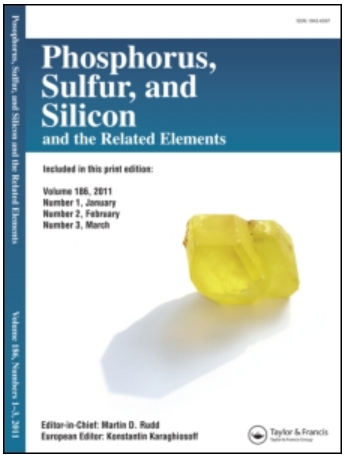

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A RETINYL PHOSPHONIC ACID DERIVATIVE AN ANALOG OF RETINYL PHOSPHATE: HOMORETINYLPHOSPHONIC ACID

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A RETINYL PHOSPHONIC ACID DERIVATIVE AN ANALOG OF RETINYL PHOSPHATE: HOMORETINYLPHOSPHONIC ACID

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The synthesis of phosphonate isosteres or homologues of natural retinylphosphate was developed starting from carbonylated terpenes, through Abramov and Wittig-Horner reactions. Homoretinylphosphonic acid (HRP) was isolated and tested for its ability to be involved in the biosynthesis of membrane glycoconjugates.

Keywords: Homoretinylphosphonic acid; phosphonate; retinylphosphate; glycoconjugate

INTRODUCTION

Membrane bioconjugates play an essential role in recognition between cells. Located in the outermost part of the membrane, these molecules possess glycidic entities anchored to external membrane proteins. They regulate the growth of the cells and their response toward exogenous molecules[1] such as hormones, drugs, neurotransmitters, toxins or viruses. Moreover, oligosaccharides linked to membrane proteins play a role in the recognition of "sites of cell arrest" and regulate in a certain way the "social life of the cells"[2]. These sugar entities protect proteins to which they are

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linked, preventing their proteolysis, and also inducing and protecting their conformation[3].

Glycoconjugates are made from six fundamental sugars: D-glucose, D-galactose, L-fucose, N-acetyl-glucose-amine, N-acetyl-galactose-amine and sialic acid. Their biosynthesis is achieved using polyisoprenic alcohols such as dolichols[4].

Oligosaccharides are synthesized by sequential addition of monosaccharides on dolichyle phosphate which is the initiation and elongation site of the chain. The sugar moiety formed is transferred onto a basic amino-acid of a protein to form N-linked oligosaccharides[5]. After maturation, the glycoprotein is subject to the action of glycosyltransferases before being transported to the membrane using secretion vesicles.

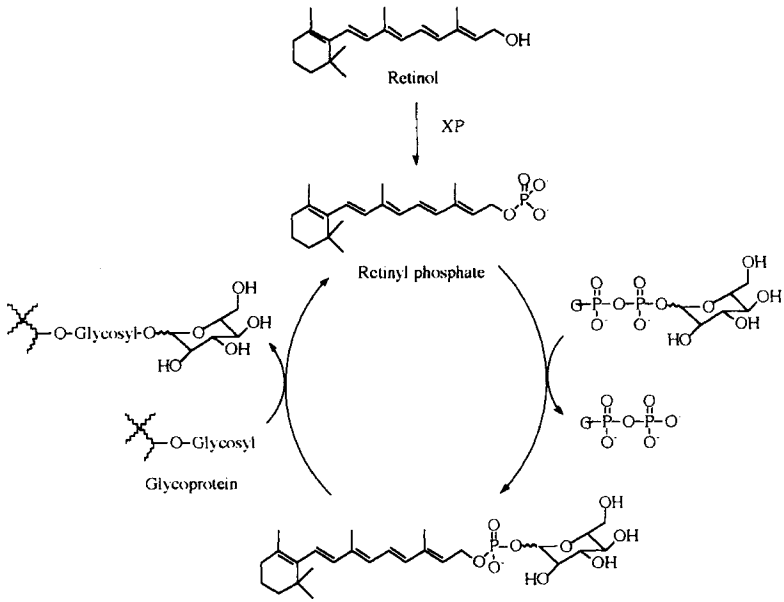
In addition to this intracellular biosynthesis pathway, L. M. Deluca[6] has shown that vitamin A may play an important role in the biosynthesis of some membrane glycoproteins. In this case, the osylation agent is retinyl phosphate (R-P).

L. M. Deluca's work has shown that (Scheme 1): (a) adenosine triphosphate (ATP) phosphorylates retinol; (b) the glycosylation of retinyl phosphate is carried out using a donor sugar, guanosine diphosphate-ose (GDP-ose). The sugar linked by R-P is either mannose or galactose; (c) mannosyl retinyl phosphate (MRP), or the corresponding galactosyl phosphate (GRP), transfers the monosaccharide to a membrane glycoconjugate.

Due to the instability of retinyl phosphate, and in order to confirm Deluca's hypothesis on the role of retinol, we have considered synthesizing retinyl phosphonic acid derivatives as analogs of the phosphate in which the C-P bond is non-hydrolyzable enzymatically. The ability of these derivatives to link a monosaccharide such as mannose or galactose was then determined.

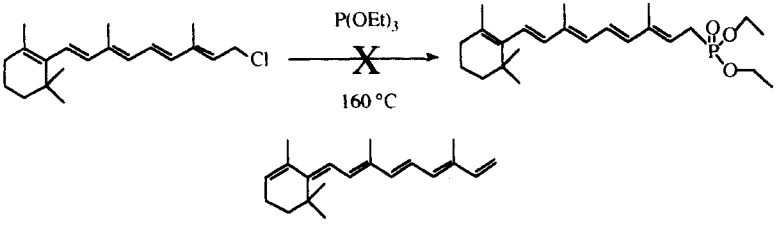
SYNTHETIC CHEMISTRY

The first analog we have synthesized is retinyl phosphonic acid. This derivative, for which the C-O-P bond is replaced by a C-P bond, constitutes a non-bioisostere analog of retinyl phosphate since the latter lacks an atom separating the C and P.



SCHEME 1

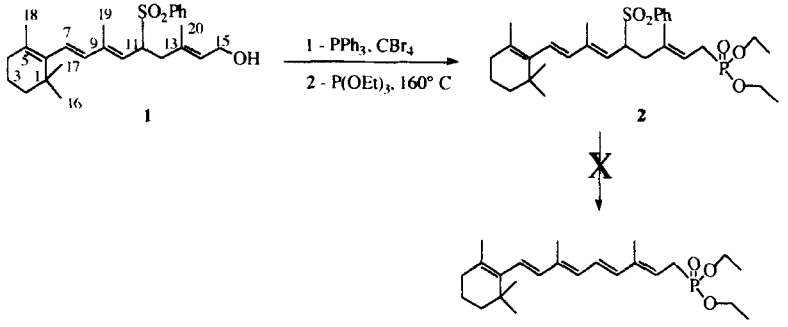
Synthesizing diethyl retinyl phosphonate by a traditional Arbuzov reaction can not be accomplished in this particular case. Like retinol, retinyl chloride is unstable at high temperature and at pH values below 6 or above 8. It dehydrochlorinates spontaneously to give the anhydroretinol derivative[7] (Scheme 2). This dehydrochlorination is promoted by the conjugated polyenic system.



Anhydroretinol

SCHEME 2

To circumvent this problem, we decided to synthesize diethyl retinyl phosphonate from the phenylsulfone **1** at C11, the molecule used by Julia for the synthesis of Vitamin A. This intermediate, in which conjugation is interrupted, can easely lead to the corresponding phosphonate **2** according to an Arbuzov reaction (Scheme 3). The dehydrodesulfonation of this derivative should give the diethyl retinyl phosphonate of interest.



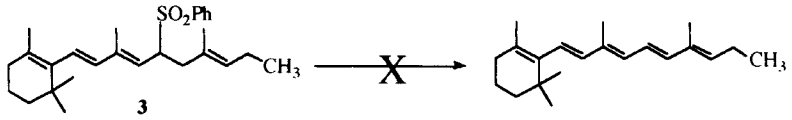
SCHEME 3

The dehydrodesulfonation described by Chabardes[9] with the O-acetylated phenylsulfone **1** consists of a deprotonation at C12 followed by the departure of the phenylsulfone group, leading to a double bond *via* a β-elimination reaction.

Unfortunatly, this reaction on phosphonate **2** did not give the expected dehydrosulfonated derivative. It seems that without an electronwithdrawing group at C15, the proton in the α position to the phenylsulfone is more acidic than the β protons. Since the protons at C12 are less labile, the dehydrodesulfonation could not take place.

In order to demonstrate the influence of inductive effects of C15 groups toward dehydrodesulfonation, we ran this reaction with derivative **3**, for which inductive effects on carbon C15 have been eliminated (Scheme 4). This derivative was obtained by reduction with DIBAH of the tosylate derivative of sulfone **1**. No reaction was observed. These results confirm Julia's[8] hypothesis on the necessity of an electron-withdrawing group on carbon C15.

According to these results, a new strategy was investigated. It consisted of an Abramov reaction on retinal, a more stable compound than retinol, in

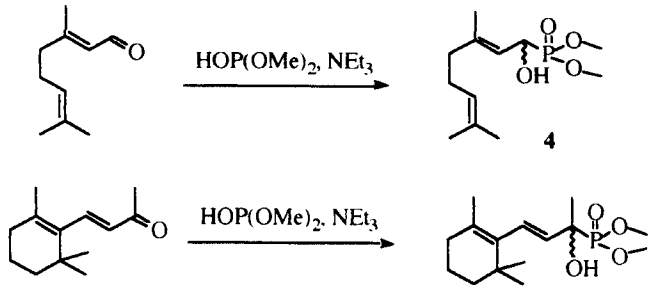


3

SCHEME 4

order to get the corresponding α-hydroxylated phosphonate. After deoxygenation, the desired retinyl phosphonate would be formed.

This reaction will be first applied on two models: citral and β-ionone (Scheme 5). These compounds were treated at room temperature with an excess of di-methylphosphite in the presence of six equivalents of triethylamine to give the corresponding dimethyl α-hydroxyphosphonates **4** and **5** in 78% and 83% yields, respectively. In both cases, only 1,2 addition was observed.



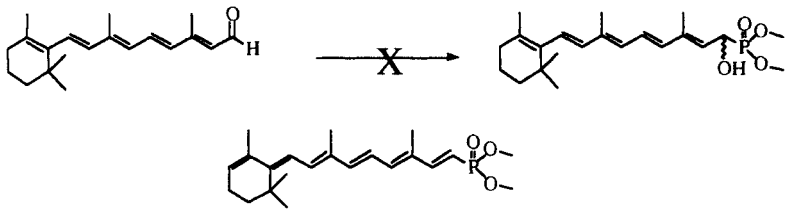
4

5

SCHEME 5

Applied to retinal, the Abramov reaction did not give the expected compound. Formation of derivative **6**, resulting from a dehydration and a shift of the double bonds was shown by NMR analysis.

Deoxygenation of α-hydroxyphosphonates **4** and **5** , following a procedure described by S. C. Dolan et al.[11], leads to dehydration with shifts in the double bonds and not to the deoxygenated compounds, as formed with the anhydro derivatives.



6

SCHEME 6

As the synthesis of retinylphosphonic acid was not possible in our hands, we synthesized its higher homologue with 21 carbons. The homoretinyl phosphonic acid is a bioisostere analog[12–13] of retinyl phosphate in which the oxygen is replaced by an sp^2 carbon.

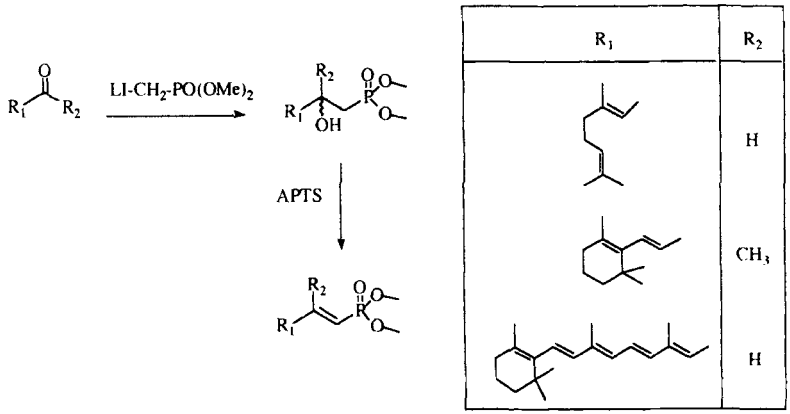
Two pathways were used for the synthesis of diethyl homoretinylphosphonate:

- The first was a condensation of α-metallated derivatives of diethyl methyl phosphonate and retinal, followed by a dehydration under acidic conditions. Two different types of organometallic compounds were used: organolithiums and organocuprates. The organolithium derivative was easily prepared at low temperature by action of butyllithium on diethyl methylphosphonate. The organocopper derivative[14] was obtained by treating this lithium derivative with CuI.
- The second was a condensation of tetraethyl methylenediphosphonate and retinal in the presence of NaH in benzene solution according to a Wittig-Horner reaction[15].

In both cases, these reactions were optimized first on two models, *i.e.* citral and β-ionone, before being applied to retinal.

Table I summarizes the results obtained using the synthesic pathway shown in Scheme 7.

In each case, the best results were obtained with α-lithium species. In the case of retinal, the formation of the dehydration compound, with shifts in the double bonds, explains the lower yield relative to the models. In all three examples, the dehydration of β-phosphonated alcohol lead with quantitative yields to the corresponding vinylphosphonates **7**, **8** and **10**.



SCHEME 7

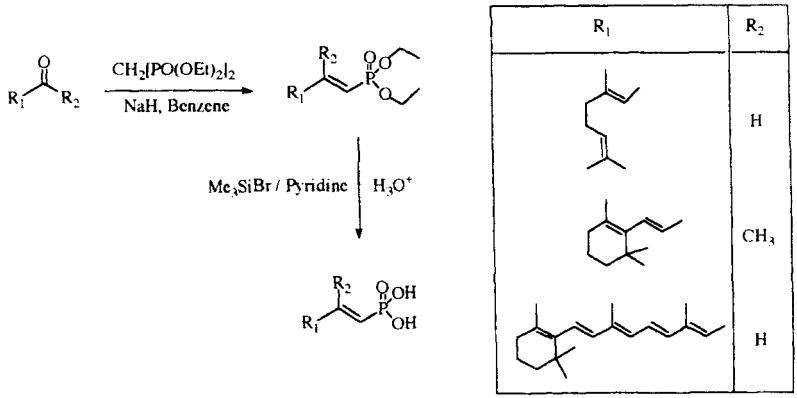
TABLE I Comparative yields between organolithiums and organocuprates

Name	*Reagents*	*Yields*	*Compound N°*
Citral	Cu CH_2-PO(OMe)$_2$	60%	**7**
	Li CH_2-PO(OMe)$_2$	74%	
β-ionone	Cu CH_2-PO(OMe)$_2$	58%	**8**
	Li CH_2-PO(OMe)$_2$	75%	
Rétinal	Cu CH_2-PO(OMe)$_2$	40%	**9 et 10**
	Li CH_2-PO(OMe)$_2$	55%	

TABLE II Results obtained by Wittig-Horner reaction

	Yields Wittig-Horner	*Compound N°*	*Deprotection yields*	*Compound N°*
Citral	98%	**11**	92%	**12**
β-ionone	68%	**13**	90%	**14**
Retinal	95%	**15**	91%	**16**

The results obtained using a Wittig-Horner as alternate strategy (Scheme 8) are given in Table II. Except for the β-ionone, all the yields were higher then 90%.



SCHEME 8

The Wittig-Horner reaction presents many advantages: it is a one-step synthesis with an easy work-up. Moreover, it proceeds with an excellent diastereoselectivity, because only the all-*trans* isomer is obtained. Phosphonic acids **12**, **14** and **16** were obtained by treatment of the corresponding diethylphosphonates with trimethylsilylbromide and pyridine in a benzene solution[16]. Phosphonic acid **16** was first isolated as a pyridinium salt and then transformed to a sodium salt using an ion exchange resin (Na^+ form). It is preferable to keep the homoretinylphosphonic acid at low temperature (-18°C) and as a monosodium salt.

BIOLOGICAL EVALUATION

Homoretinylphosphonic acid (HRP) was tested for its ability to be involved in the biosynthesis of membrane glycoconjugates. The biological evaluation was performed in the Laboratory of Biological Chemistry of Professor Andre Verbert at the University of Science and Technology of Lille (France).

The tests consisted of measuring the association of different sugars to dolichols, to analogs of dolichols, and to membrane proteins. After, determining the dimethylsulfoxide (DMSO) doses to quicken dolichols cycle (5μL) we studied the effect of homoretinyl sodium phosphonate (HRP) on

cells CHO (hamster ovarian fibroplast) with increasing doses of HPR (10, 25 and 50 μg).

Three types of saccharides have been evaluated: a) dolichol mannose phosphate (DMP), by extraction with a mixture of chloroform and methanol (CM); b) dolichols diphosphate oligosaccharides by extraction with a mixture of chloroform, methanol and water (CMW); and c) glycoproteins (GP). Table III summarizes the results.

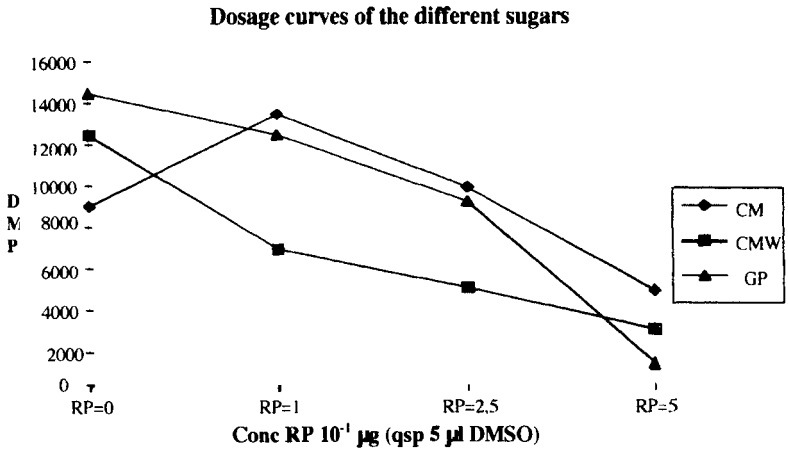
TABLE III Titration of three glycoconjugates (CM, CMW, GP)

HRP en μg	0	1	2,5	5
DMSO μl	5	4	2,5	0
CM	9000	13500	9900	4900
CMW	12500	6900	5100	3100
GP	14500	12500	9200	1460

Since a decrease of incorporation of radioactivity has been observed for increasing concentrations of homoretinyl sodium phosphonate, it is obvious that this compound inhibits the process. This result, showing that homoretinyl sodium phosphonate (HRP) is an inhibitor of membrane glycoconj-gates, correlates with De Lucas observations [6]. This author has shown that retinyl phosphate has a similar behavior, namely an inhibition with bovin pigmentary epithelial cells. This behavior demonstrates that HPR, like retinyl phosphate, has a tendancy to conjugate to mannose.

CONCLUSION

This preliminary study is very promising, because HRP has a behavior similar to RP and, consequently, its reasonable to think that HRP, like RP, is a mannose acceptor. Since enzymatic systems of the loading step of sugar are highly specific, HRP could be a competitive receptor for dolichols. As enzymatic systems that transfer carbohydrates of these charged species on oligosaccharides are not very specific, the homoretinylphosphonate-sugar could thus transfer a sugar on membrane glycoconjugates.



SCHEME 9

EXPERIMENTAL SECTION

General procedures

Reactions were monitored by TLC using aluminium plates coated with silica gel 60 F254 (Merck) and visualized using UV light, anisaldehyde or H_2SO_4 (aqueous 10% spray solution). Molybdenum blue was used to develop phosphorus containing compounds. Chromatography was performed on Merck silica gel 60H (Art.9385). ^1H NMR spectra were recorded at room temperature on an AC-250 Bruker spectrometer. Chemical shifts are reported in parts per million (ppm) using the residue solvent peaks as internal reference ($CDCl_3$: 7.24 ppm; DMSO-d6: 2.49 ppm). ^{31}P NMR spectra were recorded on a WP-200-SY Bruker spectrometer. Chemical shifts are reported in parts per million (ppm) relative to external phosphoric acid. Mass spectra were recorded using a Jeol JMS-DX 300 mass spectrometer (EI mode and FAB positive mode with a mixture of glycerol/thioglycerol (GT) v/v or/and 3-nitrobenzyl alcohol (NOBA) as the matrix). The compounds were named according to the IUPAC nomenclature.

1-hydroxy-3, 7-dimethylocta-2,6-dienyl-dimethyl phosphonate (4)

To 1 mL (5.85 mmol) of citral were added, at room temperature, 5 mL of methyl phosphite (as solvent) and 4.8 mL (35.1 mmol) of triethylamine in a round bottom flask kept dry with a calcium chloride drying tube. After stirring for 48 hours the mixture was concentrated under reduced pressure. The residue obtained was dissolved in ethylacetate and washed successively with a saturated $NaHCO_3$ solution, water, 5% citric acid solution, then water until neutral pH. The organic layer was dried over anhydrous sodium sulfate, filtrated and concentrated. The residue was chromatographed through silica gel (1/1 ether/AcOEt) to give compound (**4**) in 78% yield.

Rf= 0,33 (Ether/AcOEt 1/1). ^1H NMR ($CDCl_3$), δ (ppm): 5,3 (m large, 1H, $C_1\mathbf{H}$); 5,1 (t large, 1H, $C_2\mathbf{H}$); 4,65 (t large, 1H, $C_6\mathbf{H}$); 3,75 (2d, 6H, $J_{H\text{-}P}$= 10,5Hz, $POC\mathbf{H}_3$); 2,85 (s, 1H, $C_1O\mathbf{H}$, exch D_2O); 2 (m, 4H, $C_5\mathbf{H}_2$, $C_6\mathbf{H}_2$); 1,65 et 1,95 (2td, ratio 3:1 E/Z, 3H, $C_3C\mathbf{H}_3$); 1,58–1,5 (s, 6H, $C_8\mathbf{H}_3$, $C_8C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 27. MS (NOBA): 263 $(M+H)^+$, 285 $(M+Na)^+$, 525 $(2M+H)^+$, 547 $(2M+Na)^+$, 245 $(M+H\text{-}H_2O)^+$, 153 $(M\text{-}PO(OCH_3)_2)^+$.

1-methyl-1-hydroxy-3-(2,6,6-trimethyl-1 -cyclohex-1-enyl)-prop-2-enyl-dimethyl phosphonate (5)

Following the procedure used for the synthesis of compound **4**, 17mL (0.122 mol) of β-ionone were used to obtain compound **5** in 82% yield. Rf = 0,4 (Ether/AcOEt 1/1). ^1H NMR ($CDCl_3$), δ (ppm): 6,23 (dd, 1H, $JH_3\text{-}H_4$=16Hz, J_{3HP}=4Hz, $C_3\mathbf{H}$); 5,5 (dd, 1H, $J_{H3\text{-}H4}$=16Hz, J4HP=4,4Hz, $C_4\mathbf{H}$); 3,8 (2d, 6H, JHP=10,25Hz, $POC\mathbf{H}_3$); 2,95 (t large, 1H, $C_2O\mathbf{H}$, échange D_2O); 1,9 (t, 2H, $C_3\mathbf{H}_2$); 1,68–1,58 (2s, 6H, $C_1\mathbf{H}_3$, $C_2C\mathbf{H}_3$); 1,6–1,45 (2m, 4H, $C_5\mathbf{H}_2$, $C_4\mathbf{H}_2$); 1 (s, 6H, $C_6C\mathbf{H}_3$, $C_6C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 27.MS (NOBA): 303 $(M+H)^+$, 325 $(M+Na)^+$, 605 $(2M+H)^+$, 285 $(M+H\text{-}H_2O)^+$, 193 $(M\text{-}PO(OCH_3)_2)^+$.

3, 7-dimethyl-9-(2,6,6-trimethyl-2-cyclohex-2-enylidene)-1,3,5,7-nonatetraenyl-dimethylphosphonate (6)

Following the procedure used for the synthesis of compound **4**, 1g (3,5 mmol) of retinal was caused to react with 2.5 mL (17 mmol) of tri-

ethylamine and 5 mL of dimethylphosphite to give 0.92g of compound **6** in 70% yield. Rf = 0,35 (Ether/AcOEt 1/1). ^{1}H NMR ($CDCl_3$), δ (ppm): 7,2 (dd, 1H, J_{H1-H2}=18Hz, J_{HP}=21Hz, C_2**H**); 6,9 (d, 1H, J=12,4Hz, C_8**H**); 6,5 (m, 3H, C_4**H**, C_5**H**, C_6**H**); 6,35 (d, 1H, J=12,4Hz, C_9**H**); 5,80 (t, 1H, C_3**H**); 5,55 (t, 1H, J_{H1-H2}=18Hz, J_{HP}=18Hz, C_1**H**); 3,74 (d, 6H, J_{HP}=10Hz, POC**H**$_3$); 2,15 (m, 2H, C_4**H**$_2$); 1,95–1,85 (3s, 9H, C_3C**H**$_3$, C_7C**H**$_3$, C_2C**H**$_3$); 1,5 (2m, 2H, C_4**H**$_2$). 1,25 (s, 6H, C_6C**H**$_3$, C_6C**H**$_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 24. MS (NOBA): 377 $(M+H)^+$, 399 $(M+Na)^+$, 753 $(2M+H)^+$, 267 $(M-PO(OCH_3)_2)^+$.

General procedure for the synthesis of β-hydroxydimethylphosphonate using the organolithium method

1.1 equivalents of methyl-dimethylphosphonate were dissolved in 10 mL of anhydrous THF. The mixture was cooled to −78°C under a nitrogen atmosphere before adding 1.1 equivalents of n-BuLi (1.6M in hexane). The mixture was stirred at this temperature for 20 minutes before adding 1 equivalent of the carbonyl derivative dissolved in 5 mL of THF. The reaction was stirred at room temperature for 3 hours. Methylene dichloride was then added and washed with an ice-cold 5% citric acid solution then with water until neutral. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The compound was purified by chromatography on silica gel.

General procedure for the synthesis of β-hydroxydimethylphosphonate using the organocopper method

1.1 equivalents of methyl-dimethylphosphonate were dissolved in 10 mL of anhydrous THF. The mixture was cooled to −78°C under a nitrogen atmosphere before adding 1.1 equivalents of n-BuLi (1.6M in hexane). The mixture was stirred at this temperature for 20 minutes before adding 1 equivalent of CuI. The mixture was stirred at −30°C for 15 minutes. 1 equivalent of carbonyl derivative dissolved in 5mL of THF was then added and the reaction was stirred at room temperature for 3 hours. Methylene dichloride was then added and washed with an ice cold 5% citric acid solution then water until neutral. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The compound was purified by chromatography on silica gel.

4,8-dimethyl-2-hydroxy-3, 7-nonadienyl-dimethylphosphonate (7)

Rf = 0,42 (Ether/AcOEt 1/1). ^{1}H NMR ($CDCl_3$), δ (ppm): 5,2 (d, 1H, $C_3\mathbf{H}$, J_{H2-H3}=8,4Hz); 5 (t large, 1H, $C_7\mathbf{H}$); 4,7 (m. 1H, $C_2\mathbf{H}$); 3,75 (2d, 6H, J_{H-P}=10Hz, $POC\mathbf{H}_3$); 3,3 (s large, 1H, $C_2O\mathbf{H}$, exch D_2O); 2 (m, 6H, $C_1\mathbf{H}_2$, $C_5\mathbf{H}_2$, $C_6\mathbf{H}_2$); 1,65 et 1,6 (2s, ratio 3: 1 E/Z, 3H, $C_4C\mathbf{H}_3$); 1,55–1,5 (s, 6H, $C_9\mathbf{H}_3$, $C_9C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 29,5. MS (NOBA): 277 $(M+H)^+$, 299 $(M+Na)^+$, 533 $(2M+H)^+$, 259 $(M+H-H_2O)^+$, 167 $(M-PO(OCH_3)_2)^+$.

2-methyl-2-hydroxy-4-(2-methyl-1-cyclohex-1-enyl)-but-3-enyl-dimethylphosphonate (8)

Rf = 0,6 (Ether/AcOEt 1/1). ^{1}H NMR ($CDCl_3$), δ (ppm): 6,18 (dd, 1H, J_{H3-H4}=16Hz, $C_3\mathbf{H}$); 5,5 (d, 1H, J_{H3-H4}=16Hz, $C_4\mathbf{H}$); 4,25 (s large, 1H, $C_2O\mathbf{H}$, exch D_2O); 3,7 (m, 6H, $POC\mathbf{H}_3$); 2,13 (dd, J_{H-P}=17Hz, J_{HI-H3}=2,3Hz. $C_1\mathbf{H}_2$); 2 (t, 2H, $C_3\mathbf{H}_2$); 1,65–1,6 (2s, 6H, $C_1\mathbf{H}_3$, $C_2C\mathbf{H}_3$); 1,6–1,45 (2m, 4H, $C_5\mathbf{H}_2$, $C_4\mathbf{H}_2$); 1 (s, 6H, $C_6C\mathbf{H}_3$, $C_6C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 29,5. MS (NOBA): 317 $(M+H)^+$, 399 $(M+Na)^+$, 633 $(2M+H)^+$, 299 $(M+H-H_2O)^+$, 207 $(M-PO(OCH_3)_2)^+$.

4,8-dimethyl-2-hydroxy-10-(2,6,6,-trimethyl-1-cyclohex-1-enyl)-3,5, 7,9-decatetraenyl-dimethyl phosphonate (9)

Rf = 0,33 (Ether/AcOEt 1/1). ^{1}H NMR ($CDCl_3$), δ (ppm): 6,62 (dd, 1H, J_{H5-H6}=16Hz, $C_6\mathbf{H}$); 6,25 (d, 1H, J_{H5-H6}=16Hz, $C_5\mathbf{H}$); 6,1 (m, 3H, $C_{10}\mathbf{H}$, $C_9\mathbf{H}$, $C_3\mathbf{H}$); 5,55 (d, 1H, $C_7\mathbf{H}$); 4,95 (m, 1H, $C_2\mathbf{H}$): 3,75 (m, 6H, $POC\mathbf{H}_3$); 3,15 (s large, $C_2O\mathbf{H}$, exch D_2O); 2,35 (m, 2H, $C_1\mathbf{H}_2$); 2,1 (m, 2H, $C_3\mathbf{H}_2$); 1,95–1,7 (3s, 9H, $C_4C\mathbf{H}_3$, $C_8C\mathbf{H}_3$, $C_2C\mathbf{H}_3$) 1,6–1,45 (2m, 2H, $C_4\mathbf{H}_2$, $C_5\mathbf{H}_2$), 1,1 (s, 6H, $C_6C\mathbf{H}_3$, $C_6C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 30. MS (NOBA): 409 $(M+H)^+$, 431 $(M+Na)^+$, 817 $(2M+H)^+$, 391 $(M+H-H_2O)^+$, 299 $(M-PO(OCH_3)_2)^+$.

Synthesis of vinyl-diethylphosphonate according to a Wittig-Horner reaction

1.2 equivalents of tetraethyl-methylenediphosphonate were dissolved in 10 mL of anhydrous THF in the presence of 1.2 equivalents of NaH. The

mixture was stirred for 30 minutes before adding dropwise 1 equivalent of the carbonyl derivative dissolved in 10 mL of anhydrous benzene. The reaction was monitored by TLC. Ethyl acetate was added and the mixture was washed with water and then with a solution of $KHSO_4$. The organic layer was washed with water until neutral, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The compound was purified by chromatography on silica gel.

[1E,3E]-4,8-dimethylnona-1,3, 7-trienyl-diethylphosphonate (11)

Rf = 0,28 (Ether/AcOEt 6/4). ^1H NMR ($CDCl_3$), δ (ppm): 7,35 (ddd, 1H, $J_{H1\text{-}H2}$=16,6Hz, $J_{H2\text{-}H3}$=11,4Hz, $J_{H\text{-}P}$=21Hz, $C_2\mathbf{H}$); 5,95 (d, 1H, $J_{H3\text{-}H2}$=11,4Hz, $C_3\mathbf{H}$); 5,50 (dd, 1H, $J_{H1\text{-}H2}$=16,6Hz, $J_{H\text{-}P}$=20,2Hz, $C_1\mathbf{H}$); 5 (t large, 1H, $C_7\mathbf{H}$); 4,10 (m, 4H, $POC\mathbf{H}_2$); 2,13 (m, 4H, $C_5\mathbf{H}_2$, $C_6\mathbf{H}_2$); 1,85–1,58 (3s, 9H, $C_4C\mathbf{H}_3$, $C_8C\mathbf{H}_3$, $C_8C\mathbf{H}_3$); 1,30 (t, 6H, $POCH_2C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 21,2. MS (NOBA): 287 $(M+H)^+$, 309 $(M+Na)^+$, 573 $(2M+H)^+$, 149 $(M\text{-}PO(OCH_2CH_3)_2)^+$.

[1E,3E]-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)-buta-1,3-dienyl-diethyl phosphonate (13)

Rf = 0,5 (Ether/AcOEt 6/4). ^1H NMR ($CDCl_3$), δ (ppm): 6,4 (d, 1H, $J_{H3\text{-}H4}$=16,1Hz, $C_3\mathbf{H}$); 6 (d, 1H, J_{H3H4}=16,1Hz, $C_4\mathbf{H}$); 5,4 (d, 1H, J_{HP}=17,8Hz, $C_1\mathbf{H}$); 4 (q, 4H, $OC\mathbf{H}_2$); 2,2 (d, 3H, $C_2C\mathbf{H}_3$); 2 (t, 2H, $C_3\mathbf{H}_2$); 1,6–1,4 (2m, 4H, $C_5\mathbf{H}_2$, $C_4\mathbf{H}_2$); 1,55 (s, 3H, $C_2C\mathbf{H}_3$); 1,25 (t, 6H, $C\mathbf{H}_3\text{-}CH_2O$); 1 (s, 6H, $C_6C\mathbf{H}_3$, $C_6C\mathbf{H}_3$). ^{31}P NMR ($CDCl_3$), δ (ppm): 19,5. MS (NOBA): 327 $(M+H)^+$, 349 $(M+Na)^+$, 653 $(2M+H)^+$, 189 $(M\text{-}PO(OCH_2CH_3)_2)^+$.

4,8-dimethyl- 10-(2,6,6-trimethyl-1-cyclohex-1-enyl)-deca-1,3,5,7,9-pentaenyl-diethyl phosphonate (16)

Rf = 0,28 (Ether/AcOEt 6/4). ^1H NMR ($CDCl_3$), δ (ppm): 7,5 (ddd, 1H, J_{H1H2}=16,5Hz, J_{H2H3}=11,6Hz, JHP=20,5Hz, $C_2\mathbf{H}$); 6,8 (dd, 1H, J_{H5H6}=15Hz, J_{H6H7}=11,25Hz, $C_6\mathbf{H}$); 6,3 (d, 1H, J_{H6H5}=15Hz, $C_5\mathbf{H}$); 6,15 (m, 4H, $C_{10}\mathbf{H}$, $C_9\mathbf{H}$, $C_7\mathbf{H}$, $C_3\mathbf{H}$); 5,55 (dd, 1H, J_{H1H2}=16,5Hz, J_{HP}=19,5Hz, $C_1\mathbf{H}$); 4,05 (m, 4H, $POC\mathbf{H}_2$); 2,1 (m, 2H, $C_3\mathbf{H}_2$); 2,05-1,97

and 1,7 (3s, 9H, C_8CH_3, C_4CH_3. $C_{18}H_3$); 1,6 and 1,4 (2m, 4H, C_5H_2, C_4H_2); 1,25 (t, 6H, CH_3-CH_2O); 1 (s, 6H, C_6CH_3, C_6CH_3). ^{31}P NMR ($CDCl_3$), δ (ppm): 22. MS (NOBA): 419 $(M+H)^+$, 435 $(M+Na)^+$, 837 $(2M+H)^+$, 281 $(M-PO(OCH_2CH_3)_2)^+$.

General methodology used for the deprotection of phosphonates

1 equivalent of methyl or ethyl phosphonate was dissolved in 10 mL of anhydrous benzene. 1 equivalent of anhydrous pyridine, followed by 5 equivalents of trimethylbromosilane dissolved in 10 mL of anhydrous pen-zene were added at 0°C. The mixture was stirred at room temperature for 24 hours. Methylene dichloride was then added and the mixture was washed two times with an aqueous HCl solution then three times with water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The compound was purified by chro-matography on silica gel.

4,8-dimethylnona-1,3,7-trienylphosphonic acid (12)

MS (NOBA): 231 $(M+H)^+$, 253 $(M+Na)^+$, 461 $(2M+H)^+$, 149 $(M-PO(OH)_2)^+$. Anal. Calcd for $C_{11}H_{19}O_3P$: C, 57,38; H, 8,31. Found: C, 57,02: H, 8,36.

2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)-buta-1,3-dienylphosphonic acid (14)

MS (NOBA): 271 $(M+H)^+$, 293 $(M+Na)^+$, 540 $(2M+H)^+$. Anal. Calcd for $C_{14}H_{23}O_3P$: C, 62,21; H, 8,57. Found: C, 61,87; H, 8,63.

4,8-dimethyl-10-(2,6,6-trimethyl-1-cyclohex- 1-enyl)-deca-1,3,5,7,9-pentaenyl-phosphonic acid (16)

MS (NOBA): 362 $(M+H)^+$, 384 $(M+Na)^+$, 723 $(2M+H)^+$. Anal. Calcd for $C_{21}H_{31}O_3P$: C, 69,59; H, 8,62. Found: C, 69,23; H, 8,68.

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