Development of Novel Antiatherogenic Biaryls: Design, Synthesis, and Reactivity

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On the basis of the 5,5′-bisvanillin scaffold, a series of compounds has been synthesized presenting symmetric or dissymmetric frames on each phenolic moiety. These frames are α,β-unsaturated (fluoro)phosphonate and/or α,β-unsaturated hydrazone(s) formed by coupling aldehydic with isoniazid or hydralazine. All compounds were tested for their ability to inhibit cell-mediated low-density lipoprotein oxidation. Oxidized low-density lipoprotein induced cytotoxicity was also evaluated along with the carbonyl scavenger properties of selected compounds. The most efficient agents were found to be those possessing at least one hydralazinone frame, with the most potent being the symmetrical compound: 4,4′-dihydroxy-3,3′-dimethoxy-5,5′-biphenyl-1,1′-(diphthalazin-1-yl)methylhydrazone hydrochloride.

Introduction

Atherosclerosis is a multifactorial pathology characterized by the development of focal lesions in the vascular wall of medium and large arteries.[1] It is a chronic inflammatory disease with acute complications represented by plaque erosion and rupture, thrombosis, and cardiovascular and neurovascular events.[1] Low-density lipoproteins (LDL) become atherogenic after undergoing oxidative modifications in the intima. Oxidatively modified low-density lipoproteins (oxLDLs) play an important role in the initiation and progression of atherosclerosis by promoting cholesterol storage, endothelial dysfunction, lipid deposition, chronic inflammatory response, and toxic events.[1–3] Modification of LDLs through oxidative damage may increase their atherogenicity by increasing their receptor-mediated uptake by cells in the intima of blood vessels, leading to creation of foam cells in the subendothelium, an early feature of atherosclerotic plaque.[3] The biological effects of oxLDLs depend on their local concentration and their content of lipid peroxidation products such as lipid hydro- and lipoperoxides, oxidized phospholipids, oxysterols, and aldehydes such as 4-hydroxyalkenals and 4-hydroxynonenal (4-HNE), malondialdehyde (MDA) or acrolein.[4,5] Lipid oxidation-derived aldehydes (carbonyl precursors) may react with free amino groups and thiol residues, thereby forming protein cross-links which induces progressively protein dysfunction, also called "carbonyl stress".[6] Antioxidants are antiatherogenic as they inhibit the early steps of the LDL oxidation process,[5] but these agents are inefficient against carbonyl stress once lipid oxidation products are formed.[7] Carbonyl scavengers usually exhibit modulated antioxidant activities but inhibit the carbonyl stress by blocking the formation of protein cross-links.[7] Dinitrophenylhydrazine (DNPH) is a powerful carbonyl scavenger agent, largely utilized for the detection of reactive carbonyl compounds (RCCs).[4] DNPH prevents in vitro the formation of acrolein- and HNE-adducts on cellular proteins[8] but cannot be used in vivo because of its hemolytic, mutagenic, and carcinogenic properties.[9] Other hydrazine derivatives, such as hydralazine, also behave as carbonyl scavengers but do not exhibit strong antioxidant activity.[10] Thus, the generation of drugs sharing both antioxidant and carbonyl scavenger properties represents a new therapeutic challenge in the prevention and treatment of atherosclerosis.

Many hindered phenol-derived antioxidants possessing both of these features have been screened since the initial work on probucol.[11,12] In a previous paper,[13] we reported synthesis of phosphonocinnamic analogues. Phosphonodiesters and especially fluorophosphonoesters have shown really interesting antioxidant effects. The latter have also presented strong dual biological antioxidant and cytoprotective properties. To get insight to families of compounds sharing these two properties, we have designed biaryl compounds possessing identical or different substitutions patterns on a judiciously chosen phenolic scaffold, i.e., bisvanillin. On one hand, the 4-OH and adjacent 3-OMe functionalities are always present in order to confer to the compound the basis of a good antioxidant activity, as we have previously noticed by studying the monomeric units. On the other hand, the presence of the two aldehydic groups allows us to construct symmetric or dissymmetric entities conjugated with their phenolic moieties (Scheme 1). These entities may confer not only antioxidant properties but also cytoprotection. In that respect, compounds possessing the known phosphonodiester and/or fluorophosphonoester moiety and also a hydrazone function could be synthesized.

The hydrazones family may be interesting by various means. On one hand, hydrazines possessing strong electron withdrawing groups are known to form hydrazones sufficiently activated to be hydrolyzed at physiological pH,[14] ultimately making these type of derivatives useful through creation of dynamic libraries. On the other hand, hydrazines possessing in their frames basic centers that can be protonated (or complexed by metals) may be transformed to stable hydrazones under neutral pH conditions that could release hydrazines under acid assistant conditions.[15] Finally, delocalized systems possessing a hydrazone frame may induce cytoprotective effects by acting through completely different ways yet to be adressed.

In this paper, we report the synthesis of symmetric and dissymmetric biaryl analogues bearing (fluoro)phoshonate(s) and/or hydrazone frames (issued from isoniazid and hydralazine)

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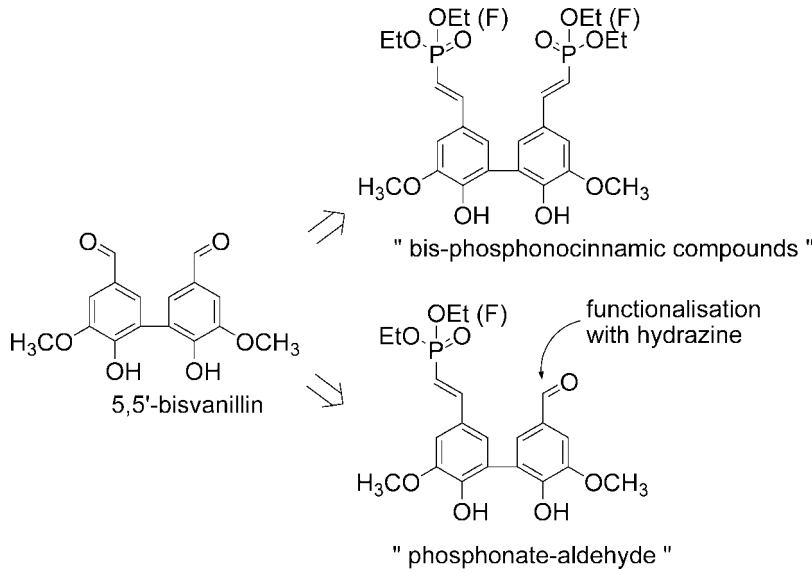
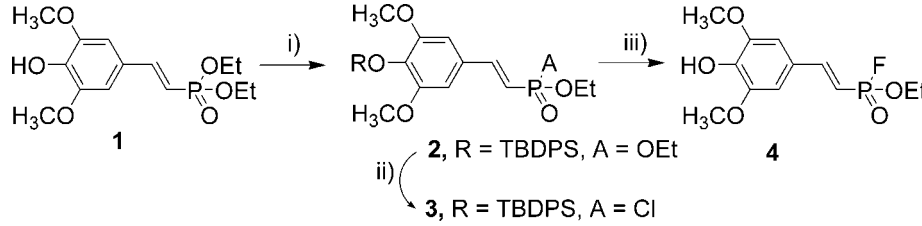
Scheme 1. Biaryl Derivatives Generated from 5,5′-Bisvanillin

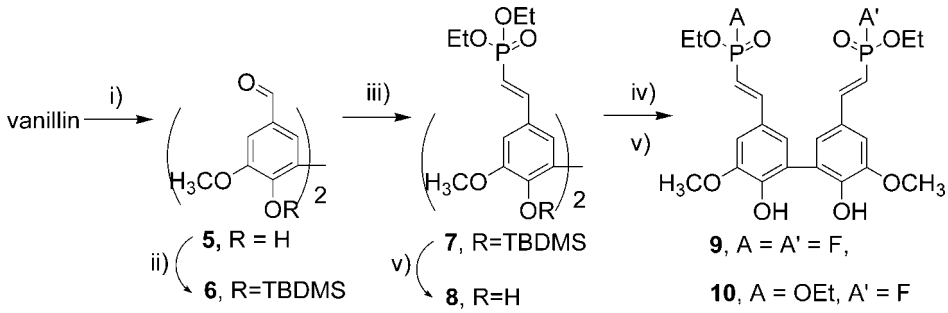


Scheme 2[a]



[a] Reagents and conditions: (i) imidazole, DMAP, TBDPSCl, DMF, room temperature, 16 h, 85%; (ii) $(COCl)_2$, room temperature, 3 h, quantitative; (iii) $Et_3N\cdot HF$, THF, room temperature, 1 h, 77%.

Scheme 3[a]



[a] Reagents and conditions: (i) $Na_2S_2O_8$, $FeSO_4$, H_2O, 5d, 50 °C, 95%; (ii) imidazole, DMAP, TBDMSCl, DMF, room temperature, 3 h, 70%; (iii) iPr_2NH, *n*-BuLi, THF, tetraethyl methylenediphosphonate, −78 °C, 3 h, 86%; (iv) $(COCl)_2$, CH_2Cl_2, room temperature, quantitative; (v) $Et_3N\cdot HF$, THF, room temperature, 1 h or 30 min respectively for **8** or **9** and **10**, 57%, 60%, and 41%, respectively.

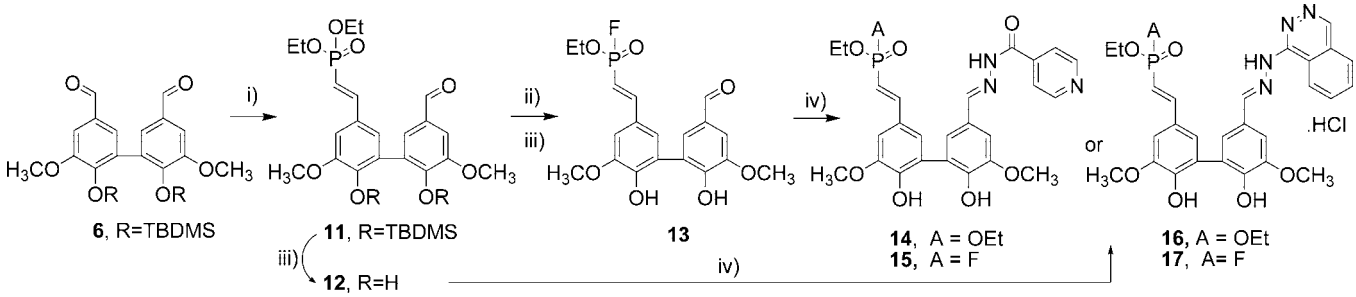
and their biological properties, indicating that they can be considered as lead structures, endowed with antioxidant and carbonyl scavenger properties.

Chemistry

To develop an efficient synthetic route to achieve phosphonodiester[16] and fluorophosphonoester biaryl compounds, we first focused our efforts on improvement of the monoaryl fluorophosphonate **4** synthesis previously described.[13] Diethylphosphonate **1** was silylated on the *p*-hydroxyphenolic position by using *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of imidazole and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in dimethylformamide (DMF). The protected diethylphosphonate **2** (^{31}P NMR 20.09 ppm) was treated with neat oxalyl chloride, and the reaction was followed by ^{31}P NMR spectroscopy. The chlorinated intermediate **3** obtained quantitatively (^{31}P NMR 29.94 ppm) was then directly reacted with the mild fluorinating reagent $Et_3N\cdot HF$[17] in tetrahydrofuran (THF), furnishing fluorophosphonate **4** (^{31}P NMR 19.50 ppm), which was also deprotected from its silyl group as illustrated in Scheme 2. Other fluorinating reagents used led to sluggish results and much lower yields.

So we developed a new shorter synthetic way to obtain the fluorophosphonate **4** with better global yield (65% instead of 23%). This method was applied to synthesis of the new biaryl derivatives as depicted in Scheme 3. First, oxidative coupling of commercial vanillin in the presence of iron sulfate afforded bisvanillin **5** with excellent yield (95%), improving by 20% on the literature report.[18] Hydroxyl groups were silylated using *tert*-butyldimethylsilyl chloride (TBDMSCl) instead of TBDPSCl

Scheme 4[a]



[a] Reagents and conditions: (i) *i*Pr$_2$NH, *n*-BuLi, THF, tetraethyl methylenediphosphonate, −78 °C, 3 h, 54%; (ii) $(COCl)_2$, room temperature, 3 h, quantitative; (iii) $Et_3N\cdot HF$, THF, room temperature, 30 min, 75% and 72% for compounds **12**, **13**, respectively; (iv) hydralazine or isoniazid, EtOH, reflux, 6 h, 81%, 69%, 93%, and 83% for compounds **14**, **15**, **16**, and **17**, respectively.

because of the inefficiency of the latter due to steric hindrance. Silylated 5,5′-bisvanillin **6** was reacted with lithiated tetraethyl methylenediphosphonate. Thermal treatment of the bis-phosphorylated intermediate gave exclusively the double *trans*-vinylic phosphonate **7**.[16] At this stage, compound **7** can be treated by $Et_3N\cdot HF$ complex to afford desilylated bisphosphonate **8.**

Compound **7** was also used in order to introduce one or two fluorine atom(s) on the phosphorus, following the chlorination−fluorination procedure. Extensive experimental conditions were checked and reactions were quantified after each final workup and purification as ^{31}P NMR spectroscopy gave in this case only qualitative but not quantitative information on the constitution of the reaction mixtures.

For the difluorinated addent **9**, best results were obtained when compound **7** reacted with 6 equiv of oxalyl chloride in methylene chloride for 40 h followed by $Et_3N\cdot HF$ treatment. Compound **9** was thus obtained in 60% yield after purification by silica-gel chromatography. Using 4.5 equiv of oxalyl chloride in CH_2Cl_2 and modifying the reaction time gave the monofluorinated adduct **10** in 41% yield along with desilylated starting material **8** (15%) and the difluorinated compound **9** (32%).

To introduce dissymmetrization on the phosphorus atoms (as for compound **10**) and on the aldehydic frame of the 5,5′-bisvanillin, we synthesized key intermediate **11**. Scheme 4 details the formation of **11** and its use in the synthesis of compounds **12**−**17**.

Protected bisvanillin **6** reacted with strictly 1 equiv of lithiated tetraethyl methylenediphosphonate. The reaction, carefully controlled by TLC, afforded mainly phosphonate-aldehyde **11** in 54% yield along with starting material (21%) and the symmetrical functionalized compound **7** (12%). Compound **11** could be either deprotected through desilylation, affording **12** in 75% yield or used in the chlorination−fluorination process, yielding compound **13** in 72% total yield after silica-gel purification.

Compounds **11**, **12**, and **13** were used in order to introduce hydrazone functionality on the remaining aldehydic frame. Two examples are presented here where the reagents are the known drugs, i.e., isoniazid[19] and hydralazine[8] (antimalarial and hypotensor, respectively). Filtration and purification on silica gel chromatography afforded in good yields compounds **14** and **16** (starting from **12**) and **15** and **17** (starting from **13**).

Finally, synthesis of symmetrical hydrazones **18** and **19** was undertaken as depicted in Scheme 5. 5,5′-Bisvanillin **5** reacted with 2 equiv of hydrazine (hydralazine or isoniazid) in ethanol under reflux. Precipitation of double hydrazones **18** and **19** during reaction afforded pure compounds **18** and **19** in good yield.

Results and Discussion

Symmetric or dissymmetric phosphonocinnamic and fluorophosphonocinnamic derivatives were synthesized. Their antioxidant properties were studied on cell-mediated LDL oxidation. LDL oxidation by vascular cells and the uptake of oxLDL by macrophages are involved in the formation of fatty streaks, which constitute the early atherosclerotic lesions.[1–3] Thus we aimed to determine the antioxidant ability of the newly synthesized compounds in inhibiting LDL oxidation mediated by human microvascular endothelial cells (HMEC-1) and the formation of foam cells.

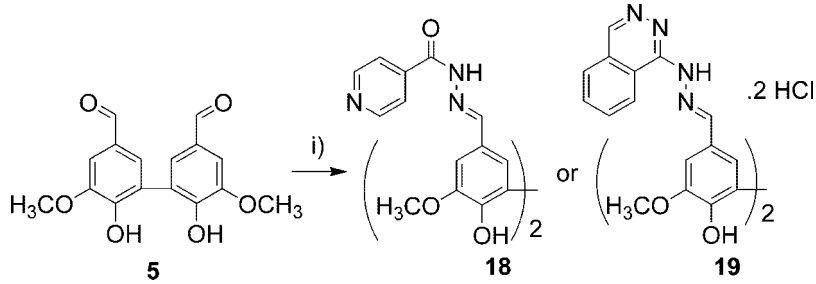
LDL oxidation was determined by using the thiobarbituric acid reactive substance (TBARS) assay and expressed as a percentage of TBARS formed in LDL in contact with HMEC-1 in the absence of antioxidant. HMEC-1 were incubated for 6 h in Roswell Park Memorial Institute (RPMI) 1640 culture medium containing native LDL (100 μg/mL) and the different agents solubilized in dimethylsulfoxide (DMSO) (less than 0.1% final concentration) tested at a final concentration of 10 μM. LDL oxidation was monitored by the TBARS content.[20] As shown in Table 1, only compounds **16**, **17**, and **19** exhibited strong antioxidant properties by comparison to probucol and α-tocopherol tested at the same concentration (10 μM).

The other agents were not (or very slightly) able to inhibit LDL oxidation by HMEC-1 in this system.

Because LDL oxidation by cultured cells renders them cytotoxic[21] and because antioxidants are able to block the LDL oxidation process by cells,[22] we evaluated the residual cytotoxicity of LDL previously oxidized by HMEC-1 in presence or absence of the synthesized biaryls agents by the MTT assay. The data were expressed as a percentage of the unstimulated control (cell incubated without LDL). The data presented in Table 1 are in agreement with the TBARS content of LDL, indicating a strong cytotoxicity of LDL incubated without any agent (less than 15% of residual cell viability), which was efficiently protected by the compounds **16**, **17**, **19**. Interestingly, compounds **8** and**12**, found noneffective as antioxidants, were able to protect in part against oxLDL cytotoxicity, thus suggesting that they may neutralize the toxic effect of newly formed oxidized lipids either through direct interactions or by blocking the apoptotic signaling of oxLDL.

Considering the compounds synthesized, results are partitioned in four groups. In the first group the 5,5′-biaryl compounds issued from the oxidative coupling of vanillin

Scheme 5[a]



5 18 19

[a] Reagents and conditions: (i) hydralazine or isoniazid, EtOH, reflux, 6 h, 77% and 87% for compounds **18** and **19**, respectively.

Table 1. Antioxidant Effect (TBARS) and Residual Cytotoxicity (MTT) of Biaryls Derivatives

	TBARS %[a]	MTT %[b]
control	0 ± 5	100 ± 5
native LDL	100 ± 5	15 ± 5
5	86.2 ± 7.4	40.5 ± 8.6
13	135.1 ± 8.9	26.8 ± 4.6
12	67.5 ± 8.4	63.5 ± 9.5
8	67.4 ± 8.6	66.0 ± 5.5
9	76.5 ± 7.9	30.2 ± 2.5
10	74.6 ± 9.4	40.9 ± 8.7
14	67.5 ± 6.9	31.2 ± 5.5
15	95.8 ± 9.1	43.3 ± 6.2
18	83.0 ± 2.1	13.5 ± 0.3
16	4.1 ± 0.6	61.2 ± 1.5
17	2.6 ± 0.6	68.9 ± 0.9
19	2.8 ± 0.1	82.0 ± 8.6
α-tocopherol	55 ± 2.1	29 ± 2.8
probucol	10 ± 0.7	92 ± 11.3

[a] The different compounds were tested at 10 μM on LDL oxidation mediated by HMEC-1 in RPMI culture medium in presence of traces of $CuSO_4$(μM). LDL oxidation was monitored after 6 h of incubation by the determination of TBARS in the culture medium. The results are expressed as % of the TBARS content in LDL-containing culture medium incubated with HMEC-1 in the absence of any agent (15 ± 2.4 nmol TBARS/mg apoB). [b] The toxicity of LDL oxidized by HMEC-1 in presence or absence of the different compounds was evaluated by the MTT test after 24 h of incubation. The results are expressed as % of the unstimulated cell control (MTT done on control cells incubated in fresh RPMI culture medium in the absence of LDL and antioxidant).

present all an aldehydic moiety. While vanillin itself is a known antioxidant, the diethyl phosphonate monomer **1** and the corresponding fluorinated adduct **4** previously synthesized and tested[13] present good antioxidant activities, compounds **5**, **12**, and **13** are much less efficient.

In the second group, the 5,5′-biaryl compounds from the oxidative coupling of vanillin present in both parts the α,β-unsaturated phosphonate moiety that might be symmetrically substituted as for compounds **8** and **9** or dissymmetrically substituted on the phosphorus atom as for compound **10**. Bis-phosphonodiester **8** presents the best result and is similar to that of compound **12**. As previously, compound **8** is less efficient than its corresponding monomer **1**.

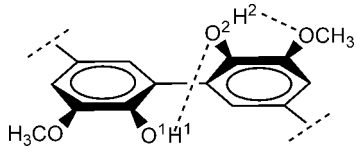
The third group of compounds present a hydrazone bond provided by the condensation between the aldehydic moiety of the 5,5′-biaryl compounds and isoniazid. Compounds are symmetrically substituted as for **18** or dissymmetrically as for **14** and **15**. The latter two possess also a α,β-unsaturated (fluoro)phosphonate moiety. Results are also very modest; the best activity is observed for the dissymmetrically substituted compound **14** possessing the diethylphosphonate functionality. Note that pure isoniazid tested alone did not exhibit any antioxidant effect at this concentration (data not shown). Thus we can say that introduction of isoniazid through a hydrazone link on compound **12** did not modify its antioxidant activity (identical activities for **12** and **14**). Finally, the fourth group of compounds (**16**, **17**, and **19**) present a hydrazone bond provided by the condensation between the aldehydic moiety (moieties) of the 5,5′-biaryl compounds and hydralazine. Gratifyingly, all compounds present an extremely potent antioxidant activity between 2.6% and 4.1% in the TBARS assay. These are the most potent antioxidants we have synthesized. These results contrast with those where the hydrazone link is obtained through introduction of isoniazid. It should be noted also that compounds **16**, **17**, and **19** present much better antioxidant and cytoprotective effects values than the corresponding parent compounds **12** and hydralazine alone. In fact, pure hydralazine tested alone did not exhibit any antioxidant effect at this concentration (data not shown). We do not know yet the reason for these excellent activities. A possible explanation could be as a result of their hydrazone link. Dolenc and al.[23] recently reported that hydrazones deriving from primary hydrazines are prone to autoxidation reactions, this depending strongly on the nature of the substituents on the two nitrogen atoms.

Concerning the first three groups of compounds, we can conclude that these 5,5′-biaryl derivatives present lower antioxidant activities than the related phosphonate or fluorophosphonate monomers **1** and **4** reported earlier. This is in agreement with the few studies reported on polyphenolic systems where the hydroxy groups do not belong in the same aromatic ring. In a recent paper, Pedulli and al.[24] reported that 2,2′-dihydroxybiphenyl systems presented low reactivity toward peroxy radicals and were weak antioxidants, attributed to the formation of twin intramolecular hydrogen bond in the starting biphenyl frame and to strong H-bond interaction between the quinone adduct and the remaining OH phenol group. The second OH group is thus less reactive, resulting in a reduction of its antioxidant activity.

To make an exploration of molecular geometry in relation to the dihedral angle of interest, a conformation sampling was carried using a short molecular dynamics on each compound synthesized. The temperature was increased from 298 to 450 K during 50 ps in order to provide a range of 5000 conformers. Software used was Discover from Accelrys and extensible and systematic force field (ESFF) in vacuum. Energy versus time graphs were plotted. Lower energy conformers (6−8) with energies varying up to 30% comparing to the lowest value, were selected and each one of them was subjected to a new dynamic (Discover/ESFF). This process is repeated until reaching the minimum energy conformer. Selected dihedral angles ($H^1-O^2-H^2-OCH_3$) and hydrogen bond lengths of potent (**16**, **17**, **19**) and less potent (**12**, **14**) derivatives are reported in Table 2.

Results indicated, as in the case of Pedulli's et al. compounds, the adoption of cisoid geometry with the two aromatic rings making a dihedral angle of ca. 50° and where each of the two

Table 2. Computational Modelling Studies of Biaryl Dihedral Angle and Hydrogen Bond Distances



	dihedral angle, deg	hydrogen bond (Å) H^1-O^2	hydrogen bond (Å) H^2-OCH_3
12	52.18	1.90	2.10
14	56.26	1.89	2.14
16	51.83	1.87	2.08
17	50.79	1.92	2.05
19	53.66	1.89	2.12

OH protons is hydrogen bonded to the oxygen atom of the other hydroxy group. So, the strong biological activities found for compounds **16**, **17**, and **19** may be due to another structural and/or physicochemical properties of these derivatives yet to be adressed.

Concerning some further biological experiments, we tested the ability of the selected compounds **8**, **15**, and **19**, as representatives of the newly synthesized derivatives, to inhibit the formation of foam cells. For this purpose, human macrophage-like U937 cells were incubated for 18 h with native LDL (100 μg/mL) in the presence of each selected agent (10 μM). The formation of foam cells was evaluated fluorimetrically after Nile Red staining, in the following reported conditions.[25] Alternatively, foam cells were microscopically characterized in terms of red lipid droplet formation after staining with the neutral lipid probe, oil red O. As shown in Figure 1, LDL incubated with U937 were oxidized (TBARS content 26.9 $\pm$ 3.4 nmol/mg protein) and taken up by the cells, resulting in a huge increase in Nile Red fluorescence (Figure 1a) and characteristic lipid droplet accumulation in the cytosol (Figure 1b). As expected, compound **19** efficiently inhibited foam cells formation, this was also partially prevented by compound **8** (in agreement with its protective effect against oxLDL toxicity). In contrast, compound **15** did not exhibit any protective effect.

We then aimed to evaluate the direct cytoprotective effect of all synthesized compounds, toward LDL oxidized by UV-irradiation, in the absence of any agent. As shown in Figure 2, the first three groups of compounds are not very efficient, with better results obtained for the bis(fluoro)phosphonate derivatives. Interestingly, the fourth group (compounds **16**, **17**, and **19**) was shown to be very potent, with compound **19** possessing the highest efficiency (more than 80% of cell survival).

Finally, we evaluated the ability of some newly synthesized agents in preventing cell death induced by 4-HNE and in neutralizing the formation of carbonyl proteins (carbonyl stress).

Previous experiments have shown that high hydralazine and isoniazid concentrations (100 μM) may protect against 4-HNE-induced cell death. We tested the protective effect of the most representative compounds on the toxicity induced by 4-HNE (50 μM).

As shown in Figure 3a, the compound **19** was highly efficient in protecting cells against 4-HNE-induced cell death. Compound **8** was mildly protective, while compound **15** was ineffective. Inhibitory effect of these agents on carbonyl stress evaluated as DNPH-labeled proteins in HMEC-1 incubated with oxLDL (200 μg/mL) or 4-HNE (50 μM) for 14 h (Figure 3b), showed also that compound **19** is very efficient in preventing the increase in carbonyl proteins, and thus the formation of adducts resulting in carbonyl stress. The other agents were almost ineffective.

Altogether, these data indicate that the newly synthesized agents (**16**, **17**, and **19**) exhibited potent antioxidant properties against cell-mediated LDL oxidation and subsequent cytotoxicity as well as the formation of foam cells, properties that are well beyond the individual considered frames (phosphonate, fluorophosphonate, or hydrazine). Our results show that at least in vitro, these compounds are efficient for preventing the early steps of atherosclerosis.

Although compound **19** exhibited antioxidant and carbonyl scavenger properties as well, it is likely that its antioxidant activity was not involved in its protective effect against carbonyl stress because **19** prevented the toxicity and the carbonyl stress mediated by 4-HNE, which is a potent carbonyl stress inducer, independently of any reactive oxygen species increase. It is noteworthy that hydralazine was also able to prevent the carbonyl stress but at higher concentrations (>100 μM).

In conclusion, on the basis of the bisvanillin scaffold for which the synthesis has been greatly improved, we have synthesized different biaryls compounds, symmetrically or dissymmetrically substituted on aromatic frames by a vinyl(fluoro)phosphonate and/or hydrazino functionalities.

Altogether, biological data indicate that the newly synthesized biaryl agents exhibit both antioxidant and carbonyl scavenger properties, thus suggesting a potential strong antiatherogenic effect. In that respect, compounds **16**, **17**, and **19** are shown to be very interesting. The reason for their strong antioxidant and cytoprotective efficiency will be addressed in terms of physicochemical properties and of biological future of these compounds (reactivity against radicals, prodrug nature of the compounds, interaction with metals, activity against others biological targets, . . .).

Concerning the biological aspect of our research program, further investigations in apoE−/− mice as an animal model for atherosclerosis, should support the antiatherogenic efficiency of these agents.

Moreover, to increase the antioxidant activities of the three first groups of compounds synthesized, we envisage to introduce a new dissymmetry on the phenolic parts of the bisvanillin in order to eliminate the strong double OH interaction leading to another interesting scaffold for further elaboration of hydrazone-like antiatherogenic derivatives.

Experimental Section

Materials. All chemicals are of the highest commercially available purity. 2-Thiobarbituric acid and diagnostic kits for total cholesterol, LDL, and triglyceride determination are purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Aldrich-Chemie (Steinheim, Germany). Commercial 2-piperidinemethanol corresponds to the racemate.

Synthesis. Organic solvents were purified when necessary by methods described by Perrin, Armarengo, and Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1986) or were purchased from Aldrich Chemie.

Melting points (mp) were obtained on a Buchi apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1725 infrared spectrophotometer, and the data are reported in inverse centimeters. UV spectra were recorded on a Cary 100/300 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with Bruker AC-200, AC-250, AC-300, and AC 400 MHz spectrometers. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS), and signals are given as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectra were recorded on an R 10-10 C Nermag (70 eV) quadripolar spectrometer using desorption chemical ionization (DCI) or electrospray (ES) techniques. Elemental analyses were performed with a Perkin-Elmer 2400 CHN analyzer.

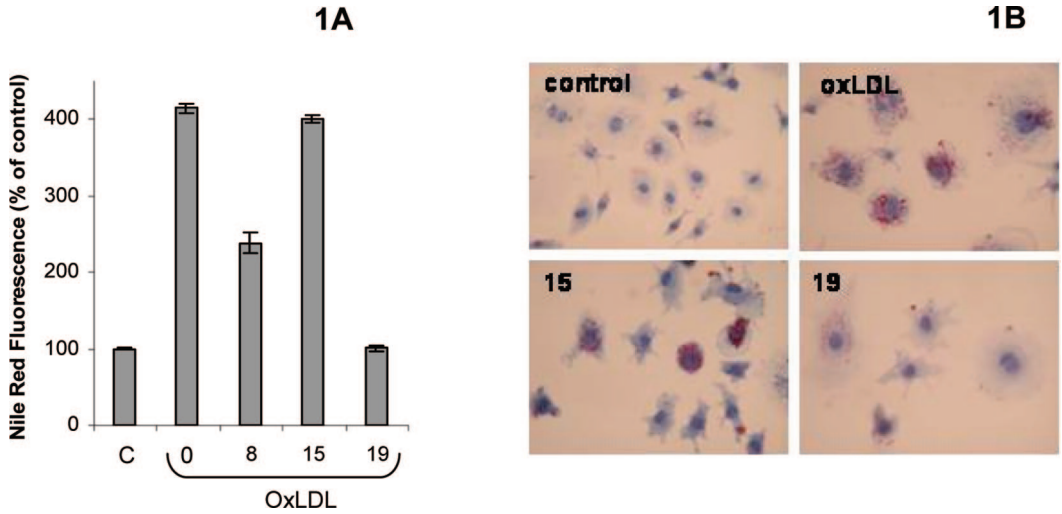


Figure 1. Inhibition of foam cell formation. (1A) Human U937 monocyte/macrophage cells were incubated with oxLDL (100 μg/mL) for 14 h, in RPMI 1640 medium containing 1 μM $CuSO_4$, in the absence (0) or in the presence of the newly synthesized biaryl compounds **8**, **15**, and **19** (10 μM). The control (C) was performed in the absence of oxLDL and of any agent. Foam cells formation was monitored fluorometrically by the uptake of Nile Red, as described in the Experimental Section. These data are a mean of three separate experiments. (1B) Pictures showing the accumulation of oil Red O-stained lipid droplets, in U937 incubated with oxLDL as in (1A), and in the presence of agents **15** and **19** (10 μM).

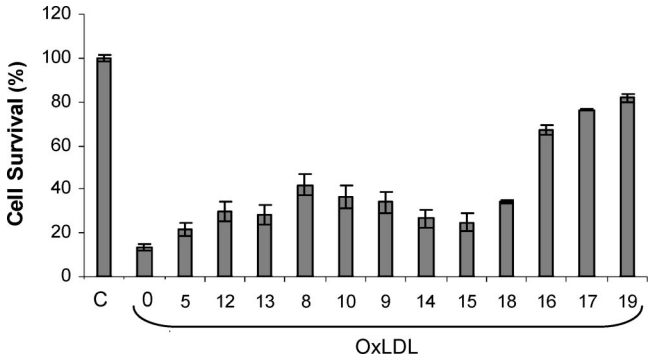


Figure 2. Direct cytoprotective effect towards oxLDL. Cytotoxic effect of UV-oxidized LDL and cytoprotective effect of the synthesized agents (10 μM). HMEC-1 was incubated for 24 h with UV-oxidized LDL (200 μg/mL), alone (0) or in the presence of the newly synthesized agents (10 μM). The cytotoxicity was evaluated by the MTT assay. Results are expressed as % of the unstimulated control (cell incubated in LDL-free medium). Each bar represents the mean for four separate determinations.

Diethyl(*E*)-2-(4-hydroxy-3,5-dimethoxyphenyl)vinylphosphonate (1). To iPr_2NH (5 mL, 36.26 mmol) dissolved in dried THF (8 mL/mmol) stirred at −78 °C under nitrogen, *n*-BuLi (1.6 M in hexane: 22.7 mL, 36.26 mmol) was added. The reaction mixture was stirred for 30 min at −78 °C under N_2. Tetraethyl methylenediphosphonate (9 mL, 36.26 mmol) in THF was added to formed lithium diisopropylamide (LDA) solution, and the reaction mixture was stirred 1 h at −78 °C. Syringaldehyde (17.6 mmol, 3.21 g) in THF was added, and mixture was stirred for 45 min at −78 °C, then allowed to warm up to room temperature and refluxed. After 2 h, saturated aqueous NH_4Cl (150 mL) was added, and the aqueous layer was extracted with Et_2O (3 × 200 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by recrystallization from CH_2Cl_2 to give **1** (3.75 g, 67%) as a white solid; mp = 181−183 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.35 (t, 6H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 3.91 (s, 6H, CH_3O); 4.13 (qd, 4H, $^3J_{H-H}$ = 7,2 Hz, $^3J_{H-P}$ = 7.2 Hz, CH_3CH_2O); 5.78 (s, 1H, OH); 6.08 (dd, 1H, $^3J_{H-H}$ = 17.2 Hz, $^2J_{H-P}$ = 17.2 Hz, CH═CH-P); 6.74 (s, 2H, H_2 + H_6); 7.39 (dd, 1H, $^3J_{H-H}$ = 17.5 Hz, $^3J_{H-P}$ = 24,5 Hz, CH═CH-P). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.42 (d, 2C, $^3J_{C-P}$ = 6.5 Hz, CH_3CH_2O); 56.34 (s, 2C, CH_3O); 61.78 (d, 2C, $^2J_{C-P}$ = 5.4 Hz, CH_3CH_2O); 104.72 (s, 2C, C_2 + C_6); 111.13 (d, 1C, $^1J_{C-P}$ = 192.0 Hz, C═C−P); 126.36 (d, 1C, $^3J_{C-P}$ = 20.3 Hz, C_1); 137.02 (s, 1C, C_4); 147.20 (s, 2C, C_3 + C_5); 148.95 (d, 1C, $^2J_{C-P}$ = 6.8 Hz, C═C−P). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 20.36 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 317.0 (MH^+, 100%), 334.0 (MNH_4^+, 36%). Anal. ($C_{14}H_{21}O_6P$) C, H calcd 53.16, 6.69; found 52.91, 6.76. IR (KBr) ν cm^{-1}: 3188 (O−H); 3082 (C−H ethyl); 1620 (C═C ethyl); 1594 (C═C arom); 1242 (*P*═O); 1162 (P−O). UV (EtOH, 22 μM): λ = 232 nm, ϵ = 27600 $mol^{-1}\cdot L\cdot cm^{-1}$.

Diethyl(*E*)-2-(4-*tert*-butyldiphenylsilyloxy-3,5-dimethoxyphenyl)vinylphosphonate (2). To vinylphosphonate (**1**) (2 g, 6.33 mmol) in dried DMF (29 mL) were added imidazole (0.86 g, 12.66 mmol), DMAP (0.77 g, 6.33 mmol), and TBDPSCl (2.6 g, 9.5 mmol) under N_2. The reaction mixture was stirred for 16 h at room temperature. Aqueous $NaHCO_3$ 5% (30 mL) was added, and the aqueous layer was extracted with Et_2O (3 × 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated under vacuo. Silica gel chromatography (EtOAc/petroleum ether 6/4) gave **2** as white crystals (5.33 g, 78%); mp =155−157 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 1,11 (s, 9H, tBu); 1,34 (t, 6H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 3.47 (s, 6H, CH_3O); 4.13 (qd, 4H, $^3J_{H-H}$ = 7.0 Hz, $^3J_{H-P}$ = 7.0 Hz, CH_3CH_2O); 6.05 (dd, 1H, $^3J_{H-H}$ = 13.8 Hz, $^2J_{H-P}$ = 17.4 Hz, CH═CH-P); 6.60 (s, 2H, H_2 + H_6); 7.36 (m, 7H, CH═CH-P + H_p and H_m phenyl); 7.72 (d, 4H, $^3J_{Ho-Hm}$ = 7.5 Hz, H_o phenyl). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.43 (d, 2C, $^3J_{C-P}$ = 6.8 Hz, CH_3CH_2O); 20.15 (s, 1C, C_q tBu); 26.17 (s, 3C, CH_3 tBu); 55.21 (s, 2C, CH_3O); 61.73 (d, 2C, $^2J_{C-P}$ = 5.3 Hz, CH_3CH_2O); 104.77 (s, 2C, C_2 + C_6); 111.18 (d, 1C, $^1J_{C-P}$ = 192.0 Hz, C═C−P); 127.09 (s, 4C, C_m phenyl); 127.51 (d, 1C, $^3J_{C-P}$ = 24.0 Hz, C_1); 129.20 (s, 2C, C_p phenyl); 134.23 (s, 1C, C_4); 135.06 (s, 4C, C_o phenyl); 136.63 (s, 2C, C phenyl); 149.13 (d, 1C, $^2J_{C-P}$ = 6.8 Hz, C═C−P); 151.06 (s, 2C, C_3 + C_5). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 20.09 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 555.0 (MH^+).

Ethyl(*E*)-2-(4-*tertio*butyldiphenylsilyloxy-3,5-dimethoxyphenyl)vinylphosphonochloridate (3). To phosphonodiester (**2**) (1.25 g, 2.26 mmol) in CH_2Cl_2 (15 mL) under N_2 was added freshly distilled oxalyl chloride (0.89 mL, 10.2 mmol). The reaction mixture was stirred at room temperature. Reaction was monitored by ^{31}P NMR until completion. CH_2Cl_2 and oxalyl chloride excess were removed under vacuo. Phosphonochloridate was obtained as yellow solid (1.23 g, 100%) and used without purification in fluorination step. ^{31}P NMR (D_2O, 81 MHz) δ ppm: 29.94 (s, P−Cl).

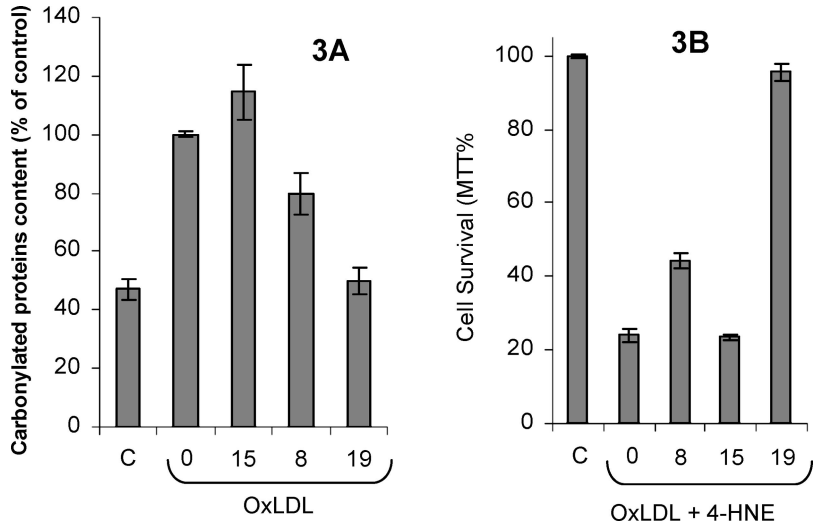


Figure 3. Direct cytoprotective effect towards 4-HNE-induced cell death proteins carbonylation at 10 μM. (3A) HMEC-1 were incubated overnight in pure fetal calf serum (FCS)-free RPMI-1640 culture medium containing 4-HNE (50 μM). The cells were carefully washed with warm PBS, scrapped, and the carbonyl protein content was determined in presence of DNPH as described in the Experimental Section. The results are expressed as % of the unstimulated control ± (structural equation modelling) SEM. These data are a mean of four separate experiments. (3B) Cytotoxicity of UV-oxidized LDL (200 μg/mL) and of 4-HNE (5 μM) was evaluated by the MTT test on HMEC-1 after 24 h of incubation. The results are expressed as % of the unstimulated control (cell incubated in LDL-free medium). Each bar represents the mean for four separate determinations.

Ethyl(*E*)-2-(4-hydroxy-3,5-dimethoxyphenyl)vinyl phosphonofluoridate (4). To phosphonochloridate (**3**) (0.3 g, 0.42 mmol) in freshly distilled THF (4.2 mL) under N_2 were added Et_3N (0.2 mL, 1.4 mmol) and $Et_3N{\cdot}3HF$ (0.11 mL, 0.7 mmol). The reaction mixture was stirred at room temperature and monitored by ^{31}P NMR and then quenched by addition of EtOAc. Precipitate formed was filtered off. Water was added to the filtrate. Aqueous layer was extracted with EtOAc (3 × 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated under vacuo. Silica gel chromatography (EtOAc/petroleum ether 4/6) gave **4** as a white solid (93 mg, 77%); mp = 132−134 °C. ^1H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.38 (t, 3H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 3.88 (s, 6H, CH_3O); 4.27 (qd, 2H, $^3J_{H-H}$ = 7.0 Hz, $^3J_{H-P}$ = 7.0 Hz, CH_3CH_2O); 5.81 (s, 1H, OH); 6.07 (dd, 1H, $^3J_{H-H}$ = 17.5 Hz, $^2J_{H-P}$ = 19.2 Hz, CH═CH-P); 6.72 (s, 2H, H_2 + H_6); 7.49 (dd, 1H, $^3J_{H-H}$ = 17.5 Hz, $^3J_{H-P}$ = 24.5 Hz, CH═CH-P). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.30 (d, 1C, $^3J_{C-P}$ = 6.0 Hz, CH_3CH_2O); 56.35 (s, 2C, CH_3O); 63.41 (d, 1C, $^2J_{C-P}$ = 6.0 Hz, CH_3CH_2O); 105.11 (s, 2C, C_2 + C_6); 106.75 (dd, 1C, $^1J_{C-P}$ = 207.0 Hz, $^2J_{C-F}$ = 33.0 Hz, C═C−P); 125.50 (d, 1C, $^3J_{C-P}$ = 25.5 Hz, C_1); 137.86 (s, 1C, C_4); 147.32 (s, 2C, C_3 + C_5); 151.62 (dd, 1C, $^2J_{C-P}$ = 7.5 Hz, $^3J_{C-F}$ = 3.8 Hz, C═C−P). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 19.50 (d, $^1J_{P-F}$ = 1023.0 Hz, P−F). ^{19}F NMR ($CDCl_3$, 188.5 MHz) δ ppm: 11.88 (d, $^1J_{F-P}$ = 1021.0 Hz, F−P). MS (DCI, NH_3, pos) *m/z*: 308.0 (MNH_4^+, 100%), 291.0 (MH^+, 38%). Anal. ($C_{12}H_{16}FO_5P$) C, H calcd 49.66, 5.56; found 49.66, 5.70. IR (KBr) ν cm^{-1}: 3270 (O−H); 3014 (C−H ethyl); 1616 (C═C ethyl); 1597 (C═C arom); 1253 (*P*═O); 1159 (P−O). UV (EtOH, 26 μM): λ = 318 nm, ϵ = 27000 $mol^{-1}{\cdot}L{\cdot}cm^{-1}$.

5,5′-Bisvanillin (5). To vanillin (10.64 g, 70 mmol) in 700 mL of water was added $FeSO_4$ (0.4 g, 1.4 mmol) under stirring. After heating during 10 min at 50 °C, $Na_2S_2O_8$ (8.93 g, 37.5 mmol) was added. The reaction mixture was stirring at 50 °C for 5 days. The brown precipitate formed was filtered off. The solid was dissolved in NaOH (2 M) solution. HCl (2 M) solution was added and precipitate formed was isolated by filtration (10 g, 95%); mp >270 °C. ^1H NMR (DMSO-d_6, 250 MHz) δ ppm: 3.94 (s, 6H, CH_3O); 7.42 (s, 4H, H_2 + H_6); 9.80 (s, 2H, CHO); 9.89 (s, 2H, OH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 56.50 (s, 2C, CH_3O); 109.70 (s, 2C, C_2); 125.00 (s, 2C, C_5); 128.23 (s, 2C, C_1); 128.62 (s, 2C, C_6); 148.60 (s, 2C, C_4); 150.90 (s, 2C, C_3); 191.60 (s, 2C, CHO). MS (FAB, MNBA) *m/z*: 303.1 (MH^+). Anal. ($C_{16}H_{14}O_6$) C, H calcd 63.52, 4.63; found 63.46, 4.61. IR (KBr) ν cm^{-1}: 3186 (O−H); 1672 (C═O); 1587 (C═C arom); 1454 (C═C arom). UV (EtOH + 0.2% DMSO, 25 μM): λ = 303 nm, ϵ = 14300 $mol^{-1}{\cdot}L{\cdot}cm^{-1}$.

5,5′-bis(4-*tert*-butyldimethylsilyloxy-3-methoxybenzaldehyde) (6). *Tert*-butyldimethylsilyl chloride (1.25 g, 8.28 mmol) and imidazole (1.13 g, 16.56 mmol) were added to a solution of 5,5′-bisvanillin **5** (1.0 g, 3.31 mmol) in DMF (10 mL) under N_2. The reaction mixture was stirred at room temperature for 3 h, then poured into 5% aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc (3 × 50 mL) and the combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated as brown oil. The residue was dissolved in EtOAc (10 mL), filtered through a short pad of silica, and concentrated. Silica gel chromatography (7/3 petroleum ether/EtOAc) gave **6** (1.24 g, 70%) as a crystals; mp = 183−185 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 0.02 (s, 12H, $Si(CH_3)_2$); 0.61 (s, 18H, tBu); 3.90 (s, 6H, CH_3O); 7.41 (d, 2H, $^4J_{H2-H6}$ = 2.0 Hz, H_2); 7.47 (d, 2H, $^4J_{H6-H2}$ = 1.8 Hz, H_6); 9.84 (s, 2H, CHO). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: −4.28 (s, 4C, $Si(CH_3)_2$); 18.34 (s, 2C, C_q tBu); 25.16 (s, 6C, CH_3 tBu); 55.20 (s, 2C, CH_3O); 108.68 (s, 2C, C_2); 129.40 (s, 2C, C_6); 129.46 (s, 2C, C_5); 129.67 (s, 2C, C_1); 149.10 (s, 2C, C_4); 151.06 (s, 2C, C_3); 190.96 (s, 2C, CHO). MS (ES, pos) *m/z*: 531.5 (MH^+).

Diethyl(*E*)-2-[4,4′-di*tertio*butyldimethylsilyloxy-1′-(*E*)-(diethoxyphosphoryl)vinyldimethoxy-5,5′-biphenyl]vinylphosphonate (7). Compound **7** was synthesized from **6** (0.2 g, 3.77 ×10^{-4} mol) as compound **1**. The residue was purified by silica gel chromatography (EtOAc) to give pure **7** (0.26 g, 86%) as a white solid; mp = 181−183 °C. ^1H NMR ($CDCl_3$) δ ppm: −0.11 (s, 6H, $SiCH_3$); −0.02 (s, 6H, $SiCH_3$); 0.57 (s, 18H, tBu); 1.29 (t, 12H, $^3J_{H-H}$ = 7.3 Hz, CH_3CH_2O); 3.79 (s, 6H, CH_3O); 4.05 (q, 8H, $^3J_{H-H}$ = 7.3 Hz, CH_3CH_2O); 6,02 (dd, 2H, $^3J_{H-H}$ = $^2J_{H-P}$ = 17.7 Hz, CH═CH-P); 6.91 (s, 2H, H_2); 7.05 (s, 2H, H_6); 7.36 (dd, 2H, $^3J_{H-H}$ = 17.4 Hz, $^3J_{H-P}$ = 22.5 Hz, CH═CH-P). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: −4.54 (s, 2C, $SiCH_3$); −4.22 (s, 2C, $SiCH_3$); 16.40 (d, 4C, $^3J_{C-P}$ = 6.0 Hz, CH_3CH_2O); 18.35 (s, 2C, C_q tBu); 25.29 (s, 6C, CH_3 tBu); 55.10 (s, 2C, CH_3O); 61.70 (d, 4C, $^2J_{C-P}$ = 5.3 Hz, CH_3CH_2O); 109.56 (s, 2C, C_2); 111.00 (d, 2C, $^1J_{C-P}$ = 191.0 Hz, C═C−P); 124.36 (s, 2C, C_6); 127.15 (d, 2C, $^3J_{C-P}$ = 23.3 Hz, C_1); 130.19 (s, 2C, C_5); 145.01 (s, 2C, C_4); 148.83 (d, 2C, $^2J_{C-P}$ = 6.8 Hz, C═C−P); 150.59 (s, 2C, C_3). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 20.53 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 799.9 (MH^+). IR (KBr) ν cm^{-1}: 2928 (C−H ethyl); 1616 (C═C ethyl); 1573 (C═C arom); 1495 (C═C arom); 1233 (*P*═O); 1155 (P−O).

Diethyl(*E*)-2-[4,4′-dihydroxy-1′-(*E*)-(diethoxyphosphoryl)vinyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonate (8). To bisphosphonate **7** (53 mg, 6.63×10^{-2} mmol) in freshly distilled THF (1 mL) under N_2 were added Et_3N (15 μL, 11.07×10^{-2} mmol) and $Et_3N \cdot 3HF$ (9 μL, 5.5×10^{-2} mmol). The reaction mixture was stirred at room temperature for 1 h and then quenched by addition of EtOAc. Aqueous HCl (1 M) was added until pH = 4. Aqueous layer was extracted with EtOAc (3 × 2 mL). The combined extracts were dried (Na_2SO_4) and concentrated under vacuum. Silica gel chromatography (EtOAc/MeOH 95/5) gave **8** as a white solid (21 mg, 57%); mp = 187−189 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.34 (t, 12H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 3.95 (s, 6H, CH_3O); 4.11 (q, 8H, $^3J_{H-H}$ = $^3J_{H-P}$ = 7.0 Hz, CH_3CH_2O); 6.10 (dd, 2H, $^3J_{H-H}$ = $^2J_{H-P}$ = 17.4 Hz, CH=CH-P); 7.02 (d, 2H, $^4J_{H2-H6}$ = 1.8 Hz, H_2); 7.10 (d, 2H, $^4J_{H6-H2}$ = 1.8 Hz, H_6); 7.43 (dd, 2H, $^3J_{H-H}$ = 17.4 Hz, $^3J_{H-P}$ = 22.6 Hz, CH=CH-P). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.40 (d, 4C, $^3J_{C-P}$ = 6.0 Hz, CH_3CH_2O); 55.19 (s, 2C, CH_3O); 61.81 (d, 4C, $^2J_{C-P}$ = 5.3 Hz, CH_3CH_2O); 108.83 (s, 2C, C_2); 111.32 (d, 2C, $^1J_{C-P}$ = 191.0 Hz, C=C−P); 123.84 (s, 2C, C_5) 124.14 (s, 2C, C_6); 127.13 (d, 2C, $^3J_{C-P}$ = 24.0 Hz, C_1); 145.18 (s, 2C, C_4); 147.41 (s, 2C, C_3); 148.64 (d, 2C, $^2J_{C-P}$ = 7.5 Hz, C=C−P). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 20.31 (s, P-OEt). MS (APCI, pos) *m/z*: 571.1 (MH^+). Anal. ($C_{26}H_{36}O_{10}P_2$) C, H calcd 54.69, 6.31; found 54.51, 6.19. IR (KBr) ν cm^{-1}: 3223 (O−H); 2980 (C−H ethyl); 1612 (C=C ethyl); 1590 (C=C arom); 1464 (C=C arom); 1235 (*P*=O); 1165 (P−O). UV (EtOH, 25 μM): λ = 296 nm, ϵ = 29000 $mol^{-1} \cdot L \cdot cm^{-1}$.

Ethyl(*E*)-2-[4,4′-dihydroxy-1′-(*E*)-(fluoroethoxyphosphoryl)vinyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonofluoridate (9). To bisphosphonodiester **7** (0.1 g, 0.125 mmol) in CH_2Cl_2 (1 mL) under N_2 was added freshly distilled oxalyl chloride (65 μL, 0.75 mmol). The reaction mixture was stirred at room temperature for 40 h. CH_2Cl_2 and oxalyl chloride excess were removed under vacuum. Phosphonochloridate was obtained as a yellow solid (0.097 g, 100%) and used without purification in fluorination step. ^{31}P NMR (D_2O, 81 MHz) δ ppm: 29.95 (s, 2P, P−Cl).

Bisfluorophosphonate **9** was synthesized from bischlorophosphonate just obtained as for compound **4**. The residue was purified by silica gel chromatography (EtOAc/MeOH 95/5) to give pure (**9**) (39 mg, 60%) as a white solid. ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.41 (t, 6H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 3.99 (s, 6H, CH_3O); 4.30 (q, 4H, $^3J_{H-H}$ = $^3J_{H-P}$ = 7.0 Hz, CH_3CH_2O); 6.12 (dd, 2H, $^3J_{H-H}$ = $^2J_{H-P}$ = 17.4 Hz, CH=CH-P); 7.05 (d, 2H, $^4J_{H2-H6}$ = 1.8 Hz, H_2); 7.15 (d, 2H, $^4J_{H6-H2}$ = 1.8 Hz, H_6); 7.58 (dd, 2H, $^3J_{H-H}$ = 17.4 Hz, $^3J_{H-P}$ = 24.4 Hz, CH=CH-P). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.34 (d, 2C, $^3J_{C-P}$ = 6.0 Hz, *C*H_3CH_2O); 56.27 (s, 2C, CH_3O); 63.42 (d, 2C, $^2J_{C-P}$ = 6.0 Hz, CH_3*C*H_2O); 107.20 (dd, 2C, $^1J_{C-P}$ = 206.0 Hz, $^2J_{C-F}$ = 33.0 Hz, *C*=*C*-P); 108.89 (s, 2C, C_2); 123.40 (s, 2C, C_5); 124.71 (s, 2C, C_6); 126.33 (d, 2C, $^3J_{C-P}$ = 25.5 Hz, C_1); 145.81 (s, 2C, C_4); 147.29 (s, 2C, C_3); 151.22 (dd, 2C, $^2J_{C-P}$ = 7.5 Hz, $^3J_{C-F}$ = 3.8 Hz, *C*=C−P). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 19.30 (d, $^1J_{P-F}$ = 1024.0 Hz, P−F). ^{19}F NMR ($CDCl_3$, 188.5 MHz) δ ppm: 11.93 (d, $^1J_{F-P}$ = 1022.0 Hz, F−P). MS (APCI pos) *m/z*: 519.0 (MH^+). Anal. ($C_{22}H_{26}F_2O_8P_2$) C, H calcd 50.93, 5.02; found 50.85, 5.52. IR (KBr) ν cm^{-1}: 3291 (O−H); 3033 (C−H ethyl); 1612 (C=C ethyl); 1590 (C=C arom); 1494 (C=C arom); 1223 (*P*=O); 1174 (P−O). UV (EtOH, 25 μM): λ = 318 nm, ϵ = 22600 $mol^{-1} \cdot L \cdot cm^{-1}$.

Diethyl(*E*)-2-[4,4′-dihydroxy-1′-(*E*)-(fluoroethoxyphosphoryl)vinyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonate (10). To bisphosphonodiester **7** (0.1 g, 0.125 mmol) in CH_2Cl_2 (1 mL) under N_2, was added freshly distilled oxalyl chloride (43 μL, 0.56 mmol). The reaction mixture was stirred at room temperature for 15 h. CH_2Cl_2 and oxalyl chloride excess were removed under vacuum. Monophosphonochloridate was obtained as yellow solid (0.068 g, 100%) and used without purification in fluoration. ^{31}P NMR (D_2O, 81 MHz) δ ppm: 20.56 (s, 1P, P-OEt); 29.95 (s, 1P, P′−Cl). Monophosphonofluoridate **10** was synthesized from monophosphonochloridate just obtained as for compound **4**. The residue was purified by silica gel chromatography (EtOAc/MeOH 95/5) to give pure **10** (28 mg, 41%) as white solid. ^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.34 (t, 6H, $^3J_{H-H}$ = 7.0 Hz, CH_3CH_2O); 1.40 (t, 3H, $^3J_{H'-H'}$ = 7.0 Hz, $CH'_3CH'_2O$); 3,95 (s, 3H, CH_3O); 3.95 (s, 3H, CH'_3O); 4.12 (q, 4H, $^3J_{H-H}$ = $^3J_{H-P}$ = 7.0 Hz, CH_3CH_2O); 4.29 (q, 2H, $^3J_{H'-H'}$ = $^3J_{H'-P'}$ = 7.0 Hz, $CH'_3CH'_2O$); 6.09 (dd, 1H, $^3J_{H-H}$ = $^2J_{H-P}$ = 17.6 Hz, CH=CH-P); 6.10 (dd, 1H, $^3J_{H'-H'}$ = $^2J_{H'-P'}$ = 17.6 Hz, CH′=CH′-P′); 7.02 (s, 1H, H_2); 7.03 (s, 1H, $H_{2'}$); 7.09 (d, 1H, $^4J_{H6-H2}$ = 1.5 Hz, H_6); 7.13 (d, 1H, $^4J_{H6'-H2'}$ = 1.8 Hz, $H_{6'}$); 7.43 (dd, 1H, $^3J_{H-H}$ = 17.3 Hz, $^3J_{H-P}$ = 22.2 Hz, CH=CH-P); 7.56 (dd, 1H, $^3J_{H'-H'}$ = 17.4 Hz, $^3J_{H'-P'}$ = 24.4 Hz, CH′=CH′-P′). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.33 (d, 2C, $^3J_{C-P}$ = 5.3 Hz, CH_3CH_2O); 16.41 (d, 1C, $^3J_{C'-P'}$ = 6.0 Hz, $C'H_3C'H_2O$); 56.20 (s, 1C, CH_3O); 56.23 (s, 1C, $C'H_3O$); 61.81 (d, 2C, $^2J_{C-P}$ = 5.3 Hz, CH_3CH_2O); 63.42 (d, 1C, $^2J_{C'-P'}$ = 6.0 Hz, $C'H_3C'H_2O$); 106.96 (dd, 1C, $^1J_{C'-P'}$ = 206.0 Hz, $^2J_{C'-F}$ = 33.0 Hz, C′=C′−P); 108.82 (s, 1C, C_2); 108.87 (s, 1C, $C_{2'}$); 111.39 (d, 1C, $^1J_{C-P}$ = 191.0 Hz, C=C−P); 123.47 (s, 1C, C_5); 123.91 (s, 1C, $C_{5'}$); 124.12 (s, 1C, C_6); 124.79 (s, 1C, $C_{6'}$); 126.25 (d, 1C, $^3J_{C-P}$ = 25.5 Hz, C_1); 127.11 (d, 1C, $^3J_{C'-P'}$ = 24.0 Hz, $C_{1'}$); 145.20 (s, 1C, C_4); 145.98 (s, 1C, $C_{4'}$); 147.34 (s, 1C, C_3); 147.46 (s, 1C, $C_{3'}$); 148.58 (d, 1C, $^2J_{C-P}$ = 6.8 Hz, C=C−P); 151.33 (dd, 1C, $^2J_{C'-P'}$ = 7.5 Hz, $^3J_{C'-F}$ = 3.8 Hz, C′=C′−P). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 19.42 (d, 1P, $^1J_{P'-F}$ = 1023.0 Hz, P′−F); 20.32 (s, 1P, P-OEt). ^{19}F NMR ($CDCl_3$, 188.5 MHz) δ ppm: 11.85 (d, $^1J_{F-P'}$ = 1022.0 Hz, F−P′). MS (APCI, pos) *m/z*: 545.3 (MH^+). Anal. ($C_{24}H_{31}FO_9P_2$) C, H calcd 52.95, 5.74; found 51.92, 5.83. IR (KBr) ν cm^{-1}: 3400 (O−H); 2987 (C−H ethyl); 1615 (C=C ethyl); 1592 (C=C arom); 1494 (C=C arom); 1270 (*P*=O); 1147 (P−O). UV (EtOH, 20 μM): λ = 318 nm, ϵ = 30700 $mol^{-1} \cdot L \cdot cm^{-1}$.

Diethyl(*E*)-2-[4,4′-di*tertio*butyldimethylsilyloxy-1′-formyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonate (11). To *i*Pr_2NH (0.25 mL, 1.88 mmol) dissolved in dried THF (15 mL) stirred at −78 °C under nitrogen *n*-BuLi (1.6 M in hexane: 1.2 mL, 1.88 mmol) was added. The reaction mixture was stirred for 30 min at −78 °C under N_2. Tetraethyl methylenediphosphonate (0.47 mL, 1.88 mmol) in THF (15 mL) was added to formed LDA solution, and the reaction mixture was stirred 1 h at −78 °C. Bisaldehyde **6** (1 g, 1.88 mmol) in THF was added and mixture was stirred for 4 h at −78 °C then allowed to warm up to room temperature, and refluxed. After 2 h, saturated aqueous NH_4Cl (8 mL) was added, and the aqueous layer was extracted with Et_2O (3 × 15 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (EtOAc/petroleum ether 6/4) to give pure **11** (0.45 g, 54%) as colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: −0.02 (s, 12H, $Si(CH_3)_2$); 0.61 (s, 9H, tBu); 0.63 (s, 9H, tBu′); 1.33 (t, 6H, $^3J_{H-H}$ = 7.1 Hz, CH_3CH_2O); 3.85 (s, 3H, CH_3O); 3.88 (s, 3H, CH'_3O); 4.10 (q, 4H, $^3J_{H-H}$ = $^3J_{H-P}$ = 7.3 Hz, CH_3CH_2O); 6.11 (dd, 1H, $^3J_{H-H}$ = $^2J_{H-P}$ = 17.5 Hz, CH=CH-P); 7.01 (d, 1H, $^4J_{H2-H6}$ = 1.9 Hz, H_2); 7.11 (d, 1H, $^4J_{H6-H2}$ = 1.9 Hz, H_6); 7.38 (d, 1H, $^4J_{H2'-H6'}$ = 1.9 Hz, $H_{2'}$); 7.43 (dd, 1H, $^3J_{H-H}$ = 17.3 Hz, $^3J_{H-P}$ = 22.4 Hz, CH=CH-P); 7.45 (d, 1H, $^4J_{H6'-H2'}$ = 1.9 Hz, $H_{6'}$); 9.82 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: −4.54 (s, 2C, $Si(CH_3)_2$); −4.32 (s, 2C, $Si(C'H_3)_2$); 16.35 (d, 2C, $^3J_{C-P}$ = 6.4 Hz, CH_3CH_2O); 18.28 (s, 1C, C_q tBu); 18.37 (s, 1C, C'_q tBu); 25.14 (s, 3C, CH_3 tBu); 25.15 (s, 3C, $C'H_3$ tBu); 55.03 (s, 1C, CH_3O); 55.07 (s, 1C, $C'H_3O$); 61.63 (d, 2C, $^2J_{C-P}$ = 5.2 Hz, CH_3CH_2O); 108.33 (s, 1C, C_2); 109.69 (s, 1C, $C_{2'}$); 111.16 (d, 1C, $^1J_{C-P}$ = 192.5 Hz, C=C−P); 124.07 (s, 1C, C_6); 127.26 (d, 1C, $^3J_{C-P}$ = 23.7 Hz, C_1); 129.31 (s, 1C, C_5); 129.57 (s, 1C, $C_{1'}$); 129.63 (s, 1C, $C_{6'}$); 130.15 (s, 1C, $C_{5'}$); 144.97 (s, 1C, C_4); 148.62 (d, 1C, $^2J_{C-P}$ = 6.7 Hz, C=C−P); 148.96 (s, 1C, $C_{4'}$); 150.54 (s, 1C, C_3); 151.01 (s, 1C, $C_{3'}$); 190.90 (s, 1C, CHO). ^{31}P NMR ($CDCl_3$, 81 MHz) δ ppm: 20.59 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 665.0 (MH^+). IR (NaCl) ν cm^{-1}: 2930 (C−H aliph); 2857 (C−H ald); 1690 (C=O); 1575 (C=C arom); 1487 (C=C arom); 1251 (*P*=O); 1158 (P−O).

Diethyl(*E*)-2-[4,4′-dihydroxy-1′-formyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonate (12). Compound **12** was synthesized from **11** (0.7 g, 1.06 mmol) as compound **8**. The residue was purified by silica gel chromatography (EtOAc) to give pure **12** (0.35 g, 75%) as a brown solid; mp = 72−74 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.34 (t, 6H, $^3J_{H-H}$ = 6.0 Hz, CH_3CH_2O); 3.93 (s, 3H,

CH_3O); 3.95 (s, 3H, CH'_3O); 4.12 (q, 4H, $^3J_{H-H} = {}^3J_{H-P} = 6.0$ Hz, CH_3CH_2O); 6.09 (dd, 1H, $^3J_{H-H} = {}^2J_{H-P} = 18.0$ Hz, CH=CH-P); 7.02 (s, 1H, H_2); 7.15 (s, 1H, H_6); 7.40 (s, 1H, $H_{2'}$); 7.45 (dd, 1H, $^3J_{H-H} = 18.0$ Hz, $^3J_{H-P} = 21.0$ Hz, CH=CH-P); 7.50 (s, 1H, $H_{6'}$); 9.82 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.40 (d, 2C, $^3J_{C-P} = 6.8$ Hz, CH_3CH_2O); 56.12 (s, 1C, CH_3O); 56.13 (s, 1C, $C'H_3O$); 61.82 (d, 2C, $^2J_{C-P} = 5.3$ Hz, CH_3CH_2O); 107.65 (s, 1C, $C_{2'}$); 108.60 (s, 1C, C_2); 110.61 (d, 1C, $^1J_{C-P} = 191.3$ Hz, C=C−P); 124.31 (s, 1C, C_6); 124.52 (s, 1C, C_4 or $C_{4'}$); 124.82 (s, 1C, $C_{4'}$ or C_4); 126.49 (d, 1C, $^3J_{C-P} = 23.3$ Hz, C_1); 128.23 (s, 1C, $C_{1'}$); 129.91 (s, 1C, $C_{6'}$); 146.83 (s, 1C, C_3); 148.17 (s, 1C, $C_{3'}$ or C_5); 148.70 (s, 1C, C_5 or $C_{3'}$); 148.90 (d, 1C, $^2J_{C-P} = 6.8$ Hz, C=C−P); 151,71 (s, 1C, $C_{5'}$); 190.92 (s, 1C, CHO). ^{31}P NMR ($CDCl_3$, 121 MHz) δ ppm: 20.51 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 454.3 (MNH_4^+). IR (KBr) ν cm^{-1}: 3353 (O−H); 2989 (C−H ethyl); 1678 (C=O); 1613 (C=C ethyl); 1589 (C=C arom); 1280 (*P*=O); 1142 (P−O). UV (EtOH, 25 μM): $\lambda = 311$ nm, $\epsilon = 20600$ mol^{-1}·L·cm^{-1}.

Ethyl(*E*)-2-[4,4′-dihydroxy-1′-formyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonofluoridate (13). To phosphonodiester **12** (0.1 g, 0.151 mmol) under N_2 was added freshly distilled oxalyl chloride (2.4 mL, 30.2 mmol). The reaction mixture was stirred at room temperature for 3 h. Oxalyl chloride excess was removed under vacuo. Phosphonochloridate was obtained as yellow solid and used without purification in fluorination step. ^{31}P NMR (D_2O, 81 MHz) δ ppm: 29.27 (s, P−Cl). Monophosphonofluoridate **13** was synthesized from phosphonochloridate just obtained as for compound **4**. The residue was purified by silica gel chromatography (EtOAc/MeOH 95/5) to give pure **13** (45 mg, 72%) as a white solid; mp = 84−86 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.33 (t, 3H, $^3J_{H-H} = 7.0$ Hz, CH_3CH_2O); 3.89 (s, 3H, CH_3O); 3.91 (s, 3H, CH'_3O); 4.22 (q, 2H, $^3J_{H-H} = {}^3J_{H-P} = 7.0$ Hz, CH_3CH_2O); 6.05 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^2J_{H-P} = 19.3$ Hz, CH=CH-P); 6.98 (d, 1H, $^4J_{H2-H6} = 1.7$ Hz, H_2); 7.09 (d, 1H, $^4J_{H6-H2} = 1.6$ Hz, H_6); 7.37 (d, 1H, $^4J_{H2'-H6'} = 1.7$ Hz, $H_{2'}$); 7.41 (d, 1H, $^4J_{H6'-H2'} = 1.8$ Hz, $H_{6'}$); 7.49 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^3J_{H-P} = 24.6$ Hz, CH=CH-P); 9.76 (s, 1H, CH′O). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 16.31 (d, 1C, $^3J_{C-P} = 6.0$ Hz, CH_3CH_2O); 56.26 (s, 1C, CH_3O); 56.35 (s, 1C, $C'H_3O$); 63.48 (d, 1C, $^2J_{C-P} = 6.0$ Hz, CH_3CH_2O); 107.02 (dd, 1C, $^1J_{C-P} = 206.0$ Hz, $^2J_{C-F} = 33.0$ Hz, C=C−P); 108.05 (s, 1C, C_2); 108.95 (s, 1C, $C_{2'}$); 123.10 (s, 1C, C_5); 123.16 (s, 1C, $C_{5'}$); 124.80 (s, 1C, C_6); 126.23 (d, 1C, $^3J_{C-P} = 26.0$ Hz, C_1); 129.19 (s, 1C, $C_{1'}$); 129.44 (s, 1C, $C_{6'}$); 146.01 (s, 1C, C_4); 147.36 (s, 1C, $C_{4'}$); 147.69 (s, 1C, C_3); 149.08 (s, 1C, $C_{3'}$); 151.31 (dd, 1C, $^2J_{C-P} = 7.9$ Hz, $^3J_{C-F} = 4.0$ Hz, C=C−P); 190.93 (s, 1C, C′HO). ^{31}P NMR ($CDCl_3$. 81 MHz) δ ppm: 19.35 (d, 1P, $^1J_{P-F} = 1024.0$ Hz, P−F). ^{19}F NMR ($CDCl_3$, 188.5 MHz) δ ppm: 11.80 (d, $^1J_{F-P} = 1024.0$ Hz, F−P). MS (DCI, NH_3, pos) *m/z*: 411.0 (MH^+, 100%); 428.0 (MNH_4^+, 72%). Anal. ($C_{19}H_{20}FO_7P$) C, H calcd 55.61, 4.91; found 54.33. 5.24. IR (KBr) ν cm^{-1}: 3375 (O−H); 2976 (C−H ethyl); 1679 (C=O); 1610 (C=C ethyl); 1590 (C=C arom); 1495 (C=C arom); 1269 (*P*=O); 1175 (P−O). UV (EtOH, 25 μM): $\lambda =$ 313 nm, $\epsilon = 20000$ mol^{-1}·L·cm^{-1}.

Diethyl(*E*)-2-[4,4′-dihydroxy-1′-isonicotinoylhydrazonomethyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonate (14). Aldehyde **12** (0.092 g, 0.21 mmol) was dissolved in absolute ethanol (8 mL). Isoniazid (37 mg, 0.21 mmol) was added. Mixture was refluxed for 6 h then warmed to room temperature and concentrated under vacuum. The residue was purified by silica gel chromatography (EtOAc) to give pure **14** (0.095 g, 81%) as an orange solid; mp = 101−103 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.18 (t, 6H, $^3J_{H-H} = 7.0$ Hz, CH_3CH_2O); 3.80 (s, 3H, CH_3O); 3.82 (s, 3H, CH'_3O); 3.93 (qd, 4H, $^3J_{H-H} = 7.1$ Hz, $^3J_{H-P} = 8.1$ Hz, CH_3CH_2O); 6.28 (dd, 1H, $^3J_{H-H} = {}^2J_{H-P} = 18.1$ Hz, CH=CH-P); 7.02 (d, 1H, $^4J_{H2-H6} = 1.8$ Hz, H_2); 7.04 (d, 1H, $^4J_{H6-H2} = 1.8$ Hz, H_6); 7.21 (dd, 1H, $^3J_{H-H} = 17.4$ Hz, $^3J_{H-P} = 22.7$ Hz, CH=CH-P); 7.23 (d, 1H, $^4J_{H2'-H6'} = 2.0$ Hz, $H_{2'}$); 7.26 (d, 1H, $^4J_{H6'-H2'} = 1.9$ Hz, $H_{6'}$); 7.74 (d, 2H, $^3J_{H2''-H3''} = 6.0$ Hz, $H_{2''}$); 8.30 (s, 1H, HC=N); 8.70 (d, 2H, $^3J_{H3''-H2''} = 3.0$ Hz, $H_{3''}$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 16.77 (d, 2C, $^3J_{C-P} = 6.2$ Hz, CH_3CH_2O); 56.27 (s, 1C, CH_3O); 56.43 (s, 1C, $C'H_3O$); 61.49 (d, 2C, $^2J_{C-P} = 5.4$ Hz, CH_3CH_2O); 107.54 (s, 1C, C_2); 109.40 (s, 1C, $C_{2'}$); 110.41 (d, 1C, $^1J_{C-P} = 188.3$ Hz, C=C−P); 121.99 (s, 2C, $C_{2''} + C_{6''}$); 123.96 (s, 2C, $C_5 + C_{5'}$); 124.85 (s, 1C, $C_{1'}$); 126.29 (d, 1C, $^3J_{C-P} = 8.3$ Hz, C_1); 125.16 (s, 1C, $C_{6'}$); 125.26 (s, 1C, C_6); 141.21 (s, 1C, $C_{1''}$); 148.81 (d, 1C, $^2J_{C-P} = 6.4$ Hz, C=C−P); 149,02 (s, 2C, $C_4 + C_{4'}$); 149.18 (s, 2C, $C_3 + C_{3'}$); 150.38 (s, 2C, $C_{3''} + C_{5''}$); 150.72 (s, 1C, HC=N); 161.73 (s, 1C, C=O). ^{31}P NMR (DMSO-d_6, 121,5 MHz) δ ppm: 20.86 (s, P-OEt). MS (DCI, NH_3, pos) *m/z*: 556.2 (MH^+). IR (NaCl) ν cm^{-1}: 3426 (O−H); 3018 (C−H ethyl); 1661 (*C*=N); 1652 (C=O); 1615 (C=C ethyl); 1552 (C=C arom); 1489 (C=C arom); 1272 (*P*=O); 1151 (P−O). UV (EtOH, 25 μM): $\lambda =$ 327 nm, $\epsilon = 22000$ mol^{-1}·L·cm^{-1}.

Ethyl(*E*)-2-[4,4′-dihydroxy-1′-isonicotinoylhydrazonomethyl-3,3′-dimethoxy-5,5′-biphenyl]vinylphosphonofluoridate (15). Compound **15** was synthesized from **13** (0.045 g, 0.11 mmol) as compound **14**. The residue was purified by silica gel chromatography (EtOAc) to give pure **12** (0.04 g, 69%) as a yellow solid. ^{1}H NMR (CD_3OD, 300 MHz) δ ppm: 1.12 (t, 3H, $^3J_{H-H} = 7.0$ Hz, CH_3CH_2O); 3.66 (s, 3H, CH_3O); 3.68 (s, 3H, CH'_3O); 3.98 (qd, 2H, $^3J_{H-H} = 7.1$ Hz, $^3J_{H-P} = 8.6$ Hz, CH_3CH_2O); 6.05 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^2J_{H-P} = 20.3$ Hz, CH=CH-P); 6.80 (d, 1H, $^4J_{H2-H6} = 1.8$ Hz, H_2); 6.82 (d, 1H, $^4J_{H6-H2} = 1.7$ Hz, H_6); 6.97 (d, 1H, $^4J_{H2'-H6'} = 1.8$ Hz, $H_{2'}$); 7.39 (d, 1H, $^4J_{H6'-H2'} = 1.8$ Hz, $H_{6'}$); 7.58 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^3J_{H-P} = 25.0$ Hz, CH=CH-P); 7.86 (d, 2H, $^3J_{H2''-H3''} = 5.3$ Hz, $H_{2''}$); 7.98 (s, 1H, HC=N); 8.56 (d, 2H, $^3J_{H3''-H2''} = 3.4$ Hz, $H_{3''}$). ^{13}C NMR (CD_3OD, 75 MHz) δ ppm: 15.91 (d, 1C, $^3J_{C-P} = 6.0$ Hz, CH_3CH_2O); 55.87 (s, 1C, CH_3O); 56.00 (s, 1C, $C'H_3O$); 63.76 (d, 1C, $^2J_{C-P} = 6.0$ Hz, CH_3CH_2O); 105.97 (dd, 1C, $^1J_{C-P} = 206.0$ Hz, $^2J_{C-F} = 32.6$ Hz, C=C−P); 107.82 (s, 1C, $C_{2'}$); 109.45 (s, 1C, C_2); 122.45 (s, 2C, $C_{2''} + C_{6''}$); 124.96 (s, 1C, C_5); 125.08 (s, 1C, $C_{5'}$); 125.35 (s, 1C, $C_{1'}$); 125.39 (d, 1C, $^3J_{C-P} = 25.8$ Hz, C_1); 125.62 (s, 1C, C_6); 125.75 (s, 1C, $C_{6'}$); 142.13 (s, 1C, $C_{1''}$); 147.17 (s, 1C, C_4); 147.58 (s, 1C, $C_{4'}$); 148.38 (s, 1C, C_3); 148.51 (s, 1C, $C_{3'}$); 149.41 (s, 1C, HC=N); 150.65 (s, 2C, $C_{3''} + C_{5''}$); 152.02 (dd, 1C, $^2J_{C-P} = 7.7$ Hz, $^3J_{C-F} = 4.1$ Hz, C=C−P); 162.16 (s, 1C, C=O). ^{31}P NMR (CD_3OD, 81 MHz) δ ppm: 22.65 (d, $^1J_{P-F} = 1017.0$ Hz, P−F). ^{19}F NMR (CD_3OD, 188.5 MHz) δ ppm: 12.75 (d, $^1J_{F-P} = 1015.0$ Hz, F−P). MS (DCI, NH_3, pos) *m/z*: 530.5 (MH^+). IR (NaCl) ν cm^{-1}: 3410 (O−H); 2927 (C−H ethyl); 1667 (*C*=N, C=O); 1615 (C=C ethyl); 1587 (C=C arom); 1494 (C=C arom); 1284 (*P*=O); 1152 (P−O). UV (EtOH, 25 μM): $\lambda =$ 325 nm, $\epsilon = 18000$ mol^{-1}· L· cm^{-1}.

Diethyl(*E*)-2-[4,4′-dihydroxy-3,3′-dimethoxy-1′-(phthalazin-1-yl)-hydrazonomethyl-5,5′-biphenyl]vinylphosphonate hydrochloride (16). Aldehyde **12** (0.092 g, 0.21 mmol) was dissolved in absolute ethanol (8 mL). Hydralazine hydrochloride (0.041 g, 0.21 mmol) was added. Mixture was refluxed for 6 h then warmed to room temperature and concentrated under vacuo. The residue was purified by silica gel chromatography (EtOAc) to give pure **16** (0.12 g, 93%) as an orange solid; mp = 195−197 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.26 (t, 6H, $^3J_{H-H} = 7.1$ Hz, CH_3CH_2O); 3.90 (s, 3H, CH_3O); 3.96 (s, 3H, CH'_3O); 4.00 (qd, 4H, $^3J_{H-H} = 7.1$ Hz, $^3J_{H-P} = 8.1$ Hz, CH_3CH_2O); 6.41 (dd, 1H, $^3J_{H-H} = {}^2J_{H-P} = 18.0$ Hz, CH=CH-P); 7.09 (d, 1H, $^4J_{H2-H6} = 1.9$ Hz, H_2); 7.24 (d, 1H, $^4J_{H6-H2} = 1.8$ Hz, H_6); 7.34 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^3J_{H-P} = 22.8$ Hz, CH=CH-P); 7.35 (d, 1H, $^4J_{H2'-H6'} = 1.9$ Hz, $H_{2'}$); 7.81 (d, 1H, $^4J_{H6'-H2'} = 1.9$ Hz, $H_{6'}$); 7.72 (m, 4H, $H_{4''-7''}$); 8.06 (s, 1H, HC=N); 8.37 (s, 1H, $H_{2''}$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 16.77 (d, 2C, $^3J_{C-P} = 6.2$ Hz, CH_3CH_2O); 56.54 (s, 1C, CH_3O); 56.63 (s, 1C, $C'H_3O$); 61.54 (d, 2C, $^2J_{C-P} = 5.4$ Hz, CH_3CH_2O); 109.22 (s, 1C, C_2); 109.63 (s, 1C, $C_{2'}$); 111.02 (d, 1C, $^1J_{C-P} = 188.5$ Hz, C=C−P); 116.72 (s, 1C, $C_{8''}$); 123.17 (s, 1C, $C_{3''}$); 124.03 (s, 1C, $C_{6'}$); 125.21 (s, 1C, C_6); 125.61 (s, 1C, $C_{7''}$); 125.63 (s, 1C, $C_{1'}$); 125.65 (d, 1C, $^3J_{C-P} = 23.9$ Hz, C_1); 126.20 (s, 1C, C_5); 126.44 (s, 1C, $C_{5'}$); 126.86 (s, 1C, $C_{4''}$); 132.13 (s, 1C, $C_{6''}$); 132.56 (s, 1C, $C_{5''}$); 137.80 (s, 1C, $C_{2''}$); 147.03 (s, 1C, C_4); 147.44 (s, 1C, $C_{4'}$); 148.06 (s, 1C, C_3); 148.54 (s, 1C, $C_{3'}$); 148.69 (d, 1C, $^2J_{C-P} = 5.6$ Hz, C=C−P); 153.99 (s, 1C, $C_{1''}$); 154.22 (s, 1C, HC=N). ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ ppm: 20.67 (s, P-OEt). MS (ES) *m/z*: 577.3 M − H^+. IR (NaCl) ν cm^{-1}: 3399 (O−H); 1650

(*C*═N); 1206 (*P*═O); 1144 (P−O). UV (EtOH, 25 μM): $\lambda = 288$ nm, $\epsilon = 25400$ mol^{-1}·L·cm^{-1}.

Ethyl(*E*)-2-[4,4′-dihydroxy-3,3′-dimethoxy-1′-(phthalazin-1-yl-)hydrazonomethyl-5,5′-biphenyl]vinylphosphonofluoridate hydrochloride (17). Compound **17** was synthesized from **13** (80 mg, 0.19 mmol) as compound **16**. The residue was purified by silica gel chromatography (EtOAc) to give pure **17** (93 mg, 83%) as a yellow solid; mp = 223−225 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ ppm: 1.23 (t, 3H, $^3J_{H-H} = 7.1$ Hz, CH_3CH_2O); 3.80 (s, 3H, CH_3O); 3.87 (s, 3H, CH'_3O); 4.12 (qd, 2H, $^3J_{H-H} = 7.2$ Hz, $^3J_{H-P} = 8.5$ Hz, CH_3CH_2O); 6.21 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^2J_{H-P} = 20.3$ Hz, CH═CH-P); 6.99 (d, 1H, $^4J_{H2-H6} = 1.6$ Hz, H_2); 7.13 (d, 1H, $^4J_{H6-H2} = 1.7$ Hz, H_6); 7.18 (d, 1H, $^4J_{H2'-H6'} = 1.8$ Hz, $H_{2'}$); 7.40 (dd, 1H, $^3J_{H-H} = 17.5$ Hz, $^3J_{H-P} = 25.0$ Hz, CH═CH-P); 7.67 (d, 1H, $^4J_{H6'-H2'} = 1.8$ Hz, $H_{6'}$); 8.00 (m, 4H, $H_{4''-7''}$); 8.39 (s, 1H, HC═N); 8.74 (s, 1H, $H_{2''}$). ^{13}C NMR (CD_3OD, 75 MHz) δ ppm: 15.92 (s, 1C, CH_3CH_2O); 55.90 (s, 1C, CH_3O); 55.93 (s, 1C, $C'H_3O$); 63.73 (d, 1C, $^2J_{C-P} = 6.8$ Hz, CH_3CH_2O); 105.08 (dd, 1C, $^1J_{C-P} = 206.3$ Hz, $^2J_{C-F} = 31.5$ Hz, C═C−P); 108.36 (s, 1C, C_2); 108.95 (s, 1C, $C_{2'}$); 123.89 (s, 1C, C_6); 124.31 (s, 1C, C_5); 125.17 (s, 1C, $C_{7''}$); 125.39 (s, 1C, $C_{4''}$); 125.64 (d, 1C, $^3J_{C-P} = 23.5$ Hz, C_1); 125.77 (s, 1C, $C_{8''}$); 126.55 (s, 1C, $C_{6'}$); 126.57 (s, 1C, $C_{5'}$); 126.60 (s, 1C, $C_{3''}$); 127.44 (s, 1C, $C_{1'}$); 132.04 (s, 1C, $C_{6''}$); 132.56 (s, 1C, $C_{5''}$); 138.87 (s, 1C, HC═N); 146.08 (s, 1C, C_4); 147.00 (s, 1C, $C_{4'}$); 148.20 (s, 1C, $C_{1''}$); 148.27 (s, 1C, C_3); 148.98 (s, 1C, $C_{3'}$); 152.26 (m, 1C, C═C−P); 154.45 (s, 1C, $C_{2''}$). ^{31}P NMR (CD_3OD, 121.5 MHz) δ ppm: 20.65 (d, $^1J_{P-F} = 1021.6$ Hz, P−F). ^{19}F NMR (CD_3OD, 188.5 MHz) δ ppm: 14.30 (d, $^1J_{F-P} = 1023.5$ Hz, F−P). MS (DCI, NH_3, pos) *m/z*: 553.2 (MH^+). IR (NaCl) ν cm^{-1}: 3399 (O−H); 2937 (C−H ethyl); 1717 (*C*═N); 1614 (C═C ethyl); 1592 (C═C arom); 1492 (C═C arom); 1272 (*P*═O); 1147 (P−O). UV (EtOH, 25 μM): $\lambda = 295$ nm, $\epsilon = 28500$ mol^{-1}·L·cm^{-1}, $\lambda = 373$ nm, $\epsilon = 15000$ mol^{-1}·L·cm^{-1}.

4,4′-Dihydroxy-3,3′-dimethoxy-5,5′-biphenyl-1,1′-dimethylisonicotinoylhydrazone (18). 5,5′-Bisvanillin **5** (0.2 g, 0.66 mmol) was suspended in absolute ethanol (26 mL). Isoniazid (0.23 g, 1.32 mmol) was added. The reaction mixture was refluxed for 6 h and filtered to give pure **18** (0.275 g, 77%) as a brown solid; mp >270 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 3.85 (s, 6H, CH_3O); 7.06 (d, 2H, $^4J_{H2-H6} = 1.8$ Hz, H_2); 7.34 (d, 2H, $^4J_{H6-H2} = 1.7$ Hz, H_6); 7.65 (d, 4H, $^3J_{H2'-H3'} = 6.1$ Hz, $H_{2'}$); 8.31 (s, 2H, HC═N); 8.61 (d, 4H, $^3J_{H3'-H2'} = 6.1$ Hz, $H_{3'}$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.97 (s, 2C, CH_3O); 107.81 (s, 2C, C_2); 121.82 (s, 4C, $C_{2'} + C_{6'}$); 125.00 (s, 2C, C_1); 125.11 (s, 2C, C_5); 125.21 (s, 2C, C_6); 140.96 (s, 2C, $C_{1'}$); 146.91 (s, 2C, C_4); 148.42 (s, 2C, C_3); 150.11 (s, 2C, HC═N); 150.45 (s, 4C, $C_{3'} + C_{5'}$); 162.11 (s, 2C, C═O). MS (DCI, NH_3, pos) *m/z*: 541.3 (MH^+). IR (KBr) ν cm^{-1}: 3215 (O−H); 2967 (C−H ethyl); 1652 (*C*═N, C═O); 1594 (C═C); 1550 (C═C arom); 1489 (C═C arom). UV (EtOH + 0.2% DMSO, 25 μM): $\lambda = 337$ nm, $\epsilon = 22000$ mol^{-1}·L·cm^{-1}.

4,4′-Dihydroxy-3,3′-dimethoxy-5,5′-biphenyl-1,1′-(diphthalazin-1-yl)methylhydrazone hydrochloride (19). 5,5′-Bisvanillin **5** (0.2 g, 0.66 mmol) was suspended in absolute ethanol (26 mL). Hydralazine hydrochloride (0.26 g, 1.32 mmol) was added. The reaction mixture was refluxed for 6 h and filtrated to give pure **19** (0.38 g, 87%) as a yellow solid; mp >270 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 3.94 (s, 6H, CH_3O); 7.41 (d, 2H, $^4J_{H2-H6} = 1.8$ Hz, H_2); 7.78 (d, 2H, $^4J_{H6-H2} = 1.8$ Hz, H_6); 8.10 (m, 8H, $H_{4'-7'}$); 8.68 (s, 2H, HC═N); 8.92 (s, 2H, $H_{2'}$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 56.55 (s, 2C, CH_3O); 109.70 (s, 2C, C_2); 119.35 (s, 2C, $C_{8'}$); 123.91 (s, 2C, $C_{3'}$); 124.26 (s, 2C, $C_{7'}$); 125.12 (s, 2C, C_6); 125.38 (s, 2C, C_1); 126.61 (s, 2C, $C_{4'}$); 128.25 (s, 2C, C_5); 128.62 (s, 2C, $C_{6'}$); 134.12 (s, 2C, $C_{5'}$); 136.27 (s, 2C, $C_{2'}$); 147.70 (s, 2C, C_4); 148.29 (s, 2C, C_3); 148.46 (s, 2C, $C_{1'}$); 153.75 (s, 2C, HC═N). MS (DCI, NH_3, pos) *m/z*: 587.2 (MH^+). IR (KBr) ν cm^{-1}: 3423 (O−H); 1675 (*C*═N); 1617 (C═C); 1594 (C═C arom); 1473 (C═C arom). UV (EtOH + 0.2% DMSO, 25 μM): $\lambda = 377$ nm, $\epsilon = 34750$ mol^{-1}·L·cm^{-1}, $\lambda = 296$ nm, $\epsilon = 21500$ mol^{-1}·L·cm^{-1}.

Pharmacological Methods. Cell Culture. HMEC-1 line was obtained from Centers for Disease Control (CDC Atlanta, GA)[26] and was a generous gift from Dr. F. Trottein (Institut Pasteur, Lille). Cells were grown in MCDB-131 supplemented with 10% heat inactivated fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Then 24 h before LDL incorporation, cells were starved in serum-free RPMI medium.

LDL Isolation and Oxidation. Effect of the Newly Synthesized Biaryl Agents on Cell Viability. LDL were isolated by ultracentrifugation from the pooled plasma of healthy normolipidemic human subjects and dialyzed against phosphate buffer saline (PBS) containing 100 μmol/L ethylene diamine tetraacetic acid (EDTA), as previously indicated.[27] To evaluate cell-mediated LDL oxidation, HMEC-1 were seeded in 24 multiwell plates. The standard culture medium (on sparse proliferative cells), was removed and replaced by serum-free RPMI-1640 containing native LDL (100 μg apoB/mL) and $CuSO_4$ (1 μM) and incubated at 37 °C for 6 h with cells or in cell-free medium, as previously used.[28] The synthesized compounds dissolved in DMSO were added to the culture medium at variable concentrations, just before beginning the oxidation and in duplicate. At the end of the incubation, LDL-containing medium was immediately used for determining thiobarbituric acid reactive substances (TBARS) formation using the fluorimetric procedures of Yagi.[20] The subsequent toxicity of cell-oxidized LDL (in the absence or presence of the different agents) was evaluated by the MTT test.[13]

Alternatively, to test the direct cytoprotective effect of the newly synthesized molecules (independently of their antioxidant properties), we used either free 4-HNE (50 μM) directly added to the culture medium, or LDL oxidized by (UV + Cu/EDTA) as previously described:[28] briefly, LDL solution [2 mg apoprotein B (apoB)/mL, containing 2 μmol/L $CuSO_4$] was irradiated for 2 h, as a thin film (5 mm) in an open beaker placed 10 cm under the UV−C source (HNS 30W OFR Osram UV−C tube, λ max 254 nm, 0.5 mW/cm^2 determined using a Scientech thermopile model 360001). At the end of the irradiation, aliquots were taken up for analyses and oxidized LDL (200 μg apoB/mL under standard conditions) were immediately incorporated in the culture medium. The cytotoxicity of 4-HNE or UV-oxidized LDL was determined after 24 h by the MTT test as described.[13]

Determination of Foam Cells. Human monocyte/macrophage U937 cells (American Type Culture Collection (ATCC), Camden NJ) were grown in RPMI culture medium containing 10% FCS and antibiotics. For the experiments, U937 were seeded in Petri dishes (100 mm diameter). At subconfluence, the standard culture medium was removed and replaced by fresh RPMI containing native LDL (100 μg/mL), $CuSO_4$ (1 μM), and the different agents used at 10 μM). After 14 h incubation, the LDL-containing culture medium was removed, the cells were fixed in 10% paraformaldehyde for 10 min in PBS, then carefully washed in PBS and stained with 0.5 μg/mL Nile Red as reported.[25] After washing in PBS, the cells were scrapped and the fluorescence was measured spectrofluorometrically (excitation and emission wavelength 460 and 550 nm, respectively). Alternatively, U937 were cultured on glass slides, and after incubation with oxLDL and the different agents were stained with 0.2% oil red O in 60% 2-propanol for 10 min, then treated with hematoxylin for 5 min to stain nuclei and observed microscopically.

Determination of the Protein Carbonyl Content. After incubation overnight with 4-HNE (50 μM) or UV-oxidized LDL (100 μg/mL), HMEC-1 were washed, scraped in PBS, and centrifuged for 10 min, 1500 rpm. The pellets were suspended in 0.7 mL PBS. The protein carbonyl content was analyzed using the conditions reported by Ichihashi and al.[29] Briefly, an aliquot of the protein samples was incubated for 1 h with an equal volume of 0.1% (w/v) 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl at room temperature. The mixture was treated with 0.5 mL of 20% trichloroacetic acid (w/v, final concentration), centrifuged, and the pellet was extracted with ethanol/ethyl acetate (1:1, v/v). The protein sample was dissolved with 2 mL of 8 M guanidine hydrochloride/13 mM EDTA/133 mM Tris solution (pH 7.4), and the UV absorbance was measured at 365 nm. The results were expressed as % of the unstimulated control expressed as arbitrary unit/mg protein.

Computational Chemistry. All molecular modeling was performed using the software programs from Accelrys Software Inc.[30] Dynamics calculations performed with the Discover_3 using ESFF force field and graphical displays generated with Insight Molecular Modeling System.

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Supporting Information Available: Elemental analysis data for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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