# Synthesis of 8-vinyladenosine 5′-di- and 5′-triphosphate: Evaluation of the diphosphate compound on ribonucleotide reductase

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# Synthesis of 8-vinyladenosine 5′-di- and 5′-triphosphate: evaluation of the diphosphate compound on ribonucleotide reductase

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**Abstract**—The synthesis of 5′-di- and 5′-triphosphate of 8-vinyladenosine to be tested on ribonucleotide reductases requires the modification of known methods. The phosphate group was introduced by treatment with an in situ generated chlorophosphite. Protection of the 2′,3′ diol with acetyl groups suppressed depurination during acid removal of the phosphotriester protecting groups. The di- and triphosphate compounds were obtained by treatment of the activated adenylic acid with phosphate or pyrophosphate anions followed by removal of the acetate protecting groups. Preliminary studies were conducted on *Escherichia coli* ribonucleotide reductase and have shown that the diphosphate compound is efficiently reduced.


## 1. Introduction

Among the large number of C-8-substituted purine nucleoside analogues prepared and so far screened against different tumor cell lines and viruses, 8-vinyladenosine has shown significant anti-tumoral and anti-viral activity.[1,2] In connection with 8-vinyladenosine, we and others[2,3] have proposed that 8-vinyladenosine, after activation by the kinase to its di- or triphosphate may act as a radical trap of the proposed C-2′ radical intermediate mediated by ribonucleotide reductase.[4] Trapping of the incipient radical may lead to inhibition of this crucial enzyme in DNA replication and thus may account for the biological activity. Evaluation of 8-vinyladenosine on ribonucleotide reductases requires the preparation of its di- and triphosphate analogues.

## 2. Results and discussion

Nucleoside di- and triphosphate are ubiquitous biological molecules and thus their analogues could have important therapeutic and diagnostic applications. Nevertheless, the preparation of di- and triphosphate of nucleoside analogues remains a difficult task and none of the methods described is universally satisfactory.[5] Basically, their synthesis can be classified in two approaches. One involves the displacement of a 5′-*O*-leaving group by a nucleophilic phosphorylating reagent, while the other consists of reacting the 5′ hydroxyl function with an electrophilic phosphorylating reagent.

Initially, we applied known and conventional methods that we expected compatible with the chemical reactivity of 8-vinyladenosine. The double bond of 8-vinyladenosine and 8-vinyl purine is electron deficient and is known to add nucleophiles.[1,2,6,7] In a first attempt, the Poulter method was chosen.[8,9] This method, based on the displacement of a 5′-*O*-tosyl group by a di- or triphosphate reagent, has been efficiently used for the preparation of natural di- and triphosphate nucleosides.

The tosyl intermediate **4** required for condensation was prepared from the known protected 8-vinyladenosine **1**[1] as shown in Scheme 1. The 5′-silyl protecting group of compound **1** was selectively cleaved in 78% yield by treatment with $CF_3COOH/H_2O$ (95:5) at 0°C. Introduction of the tosyl group as described by Poulter using tosyl chloride and 4-dimethylaminopyridine as base failed. Thus,

*Keywords*: di- and tri-phosphate synthesis; ribonucleotide reductase; sensitive base.

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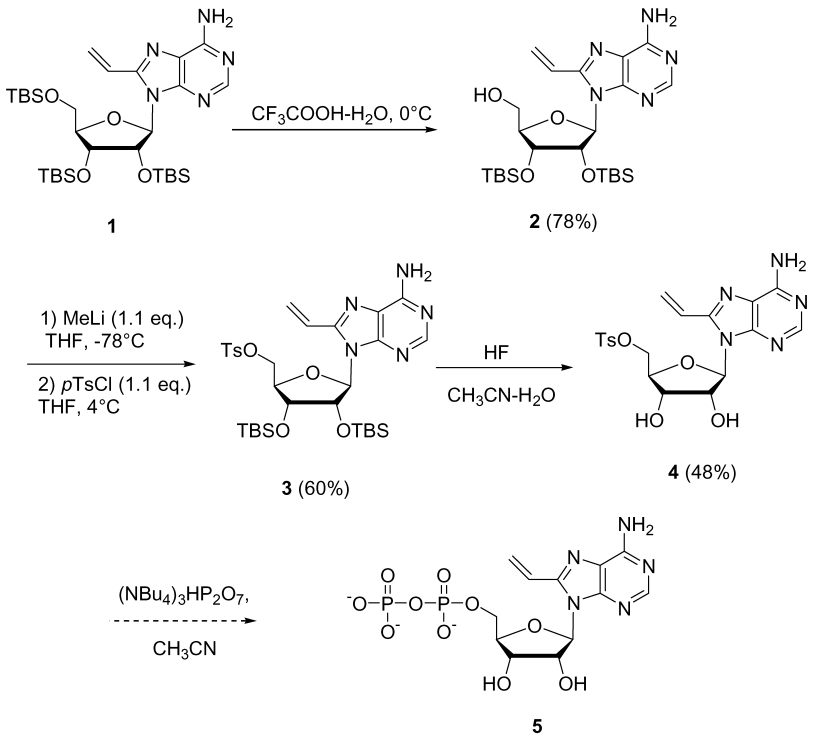



**Scheme 1.** First attempt to synthesize 8-vinyladenosine diphosphate.

the tosylate **3** was prepared by an alternative procedure. Treatment of the alcohol **2** with methyllithium in THF at low temperature and reaction with *para*-toluenesulfonyl chloride afforded compound **3** in 60% yield. Removal of the silyl protecting groups was realised in a mixture of hydrofluoric acid (2%) in water and acetonitrile at room temperature. The modest yield is explained by the difficulties encountered during purification due to the low solubility of product **4** in organic solvents. Compound **4** was treated by tris(tetra-butylammonium) hydrogen-pyrophosphate in acetonitrile but no product was detected even after two day stirring. From this first attempt, it turned out that nucleophilic attack on C-5′ of 8-vinyladenosine is dramatically retarded, since this compound remained unaffected while, in a control experiment, tosyladenosine was consumed after two days. We then focused our attention on another approach.

We first tried the procedure developed by Kovacs and Ötvös[10] for the preparation of acid-sensitive nucleotides in order to prepare the triethyl ammonium salt of the monophosphate intermediate **7** as shown in Figure 1.

Reaction in trimethyl phosphate of 8-vinyladenosine **6** with $POCl_3$ in the presence of Proton Sponge® was slow and afforded four major products in addition to other minor products; whereas, with adenosine, the reaction proceeded to completion. Again this procedure was unsuitable for substrate **6**.

We then explored the phosphoramidite chemistry by procedures previously developed for the phosphate of AZT[11] and modified our synthetic scheme in introducing first the phosphate and later the vinyl group (Schemes 2 and 3).

Selective deprotection of the 5′ protecting group of known compound **8**[1] with TFA in $CH_2Cl_2$ and water afforded the alcohol **9** in 72% yield. When compound **9** was reacted with Perich and Johns phosphitylating reagent,[12] the reduced product **11** was obtained in a low yield (30%) rather than the expected iodo compound **10**. Dehalogenation at C-8 was supported by $^1H$ NMR analysis in $CDCl_3$, a new signal was observed at $\delta$ 8.09 ppm compatible to the chemical shift assigned for the C-8 proton of adenosine derivatives. We have already observed in our laboratory the reduction of an 8-iodoadenine derivative when treated with triethyl phosphite in acidic medium.[13] Other authors have also reported

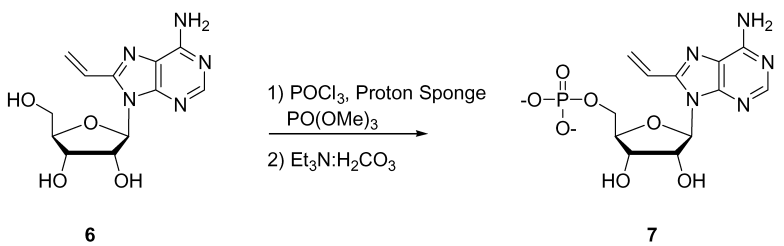
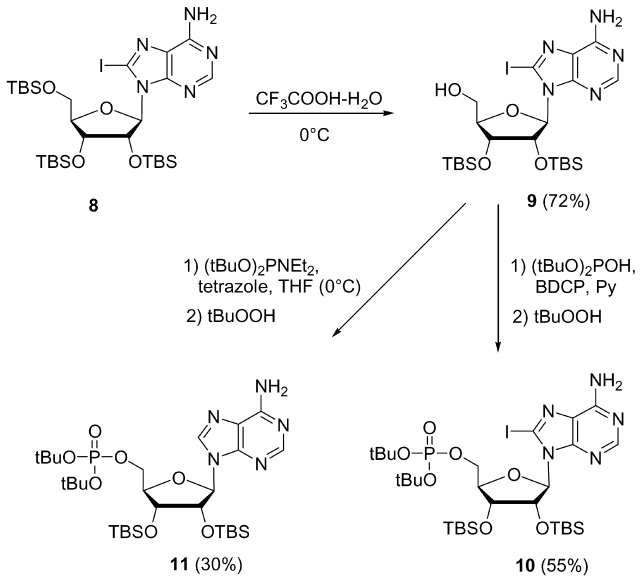



**Figure 1.** Second attempt.



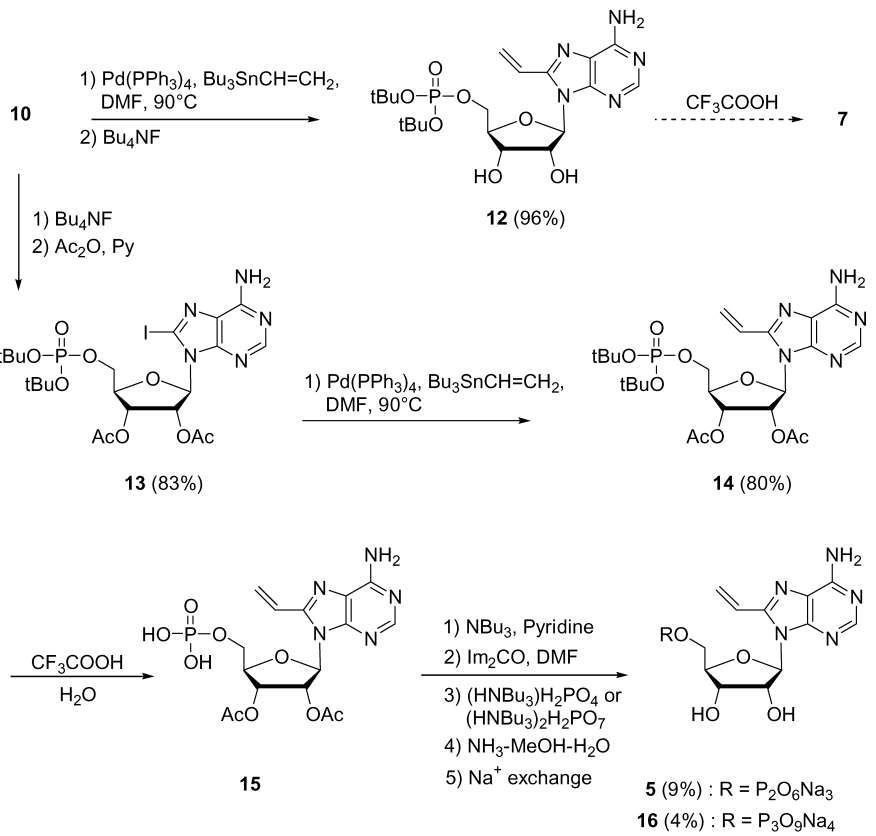

**Scheme 2.** Synthesis of 8-iodoadenosine phosphotriester intermediate.

the reduction of 8-iodoadenosine derivatives with different reagents as for example with thiourea.[14] Furthermore, oxidation of phosphite with iodine is frequently used in oligonucleotide solid phase synthesis and its mechanism has been described. We overcame this problem by introducing the phosphate group on alcohol **9** in pyridine with an in situ generated chlorophosphite.[15] Under these conditions, the phosphate triester **10** was isolated in 55% yield.

The vinyl moiety was then introduced in 80% yield under Still conditions using tributylvinyltin and a catalytic amount of $Pd(PPh_3)_4$ at 90°C.[3,16] The silyl protecting groups were cleaved with $Bu_4NF$ quantitatively. The next step was to remove the *tert*-butyl phosphate protecting groups of compound **12** but attempts with $CF_3COOH$ in water or $CH_2Cl_2$ failed. Likely depurination reaction took place during isolation of the adenylic acid as evidenced by TLC and $^1H$ NMR. Depurination in acid medium of adenosine is well known and glycosidic bond cleavage is even more pronounced for some 8-substituted adenosine derivatives.[17–21]

We anticipated that protection of the 2′,3′ diol **10** with acid resistant and electron attracting protecting groups should retard glycosidic bond cleavage during acid removal of the phosphate triester protecting groups.[22] The silyl groups of compound **10** were then removed quantitatively with fluoride anion. Protection of the hydroxyl functions by acetyl groups under standard conditions gave the diacetate **13** in 83% yield. The vinyl moiety was then introduced as described above. Removal of the *tert*-butyl groups was realised quantitatively in a mixture of TFA/$H_2O$ (95:5). The desired compound was obtained by simple evaporation of the volatiles and was sufficiently pure to be used for the next step as evidenced by TLC and $^1H$ NMR. This result showed that protection of the 2′,3′-hydroxyl functions by acetyl groups suppressed depurination during acidic treatment of the phosphotriester.

Starting from nucleoside monophosphate, many chemical methods are available for the preparation of the corresponding nucleoside di- and triphosphates.[5] For this purpose,




**Scheme 3.** Synthesis of 8-vinyladenosine di- and triphosphate.

Hoard and Ott's procedure was applied on phosphate **15**.[23] The acetyl groups were retained for monophosphate activation with carbonyl diimidazole in order to avoid formation of cyclocarbonate at 2′ and 3′.[24] The stability of the vinyl group was checked under the conditions used for removal of the acetate protecting groups. When 8-vinyladenosine was treated with ammoniac TLC and UV showed that the vinyl group remained unaffected under these conditions. After condensation with phosphate or pyrophosphate anion on the phosphorimidazolate intermediate, the ester protecting groups were removed with ammonia as previously described for solid phase synthesis of nucleoside triphosphates.[25,26] Finally, the sodium salts of the di- and triphosphate of 8-vinyladenosine **5** and **16** were obtained by ion exchange chromatography in 9 and 4% overall yield, respectively (calculated from compound **14**). The structures of products **5** and **16** were ascertained from UV, MS, $^{1}H$ and $^{31}P$ NMR analyses.

Ribonucleotide reductase catalyses the conversion of ribonucleotides to deoxyribonucleotides and thus plays a central role in DNA biosynthesis. In mammals, viruses and some bacteria, such as *Escherichia coli*, the enzyme consists of two subunits named R1 and R2, both required for the activity.[27] The protein R1 contains the binding sites for the substrates which are the purine and pyrimidine ribonucleoside diphosphates and also binding sites for nucleoside triphosphates which function as allosteric effectors.

Since 8-vinyladenosine has shown anti-tumoral and anti-viral activity, we started to investigate the reaction of its diphosphate analogue with ribonucleotide reductase. The model for this study is the *E. coli* enzyme. Figure 2 shows that in the presence of the allosteric effectors ATP and dGTP and an electron transfer chain consisting of thioredoxin and thioredoxin reductase, pure preparations of R1 and R2 are able to catalyse the reduction of 8-vinylADP **5** by NADPH.

The reaction can be monitored spectrophotometrically, since oxidation of NADPH results in the loss of its strong absorption at 340 nm. No oxidation of NADPH could be detected when protein R1 or protein R2 was omitted (data not shown). Moreover, the reduction of 8-vinylADP only occurred when dGTP was present (data not shown). These preliminary results (Fig. 2) demonstrate that 8-vinylADP is recognized by protein R1 substrate sites and efficiently reduced in the presence of dGTP, which is the specific allosteric effector that drives the reduction of the natural substrate ADP. The presence of the vinyl group at the 8-position of adenosine thus does not strongly affect the interaction of the corresponding nucleotide with ribonucleotide reductase. Similar results were previously obtained with the 8-azido analogue.[28]

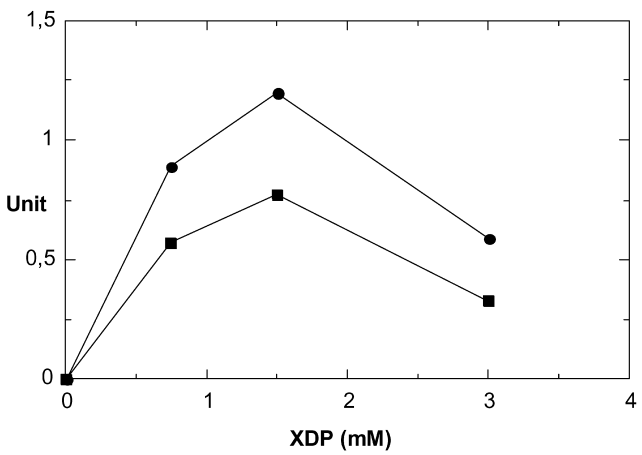



**Figure 2.** Reduction of ADP (●) or 8-vinylADP (■). Assays were performed at 37°C as described in Section 4, in the presence of 1.4 mM ATP and 0.6 mM dGTP. Units are defined as nmol of NADPH oxidized per minute.

## 3. Conclusion

In conclusion, we have described the synthesis of the diphosphate and the triphosphate derivative of the 8-vinyladenosine by modifications of known methods. This new approach may give access to a wide range of new C-8 substituted purine phosphate derivatives because of the versatility of the palladium catalysed reaction used for the introduction of the functionality. Preliminary studies were conducted with the synthesized diphosphate compound on purified *E. coli* ribonucleotide reductase and have shown that the compound is efficiently reduced. These experimental data indicate that the anti-tumoral and anti-viral activity of 8-vinyladenosine can unlikely result from inhibition of ribonucleotide diphosphate reductase. Therefore, the mechanism(s) by which 8-vinyladenosine exerts its cytotoxic effects remains to be solved. Another possible mechanism of action might be the inhibition of DNA synthesis at the polymerase level by 2′-deoxy-8-vinyladenosine 5′-triphosphate after enzymatic phosphorylation and reduction of the adenosine analogue.

## 4. Experimental

### 4.1. General

Melting points were measured on a Reichert microscope and were uncorrected. UV spectra were determined on a Hewlett–Packard 8451A. IR spectra were recorded on a Brucker FT IFS25. NMR spectra were recorded on a Brucker SY (200 or 300 MHz) apparatus. For $^{1}H$ NMR the residual proton signal of the deuteriated solvent was used as an internal reference; for $CDCl_3$ ($\delta$=7.26 ppm), $^{2}H_6$-DMSO ($\delta$=2.50 ppm). For $^{13}C$ NMR, the central $^{13}C$ signal of the deuteriated solvent was used as an internal reference; for $^{2}H_6$-DMSO ($\delta$=39.46 ppm) and for $CD_3OD$ ($\delta$=49 ppm). For $^{31}P$ NMR, the signal of $H_3PO_4$ (85%) was used as an external reference ($\delta$=0 ppm). The chemical shifts are reported in ppm downfield from TMS or external reference. MS were measured on a ZAB apparatus (Fison) by fast atom bombardment (FAB, matrix nitrobenzylalcohol) or on a Bio-Q quadrupole mass spectrometer (Fison) by electron spray mass analysis. Microanalyses were performed by the Strasbourg service of the CNRS analytical department.

Unless otherwise indicated, all reagents were obtained from commercial suppliers and were used without purification. All experiments sensitive to air or/and to moisture were carried out under an argon atmosphere in an oven dried

(120°C) glassware assembled under a stream of argon. Anhydrous solvents were freshly distilled before use: tetrahydrofurane from sodium benzophenone ketyl, pyridine from $CaH_2$ and DMF from $P_2O_5$ (distilled under reduced pressure). Analytical thin-layer chromatography was performed on silica gel precoated TLC plates (Merck, 60, F254). Flash chromatography was performed on silica gel (Merck, 60, 40–63 mesh).

**4.1.1. 8-Vinyl-9-[2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-ribo-pentofuranosyl]-adenine (2).** Compound **1** (1.91 g; 3.0 mmol) was dissolved in a mixture of trifluoroacetic acid and water at 0°C (12 mL, 95:5). The reaction was monitored by TLC (ether). When hydrolysis was completed, the reaction solution was poured into a mixture of $CH_2Cl_2$ and a saturated aqueous solution of $NaHCO_3$ (600 mL, 1:3), and decanted. The aqueous phase was extracted twice with $CH_2Cl_2$ (2×150 mL). The combined organic layers were dried ($Na_2SO_4$) and filtered. After evaporation to dryness, the crude product was dissolved in a minimum of $CHCl_3$ and chromatographed over silica gel with ether to give product **2** in 78% yield. Mp=238–240°C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ −0.62 (3H, s, Si–$CH_3$), −0.14 (3H, s, Si–$CH_3$), 0.12 (3H, s, Si–$CH_3$), 0.14 (3H, s, Si–$CH_3$), 0.73 (9H, s, Si–$C(CH_3)_3$), 0.96 (9H, s, Si–$C(CH_3)_3$), 3.70 (1H, m, H-5′), 3.93 (1H, m, H-5′), 4.15 (1H, m, H-4′), 4.33 (1H, d, $J$=4.6 Hz, H-3′), 5.07 (1H, dd, $J_1$=7.9 Hz, $J_2$=4.6 Hz, H-2′), 5.73 (2H, bs, $NH_2$), 5.75 (1H, dd, $J_1$=11.1 Hz, $J_2$=1.4 Hz, $H_{olefinic}$), 5.98 (1H, d, $J$=7.9 Hz, H-1′), 6.49 (1H, dd, $J_1$=17.2 Hz, $J_2$=1.4 Hz, $H_{olefinic}$), 6.82 (1H, dd, $J_1$=17.2 Hz, $J_2$=11.1 Hz, $H_{olefinic}$), 6.88 (1H, m, OH), 8.30 (1H, s, H-2); $^{13}$C NMR, ($CDCl_3$) $\delta$ −5.5 (p), −5.4 (p), −5.3 (p), −4.50 (p), 17.8 (q), 18.1 (q), 25.9 (p), 25.8 (p), 63.1 (s), 73.9 (t), 74.1 (t), 88.7 (t), 89.5 (t), 120.3 (q), 122.9 (t), 124.9 (s), 149.4 (q), 151.4 (q), 151.9 (t), 155.5 (q); MS (FAB$^+$) MH$^+$ 522. Anal. calcd for $C_{24}H_{43}IN_5O_4Si_2$, C 55.24, H 8.31, N 13.42; found C 55.44, H 8.46, N 13.52.

**4.1.2. 8-Vinyl-9-[2,3-di-*O*-(*tert*-butyldimethylsilyl)-5-*O*-tosyl-β-D-ribo-pentofuranosyl]-adenine (3).** Compound **2** (0.104 g, 0.2 mmol) was dissolved in THF and cooled down at −78°C (2.5 mL), and an ether solution of methyllitihum was added (0.14 mL, 0.22 mmol, 1.1 equiv.). After 15 min stirring at −78°C and further 15 min at 4°C, a solution of *p*-toluenesulfonyl chloride in THF (0.04 g, 0.22 mmol, 1.1 equiv.) was added. The reaction was followed by TLC (EtOAc). The reaction mixture was hydrolysed by water (0.1 mL), the organic phase was dried ($Na_2SO_4$) and filtered. After evaporation to dryness, the crude product was dissolved in a minimum of $CHCl_3$ and chromatographed over silica gel with ether/hexane (9:1) to give product **3** in 60% yield. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ −0.45 (3H, s, Si–$CH_3$), −0.12 (3H, s, Si–$CH_3$), 0.13 (6H, s, Si–$CH_3$), 0.74 (9H, s, Si–$C(CH_3)_3$), 0.94 (9H, s, Si–$C(CH_3)_3$), 2.41 (3H, s, $CH_3$), 4.07–4.27 (3H, m, H-4′ and H-5′), 4.46 (1H, dd, $J_1$=4.5 Hz, $J_2$=2.8 Hz, H-3′), 5.30 (1H, dd, $J_1$=5.9 Hz, $J_2$=4.5 Hz, H-2′), 5.54 (2H, bs, $NH_2$), 5.75 (1H, dd, $J_1$=11.0 Hz, $J_2$=1.5 Hz, $H_{olefinic}$), 5.85 (1H, d, $J$=5.9 Hz, H-1′), 6.49 (1H, dd, $J_1$=17.1 Hz, $J_2$=1.5 Hz, $H_{olefinic}$), 6.80 (1H, dd, $J_1$=17.1 Hz, $J_2$=11.0 Hz, $H_{olefinic}$), 7.25 (2H, d, $J$=8.1 Hz, $H_{Ar}$), 7.72 (2H, d, $J$=8.1 Hz, $H_{Ar}$), 8.16 (1H, s, H-2), MS (FAB$^+$) MH$^+$ 676.

**4.1.3. 8-Vinyl-9-(5-*O*-tosyl-β-D-ribo-pentofuranosyl)-adenine (4).** Compound **3** (0.024 g, 0.036 mmol) was suspended in acetonitrile and an aqueous solution of HF (2%) was added (1 mL). After 24 h stirring, the reaction mixture was neutralised by solid ammonium bicarbonate. After evaporation to dryness, the crude product was chromatographed over silica gel with $CHCl_3$/MeOH (9:1) to give product **4** in 48% yield. $^1$H NMR (200 MHz, $CDCl_3$+5%$CD_3OD$) $\delta$ 2.33 (3H, s, $CH_3$), 4.10–4.28 (3H, m, H-4′ and H-5′), 4.48 (1H, dd, $J_1$=5.9 Hz, $J_2$=4.5 Hz, H-3′), 4.87 (1H, dd, $J_1$=5.0 Hz, $J_2$=5.9 Hz, H-2′), 5.67 (1H, dd, $J_1$=11.2 Hz, $J_2$=1.2 Hz, $H_{olefinic}$), 5.85 (1H, d, $J$=5.0 Hz, H-1′), 6.30 (1H, dd, $J_1$=17.2 Hz, $J_2$=1.2 Hz, $H_{olefinic}$), 6.81 (1H, dd, $J_1$=17.2 Hz, $J_2$=11.2 Hz, $H_{olefinic}$), 7.25 (2H, d, $J$=8.3 Hz, $H_{Ar}$), 7.72 (2H, d, $J$=8.3 Hz, $H_{Ar}$), 8.16 (1H, s, H-2).

**4.1.4. 8-Iodo-9-[2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-ribo-pentofuranosyl]-adenine (9).** Compound **8** (1.5 g; 2.04 mmol) was dissolved in a mixture of trifluoroacetic acid and water at 0°C (25 mL, 95:5). The reaction was monitored by TLC (ether/hexane, 1:1). When hydrolysis was completed, the reaction solution was poured into a mixture of $CH_2Cl_2$ and a saturated aqueous solution of $NaHCO_3$ (600 mL, 1–3) and decanted. The aqueous phase was extracted twice with $CH_2Cl_2$ (2×150 mL). The combined organic layers were dried ($Na_2SO_4$) and filtered. After evaporation to dryness, the crude product was dissolved in a minimum of $CHCl_3$ and chromatographed over silica gel with ether/hexane (9:1) to give product **9** in 72% yield. Mp=242–243°C; $[\alpha]_D{}^{25}$=−12 (c 0.33, MeOH); UV (MeOH); $\lambda_{max}$=267 nm ($\varepsilon$=15500); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ −0.57 (3H, s, Si–$CH_3$), −0.14 (3H, s, Si–$CH_3$), 0.11 (3H, s, Si–$CH_3$), 0.14 (3H, s, Si–$CH_3$), 0.78 (9H, s, Si–$C(CH_3)_3$), 0.96 (9H, s, Si–$C(CH_3)_3$), 3.69 (1H, m, H-5′), 3.91 (1H, m, H-5′), 4.14 (1H, m, H-4′), 4.31 (1H, bd, $J$=4.4 Hz, H-3′), 5.30 (1H, dd, $J_1$=8 Hz, $J_2$=4.4 Hz, H-2′), 5.92 (2H, bs, $NH_2$), 5.96 (1H, d, $J$=8 Hz, H-1′), 8.25 (1H, s, H-2); $^{13}$C NMR, ($CDCl_3$) $\delta$ −4.72 (p), −3.86 (p), −3.67 (p), −3.50 (p), 18.7 (q), 19.1 (q), 26.8 (p), 64.0 (s), 74.3 (t), 74.8 (t), 90.4 (t), 93.7 (t), 101.7 (q), 124.4 (q), 150.8 (q), 153.1 (t), 155.4 (q); HRMS (FAB$^+$) calcd for MH$^+$ 622.1742, found 622.1741. Anal. calcd for $C_{22}H_{40}IN_5O_4Si_2$, C 42.50, H 6.49, N 11.27; found C 42.27, H 6.50, N 11.00.

**4.1.5. 8-Iodo-9-[2,3-di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-di-(*tert*-butoxyphosphoryl)-β-D-ribo-pentofuranosyl]-adenine (10).** Compound **9** (1 g, 1.6 mmol) was dried by repeated co-evaporation with dry pyridine (3×5 mL). Tris(2,4,6-tribromophenoxy)dichlorophosphorane, (BDCP, 2.81 g, 2.57 mmol) was dissolved in dry pyridine (8 mL) and di-*t*-butyl phosphonate (0.32 mL, 1.93 mmol) was added. The mixture was stirred at room temperature for 15 min and then compound **9** was added (1.0 g, 1.61 mmol). The resulting solution was stirred at room temperature for 1 h, and then a solution of *t*-butyl hydroperoxide in water (1 mL, 7.24 mmol) was added. After stirring for an additional 4 h, the mixture was diluted with $CH_2Cl_2$, washed with $NaHCO_3$ and water, dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure. The crude product was dissolved in $CHCl_3$ and purified by chromatography over silica gel with ether/acetone (8:2) to give

product **10** in 55% yield. Mp=67–68°C; $[\alpha]_D{}^{25}$=+1 (c 0.37, MeOH); UV (MeOH); $\lambda_{max}$=265 nm ($\varepsilon$=21300); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ −0.38 (3H, s, Si–$CH_3$), −0.08 (3H, s, Si–$CH_3$), 0.17 (6H, s, Si–$CH_3$), 0.79 (9H, s, Si–$C(CH_3)_3$), 0.97 (9H, s, Si–$C(CH_3)_3$), 4.06 (1H, m, H-5′), 4.40 (1H, m, H-5′), 4.22 (1H, m, H-4′), 4.60 (1H, dd, $J_1$=4.3 Hz, $J_2$=2.5 Hz, H-3′), 5.30 (1H, dd, $J_1$=6 Hz, $J_2$=4.3 Hz, H-2′), 5.25 (2H, bs, $NH_2$), 5.88 (1H, d, $J$=6 Hz, H-1′), 8.22 (1H, s, H-2); $^{13}$C NMR ($CDCl_3$) $\delta$ −4.2 (p), −3.6 (p), 18.8 (q), 19.0 (q), 26.7 (p), 26.8 (p), 30.7 (p, d, $J$=3 Hz), 66.0 (s), 72.7 (t), 73.6 (t), 83.4 (q, d, $J$=8 Hz), 84.7 (q, d, $J$=8 Hz), 93.5 (t), 102.6 (q), 124.3 (q), 151.7 (q), 153.3 (t), 155.0 (q); HRMS (FAB$^+$) calcd for MH$^+$ 814.2657, found 814.2651.

**4.1.6. 9-[2,3-di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-di-(*tert*-butoxyphosphoryl)-β-D-ribo-pentofuranosyl]-adenine (11).** Compound **9** (0.6 g, 9.65 mmol) was dissolved in dry THF (8 mL) and di-*t*-butyl phosphoramidate (0.587 mL, 1.93 mmol) was added and tetrazole (0.27 g, 3.86 mmol) were added at −20°C. The reaction temperature was allowed to reach room temperature and the resulting solution was stirred further at this temperature for 1 h before a solution of *t*-butyl hydroperoxide in water (0.66 mL, 4.8 mmol) was added. After stirring for an additional 2 h, the mixture was diluted with AcOEt, quenched with $Na_2SO_3$, washed with water, dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure. The crude product was dissolved in $CHCl_3$ and purified by chromatography over silica gel with ether/acetone (8:2) to give product **11** in 30% yield. $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ −0.24 (3H, s, Si–$CH_3$), −0.04 (3H, s, Si–$CH_3$), 0.11 (3H, s, Si–$CH_3$), 0.13 (3H, s, Si–$CH_3$), 0.79 (9H, s, Si–$C(CH_3)_3$), 0.93 (9H, s, Si–$C(CH_3)_3$), 4.40–4.10 (4H, m, H-5′, H-4′, H-3′), 5.30 (1H, dd, $J_1$=6 Hz, $J_2$=4.3 Hz, H-2′), 5.64 (2H, bs, $NH_2$), 5.97 (1H, d, $J$=6 Hz, H-1′), 8.09 (1H, s, H-8), 8.33 (1H, s, H-2).

**4.1.7. 8-Vinyl-9-[5′-*O*-di-(*tert*-butoxyphosphoryl)-β-D-ribo-pentofuranosyl]-adenine (12).** To a solution of the 8-vinyl protected adenosine derivative (0.93 g, 1.30 mmol) in dry THF (20 mL) was added $Bu_4NF$ (2.2 equiv.) and this mixture was stirred for 1 h. The reaction mixture was purified by chromatography over silica gel with acetone to give the deprotected product in 96% yield. $^1$H NMR (200 MHz, $CD_3OD$) $\delta$ 1.40 (9H, s, $C(CH_3)_3$), 1.43 (9H, s, $C(CH_3)_3$), 4.34–4.09 (3H, m, H-5′, H-4′), 4.57 (1H, dd, $J_1$=5.6 Hz, $J_2$=4.2 Hz, H-3′), 5.19 (1H, dd, $J_1$=$J_2$=5.4 Hz, H-2′), 5.79 (1H, dd, $J_1$=11.1 Hz, $J_2$=1.5 Hz, $H_{olefinic}$), 6.05 (1H, d, $J$=5.2 Hz, H-1′), 6.43 (1H, dd, $J_1$=17.2 Hz, $J_2$=1.5 Hz, $H_{olefinic}$), 7.06 (1H, dd, $J_1$=17.2 Hz, $J_2$=11.1 Hz, $H_{olefinic}$), 8.16 (1H, s, H-2).

**4.1.8. 8-Iodo-9-[2,3-di-*O*-(acetyl)-5′-*O*-di-(*tert*-butoxyphosphoryl)-β-D-ribo-pentofuranosyl]-adenine (13).** To a solution of compound **10** (0.904 g, 1.17 mmol) in dry THF (5 mL) was added $Bu_4NF$ (2.5 equiv.) and this mixture was stirred for 1 h. The reaction mixture was purified by chromatography over silica gel with acetone to give the deprotected product quantitatively. Mp>170°C (Dec.); $[\alpha]_D{}^{25}$=−4 (c 0.33, MeOH); UV (MeOH); $\lambda_{max}$=265 nm ($\varepsilon$=15600); $^1$H NMR (200 MHz, $CD_3OD$) $\delta$ 1.37 (9H, s, $C(CH_3)_3$), 1.41 (9H, s, $C(CH_3)_3$), 4.05–4.32 (3H, m, H-5′, H-4′), 4.60 (1H, dd, $J_1$=$J_2$=5.5 Hz, H-3′), 5.41 (1H, dd, $J_1$=5.5 Hz, $J_2$=4.4 Hz, H-2′), 5.94 (1H, d, $J$=4.4 Hz, H-1′), 8.22 (1H, s, H-2); $^{13}$C NMR ($CD_3OD$) $\delta$ 30.5 (p), 67.6 (s), 71.7 (t), 72.6 (t), 84.0 (q, d, $J$=8 Hz), 84.6 (q, d, $J$=6.5 Hz), 94.4 (t), 94.8 (t), 104.2 (q), 151.7 (q), 152.0 (q), 152.9 (t), 155.5 (q); HRMS (FAB$^+$) calcd for MH$^+$ 586.0938, found 586.0927. Anal. calcd for $C_{18}H_{29}IN_5O_7P$, C 36.94, H 4.99, N 11.96; found C 36.87, H 4.97, N 11.68.

To a solution of the alcohol (0.304 g, 0.52 mmol) in dry pyridine (6 mL) was added $Ac_2O$ (0.294 mL, 3.11 mmol) and the reaction solution was stirred for 3 h. The reaction mixture was diluted with $CH_2Cl_2$ (50 mL) and washed with a saturated solution of $NaHCO_3$, dried over $Na_2SO_4$, filtered and concentrated over reduced pressure. The crude product was purified by chromatography over silica gel with ether/acetone (8:2) to give compound **13** in 83% yield. Mp=74–75°C, $[\alpha]_D{}^{25}$=+16 (c 0.28, MeOH); UV (MeOH); $\lambda_{max}$=266 nm ($\varepsilon$=23900); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.42 (9H, s, $C(CH_3)_3$, 1.45 (9H, s, $C(CH_3)_3$), 2.09 (3H, s, $CH_3$), 2.15 (3H, s, $CH_3$), 4.3 (3H, m, H-5′, H-4′), 5.90 (1H, dd, $J_1$=$J_2$=5.8 Hz, H-3′), 5.95 (2H, bs, $NH_2$), 5.98 (1H, d, $J$=4.5 Hz, H-1′), 6.45 (1H, dd, $J$=4.5 Hz, $J$=5.8 Hz, H-2′), 8.26 (1H, s, H-2); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.4 (p), 21.5 (p), 30.2 30.7 (p), 65.5 (s), 70.8 (t), 71.9 (t), 81.1 (q, d, $J$=8.2 Hz), 83.7 (q, d, $J$=8.2 Hz), 90.7 (t), 85.6 (t), 100.6 (q), 123.9 (q), 151.7 (q), 153.8 (t), 155.0 (q), 170.2 (q); HRMS (FAB$^+$) calcd for MH$^+$586.0938, found 586.0927.

**4.1.9. 8-Vinyl-9-[2,3-di-*O*-(acetyl)-5′-*O*-di-(*tert*-butoxyphosphoryl)-β-D-ribo-pentofuranosyl]-adenine (14).** Compound **13** (0.255 g, 0.38 mmol) was dissolved in anhydrous DMF (5 mL) and the solution was degassed. $Pd(PPh_3)_4$ (10%, 50 mg) and vinyltributyltin (447 μL, 1.52 mmol) were added to the degassed solution under Ar. The reaction was heated to 90°C for 30 min and then the solution was diluted with $CHCl_3$ (10 mL) and washed with water (3×100 mL), brine (50 mL) and dried over $Na_2SO_4$. The crude product was purified by chromatography on silica gel with a mixture of ether/acetone (6:4) to give product **14** in 80% yield. Mp>210°C (Dec.); $[\alpha]_D{}^{25}$=+46 (c 0.29, MeOH); UV (MeOH); $\lambda_{max}$=293 nm ($\varepsilon$=15100); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.43 (9H, s, $C(CH_3)_3$), 1.46 (9H, s, $C(CH_3)_3$), 2.00 (3H, s, $CH_3$), 2.13 (3H, s, $CH_3$), 4.30 (3H, m, H-5′, H-4′), 5.75 (2H, m, $H_{olefinic}$, H-3′), 6.11 (1H, dd, $J_1$=$J_2$=6.15 Hz, H-2′), 5.91 (2H, bs, $NH_2$), 6.22 (1H, d, $J$=6.15 Hz, H-1′), 6.44 (1H, dd, $J_1$=17.0 Hz, $J_2$=1.7 Hz, $H_{olefinic}$), 6.97 (1H, dd, $J_1$=17.0 Hz, $J_2$=12.3 Hz, $H_{olefinic}$), 8.30 (1H, s, H-2); $^{13}$C NMR ($CDCl_3$) $\delta$ 20.4 (p), 20.6 (p), 29.8 (p), 65.5 (s), 70.6 (t), 72.2 (t), 81.2 (q, d, $J$=9.3 Hz), 83.0 (q, d, $J$=9.3 Hz), 83.8 (t), 85.6 (t), 119.3 (q), 123.6 (t), 124.9 (s), 148.8 (q), 150.9 (q), 152.8 (t), 155.1 (q), 169.3 169.6 (q); HRMS (FAB$^+$,) calcd for MH$^+$ 570.2328, found 570.2327.

**4.1.10. 8-Vinyl-9-[2,3-di-*O*-(acetyl)-5′-*O*-(phosphoryl)-β-D-ribo-pentofuranosyl]-adenine (15).** Compound **14** (0.054 g, 0.19 mmol) was dissolved at 0°C in a mixture of trifluoroacetic acid and water (5 mL, 95:5). After 10 min stirring, the reaction was complete (TLC: *i*-PrOH/$NH_4OH$/$H_2O$, 11:7:2) and the solvents were co-evaporated with cyclohexane (3×10 mL). The crude product was used without purification in the next step. $^1$H NMR (400 MHz,

DMSO-$d_6$) $\delta$ 2.01 (3H, s, $CH_3$), 2.13 (3H, s, $CH_3$), 4.03-4.16 (2H, m, H-5′), 4.38 (1H, m, H-4′), 5.60 (1H, dd, $J_1$=$J_2$=5.95 Hz, H-3′), 5.75 (1H, dd, $J_1$=11.2 Hz, $J_2$=1.9 Hz, $H_{olefinic}$), 6.11 (1H, dd, $J_1$=$J_2$=5.5 Hz, H-2′), 6.32 (1H, d, $J$=5.25 Hz, H-1′), 6.40 (1H, dd, $J_1$=17.0 Hz, $J_2$=1.9 Hz, $H_{olefinic}$), 7.17 (1H, dd, $J_1$=17.0 Hz, $J_2$=11.2 Hz, $H_{olefinic}$), 7.95 (2H, bs, $NH_2$), 8.26 (1H, s, H-2).

*Typical procedure for the di- and triphosphate synthesis.* The crude product **15** was dissolved in pyridine (5 mL) and tributylamine (22.8 μL, 0.095 mmol) was added, the mixture was then co-evaporated with dry pyridine (3×5 mL) and dry DMF (3×5 mL). Dry DMF (1.5 mL) and carbonyldiimidazole (0.062 g, 0.38 mmol) were added, and this mixture was stirred for 3 h before adding methanol (11.6 μL, 0.28 mmol). After 30 min, tributylammonium hydrogenphosphate or bis-tributylammonium hydrogenpyrophosphate (4 equiv.) was added and the mixture was stirred for 21 h. The solvent was removed under vacuum at room temperature and the crude product was chromatographed on a DEAE fractogel column (8×3 cm) with a linear gradient from 0 to 0.5 M of triethylammonium bicarbonate (pH 7.5) at a flow rate of 30 mL/h over a 5 h period. The appropriate fractions were collected and co-evaporated with methanol. The acetyl groups were removed with 10 mL of ammoniac (34% in water) and methanol (1:1). The solvents were evaporated and the crude product was chromatographed on a DEAE column as described before. Triethylammonium and protons were exchanged for sodium by passing the solution through a Dowex AG 50W-X2 column ($Na^+$ form) and eluting with water. After freeze-drying of appropriate fractions, the desired compounds were obtained.

**4.1.11. 8-Vinyl-9-[-β-D-ribo-pentofuranosyl]-adenine-5′-diphosphate (5).** Yield: 9%; UV ($H_2O$); $\lambda_{max}$=288 nm ($\varepsilon$=14500); $^1$H NMR (300 MHz, $D_2O$) $\delta$ 4.15 (3H, m, H-5′, H-4′), 4.42 (1H, d, $J$=6.9 Hz, H-3′), 4.74 (1H, dd, $J_1$=$J_2$=6.9 Hz, H-2′), 5.73 (1H, bd, $J$=12.0 Hz, $H_{olefinic}$), 6.00 (1H, d, $J$=6.9 Hz, H-1′), 6.44 (1H, bd, $J$=17.1 Hz, $H_{olefinic}$), 6.97 (1H, dd, $J_1$=17.1 Hz, $J_2$=12.0 Hz, $H_{olefinic}$), 8.08 (1H, s, H-2);$^{31}$P NMR (121 MHz, $D_2O$) $\delta$ −7.82 (1P, d, $J$=15.4 Hz); −8.76 (1P, d, $J$=15.4 Hz); MS ($ES^-$) 474 (MHNa−, 11), 452 ($MH_2$−, 100).

**4.1.12. 8-Vinyl-9-[-β-D-ribo-pentofuranosyl]-adenine-5′-triphosphate (16).** Yield: 4%; UV ($H_2O$); $\lambda_{max}$=288 nm ($\varepsilon$=14700); $^1$H NMR (300 MHz, $D_2O$); 4.35 (3H, m, H-5′, H-4′), 4.7 (2H, m, H-3′, H-2′), 5.73 (1H, d, $J$=12.0 Hz, $H_{olefinic}$), 6.03 (1H, d, $J$=6.9 Hz, H-1′), 6.27 (1H, d, $J$=17.4 Hz, $H_{olefinic}$), 6.97 (1H, dd, $J_1$=17.4 Hz, $J_2$=12.0 Hz, $H_{olefinic}$), 8.09 (1H, s, H-2); $^{31}$P NMR (121 MHz, $D_2O$) $\delta$ −7.77 (s, 1P), −8.94 (d, $J$=20 Hz, 1P), −20.56 (d, $J$=20 Hz, 1P); MS ($ES^-$) 554 ($MH_2Na$-, 30), 532 ($MH_2$−, 100), 452 ($MH_2$−, $-PO_3$, 55).

**4.2. Assays for *E. coli* ribonucleotide reductase activity.** ADP, ATP, dGTP and NADPH were obtained from Sigma. *E. coli* thioredoxin and thioredoxin reductase were obtained from D. Padovani (CEA, Grenoble). Protein R1 and R2 were prepared from overproducing strains of *E. coli* as previously described.[29,30]

Activity of the *E. coli* ribonucleotide reductase was assayed spectrophotometrically monitoring NADPH consumption at 340 nm ($\varepsilon_{340}$=6220 $M^{-1}$ $cm^{-1}$).[31] Standard conditions were thioredoxin 0.16 mg/mL, thioredoxin reductase 0.028 mg/mL, DTT 0.5 mM, $MgCl_2$ 10 mM, NADPH 0.32 mM, ATP 1.4 mM, dGTP 0.6 mM, R1 0.18 mg/mL, R2 0.038 mg/mL in Hepes buffer 35 mM pH 7.5. The substrates were ADP and 8-vinyl ADP **5** added at concentrations indicated in the results section. Units are defined as nmol of NADPH oxidized per minute.

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