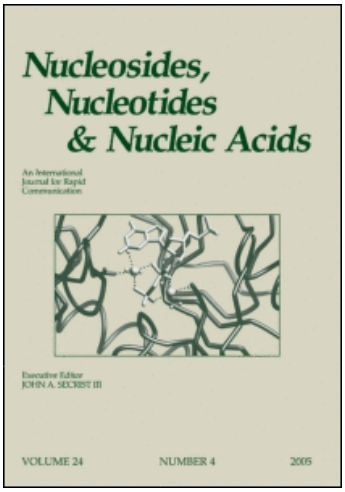

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 11 August 2010

To cite this Article Olejnik, Anna , Gdaniec, Zofia , Kierzek, Elzbieta and Kierzek, Ryszard(2010) 'The Spontaneous Rearrangement of 2,4-Dinitrophenyl Substituent in Ribonucleosides Under Neutral Conditions', Nucleosides, Nucleotides and Nucleic Acids, 29: 9, 684 — 697
To link to this Article: DOI: 10.1080/15257770.2010.507233
URL: http://dx.doi.org/10.1080/15257770.2010.507233

Nucleosides, Nucleotides and Nucleic Acids, 29:684–697, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2010.507233

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THE SPONTANEOUS REARRANGEMENT OF 2,4-DINITROPHENYL SUBSTITUENT IN RIBONUCLEOSIDES UNDER NEUTRAL CONDITIONS

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□ *In the cytidine and adenosine derivatives an isomerization of a 2,4-dinitrophenyl group between the 2′- and 3′-positions of the ribose was observed under neutral conditions. Moreover, it was shown that isomerization of the 2,4-dinitrophenyl group in conditions required to synthesize phosphoramidites and lability in aqueous ammonia make chemical synthesis of 2′-O-(2,4-dinitrophenyl) oligonucleotides impossible.*

Keywords 2,4-Dinitrophenyl; isomeration; ribonucleosides; Smiles rearrangement

INTRODUCTION

The 2,4-dinitrophenyl (DNP) group in RNA exhibits very interesting biological properties. Recently, it was reported that siRNA, containing several 2′-O-(2,4-dinitrophenyl) groups, reveals enhanced efficiency for inhibiting cancer cell growth and can easily diffuse into cells and remain there for several days without detectable degradation.[1,2] It was also proven that poly-2′-O-(2,4-dinitrophenyl)-RNA was not hydrolyzed by RNase H and its presence increased membrane permeability.[3,4] The appropriate 2′-O-(2,4-dinitrophenyl)-RNA was obtained in the reaction of 2,4-dinitrofluorobenzene with RNA, and 2,4-dinitrophenyl substituents were introduced randomly. These unique properties of the 2,4-dinitrophenyl group were the reason of our interest in the synthesis of 5′-O-dimethoxytrityl-2′-O-(2,4-dinitrophenyl)-N-acylribonucleoside-3′-O-phosphoramidites providing the opportunity to synthesize oligoribonucleotides with 2,4-dinitrophenyl modifications at definite positions.

Received 8 June 2010; accepted 1 July 2010.
This work was supported by the Ministry of Science and Higher Education, grants PBZ-MNiSW-07/I/2007 (R. K.) and N N301 3383 33 (E. K.). R. K. is the recipient of a fellowship of the Foundation for Polish Science.
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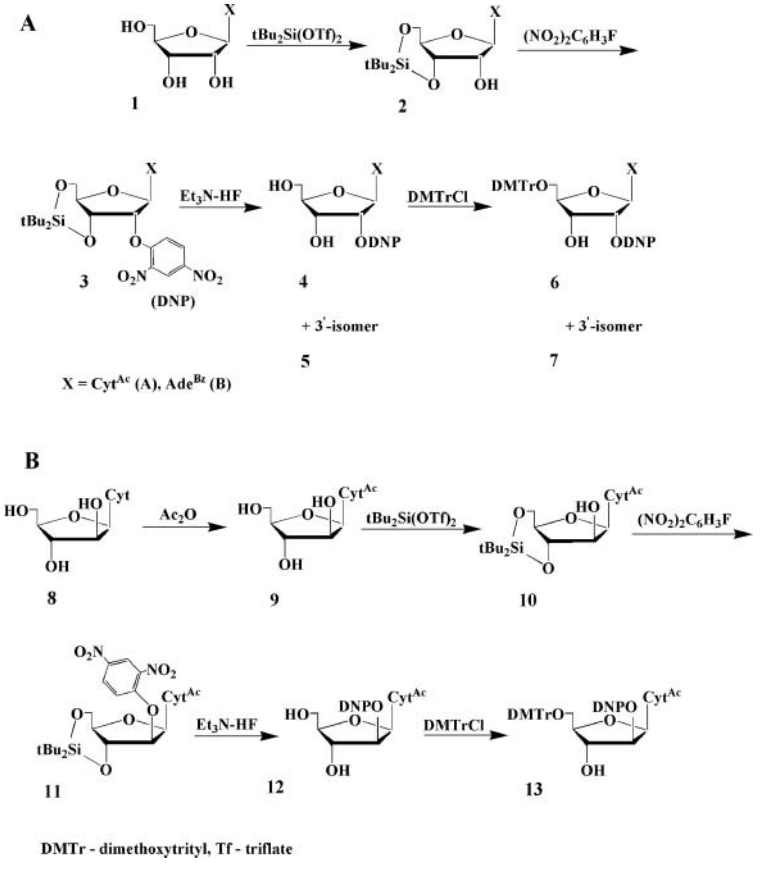
RESULTS AND DISCUSSION

The increasing demand for synthetic ribonucleotides has stimulated a search for effective routes to 2′-O-substituted ribonucleotides.[5,6] In the sythesis of 3′-O-phosphoramidites building blocks, the di-tert-butylsilylene group was used to simultaneously protect the 3′- and 5′-hydroxyls of the N-acylated nucleosides. This protection step was followed by treatment with 2,4-dinitrofluorobenzene.[7] Next, the di-tert-butylsilylene protecting group was removed from derivative **3** with fluoride, yielding derivatives **4** and **5**, which were subsequently treated with dimethoxytrityl chloride to get the appropriately protected derivatives **6** and **7**. The final products of the synthesis appeared on thin layer chromotography (TLC) as an equimolar mixture of two nucleoside derivatives **6** and **7** (Scheme 1A). In the case of the cytidine derivative, both products were separated by silica gel column chromatography.



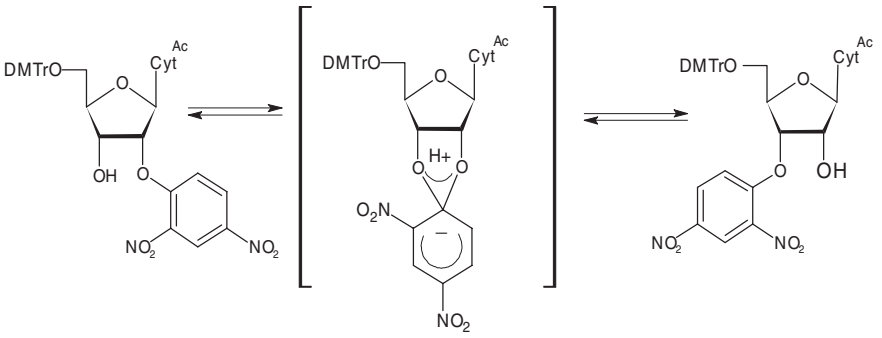
SCHEME 1 The chemical synthesis of 5′-O-dimethoxytrityl-2′-O-(2,4-dinitrophenyl)-N-acylribonucleosides (A) and 5′-O-dimethoxytrityl-2′-O-(2,4-dinitrophenyl)-4N-acetylarabinocytidine (B).

TABLE 1 The ^{1}H and ^{13}C NMR chemical shift of the sugar residues of the 2,4-dinitrophenyl derivatives of cytdidine and arabinocytidine

		^{1}H NMR [ppm]		^{13}C NMR [ppm]	
		Cytidine derivative			
Solvent	Position	2′-O-DNP (6A)	3′-O-DNP (7A)	2′-O-DNP (6A)	3′-O-DNP (7A)
$CDCl_3$	1′	6.07	5.91	88.44	92.73
	2′	5.18	4.65	84.43	76.20
	3′	4.73	4.79	68.28	79.81
	4′	4.30	4.61	82.61	83.14
	2′-OH	—	3.47	—	—
	3′-OH	3.33	—	—	—
DMSO-d_6	1′	5.75	5.86	89.28	92.73
	2′	4.58	4.65	82.31	72.54
	3′	5.50	5.18	67.72	76.20
	4′	4.12	4.37	81.58	79.81
	2′-OH	—	6.02	—	—
	3′-OH	5.97	—	—	—
		Arabinocytidine derivative			
		2′-O-DNP	3′-O-DNP	2′-O-DNP	3′-O-DNP
DMSO-d_6	1′	6.36	—	85.64	—
	2′	5.37	—	82.77	—
	3′	4.31	—	73.21	—
	4′	4.11	—	82.29	—
	3′-OH	6.22	—	—	—

The structures of the products were determined by one-dimensional nuclear magnetic resonance (NMR) (^{1}H and ^{13}C NMR) and two-dimensional NMR (^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC) spectroscopy. In the case of the cytidine derivative, the spectroscopic analysis proved that a mixture of isomers was obtained as products of the synthesis. The ^{1}H-^{1}H COSY spectra were helpful in identifying and distinguishing the signals from both isomers. The spectrum of the first isomer **6** proved that the 2,4-dinitrophenyl substituent was located at the 2′-position because a correlation between the H-3′ and the 3′-hydroxyl was observed. In the case of the second isomer **7**, the H-2′ coupled with the 2′-hydroxyl which proved that the 2,4-dinitrophenyl was located at the 3′-position. Table 1 presents ^{1}H and ^{13}C NMR chemical shifts of the sugar residues of the 2,4-dinitrophenyl derivatives of cytidine, arabinocytidine and adenosine.

Isomer **6A**, with the 2,4-dinitrophenyl group located at the 2′-position of the ribose ring, migrates faster on TLC. Surprisingly, the signal characteristic of both isomers were also observed in the NMR spectra when anhydrous DMSO-d_6 was used as the solvent even when the TLC pure isomers **6A** and **7A** were used for the analysis. In contrast, in $CDCl_3$ solution, only signals



SCHEME 2 The proposed Smiles type rearrangement of 2,4-dinitrophenyl substituted ribonucleosides.

from one isomer were observed. These experiments suggested that the 2,4-dinitrophenyl group migrated from the 2′-position to the 3′-position of ribose and vice versa under neutral conditions during the NMR experiment in DMSO-d_6 (Scheme 2). The isomerizations of the 2,4-dinitrophenyl group in the derivatives **6A** and **7A** were also confirmed by TLC analyses. Interestingly, the NMR spectra of the derivatives **2A** and **3A** showed the presence of one product only. However, after deprotection of the silyl group from derivative **3A**, the signals characteristic of the presence of a mixture of the 2′- and 3′-isomers of 2,4-dinitrophenyl appeared in the NMR spectra. This suggests that the presence of the vicinal 3′-hydroxyl was sufficient to initiate the 2,4-dinitrophenyl isomerization.

The mechanism of the migration of the 2,4-dinitrophenyl group in the ribonucleosides has not been described in the literature. However, the migration of the 4-nitrophenyl group of glucopyranose in alkaline solutions was reported.[8] The migration of this group was explained to be an intramolecular nucleophilic aromatic substitution. Moreover, Klemer and Kleefeldt observed the migration of the 2,4-dinitrophenyl group in the glucose ring under mild alkaline conditions.[9] According to the authors, the 2,4-dinitrophenyl group migrated particularly easily when it was located at the C-2′ position of glucose. In that case, the rearrangement to the C-3′ position was observed even after dissolution in acetone.

Isomerization of some protecting groups in ribonucleosides is mostly limited to acyl and (trifluoromethyl)sulfonyl esters.[10,11] Moreover, the isomerization in ribonucleosides concerns silyl ethers, including tert-butyldimethylsilyl.[12] Also extrimely fast migration of phosphotriester linkages in the oligoribonucleotides was observed.[13–15] Additionally, in the literature isomerization of the 2,4,6-trinitrophenyl group between 2′- and 3′-hydroxyl of 5′-O-triphosphate adenosine was reported and this derivative was used as a fluorescent analog to study ATP binding to heavy meromyosin ATPase.[16] Based on spectrospocic data demonstrating the formation of

Meisenheimer complex, the existence of 5′-O-triphosphate 2′,3′-O-(2,4,6-trinitrocyclohexadienylidene) adenosine at neutral and basic conditions was postulated.[17,18]

Migration of the 2,4-dinitrophenyl was also reported for aminoalcohols. Skarzewski and Skrowaczewska observed migration of the 2,4-dinitrophenyl in aminoethanol.[19] The primary product of the synthesis, the 2,4-dinitrophenyl ether of aminoethanol, rearranged to 2-(2,4-dinitrophenylamino)-ethanol in the presence of strong base. A similar rearrangement was reported when 2-(acetylamino)ethanol was treated with 2,4-dinitrofluorobenzene in the presence of sodium methoxide or potassium tert-butoxide.[20]

One of the aims of our studies was to elucidate the factors influencing the isomerization of the 2,4-dinitrophenyl substituent in ribonucleotides. The assumption was that traces of water in DMSO-d_6 might cause the migration of the 2,4-dinitrophenyl group. Therefore an NMR analysis of the purified isomers **6A** and **7A** in DMSO-d_6 with 0.1% and 1% water (v/v) was carried out. After 48 hours the isomerization of the 2′-O-(2,4-dinitrophenyl) derivative 6A results in equimolar quantities of both isomers, independent of the amount of water. The half life times of the isomerization of the 2′-O-(2,4-dinitrophenyl) derivative **6A** were 1.9 and 1.6 hours with 0.1% and 1% water in DMSO-d6, respectively. However, isomerization of the 3′-O-(2,4-dinitrophenyl) derivative **7A** was much slower. After 48 hours only ca. 20% of the 2′-isomer was observed, independent of the amount of water. In this case the half lives of the isomerization of **7A** were 6.6 and 4.9 hours with 0.1% and 1% water in DMSO-d_6, respectively.

To check whether the 2′-O-(2,4-dinitrophenyl) nucleosides can be used to synthesize the phosphoramidites and oligonucleotides, the stabilities of both **6A** and **7A** isomers in acetonitrile tetrazole solution were also analyzed. We observed isomerization of both compounds. This process was faster ($t_{1/2}$ ca. 0.9 hours) than the previously described isomerization in DMSO-d_6. The isomerization were more favorable for the 3′-isomer. Moreover, it was observed that the 2,4-dinitrophenyl group was quantitatively removed under conditions commonly used for the deprotection of oligoribonucleosides (aqueous ammonia, 16 hours at 55°C).[21]

The most probable mechanism for the isomerization is a Smiles type of rearrangement in which the aromatic ring migrates between the O-2′ and the O-3′ atoms (Scheme 2).[22] The Smiles rearrangement is facilitated by the activation of the aromatic ring via the electron-withdrawing nitro groups in the ortho and para positions. We thought that the same rearrangement could be possible for the compounds described herein. For the ribose ring in the nucleoside, in both the 2′- and 3′-endo conformers, the distance between the 2′- and the 3′-oxygen is short enough (ca. 2.8 Å) to allow the 2,4-dinitrophenyl moiety to migrate in a reversible way.

In order to check whether the isomerization of the 2,4-dinitrophenyl group also takes place in other nucleosides, the synthesis of the adenosine derivative was carried out under the same conditions as described for the cytidine derivative (Scheme 1). Two isomers of the adenosine derivatives **6B** and **7B** were obtained and identified by TLC and by ^{1}H and ^{13}C NMR (Table 1). Unfortunately, the separation of both isomers by column chromatography was impossible, and a detailed analysis of the isomerization could not be performed.

In order to gain insight into the migration of the 2,4-dinitrophenyl moiety in nucleosides, the synthesis of 5′-O-dimethoxytrityl-2′-O-(2,4-dinitrophenyl)-N4-acetylarabinocytidine was performed (Scheme 1B). The TLC analysis of the final derivative **13** showed the presence of one product only, and the analysis of the 1H-1H COSY and 1H-13C HSQC (in DMSO-d_6 and $CDCl_3$) spectra proved that the 2,4-dinitrophenyl group was located at the 2′-position of the arabinocytidine derivative (Table 1). However, even though there was an hydroxyl group at the 3′-position (the distance between the 2′- and the 3′- oxygen is 3.4–4.0 Å), migration of the substituent was not observed.

EXPERIMENTAL

General Information

Thin-layer chromatography were carried out on Merck 60 F_{254} or silanized (RF) TLC plates with variuos mitxures of dichloromethane and methanol (95:5 v/v (A) and 9:1 v/v (B)) or acetone and water (7:3 v/v (C)). Nuclear magnetic resonance spectra were recorded in DMSO-d_6 and $CDCl_3$ on Bruker Avance spectrometer (^{1}H at 400 MHz, ^{13}C at 100 MHz) and values are given in parts per milion (δ) downfiled from TMS. All reagents and solvents were purchased from commercial sources (Aldrich, Munich, Germany; Fluka, Buchs, Switzerland; Merck, Darmstadt, Germany) and were used without futher purification.

The Synthesis of 3′,5′-O-di-tert-butylsilyl-4N-acetylcytidine (2A)

To a solution of 4N-acetylcytidine (2.88 g, 10 mmol) in 100 ml of N,N-dimethylformamide 2,6-lutidine (3.48 ml, 30 mmol) was added and cooled to 0°C. To the stirring solution of di-tert-butylsilyl ditriflate (3.89 ml, 12 mmol) was added dropwise and warmed up to room temperature. The reaction progress was monitored by TLC. After 2 hours, the reaction was completed and half of the solvent was evaporated under reduced pressure. To the reaction mixture saturated aqueous solution of sodium bicarbonate was added extracted three times with dichloromethane. The combined organic layers

were dried over anhydrous sodium sulfate, filtered off, and concentrated. Purification was accomplished with silica gel column chromatography (0–4% methanol in dichloromethane). Yield: 67% (2.94 g), R_f 0.33 (A).

^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H, NH); 8.01 (d, 1H, H-5); 7.23 (d, 1H, H-6); 5.74 (d, 1H, OH); 5.68 (s, 1H, H-1′); 4.40–4.36 (m, 1H, H-2′); 4.17–4.14 (m, 1H, H-3′); 4.10–4.12 (d, 1H, H-4′); 4.05–3.94 (m, 2H, H-5′, H-5″); 2.09 (s, 3H, CH_3); 1.02 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.07 (C=O); 162.50, 154.25, 145.58, 95.69 (C-Cyt); 93.14 (C-1′); 75.46 (C-3′); 74.01 (C-2′); 72.82 (C-4′); 66.66 (C-5′); 27.31–27.00 (tBu_2Si); 24.38 (CH_3).

The Synthesis of 2′-O-(2,4-dinitrophenyl)-3′-5′-O-di-tert-butylsilyl-4N-acetylcytidine (3A)

The solution of 3′,5′-O-di-tert-butylsilyl-4N-acetylcytidine (2.94 g, 6.72 mmol) in 30 ml of N,N-dimethylformamide was cooled to -30°C and then 40% mineral oil emulsion of sodium hydride (0.62 g, 15.12 mmol) and 2,4-dinitrofluorobenzene (2.53 ml, 20.16 mmol) were added. After completion of the reaction (3 hours), saturated aqueous solution of sodium bicarbonate was added and extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuum. The product was purified by silica gel column chromatography (0–3% methanol in dichloromethane) to afford pure compound. Yield: 51% (2.06 g). R_f 0.44 (A).

^{1}H NMR (DMSO-d_6) δ 10.98 (s, 1H, NH); 8.80 (d, 1H, H-DNP); 8.50–8.52 (dd, 1H, H-DNP); 8.13 (d, 1H, H-DNP); 7.77 (d, 1H, H-5); 7.27 (d, 1H, H-6); 5.74 (d, 1H, OH); 5.68 (s, 1H, H-1′); 4.40–4.36 (m, 1H, H-2′); 4.23–4.18 (t, 1H, H-3′); 4.20–4.12 (d, 1H, H-4′); 4.05–3.94 (m, 2H, H-5′, H-5′′); 2.11 (s, 3H, CH_3); 1.04 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.19 (C=O); 162.90, 154.92 (C-Cyt); 154.23 (C-DNP); 145.94 (C-Cyt.); 140.01, 138.46, 128.40, 121.00, 117.62 (C-DNP); 96.00 (C-Cyt); 90.97 (C-1′); 79.55 (C-3′); 75.02 (C-2′); 74.52 (C-4′); 66.44 (C-5′); 27.66–26.38 (tBu_2Si); 24.41 (CH_3).

The Synthesis of 2′-O-(2,4-dinitrophenyl)-4N-acetylcytidine (4A, 5A)

To 2′-O-(2,4-dinitrophenyl)-3′,5′-O-di-tert-butylsilyl-4N-acetyl-cytidine (2.06 g, 3.40 mmol) 1 molar solution of triethylamine hydrofluoride (10.20 ml, 10.20 mmol) in pyridine was added. The reaction mixture was stirred at room temperature for 2 hours and the solution saturated aqueous sodium bicarbonate was added, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated. The crude product was purified on silica gel column chromatography (0–4% methanol in dichloromethane). Yield: 30% (0.44 g). R_f 0.14 (A).

^{1}H NMR (DMSO-d_6) δ 10.92 (s, 1H, NH); 8.76 (d, 1H, H-DNP); 8.52–8.48 (d, 1H, H-DNP); 8.45–8.43 (d, 1H, H-DNP); 7.81 (d, 1H, H-5); 7.24–7.21

(d, 1H, H-6); 5.92 (d, 1H, H-1′); 5.36 (t, 1H, H-3′); 4.61–4.58 (d, 1H, H-2′); 4.29–4.26 (m, 1H, H-4′); 3.82–3.66 (m, 2H, H-5′, H-5′’); 2.11 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.16 (C=O); 162.65, 154.86 (C-Cyt); 145.57 (C-Cyt.); 140.01, 138.88, 128.88, 123.97, 121.24, 117.71 (C-DNP); 95.78 (C-Cyt); 90.19 (C-1′); 82.50 (C-3′); 77.93 (C-2′); 72.54 (C-4′); 67.00 (C-5′); 24.42 (CH_3).

The Synthesis of 5′-O-dimethoxytrityl-2′-O-(2,4-dinitro-phenyl)-4N-acetylcytidine (6A, 7A)

To a solution of 2′-O-(2,4-dinitrophenyl)-4N-acetycytidine (0.44 g, 0.95 mmol) in 6.30 ml of pyridine the dimethoxytrityl chloride (0.37 g, 1.09 mmol) was added. The reaction was carried out at room temperature. After 3 hours the reaction was completed and to the reaction mixture saturated solution of aqueous sodium bicarbonate was added and extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and the solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography (0–3% methanol in dichloromethane). Yield: 62% (0.45 g). R_f isomer 6A 0.35 (A), R_f isomer 7A 0.30 (A).

Isomer 6A. ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H, NH); 8.75 (d, 1H, H-DNP); 8.63 (d, 1H, H-DNP); 8.53–8.51 (dd, 1H, H-DNP); 7.45 (d, 1H, H-5); 7.38–7.28 (m, 10H, H-DMTr); 7.13 (d, 1H, H-6); 6.90 (d, 3H, H-DMTr); 6.07 (s, 1H, H-1′); 5.18 (d, 1H, H-2′); 4.73 (m, 1H, H-3′); 4.30 (d, 1H, H-4′); 3.75–3.66 (m, 2H, H-5′, H-5″); 3.84 (s, 6H, OCH_3); 3.33 (s, 1H, OH-3′); 2.24 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 169.66 (C=O); 162.74, 155.48, 145.09 (C-Cyt); 143.96, 141.52, 138.75 (C-DNP); 135.19, 134.91, 130.19, 130.10 (C-DMTr); 128.16, (C-DNP); 127.30 (C-DMTr); 121.11, 118.94 (C-DNP); 113.39 (C-DMTr); 97.09 (C-Cyt); 88.44 (C-1′); 87.47 (C-DMTr); 84.43 (C-2′); 82.61 (C-4′); 68.28 (C-3′); 60.11 (C-5′); 24.98 (CH_3).

Isomer 6A. ^{1}H NMR (DMSO-d_6) δ 10.96 (s, 1H, NH); 8.76 (d, 1H, H-DNP); 8.52–8.49 (dd, 1H, H-DNP); 8.33–8.30 (d, 1H, H-DNP); 7.84–7.79 (d, 1H, H-5); 7.41–7.12 (m, 9H, H-DMTr); 7.06–7.05 (d, 1H, H-6); 6.91–6.79 (m, 4H, DMTr); 5.97 (s, 1H, OH-3′); 5.75 (d, 1H, H-1′); 5.50 (d, 1H, H-3′); 4.58 (m, 1H, H-2′); 4.12 (m, 1H, H-4′); 3.72 (s, 6H, OCH_3); 3.40–3.39 (m, 2H, H-5′, H-5″); 2.11 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.12 (C=O); 162.69, 158.24, 155.71, 144.89 (C-Cyt); 140.76, 138.88, 135.40 (C-DNP); 135.13, 129.84, 128.78, 128.86 (C-DMTr); 128.00 (C-DNP); 127.79, 127.65, 125.95, 129.84 (C-DMTr); 121.31, 117.75 (C-DNP); 113.33 (C-DMTr); 95.58 (C-Cyt); 89.28 (C-1′); 86.00 (C-DMTr); 82.31 (C-2′); 81.58 (C-4′); 67.72 (C-3′); 62.15 (C-5′); 24.42 (CH_3).

Isomer 7A. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H, NH); 8.72 (s, 1H, H-DNP); 8.27–8.23 (dd, 1H, DNP); 8.20 (d, 1H, H-DNP); 7.41–7.39 (d, 1H, H-5); 7.33–7.20 (m, 13H, H-DMTr); 6.82 (d, 1H, H-6); 5.91 (s, 1H, H-1′); 4.79 (t, 1H, H-3′); 4.65 (t, 1H, H-2′); 4.61 (d, 1H, H-4′); 3.82–3.76 (s, 6H, OCH_3); 3.75–3.72 (m, 2H, H-5′, H-5″); 3.47 (s, 1H, OH-2′); 2.25 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$) δ 170.129 (C=O); 162.66 (C-Cyt); 158.81, 156.47 (C-DMTr); 155.87, 144.44 (C-Cyt); 143.85 (C-DMTr); 140.63, 138.93 (C-DNP); 134.93, 134.69 (C-DMTr); 129.96 (C-DNP); 128.69, 128.13, 127.86, 127.30 (C-DMTr); 121.78, 116.88 (C-DNP); 113.48 (C-DMTr); 97.02 (C-Cyt); 92.73 (C-1′); 83.14 (C-4′); 79.81 (C-3′); 76.20 (C-2′); 72.54; 62.13 (C-5′); 25.00 (CH_3).

Isomer 7A. ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H, NH); 8.77 (d, 1H, H-DNP); 8.52–8.48 (dd, 1H, H-DNP); 8.33–8.29 (d, 1H, H-5); 7.83–7.79 (d, 1H, H-DNP); 7.41–7.14 (m, 9H, H-DMTr); 7.07–7.04 (d, 1H, H-6); 6.91–6.79 (m, 4H, H-DMTr); 6.02 (d, 1H, OH-2′); 5.86 (d, 1H, H-1′); 5.18 (t, 1H, H-3′); 4.65 (m, 1H, H-2′); 4.37 (m, 1H, H-4′); 3.70 (s, 6H, OCH_3); 3.55–3.52 (m, 2H, H-5′, H-5′’); 2.12 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 170.15, 171.11 (C=O); 162.69, 162.59, 154.94, (C-Cyt); 154.35 (C-DNP); 145.28 (C-Cyt); 140.15, 140.07, 138.87, 138.64 (C-DNP); 143.85 (C-DMTr); 140.63, 138.93 (C-DNP); 134.93, 134.69 (C-DMTr) 129.96 (C-DNP); 128.69, 128.13, 127.86, 127.30 (C-DMTr); 121.78 116.88 (C-DNP); 113.48 (C-DMTr); 97.02 (C-Cyt); 92.73 (C-1′); 79.81 (C-4′); 76.20 (C-3′); 72.54 (C-2′); 65.71 (C-5′); 24.41 (CH_3).

The Synthesis of 3′,5′-O-di-tert-butylsilyl-6N-benzoyladenosine (2B)

To a solution of 6N-benzoyladenosine (1.48 g, 4 mmol) in 40 ml of N,N-dimethylformamide 2,6-lutidine (1.39 ml, 12 mmol) was added and cooled to 0°C. To the stirring solution of the di-tert-butylsilyl ditriflate (1.39 ml, 5.6 mmol) was added dropwise and the solution was warmed up to room temperature. The reaction progress was monitored by TLC. After 2 hours the reaction was completed and half of the solvent was removed at reduced pressure. To the reaction mixture saturated aqueous solution of sodium bicarbonate was added and extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated. Purification was accomplished with silica gel column chromatography (0–3% methanol in dichloromethane). R_f 0.45 (A).

^{1}H NMR (DMSO-d_6) δ 11.20 (s, 1H, NH); 8.73 (s, 1H, H-2); 8.63 (s, 1H, H-8); 8.04–7.43 (m, 5H, H-arom.); 6.06 (s, 1H, OH); 5.89 (d, 1H, H-1′); 4.71–4.68 (t, 1H, H-3′); 4.61–4.59 (t, 1H, H-2′); 4.38–4.35 (m, 1H, H-4′); 4.07–3.99 (m, 2H, H-5′,H-5′′); 1.08 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 162.48 (C=O); 151.81, 143.55, 133.37 (C-Ade); 129.34–128.55 (C-arom.); 125.87, 120.14 (C-Ade); 90.64, 75.90, 74.13, 72.91, 66.99 (C-rib.); 27.71, 27.38, 27.09 (C-CH_3).

The Synthesis of 2′-O-(2,4-dinitrophenyl)-3′,5′-O-di-tert-butylsilyl-6N-benzoyladenosine (3B)

The solution of 3′,5′-O-di-tert-butylsilyl-6N-benzoyladenosine (1.48 g, 4 mmol) in 20 ml of N,N-dimethylformamide was cooled to −30°C and

40% mineral oil emulsion of sodium hydride (0.37 g, 9 mmol) and 2,4-dinitrofluorobenzene (1.50 ml, 12 mmol) were added. After 3 hours the reaction was completed and saturated aqueous solution of sodium bicarbonate was added and extracted three times with dichloromethane. Then combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuum. The product was purified by silica gel column chromatography (0–3.5% methanol in dichloromethane). Yield: 56% (1.53 g). R_f 0.13 (C).

^{1}H NMR (DMSO-d_6) δ 8.97 (s, 1H, NH); 8.84–8.79 (m, 2H, H-2; H-8); 8.59–8.25 (m, 3H, H-DNP); 7.94–7.38 (m, 5H, arom.); 6.35 (d, H1, OH); 6.04–5.99 (dd, H1, H-1′); 5.15–5.12 (t, 1H, H-3′); 4.95–4.92 (t, 1H, H-2′); 4.50–4.43 (m, 1H, H-4′); 4.15–4.00 (m, 2H, H-5′, H-5″); 1.06–1.00 (m, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 175.76 (C=O); 162.45, 154.75, 148.14, 143.91 (C-Ade); 140.40–132.35 (C-DNP); 129.91–128.45 (C-arom.); 117.86 (C-Ade); 87.96, 80.00, 75.43, 75.16, 66.66 (C-ryb.); 27.43 (C-CH_3).

The Synthesis of 2′-O-(2,4-dinitrophenyl)-6N-benzoyl-adenosine (4B, 5B)

To 2′ - O - (2,4 - dinitrophenyl) - 3′,5′ - O - di - tert - butylsilyl - 6N - benzoyl-adenosine (1.52 g, 2.25 mmol) 1 molar solution of triethylamine hydrofluoride (6.75 ml, 6.75 mmol) in pyridine was added. The reaction mixture was stirred at room temperature for 2 hours. Next the saturated aqueous solution of sodium bicarbonate was added and extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated to obtain crude product. R_f 0.58 (B).

^{1}H NMR ($CDCl_3$) δ 9.00 (d, 1H, NH); 8.38–8.34 (m, 2H, H-2; H-8); 8.02, 7.71–7.68 (m, 3H, H-DNP); 7.49–7.24 (m, 5H, H-arom.); 5.83 (d, 1H, H-1′); 4.91–4.87 (t, 1H, H-2′); 4.40 (d, 1H, H-3′); 3.93 (m, 1H, H-4′); 3.76–3.72 (m, 2H, H-5′, H-5″); 3.01 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ 170.54 (C=O); 152.31, 151.78 (C-Ade); 151.78 (C-DNP); 146.30, 144.39 (C-Ade); 140.39, 133.62, 132.80, 132.30 (C-DNP); 128.28 (C-DNP); 129.91–127.28 (C-arom.); 121.86 (C-Ade); 91.34, 87.63, 72.20, 62.97, 53.41 (C-rib.).

The Synthesis of 5′-O-dimethoxytrityl-2′-O-(2,4-dinitro-phenyl)-6N-benzoyladenosine (6B, 7B)

To a solution of 2′-O-(2,4-dinitrophenyl)-6N-benzoyladenosine (1.52 g, 2.25 mmol) in 10.2 ml of pirydine the dimethoxytrityl chloride (0.84 g, 2.47 mmol) was added. The reaction was carried out at room temperature. After 3 hours the reaction was completed, then to the mixture saturated aqueous solution of sodium bicarbonate was added and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and the solvent was evaporated under

vacuum. The crude product was purified by silica gel column chromatography (0–3.5% methanol in dichloromethane). Yield: 61% (1.15 g). R_f 0.38 (A).

^{1}H NMR (DMSO-d_6) δ 9.00–8.99 (dd, 1H, NH); 8.98–8.97 (dd, 1H, NH); 8.88–8.87 (t, 1H, H-2); 8.86–8.85 (t, 1H, H-2); 8.78 (dd, 1H, H-8); 8.77 (dd, 1H, H-8); 8.55 (s, 1H, H-DNP); 8.53 (s, 1H, H-DNP); 8.45–8.44 (t, 1H, H-DNP); 8.42–8.41 (t, 1H, H-DNP); 8.36–8.34 (d, 1H, H-DNP); 8;19–8.18 (d, 1H, H-DNP); 7.75–7.33 (m, 10H, H-arom.); 7.22–7.12 (m, 18H, H-DMTr); 6.82–6.77 (m, 8H, H-DMTr); 6.14–6.13 (d, 1H, OH); 6.13–6.11 (d, 1H, OH); 6.00–5.98 (d, 1H, H-1′); 5.96–5.95 (d, 1H, H-1′); 5.39–5.36 (q, 2H, H-3′); 5.19–5.14 (q, 1H, H-2′); 5.08–5.04 (q, 1H, H-2′); 4.35–4.34 (m, 2H, H-4′); 3.72–3.70 (m, 4H, H-5′, H-5′′); 3.31 (s, 12H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 175.55 (C=O); 158.06 (C-Ade); 155.16–145.27 (C-DMTr); 144.70 (C-Ade); 144.61–135.25 (C-DNP); 134.66 (C-Ade); 129.67–128.22 (C-arom.); 126.69 (C-Ade); 121.21–120.58 (C-DMTr); 117.14 (C-Ade); 87.91, 87.01, 85.93, 81.09, 72.06, 71.31, 62.96, 62.73 (C-rib.); 54.95 (C-OCH_3).

The Synthesis of 4N-acetylarabinocytidine (9)

Arabinocytidine (0.37 g, 1.50 mmol) was dissolved in methanol (22.5 ml) and heated under reflux. Next acetic anhydride (2.25 ml) was added to the solution. After 3 hours of stirring the mixture was evaporated with toluene to obtain the product.

^{1}H NMR (DMSO-d_6) δ 10.82 (s, 1H, NH); 8.06 (d, 1H, H-5); 7.18 -7.16 (d, 1H, H-6); 6.07 (s, 1H, OH); 5.48 (s, 1H, H-1′); 5.04 (s, 1H, H-2′); 3.92 (s, 1H, H-3′); 3.84–3.80 (m, 1H, H-4′); 3.61 (d, 2H. H-5′,5′′); 2.09 (s, 1H, CH_3). ^{13}C NMR (DMSO-d_6) δ 170.89 (C=O); 162.13, 154.46, 146.69 (C-Cyt); 94.18 (C-Cyt); 86.95, 85.73, 76.09, 63.84, 60.98 (C-rib.); 24.32 (C-CH_3).

The Synthesis of 3′,5′-O-di-tert-butylsilyl-4N-acetylarabino-cytidine (10)

To a solution of 4N-acetylarabinocytidine (0.37 g, 1.3 mmol) in 15 ml of N,N-dimethylformamide 2,6-lutidine (0.52 ml, 4.5 mmol) was added and mixture was cooled to 0°C. To the stirring solution di-tert-butylsilyl ditriflate (0.58 ml, 1.8 mmol) was added dropwise and warmed up to room temperature. The reaction progress was monitored by TLC. After 2 hours the reaction was completed and half of the solvent was removed at reduced pressure. To the reaction mixture saturated aqueous solution of sodium bicarbonate was added and extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium, sulfate, filtered off, and concentrated. Purification was accomplished with silica gel column chromatography (0–3% methanol in dichloromethane). Yield: 40% (0.22 g).

^{1}H NMR (DMSO-d_6) δ 10.86 (s, 1H, NH); 7.95 (t, 1H, H-5); 7.23 (d, 1H, H-6); 6.32–6.30 (d, 1H, OH); 5.83–5.82 (d, 1H, H-1′); 5.49 (s, 1H, H-2′); 4.36–4.28 (m, 1H, H-3′); 4.11–4.07 (m, 1H, H-.4′); 3.96–3.94 (m, 2H, H-5′,

5″); 2.10 (s, 3H, CH_3); 0.97 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.07 (C=O); 162.50, 154.25, 145.58, 95.69 (C-Cyt); 93.14 (C-1′); 75.46 (C-3′); 74.01 (C-2′); 72.82 (C-4′); 66.66 (C-5′); 27.31–27.00 (tBu_2Si); 24.38 (CH_3).

The Synthesis of the 2′-O-(2,4-dinitrophenyl)-3′,5′-O-di-tert-butylsilyl-4N-acetylarabinocytidine (11)

The solution of 3′,5′-O-di-tert-butylsilyl-4N-acetylarabinocytidine (0.22 g, 0.5 mmol) in 3 ml of N,N-dimethylformamide was cooled to −30°C and than 40% mineral oil emulsion of sodium hydride (0.05 g, 1.12 mmol) and 2,4-dinitrofluorobenzene (0.18 ml, 1.5 mmol) were added. After completion of the reaction (3 hours), saturated aqueous sodium bicarbonate solution was added and extracted three times with dichloromethane Then combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuum. The product was purified by silica gel column chromatography (0–3% methanol in dichloromethane). Yield: 70% (0.20 g).

^{1}H NMR (DMSO-d_6) δ 10.87 (s, 1H, NH); 8.68–8.77 (d, 1H, H-DNP); 8.60–8.54 (dd, 1H, H-DNP); 8.14–8.13 (d, 1H, H-DNP); 7.73–7.70 (d, 1H, H-5); 7.31–7.20 (d, 1H, H-6); 6.66 (d, 1H, OH); 5.70–5.66 (d, 1H, H-1′); 5.49 (s, 1H, H-rib.); 4.44–4.38 (m, 1H, H-ryb.); 4.24–4.19 (m, 1H, H-ryb.); 4.09–3.95 (m, 1H, H-ryb.); 2.09 (s, 3H, CH_3); 0.97 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.06 (C=O); 162.57, 154.15, 146.55 (C-Cyt); 129.68, 129.02, 121.11, 117.19, 116.43 (C-DNP); 95.85 (C-Cyt); 89.44 (C-1′); 82.78 (C-3′); 79.17 (C-2′); 74.67 (C-4′); 66.23 (C-5′); 27.20–26.89 (tBu_2Si); 21.94 (CH_3).

The Synthesis of 2′-O-(2,4-dinitrophenyl)-4N-acetylarabino-cytidine (12)

To 2′-O-(2,4-dinitrophenyl)-3′,5′-O-di-tert-butylsilyl-6N-acetyl-arabinocy tidine (0.20 g, 0.35 mmol) 1 molar solution of triethylamine hydrofluoride (1.06 ml, 1.05 mmol) in pyridine was added. The reaction mixture was stirred at room temperature for 2 hours and then to the solution saturated aqueous sodium bicarbonate was added, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated. Yield: 100% (0.20 g).

Synthesis of 5′-O-dimetoxytrityl-2′-O-(2,4-dinitrophenyl)-4N-acetylarabinocytidine (13)

To a solution of 2′-O-(2,4-dinitrophenyl)-4N-acetyarabino-cytidine (0.20 g, 0.35 mmol) in 2.5 ml pyridine the dimethoxytrityl chloride (0.13 g, 0.40 mmol) was added. The reaction was carried out at room temperature. After 3 hours the reaction was completed, then to the mixture saturated aqueous solution of sodium bicarbonate was added and the solution was extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, filtered off, and the solvent was evaporated

under vacuum. The crude product was purified by silica gel column chromatography (0–3% methanol in dichloromethane). Yield: 85% (0.17 g).

^{1}H NMR (DMSO-d_6) δ 10.96 (s, 1H, NH); 8.68 (d, 1H, H-DNP); 8.53–8.51 (dd, 1H, H-DNP); 8.33–8.30 (d, 1H, H-DNP); 8.02–8.00 (d, 1H, H-DNP); 7.71–7.67 (d, 1H, H-5); 7.38–7.7.15 (m, 9H, DMTr); 7.15–7.13 (d, 1H, H-6); 6.89–6.82 (m, 4H, DMTr); 6.36 (d, 1H, H-1′); 6.22 (d, 1H, OH-3′); 5.37 (t, 1H, H-2′); 4.31 (m, 1H, H-3′); 4.11 (m, 1H, H-4′); 3.40–3.25 (m, 2H, H-5′, H-5″); 2.10 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$) δ 170.96 (C=O); 162.49, 158.09, 154.06, 149.58 (C-Cyt); 146.10, 140.43, 138.45 (C-DNP); 135.27, 129.68, 127.38 (C-DMTr); 127.74, 121.33 (C-DNP); 116.81 (C- Cyt); 113.39 (C-DMTr); 95.23 (C-Cyt); 85.64 (C-1′); 82.77 (C-2′); 82.29 (C-4′); 73.21 (C-3′); 62.08 (C-5′); 24.35 (CH_3).

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