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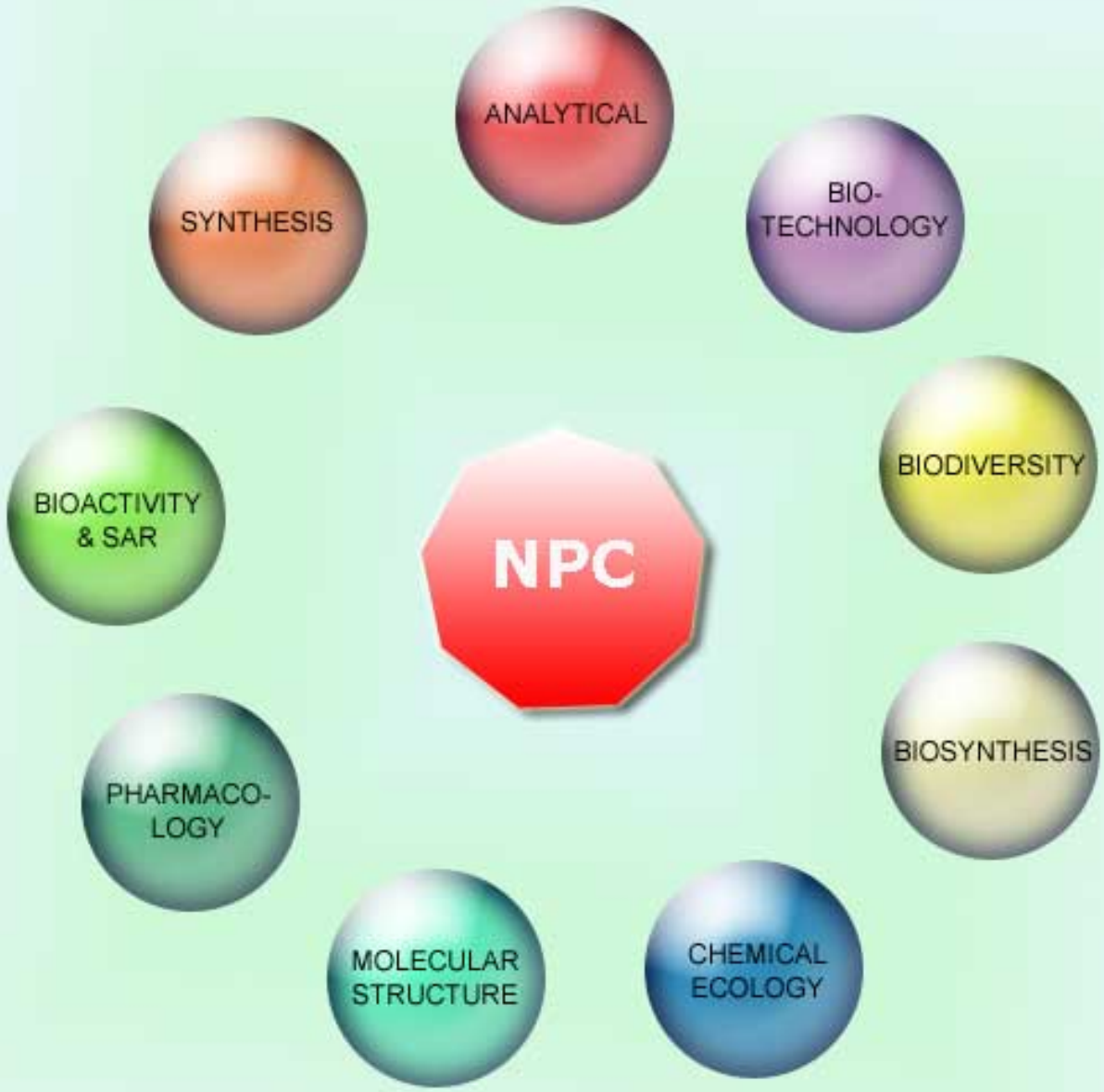

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Chemoenzymatic Synthesis and Some Biological Properties of *O*-phosphoryl Derivatives of (*E*)-resveratrol

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3-*O*-, 3,5-di-*O*- and 4'-*O*-phosphoryl derivatives of (*E*)-resveratrol have been obtained following a chemoenzymatic strategy. Variedly acylated resveratrol derivatives have been obtained first by exploiting regioselective properties of *Pseudomonas cepacea* or *Candida antarctica* lipases in organic solvents. Each acyl-resveratrol was then phosphorylated by the phosphoramidite chemistry protocol and in sequence freed of protective groups, affording the desired *O*-phosphoryl derivative. Following UV-absorption spectroscopic investigation on the interaction of the newly synthesized compounds with DNA, 4'-*O*-phosphorylresveratrol exhibited the best binding affinity. As a result of cytotoxicity tests, 3-*O*-phosphorylresveratrol was more active than resveratrol against DU 145 prostate cancer cells.

Keywords: Resveratrol derivatives, enzymatic acylation, phosphorylation, antiproliferative activity, DNA interaction.

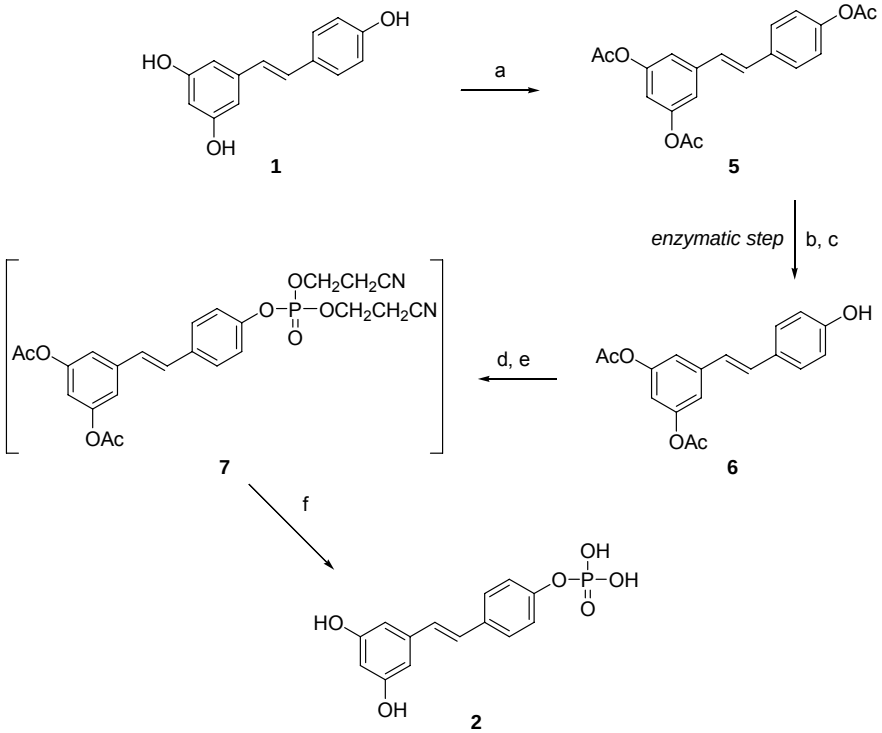
Since the pioneering discovery of the anti-cancer [1] and anti-estrogenic [2] properties of (*E*)-resveratrol [3,4',5-trihydroxy-*trans*-stilbene (**1**), indicated subsequently as resveratrol], biological effects of this compound have been widely studied and reported in the literature [3-5]. Water-soluble analogs of resveratrol are of interest as resveratrol pro-drugs [6-8] and, in general, they may be useful in biomedical and chemical studies where the poor water solubility of resveratrol makes somewhat difficult a direct utilization of the free compound. Actually, although some studies on interaction of resveratrol with nucleic acids have recently been carried out using UV-absorption, Fourier transform IR spectra analysis, and time correlated single photon counting (TCSPC) [9,10], most suitable NMR studies have been hindered so far by the low solubility of the compound in water/deuterium oxide, which is the solvent of choice in NMR experiments with these biopolymers. Chemical attachment of suitable hydrophilic groups to resveratrol would be an elegant solution to these drawbacks, provided that the consequent modification of the molecular framework of the parent compound is minimal. The phosphoryl group is very useful for this end and has been helpfully employed to convert some resveratrol analogs into the relevant water-soluble phosphoryl derivatives [11-13]. Nevertheless, although a preparation of perphosphorylated resveratrol for dermatological purpose has recently been claimed [8], synthesis aimed to obtain individual 4'-*O*- and 3-*O*-phosphoryl conjugates of resveratrol (**2** and **3**, respectively) remains a challenging problem due to the presence in the molecule of the parent compound of three equally reactive hydroxyl groups, shared between two phenyl rings and separated by a highly conjugated central carbon-carbon double bond. These structural features effectively combine to complicate many of the protection/deprotection strategies that are mostly used in organic synthesis. In this context, we devised to synthesize different phosphoryl resveratrols by following a chemoenzymatic strategy where a regioselectively acetylated resveratrol had to be prepared first by exploiting the catalytic properties of lipases working in organic solvents. The acetyl derivative would then undergo phosphorylation at the free hydroxyl(s), and final removing of the acetyl group(s) would yield the desired phosphoryl

derivative of resveratrol. We report here the application of such a strategy by which the preparation of 4'-*O*-, 3-*O*- and 3,5-di-*O*-phosphorylresveratrol (**2**, **3** and **4**, respectively) has been achieved. Some biological properties of individual compounds were also investigated and are here reported as well.

In line with the devised strategy, the key compound 3,5-di-*O*-acetylresveratrol (**6**) had to be available to achieve the synthesis of 4'-*O*-phosphorylresveratrol (**2**) (Scheme 1). To this aim, peracetylated resveratrol (**5**) was synthesized first, which was selectively freed from the acetyl group at 4'-OH following a butanolysis procedure, catalysed by *Pseudomonas cepacea* lipase [14]. After the incubation period, 3,5-di-*O*-acetylresveratrol (**6**) was isolated and identified on the basis of its ^{1}H NMR spectroscopic features.

Compound **6** was then reacted with bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite in the presence of tetrazole to give the intermediate bis(β-cyanoethyl)phosphite ester, which was converted by iodine into the relevant 3,5-(di-*O*-acetyl)-4'-*O*-bis(β-cyanoethyl)phosphorylresveratrol (**7**). Ammonia treatment of crude **7**, under argon and in the presence of sodium metabisulfite, freed it from all protective groups, affording compound **2** in good yield (83% starting from compound **6**). In the ^{13}C NMR spectrum of **2** (recorded in CD_3OD), the C-1', C-3' and C-5' resonances were downfield ($\Delta\delta$ = 5.1 ppm, each), while the C-4' resonance was shifted upfield ($\Delta\delta$ = -6.5 ppm), compared with the relevant signals in the resveratrol spectrum recorded in the same solvent. On account of the effect of phosphorylation on the carbon attached to the phenolic function and on the ortho/para carbons of the phenyl ring, these data indicated that the oxyphosphoryl group was selectively located on the C-4' of the stilbene skeleton of **2**. In accordance with this, in the ^{1}H NMR spectrum of **2**, 3'-H and 5'-H resonances were shifted downfield ($\Delta\delta$ = 0.46 ppm, both), in comparison with the relevant ones in the spectrum of resveratrol. Confirmatory evidence of the location of the phosphoryl group on the 4'-*O*- of **2** came from the analysis of ^{13}C-^{31}P coupling constants (see Experimental). According to the above data, ESI-MS(–) analysis of **2** showed a $[M–H]^-$ ion at *m/z* 307. For the synthesis of both 3-*O*- and 3,5-di-*O*-phosphorylresveratrol (**3** and **4**), 4'-*O*-acetylresveratrol (**8**) was prepared first (Scheme 2).

To this purpose, the ability of the *Candida antarctica* lipase (Novozym 435) to regioselectively acylate only 4'-OH of resveratrol was exploited [14]. Treatment of resveratrol with this lipase, working in



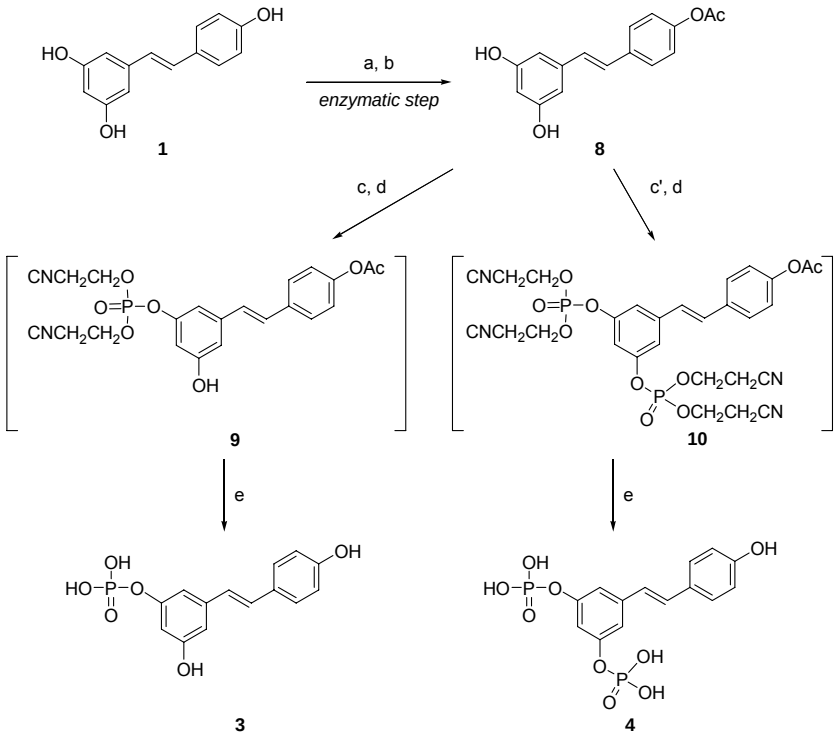
Scheme 1: Reagents and conditions: (a) Ac_2O, Py, TBME, rt, 2 h; (b) *n*-BuOH, *Pseudomonas cepacea* lipase, TBME, 40°C, 400 rpm, 2 h; (c) LiChroprep DIOL, MeOH; (d) $(i\text{-Pr})_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_3CN, rt, 2 h; (e) 0.1M I_2, $THF/H_2O/Py$; (f) 32% NH_4OH, $Na_2S_2O_5$, rt, 5 h.

t-amyl alcohol and in the presence of vinyl acetate, gave the desired **8**, which was isolated by liquid chromatography and identified by comparison of its spectroscopic properties with those reported in the literature [14]. Reaction of **8** with an almost equimolar quantity of bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite and subsequent oxidation led to 3-*O*-[bis(β-cyanoethyl)phosphoryl]-4'-*O*-acetylresveratrol (**9**), from which **3** was obtained following the same ammonia treatment as described for **2** (77% yield, starting from compound **8**). A detailed comparative analysis of ^{13}C and ^{1}H NMR spectroscopic properties of **3** and resveratrol (see Experimental) indicated that in the former the oxyphosphoryl group was located on the C-3 of the stilbene skeleton, allowing the assignment of the compound as 3-*O*-phosphorylresveratrol.

Accordingly, ESI-MS(–) analysis of **3** showed a quasimolecular ion at *m/z* 307. When **8** was reacted with 2.6 molar excess bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite and then oxidized by iodine, 3,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-4'-*O*-acetylresveratrol (**10**) was formed, which was freed of all protective groups by the above cited ammonia treatment. 3,5-Di-*O*-phosphorylresveratrol (**4**) was then obtained, which was isolated by liquid chromatography and analysed (84% yield, starting from **8**). In this case again, evidence for the presence and location of the phosphoryl groups came from the analysis of ^{13}C and ^{1}H NMR spectra of **4** and by the observed ^{13}C-^{31}P coupling constants as well. A quasimolecular ion at *m/z* 387 $[M–H]^-$ in the ESI-MS(–) spectrum of **4** was also in agreement with the expected structure.

Following ion-exchange chromatography, compounds **2**, **3** and **4** were converted into the relevant sodium salts, which all were clearly water-soluble compounds.

In previous work by Malathi et al. [9], analysis of the UV-absorption spectra of calf thymus DNA (ct-DNA) in the presence of varying concentrations of resveratrol revealed a weak complexation of the compound with nucleic acids, exhibited by an hyperchromic effect, with a shift in the λ_{max}. Thereby, we thought it would be interesting to carry out analogous investigations on the newly synthesized phosphoryl derivatives of resveratrol. To this end, the UV spectra of aqueous solutions of ct-DNA at varying concentrations were recorded in parallel experiments, in the presence of fixed amounts of each



Scheme 2: Reagents and conditions: (a) vinyl acetate, *Candida antarctica* lipase, *t*-amyl alcohol, 40°C, 400 rpm, 90 h; (b) LiChroprep DIOL, MeOH; (c) 1.3 molar excess $(i\text{-Pr})_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_3CN, rt, 2 h; (c') 2.6 molar excess $(i\text{-Pr})_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_3CN, rt, 2 h; (d) 0.1M I_2, $THF/H_2O/Py$; (e) 32% NH_4OH, $Na_2S_2O_5$, rt, 5 h.

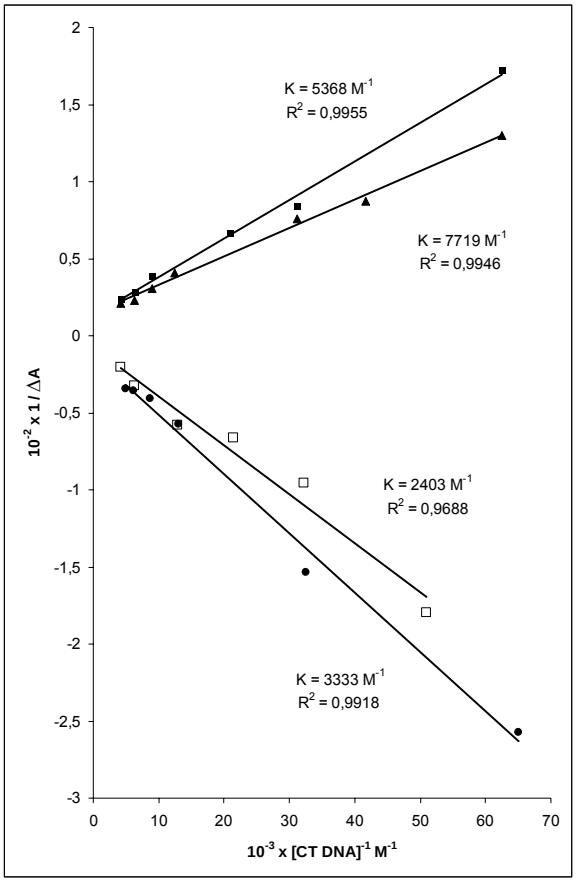


Figure 1: Benesi-Hildebrand plot of the absorption spectra of ct-DNA:**1**(●), ct-DNA:**2**(▲), ct-DNA:**3**(■) and ct-DNA:**4**(□) solutions. ΔA is the absorbance variation at 254 nm (operational details are reported under Experimental).

of the phosphoryl derivatives **2**-**4**. The Benesi-Hildebrand plot of the spectral data obtained for the three ct-DNA:drug systems are shown in Figure 1. Complex formation of mono phosphoryl compounds **2** and **3** with DNA resulted in an hyperchromic effect (8.22 and 6.53 %, respectively at 260 nm) with estimated binding constants of 7719 M^{-1} and 5368 M^{-1}, respectively. An isosbestic point at 290 nm in the spectra of ct-DNA:**2** system and another one at 291 nm found in the case of the ct-DNA:**3** system were suggestive of a 1:1 system in both cases. Differently, in the ct-DNA:**4** case, complex formation resulted in a marked decrease in absorption (3.96 % at 260 nm) associated with an estimated binding constant K= 2403 M^{-1}. No isosbestic point was observed in this case. In a parallel experiment, carried out under the same conditions as above, but in the presence of resveratrol, ct-DNA spectra showed again an hypochromic effect (3.39 % at 260 nm) with an estimated binding constant of 3333 M^{-1}. Also in this case, no isosbestic point was observed. Taken together, these results indicate that phosphorylated compounds **2**-**4**, as well as the parent compound **1**, somehow interact with ct-DNA, the monophosphoryl derivatives showing the best binding affinity. These latter have also shown an opposite chromic effect when compared with that observed in the case of either 3,5-di-*O*-phosphorylresveratrol or resveratrol itself. From these data it appears that the interaction mode of monophosphoryl compounds **2** and **3** with ct-DNA is different from that operating in the case of either resveratrol or its 3,5-di-*O*-phosphoryl derivative, but it is not simple to define unambiguously the details of the involved mechanisms. More appropriate NMR studies are needed to shed light on this topic.

Table 1: Cell growth inhibitory activity of resveratrol (**1**) and phosphoryl derivatives **2**-**4** towards prostate tumor cell lines.

Compds	GI_{50} (μM)[a]	
	BPH-1 or LNCaP	DU 145
1	>100	25 ± 0.3
2	>100	73 ± 1.5
3	>100	15 ± 0.2
4	>100	72 ± 0.9

[a] Values are the mean ± SD of four independent experiments.

Finally, it has been reported that resveratrol possesses a chemopreventive activity against prostate cancer [15], showing also differential effects on growth, cell cycle arrest, and induction of apoptosis in human prostate cancer cell lines [16-19]. In view of these findings, the newly synthesized compounds **2**-**4** were tested for cell-growth inhibitory activity towards DU 145 prostate cancer cells. With this aim, the sodium salts of compounds **2**-**4** and free resveratrol were given in parallel experiments to androgen non-responsive DU 145 human prostate cancer cells, androgen responsive LNCaP cells, and human benign prostatic hyperplasia BPH-1 cells. Normal human fibroblasts were used as non- tumor control cells.

From the results, summarized in Table 1, it was evident that compounds **1**-**4** exerted appreciable cell growth inhibitory activity against the androgen non-responsive DU 145 human prostate cancer cells, while they did not affect significantly either LNCaP or BPH-1 cell lines. Furthermore, resveratrol and compound **3** showed the best activity towards DU 145 cells, the latter being clearly more active than resveratrol. None of the tested compounds, including resveratrol, showed any appreciable toxicity toward normal human fibroblasts.

The reason for the observed higher bioactivity of **3** than that of resveratrol could be tentatively found in the increased availability of the former in the culture

medium, as a consequence of its higher water-solubility. On the other hand, the fact that the parallel 4'-*O*-phosphorylresveratrol (**2**) does not show significant activity seems to be in agreement with studies by others [19], indicating that the 4'-OH group of resveratrol is absolutely required for its activity in inhibiting cell proliferation. On the basis of the above consideration, the quite total inactivity of 3,5-di-*O*-phosphorylresveratrol (**4**) may be indirect evidence that, in addition to the 4'-OH group, at least one chemically available hydroxyl group on the dihydroxylated ring of resveratrol is required for its inhibitory activity. Possibly, the overall activity of the compounds may be explained by a right combination of more than one parameter.

The three newly synthesized compounds will find use in biological experiments where water-soluble resveratrol analogs may be preferred to the parent compound. Furthermore, they are currently used in our laboratory as model compounds in NMR studies on molecular interactions of resveratrol with specific DNA sequences.

Experimental

General: ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer. The chemical shifts are reported as δ (ppm), referenced to the following: (a) TMS as internal standard for the experiments in $CDCl_3$ and CD_3OD; (b) the resonance of the residual HOD (δ = 4.82 ppm) for ^{1}H experiments in D_2O. Unequivocal assignments of ^{1}H and ^{13}C resonances were supported by spin decoupling, NOEDS, APT experiments, and analysis of ^{13}C-^{31}P coupling constants. ESI-MS were recorded with a Finnigan LCQ Deca instrument. Spectrophotometric analyses were carried out on a Cary 500 Scan UV-VIS-NIR spectrometer, equipped with a Peltier temperature controller. All measurements were performed in a 1 cm path length cell. PLC was performed on either silica gel or LiChroprep DIOL (40-63 μm, Merck). HPLC was performed on a Hewlett-Packard 1050 chromatograph equipped with a UV detector set at 307 nm.. Resveratrol was purchased from Cayman Chemical Co. Lipases from *Candida antarctica* (Novozym 435) and *Pseudomonas cepacea* were from Fluka. Calf thymus DNA sodium salt (ct-DNA) was purchased from Sigma. Commercially purchased solvents and reagents were all of reagent quality.

Synthesis of 4'-O-phosphorylresveratrol (2): Resveratrol (**1**, 170 mg, 0.74 mmol) in *t*-butyl methyl ether (TBME) (7.5 mL) was added with pyridine (500 μL) and acetic anhydride (500 μL). The reaction mixture was kept in the dark at room temperature for 2 h and then CH_2Cl_2 (25 mL) was added. Following successive washings with 1N HCl and aq. $NaHCO_3$, 3,4',5-tri-*O*-acetylresveratrol (**5**) was isolated pure from the organic layer in quantitative yield. The ^{1}H NMR spectrum was in agreement with that reported in the literature [20]. Compound **5** underwent enzymatic alcoholysis according to Nicolosi et al. [14]. *Pseudomonas cepacea* lipase (185 mg) and *n*-BuOH (1 mL) were added to a solution of compound **5** (260 mg, 0.73 mmol) in TBME (13.5 mL, warmed up 40°C). The mixture was shaken at 400 rpm for 2 h at 40°C in the dark, then the enzyme was filtered off and the filtrate concentrated *in vacuo*. The product was chromatographed on LiChroprep DIOL with a gradient of MeOH in CH_2Cl_2 from 0 to 10%, affording pure 3,5-di-*O*-acetylresveratrol (**6**) (216 mg, 95% yield). The ^{1}H NMR spectrum of 6 was in agreement with that reported in the literature [14]. A solution of compound **6** (215 mg, 0.69 mmol) in anhydrous CH_3CN (17.5 mL) was kept in a three-neck balloon filled with argon. A solution of bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (244 mg, 0.90 mmol) in anhydrous CH_3CN (3.2 mL) and a 0.45 M tetrazole solution in the same solvent (4.0 mL) were alternately added dropwise. The stirred mixture was allowed to react for 2 h at room temperature, in the dark. The reaction mixture was then treated, under argon atmosphere, with an excess of 0.1 M iodine solution in THF/H_2O/pyridine (9:1:0.1). After the solution was evaporated *in vacuo* to small volume, CH_2Cl_2 (115 mL) was added and the resulting solution was extracted with freshly prepared 5% aqueous sodium metabisulfite (45 mL × 3) to remove residual iodine. The organic layer was washed with water and concentrated *in vacuo*. The residue was taken up in conc. ammonia (100 mL), containing sodium metabisulfite (190 mg) as antioxidant agent, and the suspension was stirred for 5 h at room temperature in the dark. After having removed ammonia *in vacuo* at room temperature, the solution was taken to dryness at 40°C. The residue was purified by semi-preparative HPLC on a Ultrasphere ODS (5 μm; 10 × 250 mm) column, eluting with a gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7.4) from 0 to 40% in 45 min, at a flow rate of 5 mL min^{-1}. After evaporation of CH_3CN *in vacuo*, the aqueous solution

was freeze dried, affording 176 mg of pure 4'-*O*-phosphorylresveratrol (**2**) (83% overall yield).

^{1}H NMR (500 MHz, CD_3OD): 7.42 (2H, d, J_{ortho} = 8.5 Hz, H-2', H-6'), 7.22 (2 H, d, J_{ortho} = 8.5 Hz, H-3', H-5'), 7.00 (1H, d, $J_{\beta,\alpha}$ = 16.2 Hz, H-β), 6.87 (1H, d, $J_{\alpha,\beta}$ = 16.2 Hz, H-α), 6.47 (2H, d, J_{meta} = 1.6 Hz, H-2, H-6), 6.17 (1H, t, J_{meta} = 1.6 Hz, H-4).
^{13}C NMR (125.7 MHz, CD_3OD,): δ 159.7 (C-3, C-5), 151.9 (d, J_{COP} = 6.7 Hz, C-4'), 140.6 (C-1), 135.5 (C-1'), 128.6 (C-2', C-6'), 128.4 (C-β or C-α), 128.3 (C-α or C-β), 121.6 (d, J_{CCOP} = 4.4 Hz, C-3', C-5'), 105.9 (C-2, C-6), 103.2 (C-4).
ESI-MS(–) *m/z*: 153 $[M - 2H]^{2-}$, 307 $[M - H]^-$.

When compound **2** was passed through a column of Dowex-50 W (Na^+ form) and the aqueous eluate was taken to dryness *in vacuo*, the relevant sodium salt was obtained.

^{1}H NMR (500 MHz, D_2O): 7.56 (2H, d, J_{ortho} = 8.4 Hz, H-2', H-6'), 7.26 (2H, d, J_{ortho} = 8.4 Hz, H-3', H-5'), 7.19 (1H, d, $J_{\beta,\alpha}$ = 16.2 Hz, H-β), 7.03 (1H, d, $J_{\alpha,\beta}$ = 16.2 Hz, H-α), 6.70 (2H, d, J_{meta} = 2.0 Hz, H-2, H-6), 6.36 (1H, t, J_{meta} = 2.0 Hz, H-4).

Synthesis of 3-O-phosphorylresveratrol (3): First, 4'-*O*-acetylresveratrol (**8**) was obtained by enzymatic acetylation [14]. A solution of **1** (460 mg, 2.0 mmol) in *t*-amyl alcohol (50 mL) was deaerated with argon and then to this was added vinyl acetate (1.9 mL, 20.5 mmol) and *Candida antarctica* (Novozym 435; 450 mg). The suspension was shaken at 400 rpm for 90 h at 40°C in the dark. The enzyme was then filtered off and the filtrate concentrated *in vacuo*. The residue was chromatographed on LiChroprep DIOL with a gradient of MeOH in CH_2Cl_2 from 0 to 10%, affording pure compound **8** (227 mg, 42% yield). The unreacted **1** was recovered in the more polar eluate (260 mg). The ^{1}H NMR spectrum was in agreement with that reported in the literature [14].

A solution of compound **8** (225 mg, 0.83 mmol) in anhydrous CH_3CN (21.5 mL) was alternately added with a solution of bis(β-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite (298 mg, 1.10 mmol) in anhydrous CH_3CN (3.9 mL) and a 0.45 M tetrazole solution in the same solvent (4.9 mL), dropwise. The reaction mixture was treated according to the procedure reported above for the synthesis of **2**. The totally deprotected product was purified by semi-preparative HPLC, as reported for compound **2**. After evaporation of CH_3CN *in vacuo*, the aqueous solution was freeze dried giving 197 mg of pure 3-*O*-phosphorylresveratrol (**3**) (77% overall yield). The chromatographic purification also afforded 23 mg of compound **4**.

^{1}H NMR (500 MHz, CD_3OD): 7.36 (2H, d, J_{ortho} = 8.5 Hz, H-2', H-6'), 7.02 (1H, d, $J_{\beta,\alpha}$ =16.2 Hz, H-β), 6.95 (1 H, br s, H-2), 6.84 (1H, d, $J_{\alpha,\beta}$ = 16.2 Hz, H-α), 6.76 (2H, d, J_{ortho} = 8.5 Hz, H-3', H-5'), 6.62 (1H, br s, H-6), 6.60 (1H, br s, H-4).
^{13}C NMR (125.7 MHz, CD_3OD): δ 159.2 (C-5), 158.4 (C-4'), 156.0 (d, J_{COP} = 5.5 Hz, C-3), 140.9 (C-1), 130.5 (C-1'), 129.7 (C-β), 128.8 (C-2', C-6'), 126.8 (C-α), 116.5 (C-3', C-5'), 110.7 (d, J_{CCOP} = 4.8 Hz, C-2), 108.8 (C-6), 107.7 (d, J_{CCOP} = 4.5 Hz, C-4).
ESI-MS(–) *m/z*: 153 $[M - 2H]^{2-}$, 307 $[M - H]^-$.

Following ion exchange chromatography on a Dowex-50 W (Na^+ form) column, the sodium salt of **3** was recovered in the aqueous eluate taken to dryness *in vacuo*.

^{1}H NMR (500 MHz, D_2O): 7.60 (2H, d, J_{ortho} = 8.6 Hz, H-2', H-6'), 7.26 (1H, d, $J_{\beta,\alpha}$ =16.4 Hz, H-β), 7.08 (1H, d, $J_{\alpha,\beta}$ = 16.4 Hz, H-α), 7.03 (1H, br s, H-2), 6.99 (2H, d, J_{ortho} = 8.6 Hz, H-3', H-5'), 6.83 (1H, br s, H-6), 6.74 (1H, br s, H-4).

Synthesis of 3,5-di-O-phosphorylresveratrol (4): The synthesis of the title compound was achieved starting from compound **8** (220 mg, 0.81 mmol) following the same procedure described above for compound **3**, except that 2.6 molar excess of bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite and 5.2 molar excess of 0.45 M tetrazole solution were added. After ammonia treatment, the residue was purified by HPLC, as reported above. The eluate was concentrated *in vacuo* and then freeze dried, affording 264 mg of pure 3,5-di-*O*-phosphorylresveratrol (**4**) (84% overall yield).

^{1}H NMR (500 MHz, CD_3OD): 7.37 (2H, d, J_{ortho} = 8.6 Hz, H-2', H-6'), 7.17 (2H, br s, H-2, H-6), 7.10 (1H, d, $J_{\beta,\alpha}$ = 16.3 Hz, H-β), 6.93 (1H, br s, H-4), 6.90 (1H, d, $J_{\alpha,\beta}$ = 16.3 Hz, H-α), 6.78 (2H, d, J_{ortho} = 8.6 Hz, H-3', H-5').
^{13}C NMR (125.7 MHz, CD_3OD): δ 158.6 (C-4'), 155.6 (d, J_{COP} = 5.6 Hz, C-3, C-5), 140.8 (C-1), 130.4 (C-β), 130.2 (C-1'), 128.9 (C-2', C-6'), 126.3 (C-α), 116.6 (C-3', C-5'), 113.9 (d, J_{CCOP} = 3.4 Hz, C-2, C-6), 112.6 (br t, C-4).
ESI-MS(–) *m/z* = 193 $[M - 2H]^{2-}$, 387 $[M - H]^-$, 409 $[M - 2H + Na]^-$.

Compound **4** was also converted into the relevant sodium salt by ion exchange chromatography on Dowex-50 W (Na^+ form).

^{1}H NMR (500 MHz, D_2O): 7.57 (2H, d, J_{ortho} = 8.1 Hz, H-2', H-6'), 7.26 (1H, d, $J_{\beta,\alpha}$ = 16.4 Hz, H-β), 7.22 (2H, br s, H-2, H-6), 7.08 (1H, d, $J_{\alpha,\beta}$ = 16.4 Hz, H-α), 6.97 and 6.96 (partially overlapped signals, 3 H altogether, H-3', H-5'and H-4).

HPLC analysis: Resveratrol and its phosphoryl derivatives **2-4** were checked for their purity by reversed-phase HPLC analysis, using a LiChrospher-100 ODS (5 μm; 4 × 250 mm) column, eluting with two different solvent systems: 1) linear gradient of CH_3CN in 0.1 M triethylammonium acetate (pH 7.4) from 0 to 40% in 45 min, and 2) linear gradient of CH_3CN (containing 0.1% trifluoroacetic acid) in H_2O (containing 0.1% trifluoroacetic acid) from 0 to 40% in 40 min. In both cases, a flow rate of 1 mL min^{-1} was used. The chromatogram profiles were as following. Elution 1): **4**, Rt = 18.1 min; **2**, Rt = 23.0 min; **3**, Rt = 28.1 min; resveratrol, Rt = 43.3 min. Elution 2): **4**, Rt = 14.9 min; **2**, Rt = 19.5 min; **3**, Rt = 23.8 min; resveratrol, Rt = 31.1 min.

UV-absorption analysis: All measurements were performed at 25°C in 10 mM sodium phosphate buffer at pH 7.0 containing 40 mM NaCl and 0.1 mM EDTA. Twice distilled and 0.22 μm filtered water was used for buffer preparation. Stock solution of ct-DNA was prepared by dissolving 1 mg/mL in the buffer, with gently inversion overnight. Prior to use, the stock solution was filtered through a 0.45 μm syringe filter (Millipore). The ct-DNA concentration was determined spectrophotometrically, using the molar extinction coefficient ε_{260} = 6600 M^{-1} cm^{-1} (per mole of nucleotide). The molar extinction coefficients of the sodium salts of 3-*O*-phosphorylresveratrol, 4'-*O*-phosphorylresveratrol, 3,5-di-*O*-phosphorylresveratrol and resveratrol in the buffer were determined as ε_{305} = 24300 M^{-1} cm^{-1}, ε_{314} = 30100 M^{-1} cm^{-1}, ε_{304} = 14600 M^{-1} cm^{-1} and ε_{305} = 28900 M^{-1} cm^{-1}, respectively. The sodium salt of resveratrol was prepared before use by dissolving resveratrol in deaerated, cold, 3 mM NaOH to make a stock solution of 1 mM [22]. The spectroscopic data were collected for a constant concentration of either resveratrol or its derivatives (substrates) and different concentrations of ct-DNA (ligand). The concentrations of substrates were the following: compound **1**, 4.04 μM; compound **2**, 16.27 μM; compound **3**, 18.26 μM; compound **4**, 17.83 μM. The concentrations of the ligand (see Figure 1) were selected in a range large enough to cover much of the binding isotherm. Before the analyses, the samples at different P/D ratios were incubated overnight at 37°C. Then they were scanned between 210-400 nm. The binding constants were determined from the Benesi-Hildebrand plot.

Cell cultures: Human benign prostatic hyperplasia BPH-1 cells, androgen-responsive LNCaP and androgen non-responsive DU-145 human prostate cancer cells, as well as human fibroblasts (not immortalised), used as non tumor control cells, were cultured, as reported previously [18]. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air and transferred to subcultures every 3 days following treatment with trypsin-EDTA. The cells were treated one day before they reached confluence.

Cell growth inhibition assay: To evaluate cell viability the MTT assay described by Mosmann was employed [21]. Briefly, BPH-1, LNCaP, DU-145 cells and human adult fibroblasts (1×10^4) were set up in flat-bottomed 200 μL microplates, and incubated at 37°C in a humidified 5% CO_2/95% air mixture for 24 h. Then, they were treated for 72 h with either individual phosphorylresveratrols (**2-4**) (as sodium salts) or with resveratrol. Mother solutions of phosphorylresveratrols were prepared by directly dissolving them in culture medium, while resveratrol was previously dissolved in the minimum amount of DMSO. In each experiment, DMSO never exceeded 0.1%, and this percentage did not interfere with cell growth. All the compounds were tested at the concentrations of 6.25, 12.5, 25, 50 and 100 μM. Four hours before the end of the incubation period, 20 μL of 0.5% MTT in phosphate buffer saline was added to each microwell. After incubation with the reagent, the supernatant was removed and replaced with 100 μL DMSO. The optical density of each sample was measured with a spectrophotometer reader at λ = 570 nm. Four replicates were performed for each sample.

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