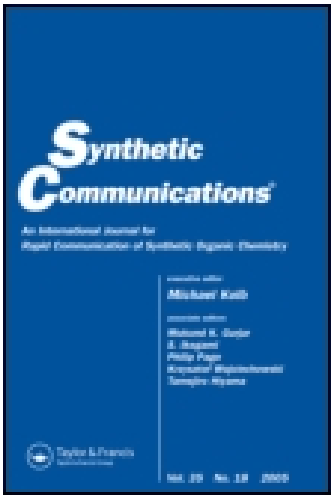

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Design and Synthesis of Some New α-Phenyl Cinnamoyl Derivatives for Selective Protection of Purine Nucleosides

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Design and Synthesis of Some New α-Phenyl Cinnamoyl Derivatives for Selective Protection of Purine Nucleosides

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Abstract: Three new α-phenylcinnamic acid derivatives [4-methoxy-α-phenylcinnamic acid, α-(4-methoxyphenyl)-cinnamic acid, and 4,4′-bismethoxy-α-phenylcinnamic acid] were synthesized, characterized, and selectively used for protecting the exocyclic amino function of purine nucleosides (2′-deoxyadenosine and 2′-deoxyguanosine) via active ester generation. The acids were first activated using *p*-nitrophenol, and these activated esters were used subsequently for the selective protection of amino groups. The *N*-protected derivatives of 2′-deoxyguanosine and 2′-deoxyadenosine have been found to be sufficiently stable toward acids, thus minimizing depurination under oligodeoxyribonucleotide synthesis protocol. The ease of syntheses of *N*-protected purine nucleosides, their stability under an acidic environment, and mild deprotection conditions are the key advantages of the new protecting groups.

Keywords: acylation, antisense, α-phenylcinnamoyl, depurination resistant

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INTRODUCTION

Chemically synthesized oligodeoxyribonucleotides (ODNs) have become extremely important tools for the study of the life sciences. More than two dozen oligonucleotide drugs are undergoing human clinical trials for the treatment of viral infections, cancers, and a range of inflammatory disorders. In addition, use of antisense gene expression modulation to produce well-defined pharmacological effects is now a routine procedure.[1] The need for very large quantities of therapeutically useful antisense ODNs can be easily anticipated by their growing demand in therapeutics.[2,3] However, a series of problems hampers the current methodology of automated solid-phase synthetic approaches on controlled pore-glass supports, specifically, the problem of depurination, which occurs because of the repeated acid treatment used for the removal of the 5′-*O*-4,4′-dimethoxytrityl (DMTr) protecting group during synthesis of ODNs.[4] Acylation of amino function in purine nucleosides facilitates the protonation at N^7,[5] which makes the glycosyl bond more prone to hydrolysis under acidic conditions.[6,7]

Depurination is the most common problem faced while using the most commonly used groups, the benzoyl, anisoyl, and isobutyryl groups. The phthaloyl group[8] for adenosine has been suggested to be depurination resistant, but because of its instability problem, it could not be used successfully in large-scale synthesis. The depurination is facilitated under acidic conditions required for removal of DMTr group during automated synthesis of ODNs. Because the growing chain of ODNs is frequently subjected to acid treatment, the risk of depurination involves protonation or deprotonation of the purine residues followed by cleavage of the glycosidic linkage, generating undesirable apurinic sites.[9,10] Depurination in the case of N^6-protected 2′-deoxyadenosine is facilitated because of the protonation at the N^7 position. In the case of partially depurinated oligomer, chain cleavage takes place at the site of depurination by double β-elimination, thus generating truncated sequences.[10] In the case of guanosine, the site of protonation is N^7, but the tendency of N^2-isobutyryl deoxyguanosine to depurinate is lower than that of N^6-benzoyl-2′-deoxyadenosine.[11] To block N^7 protonation, we had successfully used several groups earlier, namely 3-methoxy-4-phenoxy benzoyl,[12] phenoxyacetyl,[13] naphthaloyl,[14] and α-phenyl-cinnamoyl,[15] in ODN synthesis.

In continuation of our efforts toward development of new protecting groups that would resist depurination, three new derivatives of α-phenyl cinnamic acid have been synthesized by introducing a methoxy group at the *para* position of either or both benzene rings. The main purpose for the introduction of the methoxy group in these three derivatives was to decrease the $t_{1/2}$ for the deprotection step using ammonia and also to check *N*-protonation, thereby further reducing the depurination problem. More emphasis was also given to the crystallization of the protected nucleosides, which may eliminate the expensive chromatographic purification of raw materials, an important aspect in the scaling of ODN synthesis.

The conventionally used acylation reagents for amino protection are either in acid chloride or anhydride form. In the present work, the installation of the protecting group has been done by a new method. For example, instead of using corrosive and hazardous acid chloride, we utilized the safe and cheaper acid forms that have been activated via *p*-nitrophenol esters. The activated ester strategy is an established protocol in the peptide chemistry where dicyclohexylcarbodiimide is used as a coupling agent to furnish the –CO–NH– bond. To the best of our knowledge, use of the active ester approach for the *N*-protection of purine residue is not reported in the literature. Herein, the *N*-acylation of purine residue was accomplished with excellent selectivity and high yield.

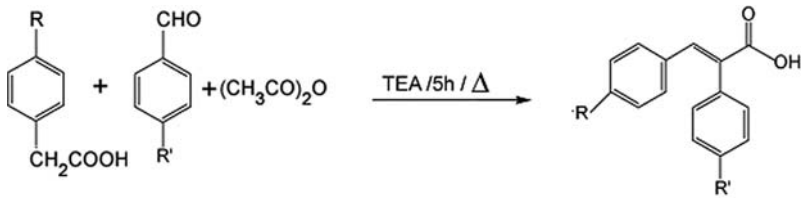
MATERIALS AND METHODS

All the deoxynucleosides and 4,4′-dimethoxytrityl chloride were purchased from Aldrich Chem. Co. Phenylacetic acid, 4-methoxy phenyl acetic acid, benzaldehyde, and 4-methoxy benzaldehyde were purchased from Merck-Schudardt, Germany. Solvents were duly purified and dried before use. Pyridine was refluxed with ninhydrin and distilled over KOH. Silica-gel G (Merck) plates were used for TLC. Plates were sprayed with sulphuric acid to ascertain the nucleoside spots. UV absorption was measured on a Hitachi 220S spectrophotometer. Elemental analyses were carried out using a Carlo Erba 1106 analyser.

α-Phenylcinnamic acid derivatives (Scheme 1) were prepared by the Perkin reaction involving phenylacetic acid, 4-methoxyphenylacetic acid, benzaldehyde, 4-methoxybenzaldehyde, and acetic acid in the presence of triethylamine as catalyst.[16]

Synthesis of 4-Methoxy α-Phenylcinnamic Acid (a)

p-Methoxy phenylacetic acid (8.3 g, 0.05 mol), benzaldehyde (5 mL, 0.05 mol), acetic anhydride (10.58 mL, 0.10 mol), and triethylamine (5 mL) were taken in a three-necked round-bottomed flask (100 mL) fitted with a



(a) R, -OCH_3; R', -H (4-methoxy-α-phenylcinnamic acid)
(b) R, -H ; R', -OCH_3 (α-(4-methoxy) phenylcinnamic acid)
(c) R, -OCH_3, R'-OCH_3 (4,4'-bismethoxy-α-phenylcinnamic acid)

Scheme 1. Synthesis of different methoxy derivatives of α-phenylcinnamic acid.

reflux condenser and a $CaCl_2$ drying tube. The reaction mixture was refluxed for 5 h. Steam distillation was done directly from the reaction flask until the distillate passing over was no longer cloudy. This process was used to remove the excess of acetic acid, which is formed as a by-product in the reaction. The distillate was discarded. The solid deposited in the flask was dissolved in minimum quantity of hot ethanol followed by quick filtration over pump. The resulting solution was cooled down to get the product in crystallized form. Yield 94% (8.5 g), mp150–152°C, λ_{max} 272 nm. Elemental analysis $C_{16}H_{13}O_2$: calc. C, 75.88%; H, 5.13%, Found C, 75.86%; H, 5.13%.

Synthesis of α-(4-Methoxyphenyl)-cinnamic Acid (b)

p-Methoxy benzaldehyde (6.8 mL, 0.05 mol), acetic anhydride (10.2 mL, 0.10 mol), and triethylamine (5 mL) were added to the equimolar amount of phenylacetic acid (6.8 g, 0.05 mol)in a three-necked flask and the reaction was carried out as previously described. Yield 88% (5.2 g), mp 168–170°C, λ_{max} 275 nm. Elemental analysis $C_{16}H_{13}O_2$: calc. C, 75.88%; H, 5.13%, Found C, 75.80%; H, 5.14%.

Synthesis of 4,4′-Bis Methoxy-α-phenylcinnamic Acid (c)

Synthesis of this acid was carried out using the previously mentioned protocol using 4-methoxy phenylacetic acid and *p*-methoxy benzaldehyde in the respective molar ratio. Yield 88% (5.2 g), mp 160–163°C, λ_{max} 29 nm. Elemental analysis $C_{17}H_{15}O_4$: calc. C, 72.12%; H, 5.3%, Found C, 72.11%; H, 5.5%.

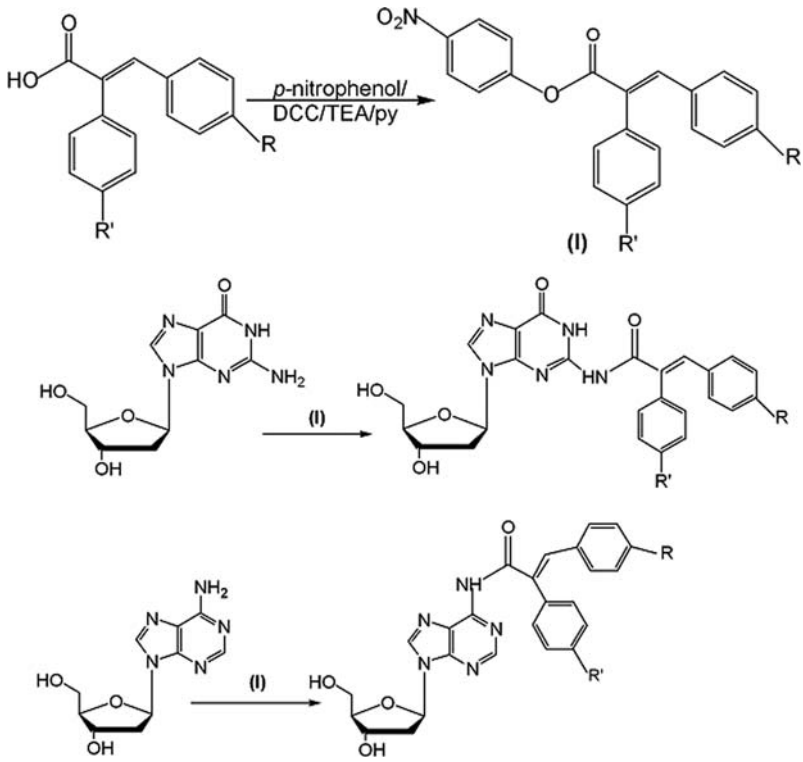
General Method for Preparation of N^6-Protected-2′-dA and N^2-Protected-2′-dG (Scheme 2)

Activation of the Respective Acids to Esters

A solution of *p*-nitrophenol (0.0048 mol) in 1,4-dioxane (5 mL) was added to a stirred solution of the appropriate acids (**a**, **b**, **c**; 0.004 mol) in dry 1,4-dioxane (5 mL) at room temperature. The reaction media was made basic by the addition of pyridine (0.5 mL) and triethylamine (0.5 mL). After stirring for 10 min dicyclohexylcarbodiimide (0.005 mol) was added and the stirring continued for 2 h. (Caution! Protection from moisture was necessary.) After completion of the reaction (monitored by TLC) the mixture was cooled to 0°C.

Protection Step

Anhydrous 2′-deoxynucleoside (0.004 mol) was dissolved in dry pyridine (5 mL) and added to the activated ester solution at 0°C. After 10 min of



Scheme 2. Protection of purine deoxynucleosides with different methoxy derivatives of α-phenylcinnamic acid.

stirring, DCC (0.005 mol) was added, and the reaction mixture was stirred for an additional 2.5 h. Completion of the reaction was checked by TLC. The precipitated dicyclohexylurea was removed by filtration, and the filtrate was concentrated under vacuum to half of its volume and then poured in a 5% sodium bicarbonate solution and extracted with dichloromethane (4 × 10 mL). The organic layer was washed with water, dried over Na_2SO_4, and filtered. The combined dichloromethane layers were concentrated and the residue crystallized from diethylether. The product data is summarized in Table 1.

General Method for Preparation of 5′-*O*-Dimethoxytrityl-*N*-protected-2′-deoxynucleosides

Each *N*-protected-2′-deoxynucleoside (1.0 mmol) was treated with 4,4′-dimethoxytrityl chloride (1.2 mmol) in pyridine (10.0 mL) in the presence of 4-dimethylamino pyridine as catalyst at room temperature (25°C) to furnish its trityl derivative. After completion of the reaction (checked by TLC), the

Table 1. Spectral properties of all the different methoxy derivatives of α-phenylcinnamic acid and the protected derivatives

Compounds	Mp (solvent)	Yield (%)	UV	^{1}H NMR	IR	Elemental analysis
4-Methoxy-α-phenyl cinnamic acid	150–152°C (EtOH)	94	272	—	—	$C_{16}O_2H_{13}$; calcd. C, 75.88%; H, 5.13%. Found C, 75.86%; H, 5.13%
N^6-4-Methoxy-α-phenyl-cinnamoyl dA	125–130°C (diethyl ether)	82	285	8.70 (H8, s), 8.32 (H2, s), 5.12 (m)	3500–3400 (S, B), 2929 (S), 1755 (S), 1580 (S), 1529 (S), 1336 (S), 1255 (S), 804 (S)	$C_{26}H_{23}N_5O_5$; calcd. C, 64.32%; H, 4.74%; N, 14.42%. Found C, 64.10%; H, 4.68%; N, 14.30%
N^2-α-Phenyl-4-methoxy-cinnamoyl-dG	142°C (diethyl ether)	80	295	12.3 (N-H, broad, s), 11.85, (N-H, broad, s), 7.81, (H8, s), 5.12 (m)	3500–3400 (S, B), 2925 (S), 1760 (S), 1580 (S), 1529 (S), 1336 (S), 1255 (S), 800 (S)	$C_{26}H_{23}N_5O_6$; calcd. C, 62.4%; H, 4.60%; N, 14.0%. Found C, 62.4%; H, 4.58%; N, 14.0%
α-(4-Methoxyphenyl)-cinnamic acid	168–170°C (EtOH)	88	275	—	—	$C_{16}O_2H_{13}$; calcd. C, 75.88%; H, 5.13%; N, 14.42%. Found C, 75.80%; H, 5.14%

N^6-α-(4-Methoxyphenyl)-cinnamoyl-dA	120–125°C (diethyl ether)	83	263, 300	8.70 (H8, s), 8.35 (H2, s), 5.12 (s)	3448-3400 (S, B), 2950 (S), 1750 (S), 1560 (S), 1525 (S), 1346 (S), 1250 (S), 802 (W)	$C_{26}H_{23}N_5O_5$; calcd. C, 64.32%; H, 4.74%; N, 14.42%. Found C, 64.15%; H, 5.13%; N, 14.3%
N^2-4-Methoxy-α-phenyl-cinnamoyl-dG	138°C (diethyl ether)	78	291, 300	12.5 (N–H, broad, s), 11.80 (N–H, broad, s), 7.5 (H8, s), 4.8 (m)	3460–3420 (S, B), 2950 (S), 1755 (S), 1550 (S), 1525 (S), 1346 (S), 1260 (S), 800 (W)	$C_{26}H_{23}N_5O_6$; calcd. C, 62.4%; H, 4.60%; N, 14.0%. Found C, 62.2%; H, 4.55%; N, 14.0%
4,4′-bis Methoxy-α-phenyl cinnamic acid	160–163°C (EtOH)	88	295	—	—	$C_{17}O_4H_{15}$; calcd. C, 72.12%; H, 5.3%; Found C, 72.11%; H, 5.23%
N^6-4,4′-bis Methoxy-α-phenyl cinnamoyl dA	180°C (diethyl ether)	79	280, 310	8.68 (H8, s), 8.35 (H2, s), 5.42 (s)	3460–3410 (S, B), 2829 (S), 2945 (S), 1746 (S)	$C_{27}H_{25}N_5O_6$; calcd.62.91%; H, 4.85%; N, 13.59%. Found C, 62.85%; H, 4.78%, N, 13.56%
N^2-4-Methoxy (4′-methoxy)-α-phenyl cinnamoyl-dG	180°C (diethyl ether)	79	280, 310	8.68 (H8, s), 8.35 (H2, s), 5.42 (s)	3460–3410 (S, B), 2890 (S), 2945 (S), 1748 (S), 1575 (S), 1526 (S), 1350 (S), 1250 (S), 780 (W)	$C_{27}H_{25}N_5O_7$; calcd. C, 61.13%; H, 4.7%; N, 13.2%. Found C, 61.1%, H, 4.7%; N, 13.1%

clear solution was evaporated to a gum under vacuum and worked up by the procedure reported earlier.[17] Yield, R_f, and λ_{max} of various derivatives is listed here.

5′-*O*-DMTr-*N*^{6}-4-Methoxy-α-phenylcinnamoyl-2′-*O*-deoxyadenosine. Yield 85%, R_f 0.76 (dichloromethane:methanol 9.5:0.5 v/v), λ_{max} 272, 286 nm.

5′-*O*-DMTr-*N*^{6}-α-(4-Methoxyphenyl)cinnamoyl-2′-deoxyadenosine. Yield 78%, R_f 0.68 (CH_2Cl_2:CH_3OH 9.5:0.5 v/v), λ_{max} 263, 280 nm.

5′-*O*-DMTr-*N*^{6}-4,4′-bis Methoxy-α-phenylcinnamoyl-2′-deoxyadenosine. Yield 68%, R_f 0.78 (CH_2Cl_2:CH_3OH 9.5:0.5 v/v), λ_{max} 275, 287, 305 nm.

5′-*O*-DMTr-*N*^{2}-4-Methoxy-α-phenylcinnamoyl-2′-deoxyguanosine. Yield 80%, R_f 0.63 (CH_2Cl_2:CH_3OH 9:1 v/v), λ_{max} 267, 285 nm.

5′-*O*-DMTr-*N*^{2}-α-(4-Methoxyphenyl)-cinnamoyl-2′-deoxyguanosine. Yield 72%, R_f 0.69 (CH_2Cl_2:CH_3OH 9:1 v/v), λ_{max} 269, 291 nm.

5′-*O*-DMTr-*N*^{2}-4,4′-bis Methoxy-α-phenylcinnamoyl-2′-deoxyguanosine. Yield 71%, R_f 0.68 (CH_2Cl_2:CH_3OH 9:1 v/v), λ_{max} 257,263, 291 nm.

Study of Deprotection Conditions of *N*-Protected dA and dG

All the six *N*-acylated derivatives, three each of dA and dG with substituted α-phenyl cinnamic acids prepared as described, were taken in small aliquots and treated with 40% aqueous ammonia solution. All the vials were kept sealed at 40°C for different time intervals. In all sets, the reaction was quenched by the addition of dilute hydrochloric acid after 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 h. The solvents from the reaction mixture were then evaporated under vacuum and again taken in CH_2Cl_2. These reaction mixtures were then subjected to semipreparative TLC (CH_2Cl_2:CH_3OH 9:1; v/v). Two major bands of relative R_f (0.65 for protected nucleoside and 0.3 for unprotected nucleoside) were obtained. The two bands were eluted separately in methanol and their absorbance (OD) was recorded. The percentage of deprotection against time was estimated as the OD of the lower band divided by the total OD of upper and lower band multiplied by 100. Graphs were manually plotted for time vs. percentage of deprotection for exact comparison (Figures 1 and 2). The results confirmed that the deprotection was complete in 4–7 h independent of the protecting group used.

Estimation of Degree of Depurination

The *N*-protected nucleosides (0.1 mmol) were dissolved in DCM (5 mL) and the unprotected nucleosides were dissolved in water (5 mL). Each solution was treated with 80% acetic acid (2 mL) at room temperature. Reaction was quenched by triethylamine–methanol (4:1 v/v) at varied time intervals. In the case of dA and its derivatives, the quenching time was 30 min, 1 h, 2 h,

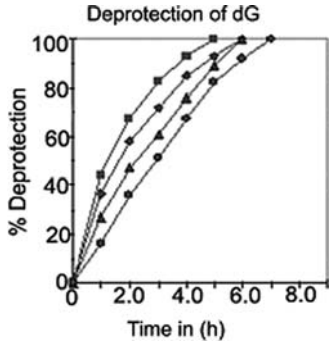


Figure 1. Deprotection of N^6-4-methoxy α-phenylcinnamoyl-dA (■); N^6-α-(4-methoxyphenyl) cinnamoyl-dA (◆); N^6-4,4′-bis methoxy-α-phenylcinnamoyl-dA (▲); N^6-benzoyl dA (●), at 40°C with 40% ammonia.

4 h, 6 h, 20 h, 30 h, and 60 h, and in the case of dG and its derivatives, the quenching time was 15 min, 30 min, 1 h, 2 h, 4 h, 10 h, 20 h, and 30 h. After quenching each fraction was evaporated to dryness and dissolved in minimum solvent (DCM for *N*-protected nucleosides and water for unprotected nucleosides). These solutions were run on semipreparative TLC. The upper band [i.e., depurinated part (free base)] and the lower band (i.e., unchanged nucleosides) were scratched and eluted with DCM in the case of *N*-protected nucleosides and with water in the case of free nucleosides. UV absorption was measured at the 288 nm wavelength for N^6-4-methoxy-α-phenylcinnamoyl dA, 290 nm for N^6-α-(4-methoxyphenyl)cinnamoyl dA, 285 nm for N^6-4,4′-bis methoxy-α-phenylcinnamoyl dA, 280 nm for N^6-bz-dA, 260 nm for dA, 285 nm for N^2-4-methoxy-α-phenylcinnamoyl dG, 290 nm for N^2-α-(4-methoxyphenyl)cinnamoyl dG, 283 nm for N^2-4,4′-bis methoxy-α-phenyl cinnamoyl dG, 260 nm for N^2-ibu-dG, and 252 nm for dG. Data is

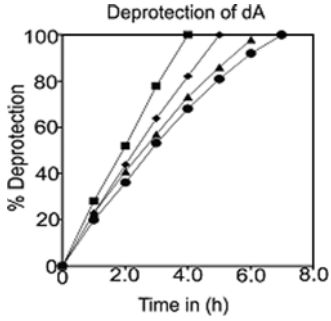


Figure 2. Deprotection of N^2-4-methoxy α-phenylcinnamoyl-dG (■); N^2-α-(4-methoxy phenyl) cinnamoyl-dG (◆); N^2-4,4′-bis methoxy-α-phenylcinnamoyl-dG (▲); N^2-benzoyl dG (●), at 40°C with 40% ammonia.

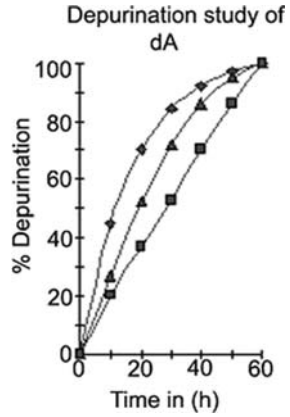


Figure 3. Depurination of *N*-protected derivatives of 2′-dA; N^6-benzoyl-dA (◆); N^6-α-phenylcinnamoyl-dA (▲); N^6-4-methoxy-α-phenylcinnamoyl-dA (■).

shown only for N^6-bz-dA and N^6-4-methoxy-α-phenylcinnamoyl dA, and a comparison with previously reported N^6-α-phenylcinnamoyl-dA (Figure 3), and for N^2-ibu-dG, N^2-4-methoxy-α-phenylcinnamoyl dG, and N^2-4-methoxy-α-phenylcinnamoyl dG (Figure 4).[15]

RESULTS AND DISCUSSION

Using the active ester protocol, all three methoxy derivatives of α-phenyl cinnamic acid were found to be regioselective for the protection of

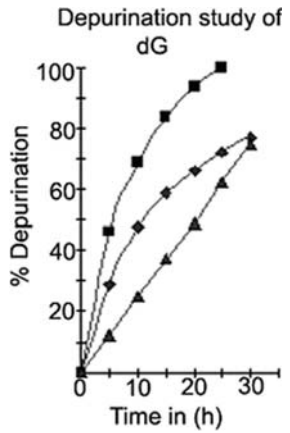


Figure 4. Depurination of *N*-protected derivatives of 2′-dG; N^2-isobutyryl-dG (■); N^2-α-phenylcinnamoyl-dG (◆); N^2-4-methoxy- α-phenylcinnamoyl-dG (▲).

exocyclic amino function in purine bases. Most important, acylation of the sugar hyroxyl group was not observed. This protocol eliminates the two-step conventional procedure that is commonly employed for the protection of purine base in nucleosides.

As reported earlier, the α-phenylcinnamoyl group[15] was found to be better at resisting depurination than benzoyl- and isobutryl-protected dA and dG, respectively (half life N^6-α-phenylcinnamoyl dA is 19.5 h; N^6-bz-dA, 10.5 h; N^2-α-phenylcinnamoyl dG, 10.5 h; and N^2-ibu-dG, 10 h). The main reason for the synthesis of these three molecules was to further slow down the depurination rates and increase the half-life beyond the earlier report.[15] Gratifyingly, this task was accomplished with 4-methoxy-α-phenylcinnamoyl derivative (**a**) exhibiting a $t_{1/2}$ of 30 h for dA (Figure 3) and a $t_{1/2}$ of 20 h for dG (Figure 4). To explain the increased half-life of α-phenylcinnamoyl dA, we postulate that the weakly acidic β-hydrogen atom of the α-phenylcinnamoyl group may form a weak intramolecular hydrogen bond with the N^7 nitogen atom. This interaction may reduce the basicity of N^7 nitrogen atom, making it less prone to attack the proton during treatment with protonated acid. However, there are two available alternative *p*-positions on the two-phenyl rings that may be used for installation of any electron-donating group, thereby rendering the β-hydrogen more available.

Keeping this rationale in mind, we have introduced two methoxy groups (**c**) that may make the β-hydrogen more available for hydrogen bonding with the N^7 of the purine nucleobase. Additionally, introduction of methoxy group may enhance the solubility of the protected nucleosides in organic solvents, enabling easy purification of the products. Although derivative **c** with two methoxy groups appears to be attractive, lower yield for the protection step with **c** was discouraging for scaling purposes and we elected not to pursue it further. The experimental data (Figures 1 and 2) confirm that the depurination sensitivity and the efficiency of deprotection were better in cases where the methoxy group was present in the main ring (derivative **a**) vis-à-vis the α-phenyl ring (derivative **b**). This extra stability shown by the derivative **a** may be attributed to the introduction of the methoxy group in the *p*-position, which enhances the availability of the β-hydrogen atom for hydrogen bonding. In addition, it is important to note that the bulky nature of the protecting group may prevent the free rotation of the glycosidic linkage, strengthening the proposed hydrogen bonding.

The cost of raw materials is of paramount importance in the synthesis of antisense ODNs. In this regard, we believe that the new active ester protocol may be more cost efficient than the old protocols. Although use of DCC as a coupling agent may not be the best choice from an industrial point of view,[18] we believe that solid-support reagents may provide an alternative.[19] Furthermore, the inherent crystalline nature of these products may assist in circumventing the expensive column chromatographic purification step.

In summary, we have developed a novel and one-pot synthetic strategy for the protection of *N*-acyl function of purine moiety of deoxyribonucleosides.

The yield was also found to be better in all the cases, a clear-cut advantage over the existing protocols for synthesizing protected monomers.

It seems that the strategy used in this communication has worked well and a conclusion may be drawn in favor of using it for further synthesis of ribo series as well as long oligomers.

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