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Synthesis of Phosphonate Analogues of Retinyl Phosphate

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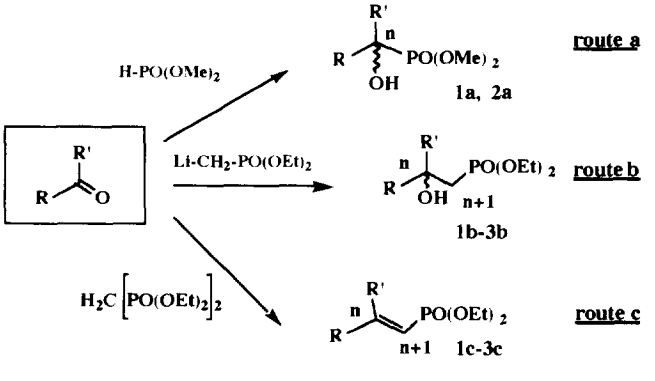
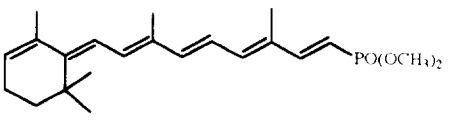
Abstract: The access to phosphonates isosteres or homologues of natural retinylphosphate-target was developed starting from carbonylated terpenes, through Abramov and Wittig-Horner reactions.

The cell surface glycoconjugates play an essential role in cellular interactions[1,2a-d] -including regulation and growth phenomena- and more specifically in cellular responses towards external agents (i.e.: drugs, hormones, toxins or viruses). The biosynthesis of glycannic moieties of membrane glycoproteins requires two terpenolic carriers: dolichols (a family of 14-24 fold polyisoprenes) and retinol (Vitamin A). In the *dolichol route*, oligosaccharidic entities are transfered through the endoplasmic reticulum on to the proteins-targets[3]. In contrast, the transfer by the *retinol way* is carried out with simple osides (mannose, galactose...) in the extracellular medium[4a-b], and would constitute a refinement step in the edification of specific epitopes of membrane glycoproteins. In both routes, the osidic precursor and the terpenol are linked by a phosphodiester bond.

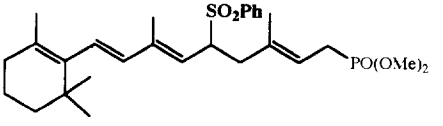


In our hypothesis, structural analogues of retinyl-osyl-phosphates would transfer unusual sugars over these epitopes, and would enhance the antigenicity of tumoral strains and subsequently increase their elimination by the immune system.

Our first attempt was to synthesize retinyl phosphonate from retinyl chloride and trialkyl phosphite, but, whatever the chlorination conditions used, retinol gave anhydroretinol as the only product. We then considered using an intermediate molecule of the synthesis of retinol following Julia's method[5]; this molecule should easily lead to the phosphonate **3d** by an Arbuzov reaction. However, all desulfonation-elimination attemps have failed. We have shown that such a reaction is possible only in the presence of a strongly attracting group (such as acetoxy) in the terminal position[6a]. We describe here three syntheses of glycosylable phosphonyl-analogues of retinylphosphates containing a non-hydrolysable C-P bond[6b]. Each method (applicable to retinal, more stable than the retinol) was investigated on two terpenes: citral and ß-ionone.



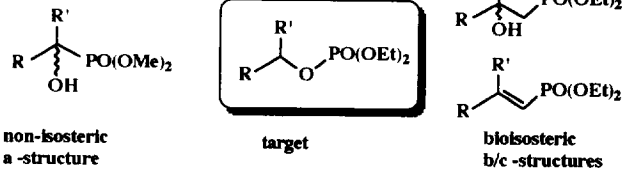
3a'

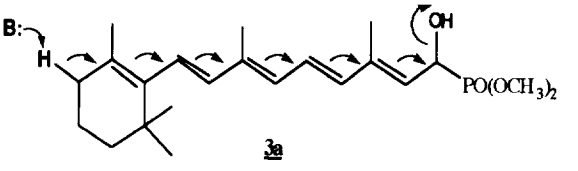


3d

In the first approach, the compounds obtained are lower homologues of the target terpenyl phosphate, while routes **b** and **c** lead to the bioisosteric structures. The Abramov reaction [7a,b] (route **a**) gives α-hydroxy-phosphonates **1a** and **2a**. Through the same work-up (dimethyl phosphite as solvent, triethylamine 5 eq), retinal gives alkenylphosphonate **3a'** by spontaneous dehydration of **3a** and intracyclic conjugation,. This reaction therefore proceeds by the elimination of a proton in the 6-position.

A Wittig-Horner reaction carried out on tetraethyl methylenediphosphonate[8a,b] (**route c**) yields alkenylphosphonates **1-3c**. The E stereochemistry of the vinyl-phosphonates has been confirmed by ^1H NMR ($^3J_{HC=CH} \approx 16.5$ Hz).





3a

In **route b**, diethyl methylenephosphonate[9a,b] anion reacts identically on the three terpenoic carbonyl compounds. The β-hydroxy phosphonates **1b**, **2b** and **3b** were obtained in 70 - 90% yields. The dehydration of the retinal adduct **3b** is very easy and leads to compound **3c**. Under acidic conditions, **1b** and **2b** give vinylphosphonates **1c** and **2c** respectively.

The structure of the different phosphonates obtained has been established by ^1H-NMR and ^{31}P-NMR (cf following Table; we can particularly notice the anisochrony of the alkoxy signals corresponding to the α– and β-hydroxy esters obtained by **routes a** and **b**). Deprotection of the phosphonoesters has been carried out by a reaction with bromotrimethylsilane followed by hydrolysis[10a,b].

The acidic form of **3c** induces, *in vitro*, a differentiation of the transformed cells similar to the effect of retinoic acid[11a-b]. Our preliminary results suggest that the homoretinylphosphonate **3c** could be a potentiating agent for induce the apoptosis phenomenom. Furthermore, this derivative would be susceptible to be mannosylated by enzymatic way as the natural retinyl-phosphate.

Thus we have shown that carbonyl compounds are the most appropriate precursors for the synthesis of terpenic derivatives containing the C-P connection. Phosphonylation through routes **b** and **c** leads to homologues of the corresponding phosphates, while the Abramov reaction which, in the case of citral and ß-ionone would yield the inferior homologue, gives in the case of retinal a conjugate system of *anhydro* type. At present, the phosphonic derivatives of **route c** are being chemically osylated.

Selected spectroscopic data of synthesized compounds [12]

Cpd	Rf	Mass	^{31}PNMR δ ppm	^{1}H NMR δ ppm ($CDCl_3$)
1a	0.33 (5/5)	263 $MH]^+$	27	5.3 (broad m, 1H, C_{10}**H**); 5.1 (broad t , 1H, C_9**H**); 4.65 (broad t , 1H, C_4**H**); 3.75 (2d,6H, J_{H-P}=10.5Hz; POC**H3**); 2.85 (sb,1H, C_{10}O**H**, exch); 2.0 (m, 4H, C_5**H2**,C_6**H2**); 1.65 and 1.95 (2 dt, ratio 3:1 E/Z; 3H, C_8**H3**); 1.58 and 1.50 (2s, 6H, C_1**H3**, C_2**H3**)
2a	0.42 (5/5)	303 $MH]^+$	27	6.23 (dd, 1H, $J_{H11-H10}$=16Hz, J_{H-P}=4Hz, C_{11}**H**); 5.55 (dd, 1H, $J_{H11-H10}$=16Hz, J_{H-P}=4.4Hz, C_{10}**H**); 3.80 (2d, 6H, J_{H-P}=10.25, POC**H3**); 2.95 (broad s, 1H, C_{12}O**H**, exch); 1.90 (t, 2H, C_6**H2**); 1.68-1.58 (2s, 2x3H, C_{12}**Me**; C_8**H3**); 1.6-1.45 (2m, 4H, C_4**H2**-C_5**H2**), 1.0 (s, 6H, C_1**H3**, C_2**H3**).
3a'	0.35 (6/4)	377 $MH]^+$	24	7.2 (dd,1H,$J_{H19-H20}$=18Hz,J_{H-P}=21.8Hz, C_{19}**H**); 6.9 (d,1H, J=12.4 Hz, C_{11}**H**); 6.5 (m, 3H, C_{14}**H**,C_{15}**H**,C_{16}**H**); 6.35 (d, 1H, J=12.4Hz, C_{10}**H**); 5.80 (t, 1H, C_6**H**); 5.55 (t, 1H, $J_{H19-H20}$=18Hz, J_{H-P}=18 Hz, C_{20}**H**); 3.74 (d, 6H, J_{H-P}=10 Hz, POC**H3**); 2.15 (m, 2H, C_5**H2**); 1.95-1.85 (3s, 3x3H, C_8**H3**, C_{13}**H3**, C_{18}**H3**); 1.5 (t, 2H, C_4**H2**; 1.25 (s,6H, C_1**H3**, C_2**H3**).
1b	0.42 (5/5)	305 $MH]^+$	29.5	5.2 (d, 1H, J=8,4 Hz, C_9**H**); 5.0 (broad t , 1H, C_4**H**); 4.7 (m, 1H, C_{10}**H**); 4.05 (m, 4H, PO**CH2**); 3.3 (broad s,1H, exch, C_{10}O**H**); 2.0 (m, 6H, C_{11}**H2**,C_5**H2**,C_6**H2**), 1.65 and 1.62 (2s, ratio 3:1 E/Z C_8**H3**); 1.55-1.5 (s, 6H, C_1**H3** C_2**H3**); 1.3 (t, 6H, **H3C**-CH_2O).
2b	0.60 (5/5)	345 $MH]^+$	29.5	6.18 (d, 1H, $J_{H10-H11}$=16Hz, C_{11}**H**); 5.50 (d, 1H, J=16Hz, C_{10}**H**); 4.25 (s, 1H, C_{12}O**H**, exch); 4.10 (m, 4H, PO**CH2**); 2.13 (dd, J_{H-P}=17Hz, $J_{H13-H11}$=2.3 Hz, C_{13}**H2**) 2.00 (t, 2H, C_6**H2**); 1.65 and 1.60 (2s, 6H, C_{12}**Me**, C_8**H3**); 1.6-1.45 (2m, 4H, C_4**H2**-C^5**H2**); 1.25 (t, 6H, **H3C**-CH_2O); 1.0 (s, 6H, C_1**H3**, C_2**H3**).
3b	0.33 (5/5)	437 $MH]^+$	30	6.62 (dd, 1H, C_{15}**H**); 6.3-5.8 (m, 5H, C_{10}**H**, C_{11}**H**, C_{14}**H**, C_{16}**H**, C_{19}**H**); 4.95 (d,1H, C_{20}**H**); 4.2-4.0 (m, 5H, C_{20}O**H** and POC**H2**); 2.35-2.10 (m, 4H, C_{21}**H2** and C_6**H2**); 2.10, 1.90,1.70 (3s, 9H, C_8**H3**, C_{13}**H3**, C_{18}**H3**); 1.6-1.4 (2m, 4H; C_4**H2**C_5**H2**); 1.25 (t, 6H, **H3C**-CH_2O); 1,05 (s, 6H, C_1**H3**, C_2**H3**).
1c	0.28 (6/4)	287 $MH]^+$	21.2	7.35(ddd,1H,$J_{H10-H11}$=16.6Hz,J_{H9-H10}=11.4Hz, J_{H-P}=21Hz, C_{10}**H**); 5.95 (d, 1H, J=11.4Hz, C_9**H**); 5.50 (dd, 1H, J=16.6 and J_{H-P}=20.2Hz, C_{11}**H**); 4.10 (m, 4H, PO**CH2**); 2.13 (m, 4H, C_5**H2**-C_6**H2**); 1.85, 1.66 and 1.58 (3s, 9H, C_1**H3**, C_2**H3** C_8**H3**); 1.30 (t, 6H, **H3C**-CH_2O).
2c	0.50 (6/4)	327 $MH]^+$	19.5	6.4 (d, 1H, $J_{H10-H11}$=16.1 Hz, C_{11}**H**); 6.0 (d, 1H, J=16.1 Hz, C_{10}**H**); 5.4 (d, 1H, J_{HP}=17.8 Hz, C_{13}**H**); 4.0 (q, 4H, O**CH2**); 2.2 (d, 3H, C_{12}-**Me**); 2.0 (t, 2H, C_6**H2**); 1.6-1.45 (2m, 4H, C_5**H2**-C_4**H2**); 1.55 (s, 3H, C_8-**H3**);1.25 (t, 6H, **H3C**-CH_2O); 1.0 (s, 6H, C_1**H3**, C_2**H3**).
3c	0.28 (6/4)	419 $MH]^+$	22	7.5 (ddd, 1H, $J_{H20-H21}$=16.5Hz, $J_{H20-H19}$=11.6Hz, J_{H-P}=20.5 Hz, C_{20}**H**); 6.8 (dd, 1H, J=15 and 11.25 Hz, C_{15}**H**); 6.3 (d,1H, J=15Hz, C_{16}**H**); 6.15 (m, 4H, C_{10}**H**, C_{11}**H**, C_{14}**H**, C_{19}**H**); 5.55 (dd, 1H, J=16.5Hz, J_{H-P}=19.5 Hz, C_{21}**H**); 4.05 (m, 4H, POC**H2**); 2.1 (m, 2H, C_6**H2**); 2.05, 1.97 and 1.70 (3s, 9H, C_8**H3**, C_{13}**H3**, C_{18}**H3**); 1.6 and 1.4 (2m, 4H; C_5**H2**, C_4**H2**); 1.25 (t, 6H, **H3C**-CH_2O); 1.00 (s, 6H, C_1**H3**, C_2**H3**).

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12. Proton nuclear magnetic resonances were determined with an AC 250 Bruker spectrometer. Chemical shifts are expressed in parts per million, with TMS as reference. Phosphorus nuclear magnetic resonances were determined with a WP 200 Bruker spectrometer, chemical shifts are expressed in parts per million, with phosphoric acid as external reference. Fast-atom bombardment mass spectra (FAB-MS) were recorded on a JEOL DX 300 mass spectrometer. Thin layer chromatography (TLC) was performed (ether-AcOEt) on a precoated aluminium sheets of silicagel 60 F_{254} (Merck n° 5554).

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