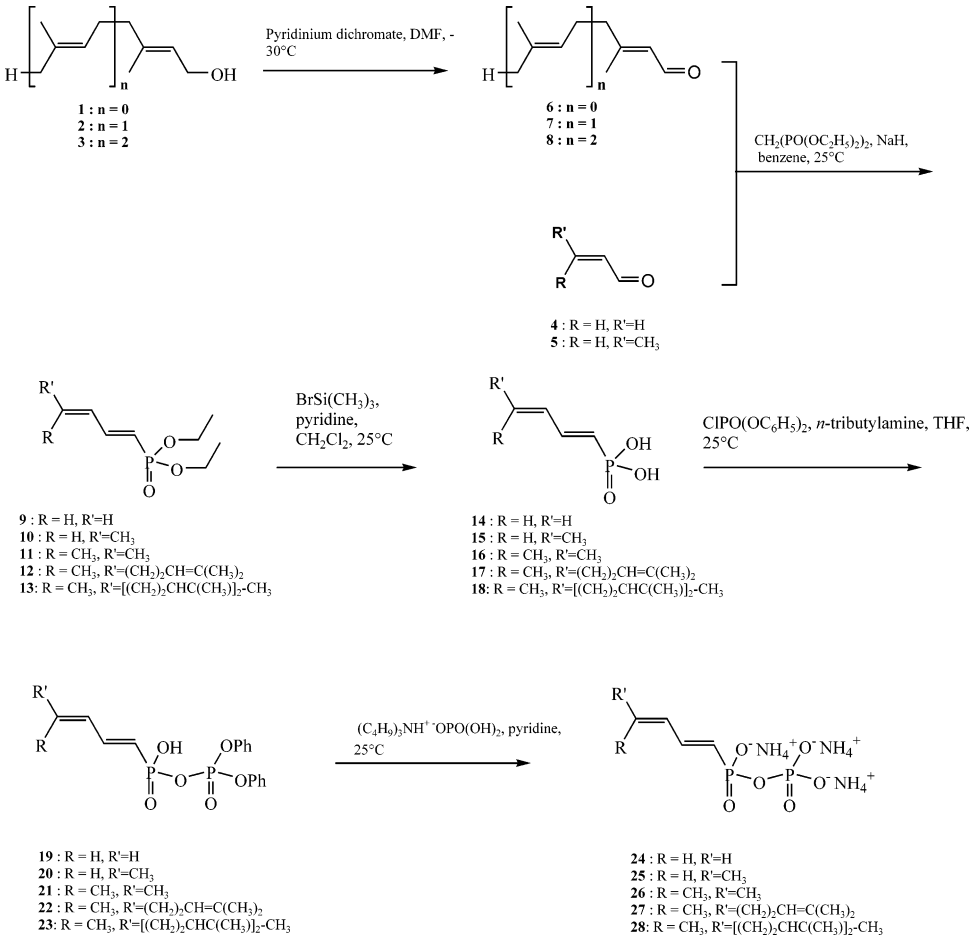
Scheme 1



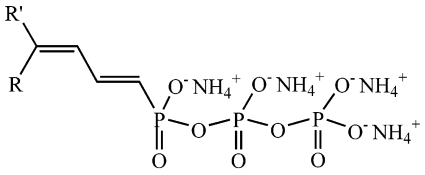
hydes **4** and **5** (Scheme 1). The alcohols were oxidized with pyridinium dichromate in DMF and gave the corresponding aldehydes **6**, **7**, and **8** in 95%, 99%, and 91% yields.[7] This reaction is very clean, and isolation of products is simple. There is no overoxidation of the aldehydes and no $E \rightarrow Z$ isomerization of the aldehyde $\alpha-\beta$ double bond that we observed with manganese dioxide[8] or the Swern reagent.[9]

Treatment of the aldehydes **(4−8)** with tetraethylmethylene diphosphonate and NaH in benzene gave via a Horner−Wadsworth−Emmons condensation the expected vinyl phosphonates (**9**−**13**) (72%, 79%, 85%, 80%, and 60% yields).[10] The resulting phosphonates were converted to the corresponding phosphonic acids (**14**−**18**) by standard treatment with bromotrimethylsilane and pyridine in CH_2Cl_2 (84%, 90%, 99% and 84% yields, respectively).[11]

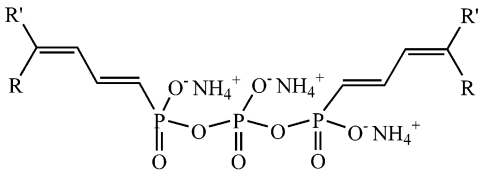
The synthesis of vinyl pyrophosphonates was performed using modified Michelson's conditions (see Experimental Section).[12] Compounds **14**−**18** were first activated by reaction of its tri-*n*-butylammonium salt in dry THF with diphenyl phosphorochloridrate and tri-*n*-butylamine producing **19**−**23**, which were then combined without any purification in dry pyridine with tri-*n*-butylammonium orthophosphate to give after purification compounds **24**−**28** in, 61%, 63%, 70%, 88%, and 49% yields, respectively.

Besides, formation of some byproducts was observed in the phosphorylation step. Compounds that had been isolated after chromatography are shown in Chart 1.

Chart 1



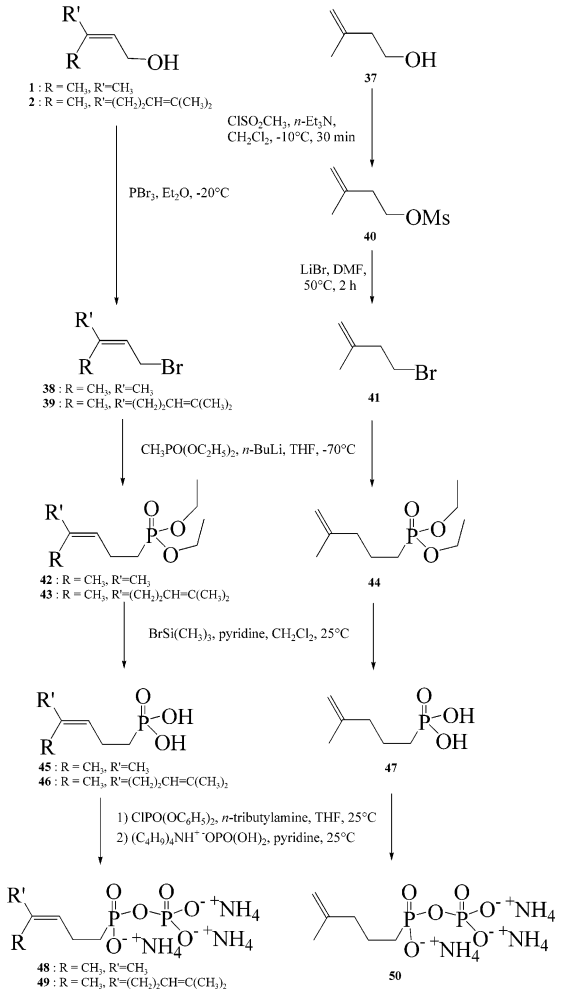
29 : R = H, R'=H
30 : R = H, R'=CH_3
31 : R = CH_3, R'=CH_3
32 : R = CH_3, R'=$(CH_2)_2CH=C(CH_3)_2$
33: R = CH_3, R'=$[(CH_2)_2CHC(CH_3)]_2$-CH_3



34 : R = H, R'=H
35 : R = H, R'=CH_3
36 : R = CH_3, R'=CH_3

Different strategies were investigated to prepare the vinylic analogue of isopentenyl pyrophosphate (IPP). However, we systematically observed the migration of the terminal double bond of the isopentenyl moiety leading to its partial conversion into the dimethylallyl isomer. The separation of both compounds being impossible because of their similar polarity, the preparation of this analogue was abandoned.

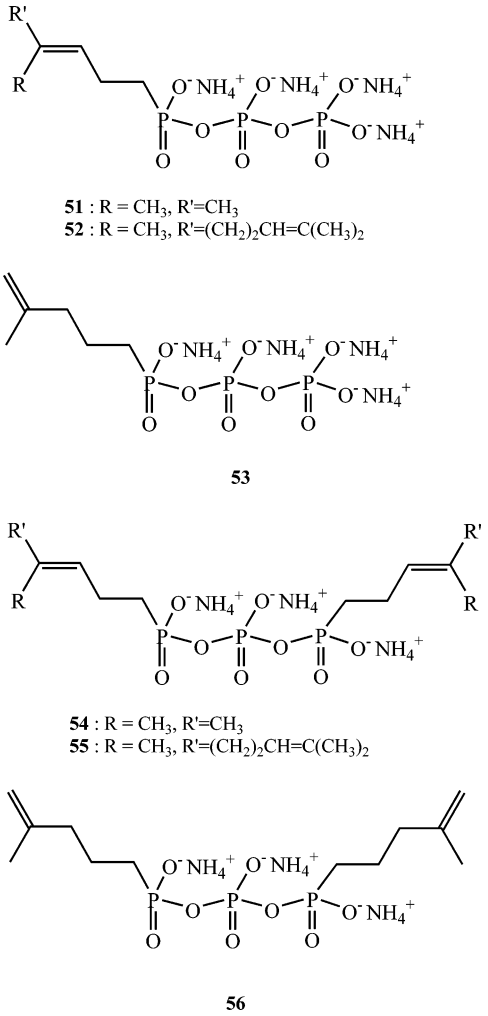
Scheme 2



Saturated pyrophosphonic analogues were also prepared. This was readily accomplished as illustrated in Scheme 2 starting from the alcohols precursors **1**, **2**, and **37**, which were brominated by phosphorus tribromide[13] in the case of 3-methylbut-3-enol (**1**) and geraniol (**2**) or mesylated[14] before being treated by lithium bromide in DMF[15] for 3-methylbut-2-enol (**37**) to give compounds **38**, **39**, and **41** in 82%, 96%, and 99% yield, respectively. Compounds **38**, **39**, and **41** were then combined with the diethyl methylphosphonate anion to obtain the phosphonic esters **42**, **43**, and **44** (59%, 70% and 64% yield),[16] and subsequent deprotection under standard conditions[11] gave compounds **45**, **46**, and **47** in 69%, 98%, and 76% yields, respectively. Phosphorylation of these compounds in Michelson's condition afforded after purification the pyrophosphonates **48**, **49**, and **50** (IPPn) in 65%, 46%, and 62% yields (byproducts obtained are shown in Chart 2).

Furthermore, we prepared nonisosteric analogues of IPP (resulting from the simple supression of the oxygen atom in the C−O−P linkage); thus, three alkyl pyrophosphonic compounds were synthesized starting from compounds **38**, **41**, and allyl bromide **57** (Scheme 3), which were converted into their diethyl phosphonic esters via a Michaelis−Arbuzov[17] reaction with triethyl phosphite[16] to afford **58**, **59**, and **60** in 58%, 67%, and 42% yields, respectively. The resulting phosphonates were then converted to their corresponding phosphonic acids **61**, **62**, and **63** (96%, 85%, and 70% yield) that were finally phosphorylated according to the same procedure described before to give compounds **64**, **65**, and **66** in 61%, 67%, and 40% yields, and byproducts obtained (compounds **67-73**) are shown in Chart 3.

Chart 2

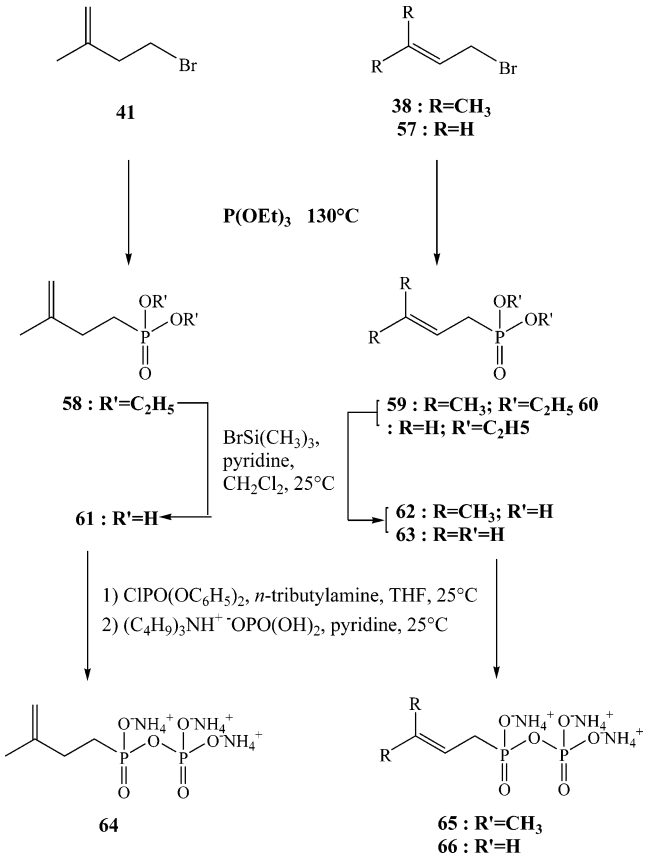


The synthesis of another class of analogue, characterized by an oxymethylpyrophosphonic group, was then performed (by inverting the locations of oxygen and carbon in the C−O linkage of the natural metabolite IPP). The preparation of this compound consisted first of the combination of 3-chloro-2-methylpropene with the sodium anion of diethyl(hydroxymethyl) phosphonate in THF to afford compound **74** in 95% yield. The resulting phosphonate was then converted to the corresponding phosphonic acid **75** in a quantitative yield. Compound **75** was finally phosphorylated to give compound **76** (41% yield) according to the same procedure we described before. (Scheme 4)

Biological Evaluation

The immunostimulating activity of compounds was determined in vitro on lymphocytes obtained from healthy donors. Upon purification over Ficoll gradients, total peripheral blood mononuclear cells (PBMC) were cultured with increasing concentrations of the different compounds and proliferation, measured on day 5 by the determination of ^{3}H-TdR uptake, and were compared to that achieved with IPP. At high concentration, i.e., 500 μM, compounds **24**, **25**, **26**, **48**, and **50** were 3−5

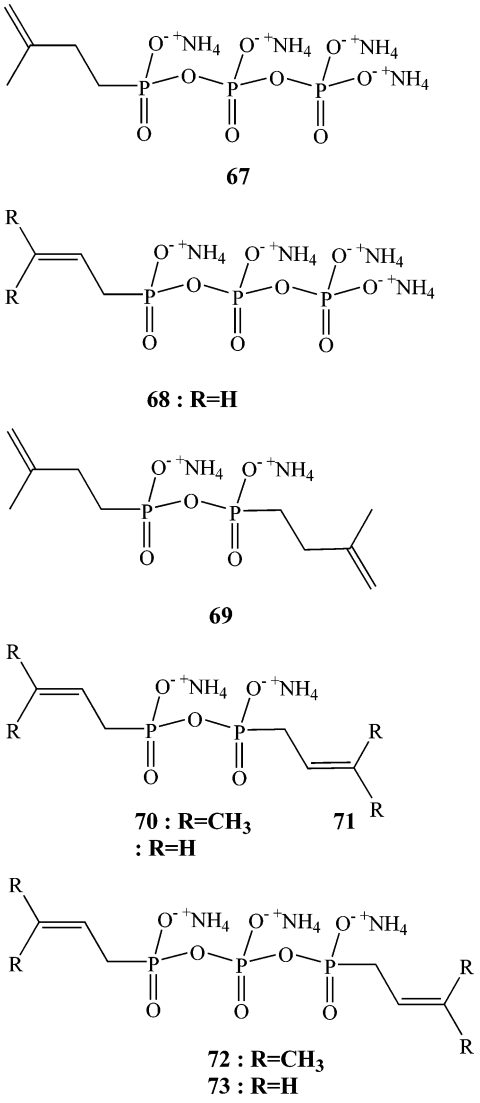
Scheme 3



times more efficient than IPP at inducing T cell proliferation. In contrast, compounds **27**, **28**, **49**, **64**, **65**, **66**, and **76** were not or poorly active. More importantly, whereas IPP was poorly efficient in the tests at concentration below 100 μM, an increasing response was observed with compounds **24**, **25**, and **26** with doses ranging from 1 to 500 μM (Figure 1A). Yet, a clear stimulation was detected with compounds **48** and **50**, detectable as low as 100 nM, which then dropped around 1 μM to reach a plateau up to 500 μM (Figure 1B). In these respects, compounds **48** and **50** were far more active than IPP, in terms of both stimulating activity and dose response.

Since phosphoantigens are selective activators of $\gamma 9\delta 2$ T cells, we then tested whether proliferation induced by our compounds was accompanied by in vitro expansion of cells expresing a Vγ9/Vδ2 TCR. This was perfomed by two-color fluorescence analysis in flow cytometry using CD3 and Vγ9 specific antibodies on cells after 5 days of stimulation in vitro. As illustrated in Figure 2a, the percentage of $\gamma 9\delta 2$ T cells was in the range of 2% among PBMC of healthy donors before stimulation and remained unchanged after stimulation with PHA. In contrast, the percentage of CD3 lymphocytes expressing a Vγ9/Vδ2 TCR reached 30−40% after stimulation with compounds **24**, **25**, **26** or **48** and **50**. Consistently, large dividing cells, defined on light scatters by flow cytometry, were all double-stained with anti-CD3 and anti-Vγ9 antibodies, as illustrated in Figure 2b on cells after 5 days of stimulation with compound **50**, whereas small lymphocytes were CD3 positive but Vγ9 negative. This demonstrated that the active pyrophosphonate analogues are strong and selective activators of $\gamma 9\delta 2$ T cells.

Chart 3



Discussion

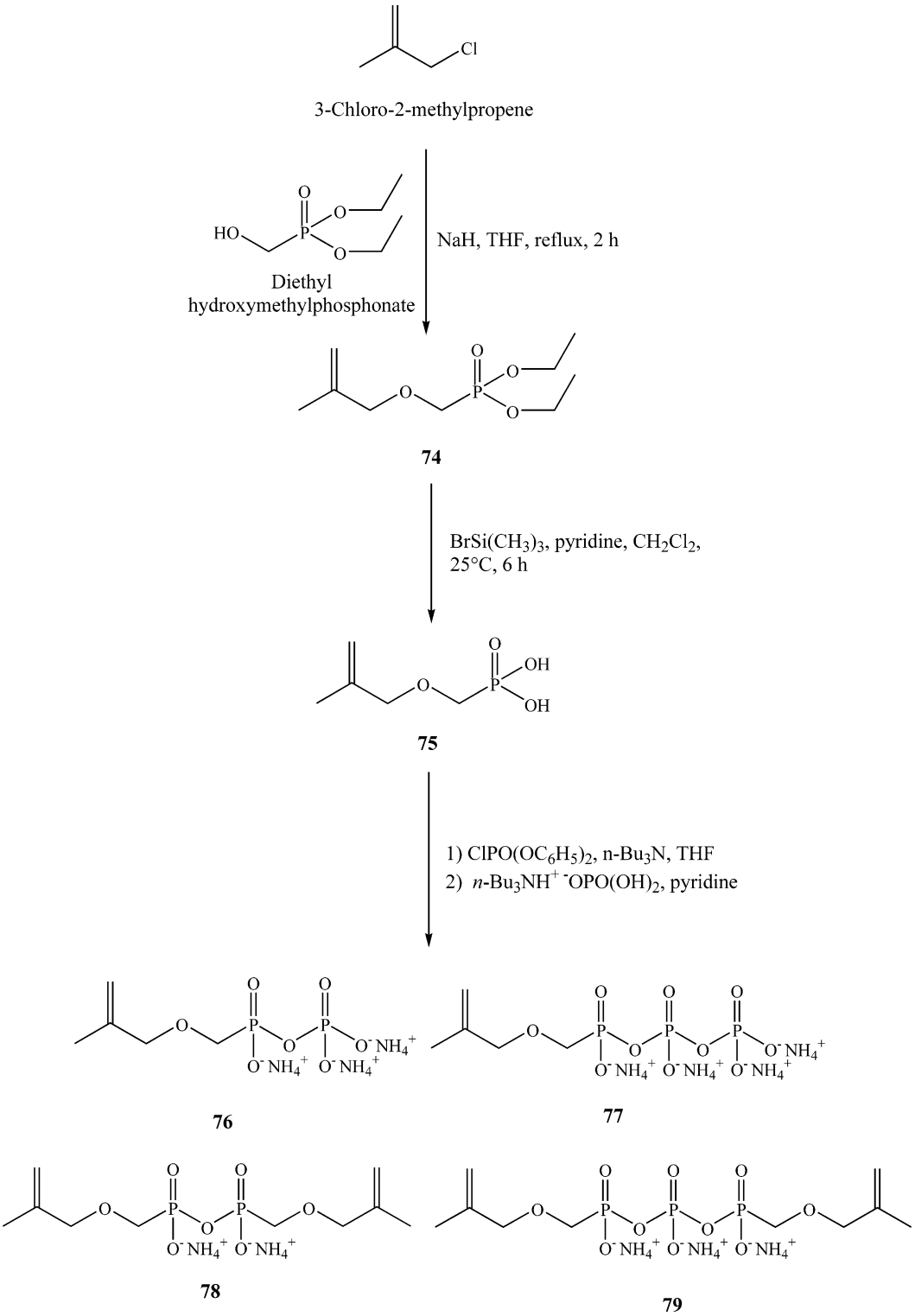
Three different groups of pyrophosphonic compounds were prepared, and their effect on the stimulation of $\gamma 9\delta 2$ T cells was evaluated and compared with that of the potential natural metabolite isopentenyl pyrophosphate (IPP).

The first series was characterized by a vinyl pyrophosphonic group (compounds **24**−**28**); the vinyl group had already been used as a substitute for the C−O linkage in the pyrophosphate moiety and had been shown to be well tolerated.[18] Furthermore, to evaluate the importance of the unsaturation degree of the C1−C2 bond, methyl phosphonate has often been considered to be isosteric to the phosphate group and widely used in therapeutic chemistry,[19] and given that, saturated pyrophosphonic analogues were also prepared (compounds **48**−**50**).

Three other alkyl pyrophosphonate compounds, nonisosteric analogues of IPP (**64**−**66**), were also prepared to get structural information that would help us to build a more detailed structure−activity profile of these phosphoantigens.

Finally, aside from rendering the phosphorus not liable to hydrolysis by normal route, the fundamental

Scheme 4



structural substitution of a phosphate group by a phosphonate group induces changes that must be considered in the biological activity aspect. For example, significant decrease in the acidity of the phosphorus-containing function is observed upon the introduction of an electron-donating alkyl group. This could result in the existence of a different state of dissociation for the analogue compared to the natural compound in the physiological medium.[4] Thus, we migth expect a better "isostery" with the introduction of a "withdrawing" group (compound **76**).

The results of the biological evaluation performed on these components demonstrate that pyrophosphonate analogues of natural phosphoantigens can be much stronger activators of human $\gamma 9\delta 2$ T cells than their pyrophosphate counterparts. This is typically illustrated by the activity of compounds **48** and **50**, which are pyrophosphonate analogues of DMAPP and IPP, respectively. Importantly, our results indicate that pyrophosphonates analogues with longer allylic or alkyl side chains such as **27**, **28**, and **49** have no biological activity. Similarly, analogues such as **64**, **65**, and **66**, which have

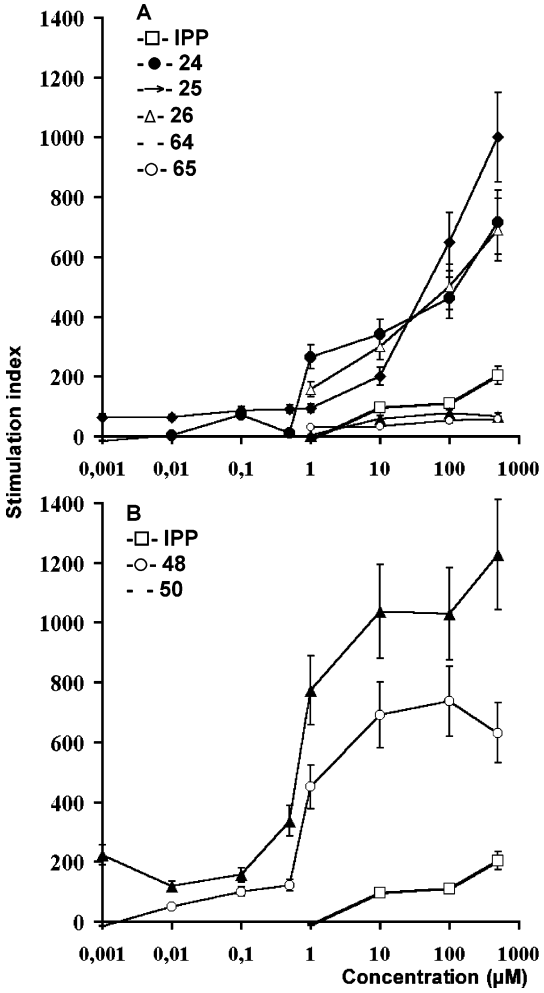


Figure 1. Comparison of the immunostimulating activity of seven pyrophosphonates with that of isopentenyl pyrophosphate (IPP).

a shorter side chain, are not active. This suggests that the size of the allyl or alkyl side chain plays an essential role in the direct interaction of phosphoantigens with the particular Vγ9Vδ2 TCR. Finally, it is worth noting that **76**, which is the true IPP isostere, is not active on γ9δ2 T cells. On the basis of in silico modeling (data not shown), this observation suggests that the conformation of the IPP derivative is essential for the interaction with the Vγ9Vδ2 TCR to support T cell activation. The general conformation of compound **50**, the pyrophosphonate analogue of IPP, looks rather similar to that of 3-formyl-1-butyl pyrophosphate, another potent natural activator of γ9δ2 T cells produced by mycobacteria.[5]

Altogether, our findings indicate that isoprenoid pyrophosphonates are more potent activators of γ9δ2 T cell activation than their pyrophosphate isosteres. They also indicate that the activity of phosphoantigens is dependent on the length of the side chain, a point that was not formely stressed. Additionally, activity seems dependent on the conformation of the Vγ9Vδ2 TCR ligand and, as deduced from the biological activity of IPP versus IPPn, on their resistance to apyrase and phosphatase ecto-enzyme activities. Experiments in progress support this last conclusion.

On the basis of their remarkable activity in vitro and their access to chemical synthesis compared to compounds extracted from cultures of mycobacteria, **48** and **50** are good candidates as specific immunoregulatory molecules for the treatment of infectious diseases and several forms of cancer.

Experimental Section

Reactions were monitored by TLC using aluminum-coated plates with silica gel 60 F_{254} (Merck). Column chromatography was carried with Carlo Erba silica gel 60 A (35−70 μm). All solvents were dried and distilled before use.[19] ^{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 using $CDCl_3$ or D_2O as solvent. Chemical shifts are given using residual solvent peaks as a reference relative to TMS. Elemental analyses were performed by the analytical department, and results were within ±0.4 of theoretical values. 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. To a solution of pyridinium dichromate (4.45 mmol, 1.2 equiv) in DMF (30 mL), which was cooled to −30 °C and under nitrogen, the alcohol **1**, **2**, or **3** (3.56 mmol, 1 equiv) was added, and the mixture was stirred for 3 h and 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.

3-Methylbut-2-enal (6): 95% yield; R_f = 0.8, SiO_2, petroleum ether/ethyl ether, 95:5; ^{1}H NMR ($CDCl_3$) δ 1.96 (d, J_{5-2} 1.3 Hz, 3H, H-5), 2.15 (d, J_{4-2} 1.3 Hz, 3H, H-4), 5.86 (d, J_{2-1} 8.2 Hz, 1H, H-2), 9.93 (d, 1H, H-1).

3,7-Dimethylocta-2,6-dienal (7): 99% yield; R_f = 0.46, SiO_2, petroleum ether/ethyl ether, 8:2; ^{1}H NMR ($CDCl_3$) δ 1.6 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.16 (s, 3H, H-9), 2.0−2.28 (m, 4H, H-4 and H-5), 4.93−5.15 (m, 1H, H-6), 5.88 (d, J_{2-1} 8.0 Hz, 1H, H-2), 9.99 (d, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 17.50 (1C, C-10), 22.10−22.19 (2C, C-8 and C-9), 22.98 (1C, C-5), 40.78 (1C, C-4), 122.96 (1C, C-6), 128.69 (1C, C-2), 132.01 (1C, C-7), 165.89 (1C, C-3), 192.45 (1C, C-1).

(*E*,*E*)-3,7,11-Trimethyl-2,6,10-dodecatrienal (8): 71% yield; R_f = 0.8, SiO_2, petroleum ether/ethyl ether, 8:2; ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.62 (s, 3H, H-14), 1.92 (m, 2H, H-9), 1.97 (m, 2H, H-5), 2.1 (s, 2H, H-8), 2.15 (m, 5H, H-4 and CH_3), 4.99 (m, 1H, H-10), 5.01 (m, 1H, H-6), 5.82 (d, J_{2-1} 9.0 Hz, 1H, H-2), 9.92 (d, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ 16.48 (1C, C-15), 18.04−18.12 (2C, C-13 and C-14), 26.08 (1C, C-12), 26.13−27.02 (2C, C-5 and C-9), 40.05−41.04 (2C, C-4 and C-8), 122.51−124.52 (2C, C-6 and C-10), 127.84 (1C, C-2), 131.93 (1C, C-11), 136.99 (1C, C-7), 164.39 (1C, C-3), 191.29 (1C, C-1).

General Procedure for the Horner−Wadsworth−Emmons Reaction. To a suspension of sodium hydride (2.98 mmol, 1.2 equiv) in dry benzene (20 mL) was added tetraethylmethylene diphosphonate (2.98 mmol, 1.2 equiv) at room temperature and under nitrogen. After 15 min, a solution of the aldehyde **4**−**8** (2.48 mmol, 1 equiv) 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.

Diethyl ((*E*)-1,3-butadienyl)phosphonate (9): 72% yield; R_f = 0.39, SiO_2, ethyl ether; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J_{6-5} 7.1 Hz, 6H, H-6), 3.98−4.16 (q, 4H, H-5), 5.45 (d, J_{4-3} 9.6 Hz, 1H, H-4), 5.55 (d, $J_{4'-3}$ 16.8 Hz, 1H, H-4′), 5.71 (dd, J_{1-P} 19.1 Hz, J_{1-2} 16.9 Hz, 1H, H-1), 6.40 (ddd, J_{3-2} 10.6 Hz, 1H, H-3), 7.08 (ddd, J_{2-P} 20.8 Hz, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 19.9; ^{13}C NMR ($CDCl_3$) δ 16.75 (2C, C-6), 62.17 (2C, C-5), 118.44 (d, J_{1-P} 190.4 Hz, 1C, C-1), 128.32 (1C, C-4), 136.11 (d, J_{3-P}

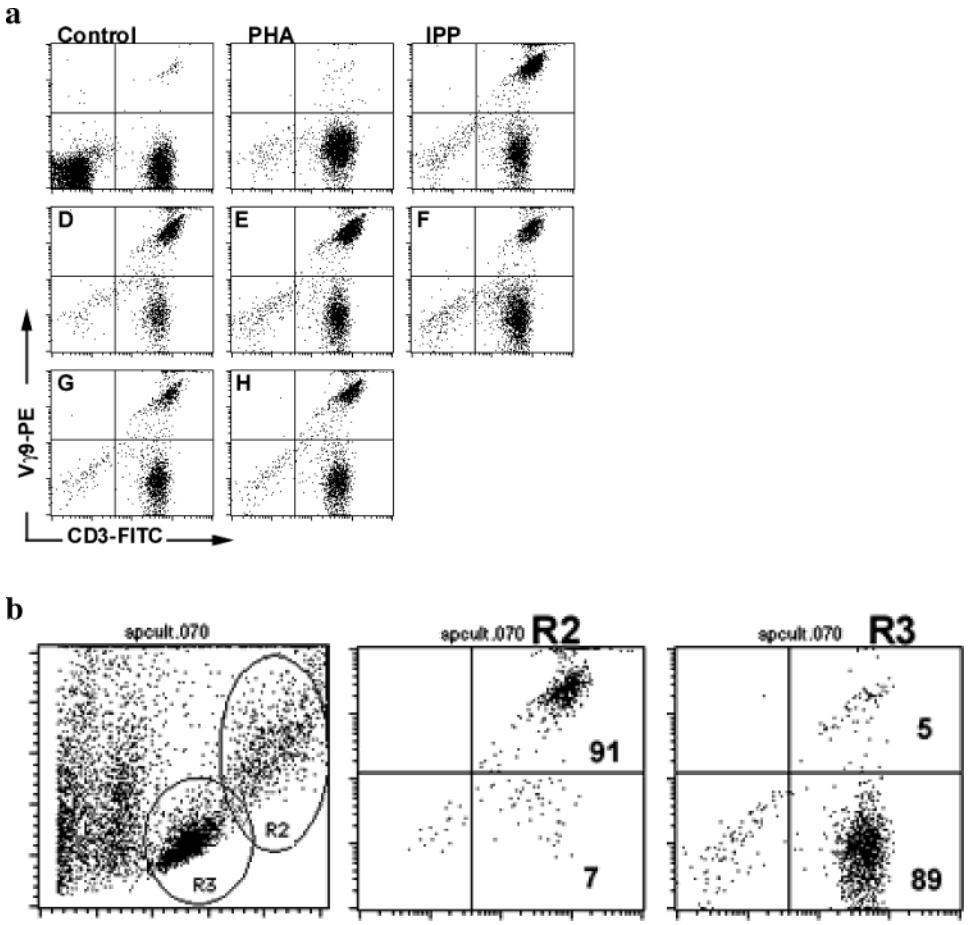


Figure 2. Fluorescence analysis in flow cytometry: (a) stimulation by isopentenyl pyrophosphate and compounds **24** (D), **25** (E), **26** (F), **48** (G), **50** (H); (b) demonstration of the selective activation of Vγ9Vδ2 T cells by compound **50**.

27.1 Hz, 1C, C-3), 149.25 (d, J_{2-P} 5.7 Hz, 1C, C-2); MS FAB$^-$ (GT) *m*/*z* 191 (M + H)$^+$, 213 (M + Na)$^+$. Anal. ($C_8H_{15}O_3P$) C, H.

Diethyl ((*E*)-1,3-pentadienyl)phosphonate (10): 79% yield; R_f = 0.35, SiO_2, ethyl ether; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J_{7-6} 4.7 Hz, 6H, H-7), 5.24 (d, J_{5-4} 5.2 Hz, 3H, H-5), 3.95−4.20 (qt, 4H, H-6), 5.56 (dd, J_{1-P} 19.5 Hz, J_{1-2} 16.9 Hz, 1H, H-1), 6.0−6.25 (m, 2H, H-3 and H-4), 7.07 (ddd, J_{2-P} 20.9 Hz, J_{2-3} 9.4 Hz, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 21.2; ^{13}C NMR ($CDCl_3$) δ 16.76 (2C, C-7), 18.83 (1C, C-5), 62 (2C, C-6), 114.66 (d, J_{1-P} 191.6 Hz, 1C, C-1), 131.22 (d, J_{3-P} 26.3 Hz, 1C, C-3), 138.96 (1C, C-4), 149.59 (d, J_{2-P} 6.1 Hz, 1C, C-2); MS FAB$^+$ (NOBA) *m*/*z* 205 (M + H)$^+$, 227 (M + Na)$^+$. Anal. ($C_9H_{17}O_3P$) C, H.

Diethyl ((*E*)-4-methyl-1,3-pentadienyl)phosphonate (11): 85% yield; R_f = 0.46, SiO_2, ethyl ether; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J_{8-7} 7.1 Hz, 6H, H-8), 1.85 (6H, H-5 and H-6), 3.95−4.15 (q, 4H, H-7), 5.51 (dd, J_{1-2} 16.6 Hz, J_{1-P} 20.2 Hz, 1H, H-1), 5.94 (d, J_{3-2} 10.9 Hz, 1H, H-3), 7.35 (ddd, J_{2-P} 21.0 Hz, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 19.4; ^{13}C NMR ($CDCl_3$) δ 16.71−16.77 (2C, C-8), 16.24 (1C, C-6), 26.74 (d, J_{5-P} 1.2 Hz, 1C, C-5), 61.86−61.92 (2C, C-7), 111.97 (d, J_{1-P} 192.0 Hz, 1C, C-1), 125.17 (d, J_{3-P} 26.34 Hz, 1C, C-3), 145.66 (d, J_{2-P} 6.6 Hz, 1C, C-2), 145.67 (1C, C-4); MS FAB$^+$ (NOBA) *m*/*z* 219 (M + H)$^+$, 241 (M + Na)$^+$. Anal. ($C_{10}H_{19}O_3P$) C, H.

Diethyl ((*E*,*E*)-4,8-dimethyl-1,3,7-nonatrienyl)phosphonate (12): 80% yield; R_f = 0.6, SiO_2, ethyl ether; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J_{13-12} 6.7 Hz, 6H, H-13), 1.60 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.05−2.15 (m, 2H, H-6), 2.18−2.32 (m, 2H, H-5), 3.95−4.20 (q, 4H, H-12), 4.95−5.11 (m, 1H, H-7), 5.53 (dd, J_{1-2} 16.6 Hz, J_{1-P} 20.3 Hz, 1H, H-1), 5.96 (d, J_{3-2} 11.3 Hz, 1H, H-3), 7.32 (ddd, J_{2-P} 20.9 Hz, 1H, H-3); ^{31}P NMR ($CDCl_3$) δ 21.0; ^{13}C NMR ($CDCl_3$) δ 16.74−16.80 (2C, C-13), 17.72−18.08 (2C, C-10 and C-11), 26.07 (1C, C-9), 26.64 (1C, C-6), 40.50 (1C, C-5), 61.90−61.96 (2C, C-12), 114.27 (d, J_{1-P} 192.0 Hz, 1C, C-1), 123.72 (1C, C-7), 124.69 (d, J_{3-P} 26.7 Hz, 1C, C-3), 132.58 (1C, C-8), 144.69 (d, J_{2-P} 6.7 Hz, 1C, C-2), 149.22 (1C, C-4); MS FAB$^-$ (GT) *m*/*z* 287 (M + H)$^+$, 309 (M + Na)$^+$, 259 (M − CH_2CH_3 + H)$^+$. Anal. ($C_{15}H_{27}O_3P$) C, H.

Diethyl ((*E*,*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraenyl)phosphonate (13): 60% yield; R_f = 0.8, SiO_2, ethyl ether; ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 6H, H-18 and 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_{1-2} 16.6 Hz, J_{1-P} 20.24 Hz, 1H, H-1), 5.9 (d, J_{3-2} 11.18 Hz, 1H, H-3), 7.3 (ddd, J_{2-3} 11.18 Hz, J_{2-1} 16.6 Hz, J_{2-P} 21.0 Hz, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 21.05. ^{13}C NMR ($CDCl_3$) δ 14.78 (CH_3), 15.1−15.17 (C-18 and C-20), 16.44 (C −14), 16.89 (CH_3), 24.46 (C-15), 24.93 (C-9), 25.43 (C-10), 38.44 (C-6), 38.44 (C-5), 60.32−60.37 (C-17 and C-19), 112 (d, J_{1-P} 192.17 Hz, C-1), 121.98 (C-11), 122.97 (d, J_{3-P} 26.36 Hz, C-3), 123.20 (C-7), 130.15 (C-8), 134.15 (C-12), 144.17 (d, J_{2-P} 6.84 Hz, C-2), 147.73 (C-4); MS FAB$^+$ (NOBA) *m*/*z* 355 (M + H)$^+$, 377 (M + Na)$^+$. Anal. ($C_{20}H_{35}O_3P$) C, H.

General Procedure for the Conversion of Vinyl Phosphonates to Vinyl Phosphonic Acids. To a solution of compounds **9**−**13** (1.61 mmol, 1 equiv) in CH_2Cl_2 (30 mL) were added successively pyridine (16.1 mmol, 10 equiv) and bromotrimethylsilane (8.1 mmol, 5 equiv). 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 and 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 to give pure vinyl phosphonic acid **14**−**18**.

(*E*)-Buta-1,3-dienylphosphonic acid (14): 85% yield; R_f = 0.4, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$)

δ 5.44 (d, J_{4-3} 10.0 Hz, 1H, H-4), 5.96 (dd, J_{1-P} 18.7 Hz, J_{1-2} 17.0 Hz, 1H, H-1), 6.52 (ddd, J_{3-2} 10.5 Hz, J_{3-4} 10.0 Hz, $J_{3-4'}$ 16.9 Hz, 1H, H-3), 6.98 (ddd, J_{2-P} 20.8 Hz, H-2), 10.31 (s, 2H, POH); ^{31}P NMR ($CDCl_3$) δ 19.2; ^{13}C NMR ($CDCl_3$) δ 122.14 (d, J_{1-P} 190.9 Hz, 1C, C-1), 123.77 (1C, C-4), 136.74 (d, J_{3-P} 27.0 Hz, 1C, C-3), 146.01 (d, J_{2-P} 5.4 Hz, 1C, C-2); MS FAB$^+$ (NOBA) *m/z* 135 $(M + H)^+$.

(*E*,*E*)-Penta-1,3-dienylphosphonic acid (15): 90% yield; R_f = 0.38, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CO(CD_3)_2$) δ 1.83 (d, J_{5-4} 6.0 Hz, 3H, H-5), 5.75 (dd, J_{1-P} 18.9 Hz, J_{1-2} 16.9 Hz, 1H, H-1), 6.08−6.25 (m, 2H, H-3 and H-4), 6.96 (ddd, J_{2-P} 21.1 Hz, J_{2-1} 16.86 Hz, J_{2-3} 9.7 Hz, 1H, H-2), 10.15 (s, 2H, PO*H*); ^{31}P NMR ($CO(CD_3)_2$) δ 20.4; ^{13}C NMR ($CO(DCl_3)_2$) δ 17.94 (1C, C-5), 118.41 (d, J_{1-P} 192.9 Hz, 1C, C-1), 131.49 (d, J_{3-P} 26.8 Hz, 1C, C-3), 137.41 (1C, C-4), 146.20 (d, J_{2-P} 5.7 Hz, 1C, C-2); MS FAB$^+$ (NOBA) *m/z* 149 $(M + H)^+$, 171 $(M + Na)^+$.

(*E*)-4-Methylpenta-1,3-dienylphosphonic acid (16): 99% yield; R_f = 0.28, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) δ 1.79 (s, 6H, H-5 and H-6), 5.79 (dd, J_{1-2} 16.8 Hz, J_{1-P} 17.3 Hz, 1H, H-1), 5.85 (d, J_{3-2} 11.0 Hz, 1H, H-3), 7.08 (ddd, J_{2-P} 19.4 Hz, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 23.2; ^{13}C NMR ($CDCl_3$) δ 17.45 (1C, C-6), 27.84 (1C, C-5), 115.97 (d, J_{1-P} 192.5 Hz, 1C, C-1), 125.39 (d, J_{3-P} 26.7 Hz, 1C, C-3), 142.50 (d, J_{2-P} 6.4 Hz, 1C, C-2), 145.67 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 163 $(M + H)^+$, 185 $(M + Na)^+$.

(*E*,*E*)-4,8-Dimethyl-1,3,7-nonatrienylphosphonic acid (17): 85% yield; R_f = 0.45, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 1.92−2.16 (m, 4H, H-5 and H-6), 5.0−5.15 (m, 1H, H-7), 5.66 (dd, J_{1-2} 17.9 Hz, J_{1-P} 18.5 Hz, 1H, H-1), 5.8−5.95 (m, 1H, H-3), 7.10−7.40 (m, 1H, H-2), 9.87 (s, 2H, PO*H*); ^{31}P NMR ($CDCl_3$) δ 23.4; ^{13}C NMR ($CDCl_3$) δ 17.87−18.24 (2C, C-10 and C-11), 26.18 (1C, C-9), 26.72 (1C, C-6), 40.63 (1C, C-5), 118.37 (d, J_{1-P} 192.6 Hz, 1C, C-1), 123.85 (1C, C-7), 124.79 (d, J_{3-P} 26.9 Hz, 1C, C-3), 132.61 (1C, C-8), 141.59 (d, J_{2-P} 6.3 Hz, 1C, C-2), 148.37 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 231 $(M + H)^+$, 253 $(M + Na)^+$.

(*E*,*E*,*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraenylphosphonic acid (18): 84% yield; R_f = 0.59, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) 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 ($CDCl_3$) δ 21.7; ^{13}C NMR ($CDCl_3$) 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-P} 184.5 Hz, 1C, C-1), 123.71−124.67 (2C, C-7 and C-11), 124.79 (d, J_{3-P} 24.6 Hz, 1C, C-3), 131.79−136.23 (2C, C-8 and C-12), 144.33 (d, J_{2-P} 6,0 Hz, 1C, C-2), 149.04 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 299 $(M + H)^+$, 321 $(M + Na)^+$.

General Method for the Phosphorylation of Vinylphosphonic Acids. 1. Preparation of the Activated Phosphonoanhydride. To a solution of compounds **14**−**18** (1.36 mmol, 1 equiv) in methanol (20 mL) was added tri-*n*-butylamine (1.36 mmol, 1 equiv). The mixture was stirred at room temperature for 30 min, and 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 (4.1 mmol, 3 equiv) was dissolved in dry THF (20 mL), and then diphenylphosphorochloridate (1.36 mmol, 1 equiv) and tri-*n*-butylamine (4.1 mmol, 3 equiv) were added successively. The mixture was stirred at room temperature under nitrogen for 2 h.

2. Preparation of the Tri-*n*-butylammonium Orthophosphate. To a solution of orthophosphoric acid (4.1 mmol, 3 equiv) in methanol (20 mL) was added tri-*n*-butylamine (4.1 mmol, 3 equiv). 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).

3. Coupling Procedure. To a stirred solution of the tri-*n*-butylammonium orthophosphate (4.1 mmol, 3 equiv) 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 2 additional hours and then the solvent was removed. Column chromatography with 2-propanol/aqueous ammonia (27%) (6:4) gave **24**−**28**.

(*E*)-Triammonium 1,3-butadienylpyrophosphonate (24): 61% yield; R_f = 0.16, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 5.23 (d, J_{4-3} 9.8 Hz, 1H, H-4), 5.38 (d, $J_{4'-3}$ 16.8 Hz, 1H, H-4′), 5.90 (dd, J_{1-P} 18.3 Hz, J_{1-2} 17.04 Hz, 1H, H-1), 6.36 (ddd, J_{3-2} 10.4 Hz, 1H, H-3), 6.72 (ddd, J_{2-P} 20.0 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −8.5 (d, $J_{\beta-\alpha}$ 23.5 Hz, P-β), 6.6 (d, P-α); ^{13}C NMR (D_2O) δ 122.48 (1C, C-4), 125.24 (d, J_{1-P} 183.4 Hz, 1C, C-1), 137.26 (d, J_{3-P} 26.1 Hz, 1C, C-3), 143.59 (d, J_{2-P} 4.9 Hz, 1C, C-2); MS FAB$^-$ (GT) *m/z* 232 $(M - 2NH_4 + 2H)^+$, 215 $(M - 3NH_4 + 4H)^+$. Anal. ($C_4H_{17}N_3O_6P_2$) C, H, N.

(*E*,*E*)-Triammonium 1,3-pentadienylpyrophosphate (25): 63% yield; R_f = 0.18, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.67 (d, J_{5-4} 6.4 Hz, 3H, H-5), 5.74 (dd, J_{1-P} 18.6 Hz, J_{1-2} 17.0 Hz, 1H, H-1), 5.85−6.20 (m, 2H, H-3 and H-4), 6.68 (ddd, J_{2-P} 20.3 Hz, J_{2-3} 9.7 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −6.4 (d, $J_{\beta-\alpha}$ 23.1 Hz, P-β), 6.8 (d, P-α); ^{13}C NMR (D_2O) δ 17.88 (1C, C-5), 121.62 (d, J_{1-P} 185.1 Hz, 1C, C-1), 129.32 (d, J_{3-P} 25.5 Hz, 1C, C-3), 136.52 (1C, C-4), 143.66 (d, J_{2-P} 5.0 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 246 $(M - 2NH_4 + 3H)^+$, 229 $(M - 3NH_4 + 4H)^+$. Anal. ($C_5H_{19}N_3O_6P_2$) C, H, N.

(*E*)-Triammonium 4-methyl-1,3-pentadienylpyrophosphonate (26): 70% yield; R_f = 0.25, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.71 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 5.72 (dd, J_{1-2} 16.8 Hz, J_{1-P} 19.5 Hz, 1H, H-1), 5.88 (d, J_{3-2} 10.9 Hz, 1H, H-3), 7.01 (ddd, J_{2-P} 20.6 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −6.61 (d, J_{P2-P1} 23.1 Hz, 1P, P-2), 7.43 (d, J_{P1-P2} 23.1 Hz, 1P, P-1); ^{13}C NMR (D_2O) δ 18.22−25.64 (2C, C-5 and C-6), 121.50 (d, J_{1-P} 184.2 Hz, 1C, C-1), 124.90 (d, J_{3-P} 25.8 Hz, 1C, C-3), 140.11 (d, J_{2-P} 5.7 Hz, 1C, C-2), 143.82 (1C, C-4); MS FAB$^-$ (GT) *m/z* 241 $(M - 3NH_4 + 2H)^-$. Anal. ($C_6H_{21}N_3O_6P_2$) C, H, N.

(*E*,*E*)-Triammonium 4,8-dimethyl-1,3,7-nonatrienylpyrophosphonate (27): 58% yield; R_f = 0.28, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.48 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.95−2.15 (m, 4H, H-5 and H-6), 4.95−5.12 (m, 1H, H-7), 5.74 (dd, J_{1-2} 16.9 Hz, J_{1-P} 19.4 Hz, 1H, H-1), 5.88 (d, J_{3-2} 11.1 Hz, 1H, H-3), 6.98 (ddd, J_{2-P} 20.5 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −5.70 (d, $J_{\beta-\alpha}$ 21.3 Hz, P-β), 7.11 (d, P-α); ^{13}C NMR (D_2O) δ 16.65−17.28 (2C, C-10 and C-11), 25.16 (1C, C-9), 25.93 (1C, C-6), 39.55 (1C, C-5), 120.91 (d, J_{1-P} 183.9 Hz, 1C, C-1), 124.24 (1C, C-7), 124.70 (d, J_{3-P} 26.3 Hz, 1C, C-3), 134.21 (1C, C-8), 143.95 (d, J_{2-P} 6.2 Hz, 1C, C-2), 147.58 (1C, C-4); MS FAB$^-$ (GT) *m/z* 309 $(M - 3NH_4 + 2H)^-$. Anal. ($C_{11}H_{29}N_3O_6P_2$) C, H, N.

(*E*,*E*,*E*)-Triammonium 4,8,12-trimethyl-1,3,7,11-tridecatetraenylpyrophosphonate (28): 49% yield; R_f = 0.35, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) 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), 5.5−578 (m, 1H, H-1), 5.98 (d, J_{3-P} 11.0 Hz, 1H, H-3), 6.88−7.13 (m, 1H, H-2); ^{31}P NMR (D_2O) δ -9.9 (d, $J_{\beta-\alpha}$ 22.2 Hz, 1P, P-β), 8.42 (d, $J_{\alpha-\beta}$ 22.2 Hz, 1P, P-α); ^{13}C NMR (D_2O) 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-P} 188.9 Hz, 1C, C-1), 124.36−124.80 (2C, C-7 and C-11), 125.04 (d, J_{3-P} 21.2 Hz, 1C, C-3), 132.56−136.38 (2C, C-8 and C-12), 140.52 (1C, C-2), 146.90 (1C, C-4); MS FAB$^-$ (GT) *m/z* 377 $(M - 3NH_4 + 2H)$. Anal. ($C_{16}H_{37}N_3O_6P_2$) C, H, N.

Tetraammonium (*E*)-buta-1,3-dienyltriphosphonate (29): 7% yield; R_f = 0.08, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 5.20 (d, J_{4-3} 10.1 Hz, 1H, H-4), 5.33 (d, $J_{4'-3}$ 16.9 Hz, 1H, H-4′), 5.79 (dd, J_{1-P} 19.5 Hz, J_{1-2} 16.98 Hz, 1H, H-1), 6.28 (ddd, J_{3-2} 10.3 Hz, 1H, H-3), 6.68 (ddd, J_{2-P} 20.7 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −21.5 (m, P-β), −8.5 (m, P-γ), 7.6 (d, $J_{\alpha-\beta}$ 22.5 Hz, P-α); ^{13}C NMR (D_2O) δ 123.49 (d, J_{1-P} 186.4 Hz, 1C, C-1), 123.90 (1C, C-4), 136.71 (d, J_{3-P} 26.7 Hz, 1C, C-3), 144.92 (d, J_{2-P} 5.7 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 395 $(M - 4NH_4 + 5H)^+$.

Tetraammonium (*E*)-penta-1,3-dienyltriphosphonate (30): 7% yield; R_f = 0.1, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.68 (d, J_{5-4} 6.4 Hz, 3H, H-5), 5.76 (dd, J_{1-P} 18.7 Hz, J_{1-2} 17.0 Hz, 1H, H-1), 5.87−6.22 (m, 2H, H-3 and H-4), 6.69 (ddd, J_{2-P} 20.5 Hz, J_{2-3} 9.7 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −21.3 (m, P-β), −6.5 (m, P-γ), 7.4 (d, $J_{\alpha-\beta}$ 22.3 Hz, P-α); ^{13}C NMR (D_2O) δ 17.87 (1C, C-5), 121.63 (d, J_{1-P} 185.1 Hz, 1C, C-$_1$), 129.32 (d, J_{3-P} 25.5 Hz, 1C, C-3), 136.51 (1C, C-4), 143.67 (d, J_{2-P} 5.0 Hz, 1C, C-2); MS FAB$^+$ (GT) *m*/*z* 309 (M − 4NH$_4$ + 5H)$^+$.

Tetraammonium (*E*)-4-methylpenta-1,3-dienyltriphosphonate (31): 7% yield; R_f = 0.15, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.72 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 5.72 (dd, J_{1-2} 16.8 Hz, J_{1-P} 20.3 Hz, 1H, H-1), 5.90 (d, J_{3-2} 11.1 Hz, 1H, H-3), 7.10 (ddd, J_{2-P} 21.1 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −21.4 (dd, $J_{\beta-\alpha}$ 21.2 Hz, $J_{\beta-\gamma}$ Hz, P-β), −5.5 (d, P-γ), 9.10 (d, P-α); ^{13}C NMR (D_2O) δ 18.24−25.66 (2C, C-5 and C-6), 121.53 (d, J_{1-P} 183.5 Hz, 1C, C-1), 124.91 (d, J_{3-P} 25.4 Hz, 1C, C-3), 140.13 (d, J_{2-P} 5.7 Hz, 1C, C-2), 143.82 (1C, C-4); MS FAB$^+$ (GT) *m*/*z* 323 (M − 4NH$_4$ + 5H)$^+$.

Tetraammonium (*E*,*E*)-4,8-dimethylnona-1,3,7-trienyltriphosphonate (32): 12% yield; R_f = 0.15, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.62 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.13−2.22 (m, 4H, H-5 and H-6), 5.86 (dd, J_{1-2} 16.8 Hz, J_{1-P} 20.2 Hz, 1H, H-1), 6.03 (d, J_{3-2} 11.0 Hz, 1H, H-3), 7.19 (ddd, J_{2-P} 21.0 Hz, 1H, H-2); ^{31}P NMR (D_2O) δ −21.2 (dd, $J_{\beta-\alpha}$ 22.1 Hz, $J_{\beta-\gamma}$ 20.0 Hz, P-β), −6.3 (d, P-γ), 8.5 (d, P-α); ^{13}C NMR (D_2O) δ 16.66−17.28 (2C, C-10 and C-11), 25.17 (1C, C-9), 25.93 (1C, C-6), 39.56 (1C, C-5), 121.93 (d, J_{1-P} 184.0 Hz, 1C, C-1), 124.25 (1C, C-7), 124.73 (d, J_{3-P} 26.4 Hz, 1C, C-3), 134.26 (1C, C-8), 143.69 (d, J_{2-P} 62 Hz, 1C, C-2), 147.84 (1C, C-4); MS FAB$^+$ (GT) *m*/*z* 406 (M − 3NH$_4$ + 4H)$^+$, 389 (M − 4NH$_4$ + 5H)$^+$.

Tetraammonium (*E*,*E*,*E*)-4,8,12-trimethylhexadeca-1,3,7,11-tetraenyltriphosphonate (33): 18% yield; R_f = 0.25, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) 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_{3-2} 11.0 Hz, 1H, H-3), 6.86−7.12 (m, 1H, H-2); ^{31}P NMR (D_2O) δ −21.3 (dd, $J_{\beta-\alpha}$ 23.1 Hz, $J_{\beta-\gamma}$ 20.0 Hz, P-β), −6.3 (d, P-γ), 8.6 (d, P-α); ^{13}C NMR (D_2O) δ15.83−16.72−16.91−17.52 (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-P} 189.2 Hz, 1C, C-1), 124.34−124.81 (2C, C-7 and C-11), 125.14 (d, J_{3-P} 22.3 Hz, 1C, C-3), 132.40−136.43 (2C, C-8 and C-12), 140.58 (1C, C-2), 146.92 (1C, C-4); MS FAB$^+$ (GT) *m*/*z* 457 (M − 4NH$_4$ + 3H)$^-$.

Triammonium α,γ-(buta-1,3-dienyl)triphosphonate (34): 5% yield; R_f = 0.25, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 5.22 (d, J_{4-3} 9.7 Hz, 2H, H-4), 5.35 (d, $J_{4'-3}$ 16.7 Hz, 2H, H-4′), 5.90 (dd, J_{1-P} 18.3 Hz, J_{1-2} 17.0 Hz, 2H, H-1), 6.40 (ddd, J_{3-2} 10.4 Hz, 2H, H-3), 6.72 (ddd, J_{2-P} 20.1 Hz, 2H, H-2); ^{31}P NMR (D_2O) δ −20.1 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 22.4 Hz, 1P, P-β), 7.5 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 123.49 (d, J_{1-P} 186.5 Hz, 2C, C-1), 123.91 (2C, C-4), 136.72 (d, J_{3-P} 26.7 Hz, 2C, C-3), 144.94 (d, J_{2-P} 5.8 Hz, 2C, C-2); MS FAB$^+$ (GT) *m*/*z* 331 (M − 3NH$_4$ + 4H)$^+$.

Triammonium α,γ-(penta-1,3-dienyl)triphosphonate (35): 2% yield; R_f = 0.5, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.68 (d, J_{5-4} 6.4 Hz, 6H, H-5), 5.75 (dd, J_{1-P} 18.6 Hz, J_{1-2} 17.1 Hz, 2H, H-1), 5.86−6.22 (m, 4H, H-3 and H-4), 6.68 (ddd, J_{2-P} 20.4 Hz, J_{2-3} 9.74 Hz, 2H, H-2); ^{31}P NMR (D_2O) δ −20.1 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 22.5 Hz, 1P, P-β), 7.5 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 17.89 (2C, C-5), 121.63 (d, J_{1-P} 185.1 Hz, 2C, C-1), 129.33 (d, J_{3-P} 25.3 Hz, 2C, C-3), 136.51 (2C, C-4), 143.66 (d, J_{2-P} 5.4 Hz, 2C, C-2); MS FAB$^+$ (GT) *m*/*z* 359 (M − 3NH$_4$ + 4H)$^+$.

Triammonium α,γ-(4-methylpenta-1,3-dienyl)triphosphonate (36): 4% yield; R_f = 0.4, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.70 (s, 6H, 2CH_3), 1.73 (s, 6H, 2CH_3), 5.70 (dd, J_{1-2} 16.8 Hz, J_{1-P} 19.5 Hz, 2H, H-1), 5.90 (d, J_{3-2} 10.9 Hz, 2H, H-3), 6.69 (ddd, J_{2-P} 20.6 Hz, 2H, H-2); ^{31}P NMR (D_2O) δ -20.3 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 22.2 Hz, 1P, P-β), 7.2 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 18.21−25.61 (4C, C-5 and C-6), 121.52 (d, J_{1-P} 184.2 Hz, 2C, C-1), 124.91 (d, J_{3-P} 25.8 Hz, 2C, C-3), 140.05 (d, J_{2-P} 5.8 Hz, 2C, C-2), 143.84 (2C, C-4); MS FAB$^+$ (GT) *m*/*z* 387 (M − 3NH$_4$ + 4H)$^+$.

General Method for the Bromination of Alcohols with Phosphorus Tribromide. To a solution of the alcohol **1** or **2** (57.63 mmol, 1 equiv) in dichloromethane (50 mL), phosphorus tribromide (28.81 mmol, 0.5 equiv) diluted in 10 mL of the same solvent is added at −20 °C. The mixture is magnetically stirred under nitrogen atmosphere for 3 h. After hydrolysis with 50 mL of water at −40 °C, the aqueous layer is extracted with ether (3 × 50 mL), washed with saturated aqueous ammonium chloride solution (3 × 50 mL), dried over sodium sulfate, and concentrated in vacuo to afford **38** and **39**.

1-Bromo-3-methyl-2-butene (38): 82% yield; R_f = 0.8, petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 3H, CH_3), 1.8 (s, 3H, CH_3), 4.05 (d, J_{1-2} 8.5 Hz, 2H, H-1), 5.55 (t, 1H, H-2).

1-Bromo-3,7-dimethyl-2,6-decadiene (39): 96% yield; R_f = 0.8, petroleum ether/ether, 9:1); ^{1}H NMR ($CDCl_3$) δ 1.64 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.05−2.2 (m, 4H, H-4 and H-5), 4.05 (d, J_{1-2} 8.4 Hz, 2H, H-1), 5.02−5.15 (m, 1H, H-6), 5.58 (t, 1H, H-2).

Method for the Bromination of 3-Methyl-but-3-enol. 2-Methyl-4-*o*-mesylbutene (40). In a three-necked flask of 250 mL equipped with a magnetic stirrer and under nitrogen atmosphere were placed 3-methylbut-3-enol (11.79 g, 137 mmol, 1 equiv) dissolved in 100 mL of dichloromethane and triethylamine (20.81 g, 205.36 mmol, 1.5 equiv) at −5 °C. A solution of chloromethylsulfonate (17.25 g, 180.67 mmol, 1.1 equiv) in 20 mL of dichloromethane is then added within a period of 5 min. After 30 min, the mixture is allowed to warm to room temperature and then hydrolyzed with 100 mL of water and ice. The organic layer was washed with a solution of 10% HCl and then with 100 mL of saturated $NaHCO_3$, dried over sodium sulfate, and concentrated in vacuo to give compound **40** pure as a colorless oil: 99% yield; R_f = 0.5, petroleum ether/ether, 6:4; ^{1}H NMR ($CDCl_3$) δ 1.8 (s, 3H, H-5), 2.48 (t, J_{3-4} 6.7 Hz, 2H, H-3), 3.03 (s, 3H, H-6), 4.35 (t, 2H, H-4), 4.8 (s, 1H, H-1), 4.9 (s, 1H, H-1).

4-Bromo-2-methylbutene (41). To a solution of lithium bromide (15 g, 182 mmol, 2 equiv) in 150 mL of *N*,*N*-dimethylformamide magnetically stirred under reflux and nitrogen atmosphere was added 4-mesyl-2-methylbutene (13.47 g, 91 mmol, 1 equiv). After 2 h, 150 mL of water was added and the organic layer was extracted with ether (3 × 50 mL). The organic extracts were assembled, washed with saturated aqueous sodium chloride solution (2 × 100 mL), dried over sodium sulfate, and concentrated in a vacuum to afford a yellowish oil: 100% yield; ^{1}H NMR ($CDCl_3$) δ 1.7 (s, 3H, H-5), 2.52 (t, J_{3-4} 7.35 Hz, 2H, H-3), 3.44 (t, 2H, H-4), 4.7 (s, 1H, H-1), 4.8 (s, 1H, H-1).

General Procedure for the Phosphonation of Bromide Derivatives with Diethylmethyl Phosphonate. To a solution of diethylmethyl phosphonate (14.75 mmol, 1.1 equiv) in 50 mL of tetrahydrofurane was slowly added 1.6 M *n*-butyllithium in hexane (16.09 mmol, 1.2 equiv) at −78 °C. The mixture was magnetically stirred under nitrogen atmosphere for 1 h and then compound **38**, **39**, or **41** (13.41 mmol, 1 equiv) was added. After 4 h of stirring, the mixture was diluted in 50 mL of ether, washed with aqueous HCl (1 N, 3 × 100 mL) and then with brine (2 × 50 mL), dried over sodium sulfate, concentrated, and chromatographed on silica gel with ethyl acetate as eluent to afford **42**−**56**.

Diethyl (4-methyl-3-pentenyl)phosphonate (42): 59% yield; R_f = 0.4, ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J_{8-7} 6.7 Hz, 6H, H-8), 1.62 (s, 3H, CH_3), 1.65−1.88 (m, 2H, H-2), 1.69 (s, 3H, CH_3), 2.13−2.35 (m, 2H, H-1), 3.97−4.2 (qd, 4H, H-7), 5.0−5.18 (m, 1H, H-3); ^{31}P NMR ($CDCl_3$) δ 33.2; ^{13}C NMR ($CDCl_3$) δ 16.61−16.70 (2C, C-8), 18.44 (1C, C-5), 21.99 (d, J_{2-P} 4.1 Hz, 1C, C-2), 25.11 (1C, C-6), 26.61 (d, J_{1-P} 138.3 Hz, 1C, C-1), 61.80−61.89 (2C, C-7),122.63 (d, J_{3-P} 18.3 Hz, 1C, C-3), 133.69 (1C, C-4); MS FAB$^+$ (NOBA) *m*/*z* 221 (M + H)$^+$. Anal. ($C_{10}H_{21}O_3P$) C, H.

Diethyl ((*E*)-4,8-dimethyl-3,7-nonadienyl)phosphonate (43): 70% yield; R_f = 0.5, ethyl acetate; ^{1}H NMR ($CDCl_3$) δ

1.31 (t, J_{13-12} 7.1 Hz, 6H, H-13), 1.58 (s, 3H, H-11), 1.6 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.66−1.88 (m, 2H, H-2), 1.9−2.12 (m, 4H, H-5 and H-6), 2.18−2.38 (m, 2H, H-1), 3.97−4.18 (q, 4H, H-12), 5.06 (m, 1H, H-7), 5.1 (m, 1H, H-3); ^{31}P NMR ($CDCl_3$) δ 33.2; ^{13}C NMR ($CDCl_3$) δ 16.22−16.38 (2C, C-13), 16.91−18.08 (2C, C-10 and C-11), 21.39 (d, J_{2-P} 4.6 Hz, 1C, C-2), 26.08 (1C, C-9), 26.35 (d, J_{1-P} 138.8 Hz, 1C, C-1), 22.97 (1C, C-6), 39.97 (1C, C-5), 61.78−61.85 (2C, C-12), 123.41 (d, J_{3-P} 17.3 Hz, 1C, C-3), 123.82 (1C, C-7), 131.88 (1C, C-8), 136.76 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 289 (M + H)$^+$. Anal. ($C_{15}H_{29}O_3P$) C, H.

Diethyl (4-methyl-4-pentenyl)phosphonate (44): 64% yield; R_f = 0.35, ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J_{8-7} 3.5 Hz, 6H, H-8), 1.72 (s, 3H, H-6), 1.6−1.82 (m, 4H, H-2 and H-3), 2.1 (m, 2H, H-1), 4.0−4.20 (q, 4H, H-7), 4.7 (s, 1H, H-5), 4.76 (s, 1H, H-5); ^{31}P NMR ($CDCl_3$) δ 33.7; ^{13}C NMR ($CDCl_3$) δ 16.63−16.72 (2C, C-8), 21.64 (d, J_{2-P} 4.4 Hz, 1C, C-2), 21.98 (1C, C-6), 27.41 (d, J_{1-P} 131.7 Hz, 1C, C-1), 34.78 (d, J_{3-P} 16.5 Hz, 1C, C-3), 61.82−61.93 (2C, C-7), 110.78 (1C, C-5), 148.13 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 221 (M + H)$^+$. Anal. ($C_{10}H_{21}O_3P$) C, H.

Phosphonic acids were obtained according to the same procedure used for compounds 14−18. 4-Methyl-3-pentenylphosphonic acid (45): 69% yield; R_f = 0.29, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 3H, H-5), 1.65−1.88 (m, 2H, H-2), 1.68 (s, 3H, H-6), 2.15−2.32 (m, 2H, H-1), 5.04−5.2 (m, 1H, H-3), 8.0 (s, 2H, PO*H*); ^{31}P NMR ($CDCl_3$) δ 34.5; ^{13}C NMR ($CDCl_3$) δ 18.05 (1C, C-5), 21.81 (d, J_{2-P} 4.1 Hz, 1C, C-2), 24.76 (1C, C-6), 26.87 (d, J_{1-P} 142.3 Hz, 1C, C-1), 122.51 (d, J_{3-P} 19.5 Hz, 1C, C-3), 133.75 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 165 (M + H)$^+$, 187 (M + Na)$^+$.

(*E*)-4,8-Dimethyl-3,7-nonadienylphosphonic acid (46): 98% yield; R_f = 0.4, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) δ 1.55−1.88 (m, 2H, H-2), 1.62 (s, 6H, 2CH_3), 1.7 (s, 3H, CH_3), 1.9−2.1 (m, 4H, H-5 and H-6), 2.17−2.41 (m, 2H, H-1), 5.0−5.22 (m, 2H, H-3 and H-7), 9.45 (s, 2H, PO*H*); ^{31}P NMR ($CDCl_3$) δ 36.8; ^{13}C NMR ($CDCl_3$) δ 15.68−18.1 (2C, C-10 and C-11), 21.25 (d, J_{2-P} 4.5 Hz, 1C, C-2), 26.10 (1C, C-9), 26.6 (d, J_{1-P} 142.9 Hz, 1C, C-1), 26.84 (1C, C-6), 39.99 (1C, C-5), 123.33 (d, J_{3-P} 18.6 Hz, 1C, C-3), 124.58 (1C, C-7), 131.87 (1C, C-8), 136.8 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 233 (M + H)$^+$, 255 (M + Na)$^+$.

4-Methyl-4-pentenylphosphonic acid (47): 76% yield; R_f = 0.4, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CDCl_3$) δ 1.73 (s, 3H, H-6), 1.6−1.85 (m, 4H, H-2 and H-3), 2.11 (m, 2H, H-1), 4.71 (s, 1H, H-5), 4.77 (s, 1H, H-5), 8.39 (s, 2H, PO*H*); ^{31}P NMR ($CDCl_3$) δ 37.7; ^{13}C NMR ($CDCl_3$) δ 21.51 (d, J_{2-P} 4.2 Hz, 1C, C-2), 21.73 (1C, C-6), 27.77 (d, J_{1-P} 133.2 Hz, 1C, C-1), 38.61 (d, J_{3-P} 17.5 Hz, 1C, C-3), 110.13 (1C, C-5), 147.63 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 165 (M + H)$^+$, 187 (M + Na)$^+$.

Pyrophosphonates were obtained according to the same procedure used for compounds 24−28. Triammonium 4-methyl-3-pentenylpyrophosphonate (48): 50% yield; R_f = 0.35, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.52 (s, 3H, H-5), 1.55−1.6 (m, 2H, H-2), 1.57 (s, 3H, H-6), 2.0−2.22 (m, 2H, H-1), 5.14 (m, 1H, H-3); ^{31}P NMR (D_2O) δ −7.0 (d, $J_{\beta-\alpha}$ 25.2 Hz, P-β), 19.5 (d, P-α); ^{13}C NMR (D_2O) δ 17.24 (1C, C-5), 21.93 (d, J_{2-P} 3.9 Hz, 1C, C-2), 24.02 (1C, C-6), 28.29 (d, J_{1-P} 135.1 Hz, 1C, C-1), 124.87 (d, J_{3-P} 18.8 Hz, 1C, C-3), 133.82 (d, J_{4-P} 1.5 Hz, 1C, C-4); MS FAB$^+$ (GT) *m/z* 279 (M − NH_4 + 2H)$^+$, 262 (M − 2NH_4 + 3H)$^+$, 245 (M − 3NH_4 + 4H)$^+$. Anal. ($C_6H_{23}N_3O_6P_2$) C, H, N.

(*E*)-Triammonium 4,8-dimethyl-3,7-nonadienylpyrophosphonate (49): 46% yield; R_f = 0.47, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.42 (s, 3H, H-11), 1.46 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.5−1.65 (m, 2H, H-2), 1.73−1.95 (m, 4H, H-5 and H-6), 1.95−2.15 (m, 2H, H-1), 4.99 (m, 1H, H-7), 5.09 (m, 1H, H-3); ^{31}P NMR (D_2O) δ −6.4 (d, $J_{\beta-\alpha}$ 25.1 Hz, P-β), 19.3 (d, P-α); ^{13}C NMR (D_2O) δ 15.56 (1C, C-10), 17.3 (1C, C-11), 21.99 (d, J_{2-P} 4.4 Hz, 1C, C-2), 25.18 (1C, C-9), 26.15 (1C, C-6), 28.39 (d, J_{1-P} 134.4 Hz, 1C, C-1), 39.15 (1C, C-5), 124.74 (1C, C-7), 125.08 (d, J_{3-P} 18.8 Hz, 1C, C-3), 133.87 (1C, C-8), 136.8 (1C, C-4); MS FAB$^+$ (GT) *m/z* 330 (M − 2NH_4 + 5H)$^+$, 313 (M − 3NH_4 + 4H)$^+$. Anal. ($C_{11}H_{31}N_3O_6P_2$) C, H, N.

Triammonium 4-methyl-4-pentenylpyrophosphonate (50): 52% yield; R_f = 0.5, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.52−1.71 (m, 4H, H-2 and H-3), 1.62 (s, 3H, H-6), 2.0 (m, 2H, H-1), 4.66 (s, 1H, H-5), 4.7 (s, 1H, H-5); ^{31}P NMR (D_2O) δ −7.0 (d, $J_{\beta-\alpha}$ 25.1 Hz, P-β), 20.3 (d, P-α); ^{13}C NMR (D_2O) δ 21.2 (d, J_{2-P} 4.0 Hz, 1C, C-2), 21.86 (1C, C6), 27.67 (d, J_{1-P} 136.7 Hz, 1C, C-1), 38.65 (d, J_{3-P} 18.4 Hz, 1C, C-3), 110.03 (1C, C-5), 147.92 (1C, C-4); MS FAB$^+$ (GT) *m/z* 262 (M − 2NH_4 + 3H)$^+$, 245 (M − 3NH_4 + 4H)$^+$. Anal. ($C_6H_{23}N_3O_6P_2$) C, H, N.

Tetraammonium 4-methylpent-3-enyltriphosphonate (51): 15% yield; R_f = 0.21, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.54 (s, 3H, H-5), 1.56−1.62 (m, 2H, H-2), 1.58 (s, 3H, H-6), 2.10−2.28 (m, 2H, H-1), 5.15 (m, 1H, H-3); ^{31}P NMR (D_2O) δ −21.1 (dd, $J_{\beta-\alpha}$ 24.0 Hz and $J_{\beta-\gamma}$ 19.6 Hz, P-β), −5.7 (d, P-γ), 21.9 (d, P-α); ^{13}C NMR (D_2O) δ 17.20 (1C, C-5), 22.12 (d, J_{2-P} 4.0 Hz, 1C, C-2), 24.10 (1C, C-6), 28.79 (d, J_{1-P} 135.7 Hz, 1C, C-1), 124.96 (d, J_{3-P} 18.9 Hz, 1C, C-3), 133.96 (1C, C-4); MS FAB$^+$ (GT) *m/z* 325 (M − 4NH_4 + 5H)$^+$.

Tetraammonium (*E*)-4,8-dimethylnona-3,7-dienyltriphosphonate (52): 9.61% yield; R_f = 0.29, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.45 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 1.54−1.69 (m, 2H, H-2), 1.77−1.99 (m, 4H, H-5 and H-6), 2.0−2.19 (m, 2H, H-1), 5.03 (m, 1H, H-7), 5.12 (s, 1H, H-3); ^{31}P NMR (D_2O) δ −20.7 (dd, $J_{\beta-\alpha}$ 23.9 Hz and $J_{\beta-\gamma}$ 20.6 Hz, P-β), −5.4 (d, P-γ), 21.4 (d, P-α); ^{13}C NMR (D_2O) δ 15.51 (1C, C-10), 17.27 (1C, C-11), 21.78 (d, J_{2-P} 3.8 Hz, 1C, C-2), 25.13 (1C, C-9), 26.10 (1C, C-6), 28.37 (d, J_{1-P} 135.2 Hz, 1C, C-1), 39.09 (1C, C-5), 124.68 (d, J_{3-P} 15.5 Hz, 1C, C-3), 124.73 (1C, C-7), 133.82 (1C, C-8), 137.06 (1C, C-4); MS FAB$^+$ (GT) *m/z* 393 (M − 4NH_4 + 5H)$^+$.

Tetraammonium 4-methylpent-4-enyltriphosphonate (53): 14% yield; R_f = 0.26, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.48−1.52 (m, 4H, H-2 and H-3), 1.58 (s, 3H, H-6), 1.98 (m, 2H, H-1), 4.62 (s, 1H, H-5), 4.7 (s, 1H, H-5); ^{31}P NMR (D_2O) δ −21.0 (dd, $J_{\beta-\alpha}$ 24.4 Hz and $J_{\beta-\gamma}$ 20.3 Hz, P-β), −5.9 (d, P-γ), 22.1 (d, P-α); ^{13}C NMR (D_2O) δ 21.08 (d, J_{2-P} 4.3 Hz, 1C, C-2), 21.83 (1C, C-6), 27.64 (d, J_{1-P} 137.1 Hz, 1C, C-1), 38.45 (d, J_{3-P} 18.3 Hz, 1C, C-3), 110.16 (1C, C-5), 147.59 (1C, C-4); MS FAB$^+$ (GT) *m/z* 325 (M − 4NH_4 + 5H)$^+$.

Triammonium α,γ-(4-methylpent-3-enyl)triphosphonate (54): 4% yield; R_f = 0.7 SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.47 (s, 6H, H-6), 1.53 (s, 6H, H-5), 1.54−1.68 (m, 4H, H-2), 1.95−2.2 (m, 4H, H-1), 5.08 (m, 2H, H-3); ^{31}P NMR (D_2O) δ −21.88 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 25.5 Hz, P-β), 20.91 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 17.24 (2C, C-5), 21.98 (d, J_{2-P} 4.0 Hz, 2C, C-2), 25.11 (2C, C-6), 28.1 (d, J_{1-P} 131.1 Hz, 2C, C-1), 124.72 (d, J_{3-P} 21.2 Hz, 2C, C-3), 133.9 (d, J_{4-P} 1.2 Hz, 2C, C-4); MS FAB$^+$ (GT) *m/z* 391 (M − 3NH_4 + 4H)$^+$.

Triammonium α,γ-[(*E*)-4,8-dimethylnona-3,7-dienyl]-triphosphonate (55): 5% yield; R_f = 0.75, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.46 (s, 6H, H-11), 1.53 (s, 12H, H-9 and H-10), 1.69−2.0 (m, 8H, H-5 and H-6), 2.08−2.12 (m, 4H, H-1), 4.95−5.05 (m, 2H, H-7), 5.06−5.17 (m, 2H, H-3); ^{31}P NMR (D_2O) δ −22.2 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 25.6 Hz, 1P, P-β), 20.7 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 15.53 (2C, C-10), 17.32 (2C, C-11), 21.85 (d, J_{2-P} 3.8 Hz, 2C, C-2), 25.27 (2C, C-9), 26.18 (1C, C-6), 29.02 (d, J_{1-P} 134.9 Hz, 2C, C-1), 40.18 (2C, C-5), 124.80 (d, J_{3-P} 15.7 Hz, 2C, C-3), 124.81 (2C, C-7), 133.89 (2C, C-8), 137.10 (2C, C-4); MS FAB$^+$ (GT) *m/z* 527 (M − 3NH_4 + 4H)$^+$.

Triammonium α,γ-(4-methylpent-4-enyl)triphosphonate (56): 12.5% yield; R_f = 0.66, SiO_2, 27% aqueous ammonia)/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.5−1.71 (m, 8H, H-2 and H-3), 1.62 (s, 6H, H-6), 2.01 (m, 4H, H-1), 4.67 (s, 2H, H-5), 4.70 (s, 2H, H-5); ^{31}P NMR (D_2O) δ −22.1 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 25.6 Hz, 1P, P-β), 21.7 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 21.14 (d, J_{2-P} 4.1 Hz, 2C, C-2), 21.85 (2C, C-6), 27.75 (d, J_{1-P} 137.4

Hz, 2C, C-1), 38.52 (d, J_{3-P} 18.7 Hz, 2C, C-3), 110.24 (2C, C-5), 147.55 (2C, C-4); MS FAB$^+$ (GT) *m/z* 391 $(M - 3NH_4 + 4H)^+$.

General Michaelis−Arbuzov Procedure of Phosphonation. In a round-bottomed flask equipped with a reflux condenser and a magnetic stirrer and under nitrogen atmosphere is introduced triethyl phosphite (64 mmol, 1 equiv), compound **38**, **41**, or **57** (58 mmol, 1 equiv) is then added, and the mixture is heated to 130 °C during 3 h. The crude is then concentrated in vacum in order to eliminate the remains of triethyl phosphite and purified by column chromatography on silica gel with ether as eluent.

Diethyl (3-methyl-3-butenyl)phosphonate (58): 67% yield; $R_f = 0.43$, ether; ^{1}H NMR ($CDCl_3$) δ 1.22−1.35 (t, J_{7-6} 7.0 Hz, 6H, H-7), 1.73 (s, 3H, H-5), 1.77−1.95 (m, 2H, H-3), 2.20−2.37 (m, 2H, H-4), 4.00−4.18 (q, 4H, H-6), 4.60−4.77 (m, 2H, H-4); ^{31}P NMR ($CDCl_3$) δ 20.6; ^{13}C NMR ($CDCl_3$) δ 16.53−16.58 (2C, C-7), 22.29 (1C, C-5), 24.23 (d, J_{1-P} 141.2 Hz, 1C, C-1), 30.28 (d, J_{2-P} 4.3 Hz, 1C, C-2), 61.61 (2C, C-6), 110.34 (1C, C-4), 144.44 (d, J_{3-P} 17.8 Hz, 1C, C-3); MS FAB$^+$ (NOBA) *m/z* 207 $(M + H)^+$.

Diethyl (3-methyl-2-butenyl)phosphonate (59): 58% yield; $R_f = 0.45$, ether; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J_{7-6} 7.0 Hz, 6H, H-7), 1.67 (d, J_{4-2} 3.9 Hz, 3H, H-4), 1.76 (d, J_{5-2} 5.3 Hz, 3H, H-5), 2.57 (dd, J_{1-P} 22.0 Hz, J_{1-2} 7.5 Hz, 2H, H-1), 4.15 (q, 4H, H-6), 5.19 (m, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 30.1; ^{13}C NMR ($CDCl_3$) δ 16.55−16.61 (2C, C-7), 18.01 (d, J_{5-P} 2.5 Hz, C-5), 25.85 (1C, C-4), 26.56 (d, J_{1-P} 138.2 Hz, 1C, C-1), 61.78−61.85 (2C, C-6), 112.75 (d, J_{2-P} 11.3 Hz, 1C, C-2), 136.62 (d, J_{3-P} 14.5 Hz, 1C, C-3); MS FAB$^+$ (NOBA) *m/z* 207 $(M + H)^+$.

Diethyl (allyl)phosphonate (60): 42% yield; $R_f = 0.39$, ether; ^{1}H NMR ($CDCl_3$) δ 1.38 (t, J_{5-4} 7.1 Hz, 6H, H-5), 2.63 (dd, J_{3-2} 7.3 Hz, J_{3-P} 22.0 Hz, 2H, H-3), 4.16 (qt, 4H, H-4), 5.15−5.30 (m, 2H, H-1), 5.73−5.82 (m, 1H, H-2); ^{31}P NMR ($CDCl_3$) δ 28.4; ^{13}C NMR ($CDCl_3$) δ 16.80−16.86 (2C, C-5), 32.19 (d, J_{3-P} 139.4 Hz, 1C, C-3), 62.29−62.37 (2C, C-4), 120.31 (d, J_{1-P} 14.5 Hz, 1C, C-1), 127.92 (d, J_{2-P} 11.1 Hz, 1C, C-2); MS FAB$^+$ (NOBA) *m/z* 179 $(M + H)^+$, 200 $(M + Na)^+$.

Phosphonic acids were obtained according to the same procedure used for compounds 14−18. 3-Methyl-3-butenylphosphonic acid (61): 96% yield; $R_f = 0.32$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CO(CD_3)_2$) δ 1.74 (s, 3H, H-5), 1.80−2.0 (m, 2H, H-2), 2.22−2.40 (m, 2H, H-1), 4.71−4.80 (m, 2H, H-4), 10.54 (s, 2H, PO*H*); ^{31}P NMR ($CO(CD_3)_2$) δ 24.0; ^{13}C NMR ($CO(DCl_3)_2$) δ 22.65 (1C, C-5), 26.98 (d, J_{1-P} 147.3 Hz, 1C, C-1), 28.99 (d, J_{2-P} 4.2 Hz, 1C, C-2), 110.68 (1C, C-4), 144.89 (d, J_{3-P} 17.9 Hz, 1C, C-3); MS FAB$^+$ (NOBA) *m/z* 151 $(M + H)^+$, 173 $(M + Na)^+$.

3-Methyl-2-butenylphosphonic acid (62): 85% yield; $R_f = 0.33$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CO(CD_3)_2$) δ 1.66−1.76 (m, 6H, H-4 and H-5), 2.55 (m, J_{1-P} 20.0 Hz, 2H, H-1), 5.07−5.22 (m, 1H, H-2), 10.86 (s, 2H, PO*H*); ^{31}P NMR ($CO(CD_3)_2$) δ 33.1; ^{13}C NMR ($CO(DCl_3)_2$) δ 17.48 (1C, C-5), 25.46 (1C, C-4), 27.85 (d, J_{1-P} 139.2 Hz, 1C, C-1), 114.28 (d, J_{2-P} 10.9 Hz, 1C, C-2), 135.75 (d, J_{3-P} 14.3 Hz, 1C, C-3); MS FAB$^+$ (NOBA) *m/z* 151 $(M + H)^+$, 173 $(M + Na)^+$.

Allylphosphonic acid (63): 50% yield; $R_f = 0.29$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CO(CD_3)_2$) δ 2.15 (dd, J_{1-2} 7.53 Hz, J_{1-P} 20.7 Hz, 2H, H-1), 4.84−4.95 (m, 2H, H-3), 5.45−5.70 (m, 1H, H-2), 10.53 (s, 2H, PO*H*); ^{31}P NMR ($CO(CD_3)_2$) δ 28.3; ^{13}C NMR ($CO(DCl_3)_2$) δ 27.70 (d, J_{3-P} 135.2 Hz, 1C, C-3), 124.28 (d, J_{1-P} 14.2 Hz, 1C, C-1), 132.11 (d, J_{2-P} 10.1 Hz, 1C, C-2); MS FAB$^+$ (NOBA) *m/z* 123 $(M + H)^+$.

Pyrophosphonates were obtained according to the same procedure described before (see compounds 24−28). Triammonium 3-methyl-3-butenylpyrophosphonate (64): 61% yield; $R_f = 0.16$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.50 (s, 3H, H-5), 1.62−1.75 (m, 2H, H-2), 1.95−2.15 (m, 2H, H-1), 4.45−4.65 (m, 2H, H-5); ^{31}P NMR (D_2O) δ −8.4 (d, $J_{\beta-\alpha}$ 25.0 Hz, P-β), 21.2 (d, P-α); ^{13}C NMR (D_2O) δ 21.78 (1C, C-5), 26.62 (d, J_{1-P} 137.0 Hz, 1C, C-1), 31.15 (d, J_{2-P} 3.9 Hz, 1C, C-2), 109.40 (1C, C-4), 147.75 (d, J_{3-P} 18.5 Hz, 1C, C-3); MS FAB$^+$ (GT) *m/z* 231 $(M - 3NH_4 + 4H)^+$. Anal. ($C_5H_{21}N_3O_6P_2$) C, H, N.

Triammonium 3-methyl-2-butenylpyrophosphonate (65): 61% yield; $R_f = 0.15$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 1.44 (d, J_{4-2} 5.2 Hz, 3H, H-4), 1.55 (d, J_{5-2} 3.8 Hz, 3H, H-5), 2.33 (dd, J_{1-P} 21.7 Hz, J_{1-2} 7.8 Hz, 2H, H-1), 5.02 (m, 1H, H-2); ^{31}P NMR (D_2O) δ −7.0 (d, $J_{\beta-\alpha}$ 26.0 Hz, P-β), 16.9 (d, P-α); ^{13}C NMR (D_2O) δ 17.66 (1C, C-5), 25.42 (1C, C-4), 28.90 (d, J_{1-P} 136.3 Hz, 1C, C-1), 114.91 (d, J_{2-P} 10.8 Hz, 1C, C-2), 136.63 (d, J_{3-P} 14.5 Hz, 1C, C-3); MS FAB$^+$ (GT) *m/z* 231 $(M - 3NH_4 + 4H)^+$. Anal. ($C_5H_{21}N_3O_6P_2$) C, H, N.

Triammonium allylpyrophosphonate (66): 40% yield; $R_f = 0.22$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR (D_2O) δ 2.52 (dd, J_{1-P} 21.7 Hz, J_{1-2} 7.48 Hz, 2H, H-1), 4.92−5.12 (m, 2H, H-3), 5.62−5.90 (m, 1H, H-2); ^{31}P NMR (D_2O) δ −6.8 (d, $J_{\beta-\alpha}$ 24.3 Hz, P-β), 14.9 (d, P-α); ^{13}C NMR (D_2O) δ 34.28 (d, J_{1-P} 133.5 Hz, 1C, C-1), 118.54 (d, J_{3-P} 13.9 Hz, 1C, C-3), 131.53 (d, J_{2-P} 10.8 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 203 $(M - 3NH_4 + 4H)^+$. Anal. ($C_3H_{17}N_3O_6P_2$) C, H, N.

Tetraammonium 3-methylbut-3-enyltriphosphonate (67): 15% yield; $R_f = 0.1$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.51 (s, 3H, H-5), 1.60−1.72 (m, 2H, H-2), 1.92−2.13 (m, 2H, H-1), 4.45−4.60 (2m, 2H, H-4); ^{31}P NMR (D_2O) δ −21.2 (dd, $J_{\beta-\alpha}$ 24.0 Hz, $J_{\beta-\gamma}$ 21.2 Hz, P-β), −6.1 (d, P-γ), 21.3 (d, P-α); ^{13}C NMR (D_2O) δ 21.83 (1C, C-5), 26.77 (d, J_{1-P} 137.1 Hz, 1C, C-1), 31.21 (d, J_{2-P} 3.9 Hz, 1C, C-2), 109.40 (1C, C-4), 147.82 (d, J_{3-P} 18.5 Hz, 1C, C-3); MS FAB$^+$ (GT) *m/z* 311 $(M - 4NH_4 + 5H)^+$.

Tetraammonium allyltriphosphonate (68): 10% yield; $R_f = 0.15$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 2.55 (dm, J_{1-P} 21.9 Hz, J_{1-2} 7.5 Hz, 2H, H-1), 5.00−5.17 (m, 2H, H-3), 5.60−5.90 (m, 1H, H-2); ^{31}P NMR (D_2O) δ −21.7 (m, P-β), −7.6 (m, P-γ), 16.6 (m, P-α); ^{13}C NMR (D_2O) δ 34.81 (d, J_{1-P} 128.9 Hz, 1C, C-1), 117.88 (d, J_{3-P} 13.5 Hz, 1C, C-3), 131.96 (d, J_{2-P} 10.7 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 283 $(M - 4NH_4 + 5H)^+$.

Diammonium α,β-(3-methylbut-3-enyl)pyrophosphonate (69): 10% yield; $R_f = 0.5$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.65 (s, 3H, H-5), 1.70−1.80 (m, 2H, H-2), 2.10−2.15 (m, 2H, H-1), 4.68 (2m, 2H, H-4); ^{31}P NMR (D_2O) δ 20.0; ^{13}C NMR (D_2O) δ 21.80 (2C, C-5), 26.63 (d, J_{1-P} 137.0 Hz, 2C, C-1), 31.14 (d, J_{2-P} 4.0 Hz, 2C, C-2), 109.32 (2C, C-4), 147.71 (d, J_{3-P} 18.5 Hz, 2C, C-3); MS FAB$^-$ (NOBA) *m/z* 303 $(M - 2NH_4 + Na)^-$, 281 $(M - 2NH_4 + H)^-$.

Diammonium α,β-(3-methylbut-2-enyl)pyrophosphonate (70): 13% yield; $R_f = 0.46$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.54 (m, 3H, H-4), 1.60 (m, 3H, H-5), 2.50 (m, J_{1-P} 21.0 Hz, J_{1-2} 7.8 Hz, 2H, H-1), 5.03−5.20(m, 1H, H-2); ^{31}P NMR (D_2O) δ 17.3; ^{13}C NMR (D_2O) δ 17.61 (1C, C-5), 25.41 (1C, C-4), 29.43 (d, J_{1-P} 144.4 Hz, 1C, C-1), 115.33 (d, J_{2-P} 10.7 Hz, 1C, C-2), 136.32 (d, J_{3-P} 14.5 Hz, 1C, C-3); MS FAB$^+$ (NOBA) *m/z* 305 $(M - 2NH_4 + Na + 2H)^+$, 283 $(M - 2NH_4 + 3H)^+$.

Diammonium α,β-allylpyrophosphonate (71): 6% yield; $R_f = 0.55$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 2.35−2.57 (m, 2H, H-1), 4.95−5.15 (m, 2H, H-3), 5.55−5.85 (2m, 1H, H-2); ^{31}P NMR (D_2O) δ 15.6; ^{13}C NMR (D_2O) δ 34.73 (d, J_{1-P} 132.7 Hz, 1C, C-1), 117.96 (d, J_{3-P} 13.5 Hz, 1C, C-3), 132.02 (d, J_{2-P} 10.7 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 227 $(M - 2NH_4 + 3H)^+$.

Triammonium α,γ-(3-methylbut-2-enyl)triphosphonate (72): 17% yield; $R_f = 0.25$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.53 (m, 3H, H-4), 1.60 (m, 3H, H-5), 2.32−2.55 (m, J_{1-P} 22.0 Hz, J_{1-2} 7.7 Hz, 2H, H-1), 5.05−5.17 (m, 1H, H-2); ^{31}P NMR (D_2O) δ −21.7 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 26.0 Hz, P-β), 18.1 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 17.45 (1C, C-5), 25.23 (1C, C-4), 28.76 (d, J_{1-P} 135.7 Hz, 1C, C-1), 115.48 (d, J_{2-P} 10.6 Hz, 1C, C-2), 136.04 (d, J_{3-P} 14.3 Hz, 1C, C-3); MS FAB$^+$ (GT) *m/z* 391 $(M - 3NH_4 + 4H)^+$.

Triammonium α,γ-allyltriphosphonate (73): 6% yield; $R_f = 0.45$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 2.30−2.60 (m, 2H, H-1), 4.92−5.12 (m, 2H, H-2), 5.50−5.80 (2m, 1H, H-3); ^{31}P NMR (D_2O) δ −22.5 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 25.0 Hz, P-β), 16.2 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 34.25 (d, J_{1-P} 133.0 Hz, 1C, C-1), 118.24 (d, J_{3-P} 13.8 Hz, 1C,

C-3), 131.6 (d, J_{2-P} 10.7 Hz, 1C, C-2); MS FAB$^+$ (GT) *m/z* 307 $(M - 3NH_4 + 4H)^+$.

Diethyl (4-Methyl-2-oxa-4-pentenyl)phosphonate (74). To a solution of diethyl (hydroxymethyl)phosphonate (3 g, 17.8 mmol, 1 equiv) in 50 mL of THF, sodium hydride (0.43 g, 17.8 mmol, 1 equiv) was slowly added, and the mixture was stirred at room temperature until the suspension of sodium hydride disappeared (30 min). 3-Chloro-2-methylpropene (1.62 g, 17.8 mmol, 1 equiv) was then added, and 1 h of stirring later the mixture was hydrolyzed by adding 100 mL of water. After extraction with ether (3 × 50 mL), the organic layers were assembled and washed with brine (100 mL) and then dried over sodium sulfate. The solvent was evaporated in vacuo and the crude product was purified by column chromatography on silica gel with ethyl acetate to give compound **74**: 95% yield; $R_f = 0.4$, ethyl acetate; ^{1}H NMR ($CDCl_3$) δ 1.22−1.35 (t, J_{8-7} 7.7 Hz, 6H, H-8), 1.88 (s, 3H, H-6), 3.67−3.77 (d, J_{1-P} 9.0 Hz, 2H, H-1), 4.0−4.10 (m, 2H, H-3), 4.10−4.18 (q, 4H, H-7), 4.90−5.02 (m, 2H, H-5); ^{31}P NMR ($CDCl_3$) δ 20.6; ^{13}C NMR ($CDCl_3$) δ 16.82−16.88 (2C, C-8), 19.63 (1C, C-6), 62.73−62.80 (2C, C-7), 63.71 (d, J_{1-P} 167.3 Hz, 1C, C-1), 77.34 (d, J_{3-P} 13.1 Hz, 1C, C-3), 114.10 (1C, C-5), 141.35 (1C, C-4); MS FAB$^+$ (NOBA)-*m/z* 223 $(M + H)^+$. Anal. ($C_9H_{19}O_4P$) C, H.

4-Methyl-2-oxa-4-pentenylphosphonic acid (75): 99% yield; $R_f = 0.29$, 27% aqueous ammonia/2-propanol, 4:6; ^{1}H NMR ($CO(CD_3)_2$) δ 1.88 (s, 3H, H-6), 3.67−3.77 (m, 2H, H-1), 4.0−4.10 (m, 2H, H-3), 4.90−5.02 (m, 2H, H-5), 10.69 (s, 2H, PO*H*); ^{31}P NMR ($CO(CD_3)_2$) δ 23.6; ^{13}C NMR ($CO(DCl_3)_2$) δ 19.00 (1C, C-6), 64.78 (d, J_{1-P} 165.1 Hz, 1C, C-1), 76.56 (d, J_{3-P} 11.7 Hz, 1C, C-3), 112.64 (1C, C-5), 142.21 (1C, C-4); MS FAB$^+$ (NOBA) *m/z* 167 $(M + H)^+$.

Triammonium (4-methyl-2-oxa-4-pentenyl)pyrophosphonate (76): 41% yield; $R_f = 0.25$, 27% aqueous ammonia/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.60 (s, 3H, H-6), 3.59 (d, J_{1-P} 8.8 Hz, 2H, H-1), 3.92 (s, 2H, H-3), 4.83−4.92 (m, 2H, H-5); ^{31}P NMR (D_2O) δ −6.3 (d, $J_{\beta-\alpha}$ 25.0 Hz, P-β), 8.7 (d, P-α); ^{13}C NMR (D_2O) δ 20.52 (1C, C-6), 67.71 (d, J_{1-P} 162.9 Hz, 1C, C-1), 78.18 (d, J_{3-P} 11.3 Hz, 1C, C-3), 114.85 (1C, C-5), 143.99 (1C, C-4); MS FAB$^+$ (GT) *m/z* 247 $(M - 3NH_4 + 4H)^+$. Anal. ($C_5H_{21}N_3O_7P_2$) C, H, N.

Tetraammonium 4-methyl-2oxa-pent-4-enyltriphosphonate (77): 15% yield; $R_f = 0.14$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.62 (s, 3H, H-6), 3.67−3.77 (d, J_{1-P} 10.1 Hz, 2H, H-1), 3.94 (s, 2H, H-3), 4.86−4.92 (m, 2H, H-5); ^{31}P NMR (D_2O) δ −21.2 (dd, $J_{\beta-\alpha}$ 26.0 Hz, $J_{\beta-\gamma}$ 19.5 Hz, P-β), −6.60 (d, P-γ), 10.2 (d, P-α); ^{13}C NMR (D_2O) δ 19.01 (1C, C-6), 65.83 (d, J_{1-P} 163.6 Hz, 1C, C-1), 76.75 (d, J_{3-P} 11.3 Hz, 1C, C-3), 113.61 (1C, C-5), 142.33 (1C, C-4); MS FAB$^+$ (GT) *m/z* 327 $(M - 4NH_4 + 5H)^+$.

Diammonium α,γ-(4-methyl-2-oxa-pent-4-enyl)pyrophosphonate (78): 18% yield; $R_f = 0.6$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.62 (s, 6H, H-6), 3.55−3.61 (d, J_{1-P} 8.3 Hz, 2H, H-1), 3.93 (s, 4H, H-3), 4.85−4.93 (m, 4H, H-5); ^{31}P NMR (D_2O) δ 9.9; ^{13}C NMR (D_2O) δ 19.10 (2C, C-6), 65.98 (d, J_{1-P} 164.0 Hz, 2C, C-1), 76.81 (d, J_{3-P} 11.3 Hz, 2C, C-3), 113.62 (2C, C-5), 142.45 (2C, C-4); MS FAB$^+$ (GT) *m/z* 315 $(M - 2NH_4 + 3H)^+$.

Triammonium α,γ-(4-methyl-2-oxa-pent-4-enyl)triphosphonate (79): 9% yield; $R_f = 0.41$, SiO_2, 27% aqueous ammonia)/2-propanol, 5:5; ^{1}H NMR (D_2O) δ 1.63 (s, 6H, H-6), 3.39 (d, J_{1-P} 8.6 Hz, 2H, H-1), 3.95 (s, 2H, H-3), 4.85−4.95 (m, 2H, H-5); ^{31}P NMR (D_2O) δ −21.9 (t, $J_{\beta-\alpha} = J_{\beta-\gamma}$ 25.0 Hz, P-β), 10.1 (d, 2P, P-α and P-γ); ^{13}C NMR (D_2O) δ 18.95 (2C, C-6), 65.85 (d, J_{1-P} 163.7 Hz, 2C, C-1), 76.77 (d, J_{3-P} 11.3 Hz, 2C, C-3), 113.67 (2C, C-5), 142.45 (2C, C-4); MS FAB$^+$ (GT) *m/z* 395 $(M - 3NH_4 + 4H)^+$.

Activation of Human PBMC. Human peripheral white blood cells were isolated from total blood from healthy donors by centrifugation over Ficoll-Paque Plus (Pharmacia, France) gradients. Blood samples were obtained from the Etablissement Français du Sang according to the French regulation. Isolated PBMC samples were seeded in triplicate at 2×10^6 cells/mL into 96-well flat-bottom tissue culture plates (Falcon) in RPMI 1640 (Gibco, France) supplemented with 10% heat-inactivated FCS, 20 mM glutamine, 10 mM Hepes, 1 mM sodium pyruvate, 10 μg/mL penicillin−streptomycin, and various concentrations of the compounds to be tested. After 4 days, 0.5 μCi of [^{3}H]thymidine (^{3}H-TdR, Amersham, France) was added to each well, and radioactivity incorporated into DNA was counted after further incubation for 24 h at 37 °C, 5% CO_2, upon cell harvesting (Top Count, Packard Instruments, France). The stimulation index was calculated as SI = [(cpm with compound − cpm in control)/(cpm in control)] × 100.

PBMC phenotypes were determined prior to or after stimulation by two-color fluorescence analysis on a FACS-Calibur cell analyzer (Becton-Dickinson, France) using FITC-conjugated anti-CD3 antibody and biotin conjugated antihuman Vγ9 antibody and revelation using streptavidin−phycoerythrin (ST-PE). Results were analyzed using the Cellquest software.

Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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