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TRANSPROTECTION OF N-BENZOYLATED NUCLEOBASE DERIVATIVES BY DIALKYLAMINOMETHYLENE GROUP

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Abstract: The use of (chloromethylene)dialkylammonium chloride in selective transprotection of N-benzoylated nucleobase derivatives is reported along with the evaluation of the stability of various protecting groups widely used in nucleoside and/or nucleotide chemistry.

Introduction

(Chloromethylene)dimethylammonium chloride ($[\text{Me}_2\text{N}=\text{CHCl}]^+\text{Cl}^-$, CDMC)^{1,2} is a useful and versatile reagent in nucleic acids chemistry. It is effective in chlorination of nucleosides both at the oxo group of heterocyclic bases^{3,4} and at the 5'-hydroxyl group^{5,6}, and also in O-formylation of the glycosyl residue of nucleosides⁷. CDMC was also used as the activating agent for the preparation of esters of carboxylic and sulfonic acids² and, recently, for the preparation of esters of PMEAs [9-(2-phosphonomethoxyethyl)adenine]⁸. In the latter case, the formation of 6-N-(dimethylaminomethylene)adenine derivative was observed⁹. On performing similar reaction with 6-N-benzoylated PMEAs we found, surprisingly, a change in the UV spectra of the adenine moiety. Significant bathochromic shift, with respect to the starting compound, revealed the formation of the 6-N-dimethylaminomethylene derivative.

This paper describes our study of this reaction with various N-acylated nucleosides, with particular attention to the stability of the O-protecting groups generally used in nucleoside and/or nucleotide chemistry.

Experimental Section

Unless stated otherwise, the solvents were evaporated at 40 °C and 2 kPa. The products were dried over phosphorus pentoxide at 50 - 70 °C and 13 Pa. Their purity was checked by chromatographic methods (TLC, HPLC), spectral methods (NMR, MS, UV), and by elemental analysis.

All the described reactions were monitored by TLC on Silufol UV 254 foils (Kavalier Glassworks, Votice, Czech Republic).

Preparative column chromatography (column dia 50 mm) was carried out on 20 - 40 µm spherical silica gel (Tessek, Prague); the amount of adsorbent was 20 - 40 times the weight of the separated mixture. Elution was performed at the flow rate 40 ml / min. TLC was carried out with the following solvent systems: toluene - ethyl acetate 1 : 1; chloroform - ethanol 9 : 1; ethyl acetate - acetone - ethanol - water 12 : 2 : 2 : 1.

Preparative chromatography (column dia 25 mm) on reverse phase was carried out on a spherical octadecylsilica gel 20 - 40 µm (Tessek, Prague). Compounds were eluted with a linear gradient of methanol in water at the flow rate 15 ml / min.

HPLC analyses were performed on a reverse phase Separon SGX C18 7 µm (Laboratorní přístroje, Prague); isocratic elution with 0.1M triethylammonium acetate or gradient of methanol in 0.1M triethylammonium acetate.

The UV spectra were measured on Beckman DU 65 UV spectrophotometer in 0.01M HCl (pH 2) or 0.01M NaOH (pH 12) in methanol-water mixture (1:1, v/v).

Mass spectra (m/z) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB (ionisation with Xe, accelerating voltage 8 kV) or SIMS (ionization with Cs⁺, accelerating voltage 35 kV). Glycerol and thioglycerol were used as matrices.

¹H NMR spectra were measured on Varian Unity 500 instrument (¹H at 500 MHz, ¹³C at 125.7 MHz) in hexadeuteriodimethylsulfoxide and referenced to the solvent signal standard (δ_H = 2.5; δ_C = 39.7).

Preparation of reagents and starting compounds

(Chloromethylene)dimethylammonium chloride (CDMC) and (chloromethylene)dibutylammonium chloride (CDBC).

Freshly distilled thionyl chloride (14.6 ml, 0.2 mol) was added to the solution of dimethylformamide (15.5 ml, 0.2 mol) or dibutylformamide (36.4 ml, 0.2 mol) in

dichloromethane (50 ml) under stirring and cooling in an ice-bath. Resulting mixture was stirred at room temperature overnight under exclusion of moisture and then diluted by dichloromethane to the final volume of 100 ml. 2M stock solutions were stored at room temperature without any detectable changes over several months.

(S)-6-N-Benzoyl-9-(2,3-dibenzoyloxypropyl)adenine (Entry 1).

Suspension of (S)-6-N-benzoyl-9-(2,3-dihydroxypropyl)adenine¹⁰ (0.85 g, 2.7 mmol) in dichloromethane (50 ml) was treated overnight with benzoyl cyanide¹¹ (0.80 g, 6.1 mmol) and triethylamine (0.07 ml, 0.5 mmol) under vigorous stirring at room temperature. Dry methanol (1 ml) was added to destroy excess benzoyl cyanide, and after several minutes of standing, the solution was applied on a silica gel column. Column was eluted with a chloroform-ethanol mixture (98:2) to give (S)-6-N-benzoyl-9-(2,3-dibenzoyloxypropyl)adenine (1 g, 71 %, foam). Anal. calcd. for C₂₉H₂₃N₅O₅ (521.53): C, 66.79; H, 4.45; N, 13.43. Found: C, 66.68; H, 4.50; N, 13.62.

2',3'-Di-O-acetyl-6-N-benzoyl-5'-O-tert-butylidiphenylsilyladenosine (Entry 3).

Acetanhydride (0.15 ml, 1.5 mmol) was added to a stirred solution of 6-N-benzoyl-5'-O-tert-butylidiphenylsilyladenosine^{12,13} (0.30 g, 0.5 mmol) in anhydrous pyridine (5 ml). The reaction mixture was kept overnight at room temperature under exclusion of moisture. The course of the reaction was followed by TLC in chloroform - ethanol mixture (9 : 1). Reaction was quenched by methanol (2 ml) and the mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 ml) and extracted with water (3 x 50 ml). The ethyl acetate layer was dried over magnesium sulphate, filtered, and concentrated *in vacuo*. Chromatography on reverse phase (70% - 100% methanol) afforded 0.32 g (92 %) of 2',3'-di-O-acetyl-6-N-benzoyl-5'-O-tert-butylidiphenylsilyladenosine. Anal. calcd. for C₃₇H₃₉N₅O₇Si (693.84): C, 64.05; H, 5.67; N, 10.09. Found: C, 63.63; H, 5.70; N, 9.68. MS (FAB) 694 (M + H⁺).

Diisopropyl 6-N-(4-methoxybenzoyl)-9-N-(2-phosphonomethoxyethyl)adenine (Entry 4).

4-Methoxybenzoyl chloride (5.3 g, 30 mmol) was added at -78 °C to a stirred solution of diisopropyl 9-N-(2-phosphonomethoxyethyl)adenine¹⁴ (3.5 g, 9.8 mmol) and pyridine

(2.6 ml, 30 mmol) in dry dichloromethane (100 ml). The reaction mixture was stirred overnight at room temperature under exclusion of moisture. The course of the reaction was followed by TLC in chloroform - ethanol mixture (9 : 1). The reaction was quenched by water (1 ml), and after 30 minutes the mixture was concentrated *in vacuo*. The primarily formed bis-N-(4-methoxybenzoyl) derivative was converted to the mono-acylated compound by treatment with 2M solution of ammonia in water - pyridine mixture (100 ml, 1 : 4, v/v) for 2 h. The reaction mixture was concentrated *in vacuo* and dried by co-evaporation with toluene (3 x 20 ml). Chromatography on a silica gel column in chloroform - ethanol mixture (98 : 2) followed by crystallization from acetone afforded desired anisoyl derivative (2.5 g, 52 %), m.p. 71.5-73.5 °C. Anal. calcd. for $C_{22}H_{30}N_5O_6P$ (491.49): C, 53.76; H, 6.15; N, 14.25. Found: C, 53.48; H, 6.27; N, 13.98. MS (FAB) 492 ($M + H^+$).

2-N-Benzoyl-9-(2-benzoyloxyethoxymethyl)guanine (Entry 6).

9-(2-Hydroxyethoxymethyl)guanine¹⁵ (1.13 g, 5 mmol) was N-benzoylated according to Jones procedure¹². 2-N-Benzoyl derivative (1.32 g, 4 mmol) was subsequently treated with benzoyl cyanide¹¹ (1.5 equiv) and triethylamine (0.15 equiv.) in DMF (20 ml) as described for Entry 1. Evaporation of reaction mixture and purification on a silica gel column in chloroform-ethanol mixture (98:2) afforded desired dibenzoyl derivative (1.37 g, 63 %). Anal. calcd. for $C_{22}H_{19}N_5O_5$ (433.42): C, 60.97; H 4.42; N 16.16. Found: C, 60.83; H, 4.35; N, 16.11.

2'-O-Benzoyl-2-N-dibutylaminomethylene-3',5'-O-tetraisopropylidisiloxanylguanosine (Entry 7).

3',5'-O-Tetraisopropylidisiloxanylguanosine¹⁶ (0.53 g, 1 mmol) was treated with N,N-dibutylformamide dimethyl acetal¹⁷ (1.5 equiv) in DMF (10 ml) at room temperature overnight. TLC in $CHCl_3$ -ethanol (9:1) revealed complete conversion of the starting material. After evaporation *in vacuo*, a 70% aqueous pyridine (10 ml) was added and after 20 min the clear solution was once again evaporated. Gummy residue was dried by co-evaporation with DMF (3 times), dissolved in dichloromethane (5 ml), and benzoylated with benzoyl cyanide¹¹ (2.4 equiv.) and triethylamine (0.24 equiv.) as described for Entry 1. Chromatography on a silica gel column in toluene-ethyl acetate mixture (9:1) afforded guanine derivative (0.58 g, 75 %). Anal. calcd. for $C_{38}H_{60}N_6O_7Si_2 \cdot 0.3 H_2O$ (774.50): C, 58.86; H, 7.86; N, 10.78. Found: C, 58.51; H, 7.86; N, 10.70.

4-N-2'-O-Dibenzoyl-3',5'-O-tetraisopropylidisiloxanylcytidine (Entry 9).

4-N-Benzoyl-3',5'-O-tetraisopropylidisiloxanylcytidine¹⁶ (0.59 g, 1 mmol) was treated with benzoyl cyanide¹¹ in dichloromethane (5 ml) as described for Entry 1. Chromatography on a silica gel column in toluene-ethyl acetate mixture (9:1) afforded desired compound (0.58 g, 84 %). Anal. calcd. for $C_{35}H_{47}N_3O_8Si_2 \cdot 0.5 H_2O$ (702.95): C, 59.77; H, 6.88; N, 6.02. Found: C, 59.43; H, 6.79; N, 5.95. MS (FAB) 711 (M + Na), 695 (M + H⁺).

3-N-2',3'-O-Tribenzoyl-5'-O-triphenylmethyluridine (Entry 10).

Benzoyl chloride (0.87 ml, 7.5 mmol) was added to a solution of 5'-O-triphenylmethyluridine¹⁸ (0.73 g, 1.5 mmol) and diisopropylethylamine (0.90 ml, 7.5 mmol) in anhydrous pyridine (7 ml) at room temperature. After standing overnight the mixture was cooled down in an ice-bath, methanol (2 ml) was added to destroy excess benzoyl chloride, and the solution was concentrated under reduced pressure. Chromatography of the residue on a silica gel column in toluene-ethyl acetate mixture (9:1) afforded desired product (0.98 g, 82 %). Anal. calcd. for $C_{49}H_{38}N_2O_9$ (798.84): C, 73.67; H, 4.79; N, 3.51. Found: C, 73.18; H, 4.90; N, 3.27. MS (FAB) 821 (M + Na), 799 (M + H⁺).

5'-O-tert-Butyldimethylsilyl-3-N-2',3'-O-tribenzoyluridine (Entry 11).

5'-O-tert-Butyldimethylsilyluridine¹⁹ (0.72 g, 2 mmol) was benzoylated according to the method described in Entry 8. The crude product was purified on a silica gel column in toluene-ethyl acetate mixture (8:2) to afford desired derivative (1.07 g, 80 %). Anal. calcd. for $C_{36}H_{38}N_2O_9Si$ (670.79): C, 64.46; H, 5.71; N, 4.18. Found: C, 64.31; H, 5.70; N, 4.10. MS (FAB) 671 (M + H⁺).

3-N-Benzoyl-2',3',5'-tri-O-acetyluridine (Entry 12).

2',3',5'-Tri-O-acetyluridine²⁰ (0.56 g, 1.5 mmol) was N-benzoylated according to the method described in Entry 8. The crude 3-N-benzoyl derivative was purified on a silica gel column in toluene-ethyl acetate mixture (7:3) to afford desired compound (0.60 g, 84 %). Anal. calcd. for $C_{22}H_{22}N_2O_{10} \cdot 0.1 C_6H_5CH_3$ (483.63): C, 56.44; H, 4.80; N, 5.76. Found: C, 56.71; H, 4.79; N, 5.62. MS (FAB) 675 (M + H⁺).

Typical procedure for N-transprotection:**2',3'-Di-O-acetyl-5'-O-*tert*-butyldiphenylsilyl-6-N-dimethylaminomethylene adenosine** (Entry 3, Method A-1).

The 2M solution of CDMC in dichloromethane (0.5 ml, 1 mmol) was added at room temperature to a stirred solution of 2',3'-di-O-acetyl-6-N-benzoyl-5'-O-*tert*-butyldiphenylsilyl-adenosine (0.14 g, 0.2 mmol) in dichloromethane (4 ml). The course of the reaction was followed by TLC in chloroform - ethanol mixture (9 : 1). After 15 minutes the reaction mixture was cooled down to 0 °C and 2M triethylammonium hydrogen carbonate buffer pH 7.5 (5 ml) was added. The mixture was diluted with chloroform (50 ml) and extracted with water (3 x 30 ml). The chloroform layer was dried with magnesium sulphate, filtered and concentrated *in vacuo*. Chromatography on a silica gel column in chloroform - ethanol mixture (95 : 5) afforded 6-N-dimethylaminomethylene derivative as a foam (0.11 g, 85 %). Anal. calcd. for C₃₃H₄₀N₆O₆Si (644.32): C, 61.47; H, 6.25; N, 13.03. Found: C, 61.20; H, 6.42; N, 12.73. MS (FAB) 645 (M + H⁺).

Typical procedure for N-deprotection:**(*S*)-9-(2,3-Dibenzoyloxypropyl)adenine hydrochloride** (Entry 1, Method A-2).

The 2M solution of CDMC in dichloromethane (6.8 ml, 13.6 mmol) was added at room temperature to a stirred solution of (*S*)-6-N-benzoyl-9-(2,3-dibenzoyloxypropyl)adenine (1.4 g, 2.7 mmol) in dichloromethane (50 ml). The course of the reaction was followed by TLC in chloroform - ethanol mixture (9 : 1). After 10 minutes the reaction was quenched at 0 °C by addition of methanol (10 ml) and after 90 min concentrated *in vacuo*. Crystallization of the residue from acetone afforded 1.06 g (87 %) of (*S*)-9-(2,3-dibenzoyloxypropyl)adenine hydrochloride, m.p. 163-165 °C. Anal. calcd. for C₂₂H₁₉N₅O₄ (417.43) .HCl: C, 58.17; H, 4.57; N, 16.76; Cl, 7.84. Found: C, 57.78; H, 4.48; N, 15.57; Cl 7.96. MS (FAB) 418 (M + H⁺).

N-Deprotection of diisopropyl 6-N-(4-methoxybenzoyl)-9-N-(2-phosphomethoxyethyl)adenine (Entry 4, Method A-3).

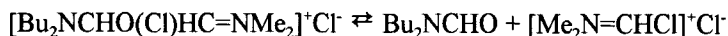
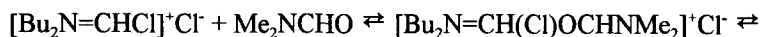
The 2M solution of CDMC in dichloromethane (2.5 ml, 5 mmol) was added at room temperature to a stirred solution of diisopropyl 6-N-(4-methoxybenzoyl)-9-N-(2-phosphomethoxyethyl)adenine (0.5 g, 1.0 mmol) in dichloromethane (20 ml). The course of the

reaction was followed by TLC in chloroform - ethanol mixture (9 : 1). After 10 minutes the reaction was quenched by methanol (10 ml). The removal of primary formed 6-N-dimethylaminomethylene derivative was checked by TLC in chloroform - ethanol mixture (9 : 1) and in ethyl acetate - acetone - ethanol - water 12 : 2 : 2 : 1 mixture. After the complete conversion (90 min at room temperature) the mixture was diluted with methanol-water-triethylamine mixture (5:5:2, 15 ml), and concentrated *in vacuo*. Chromatography on reverse phase (0-50% methanol in water) followed by crystallization from acetone afforded 0.34 g (95 %) of diisopropyl 9-N-(2-phosphonomethoxyethyl)adenine, m.p. 128-129 °C. Anal. calcd. for C₁₄H₂₄N₅O₄P (357.35): C, 47.06; H, 6.77; N, 19.60. Found: C, 47.59; H, 6.77; N, 19.55. MS (FAB) 358 (M + H⁺).

Results and Discussion

On the basis of UV, mass, and ¹H NMR spectroscopy measurements we found that N-benzoyladenine, -guanine and -cytosine derivatives undergo on the treatment with CDMC or CDBC in dichloromethane, or CDMC in DMF replacement of N-benzoyl group by dialkylaminomethylene moiety (see Table 1 and Table 2, Entry 1,2,3,6,8,9). Under these experimental conditions, the 3-N-benzoyl group of uracil derivatives is stable (Entry 10-12).

The structure of the CDMC reagent was discussed in several papers^{21,22,23,24,25}. When transprotection was performed with CDBC in dimethylformamide (Method D), an interesting exchange reaction was observed, and N-dimethylaminomethylene derivatives were isolated as the only products (Entry 6, 8). In this case, an exchange equilibrium reaction between CDBC and large excess of dimethylformamide, leads to the formation of CDMC, as shown in the following simplified equations:



Treatment of the N-dibutylaminomethylene derivative with excess CDMC in dichloromethane did not lead to any exchange reaction of the amidine function (Entry 7).

The unprotected exocyclic amino group of adenine derivative reacted with both reagents smoothly under formation of corresponding 6-N-dialkylaminomethylene derivatives (Entry 5).

TABLE 1. Transprotection of Purine-Containing Compounds.

Entry	Adenine starting compounds		Method ^a	Adenine products	
	R ¹	R ²		R ³	R ⁴ [MS FAB <i>m/z</i>]
1	Benzoyl	(S)-2,3-Dibenzoyloxypropyl	A	Dmam	R ² [473 (M+H) ⁺]
			A-2	H ₂ .HCl	R ² [418 (M+H) ⁺]
			B	Dbam	R ² [418 (M+H-Bu ₂ NCH) ⁺]
2	Benzoyl	2-Bromoethyl ²⁶	A	Dmam	R ² [242, 244 (M+H-Me ₂ NCH) ⁺]
			B	Dbam	R ² [381, 383 (M+H) ⁺]
3	Benzoyl	2,3-Di-O-acetyl-5-O- <i>tert</i> -butyldiphenylsilyl-β-D-ribofuranosyl	A-1	Dmam	R ² [645 (M+H) ⁺]
4	Anisoyl	2-Diisopropylphosphono-methoxyethyl	A-3	H ₂	R ² [358 (M+H) ⁺]
5	H	2-Diisopropylphosphono-methoxyethyl ¹⁴	A	Dmam	R ² [ND] ^b
			B	Dbam	R ² [ND]

TABLE 1. Transprotection of Purine-Containing Compounds (Continuation).

Entry	Guanine starting compounds		Method ^a	Guanine products	
	R ¹	R ²		R ³	R ⁴ [MS FAB <i>m/z</i>]
6	Benzoyl	2-Benzoyloxyethoxymethyl	C, D	Dmam	R ² [385 (M+H) ⁺]
7	Dbam ^c	2-O-Benzoyl-3,5-O-tetraiso-propyldisiloxyanyl-β-D-ribofuranosyl	A	Dbam	2-O-Benzoyl-5-chloro-5-deoxy-3-O-(hydroxytetraisopropyldisiloxyanyl)-β-D-ribofuranosyl [805 (M+H) ⁺ ; 666 (M+H-Bu ₂ NCH) ⁺]

^a Method A: CDMC-CH₂Cl₂; Method B: CDBC-CH₂Cl₂; Method C: CDMC-DMF; Method D: CDBC-DMF

^b ND not determined; ^c 2-N-Dbam derivative was used as the starting compound.

The UV spectra of all products were found to be in a good agreement with the appropriate N-dimethylaminomethylene²⁸ or N-dibutylaminomethylene²⁹ derivatives prepared as standards from ribonucleosides³⁰.

The ¹H NMR spectra of the products showed signals assigned to the hydrogen atom of the N=CH group of amidine moiety; only in the case of N-dibutylaminomethylene-guanosine derivative both E- and Z-isomers were found, the other dialkylaminomethylene derivatives were single isomers (E-Z configuration not determined; see Table 3).

The mechanism proposed for the transprotection of N-benzoylated adenine derivatives is shown in the Scheme 1. (a similar mechanism is suggested for 2-N-benzoylguanine and 4-N-benzoylcytosine derivatives, respectively).

Surprisingly, the N,O-peracetylated adenosine, guanosine or cytidine did not undergo transprotection by the action of CDMC and CDBC at all, and the starting protected

TABLE 2. Transprotection of Pyrimidine-Containing Compounds.

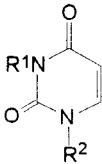
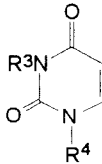
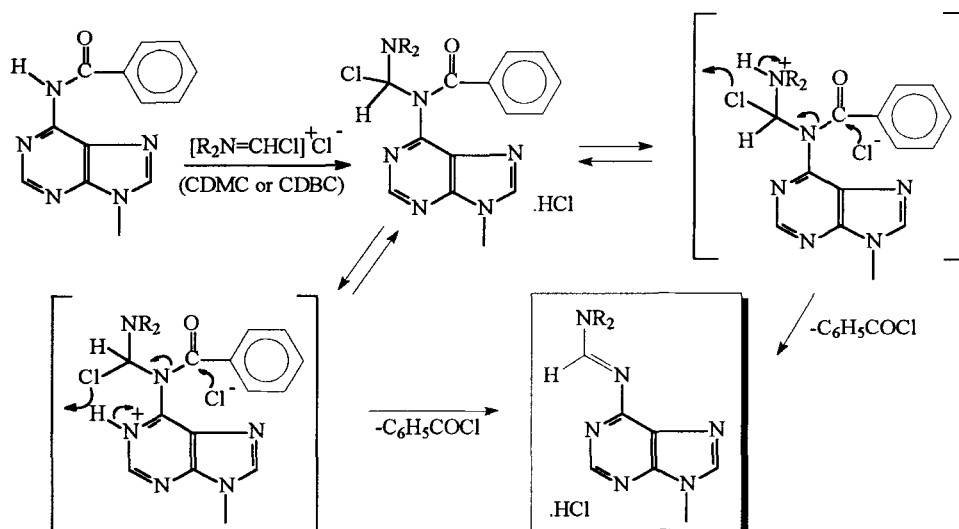
Entry	Cytosine starting compounds		Method ^a	Cytosine products	
	R ¹	R ²		R ³	R ⁴ [MS FAB <i>m/z</i>]
8	Benzoyl	2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl ²⁷	A-2	H ₂ .HCl	R ² [ND]
			C, D	Dmam	R ² [556 (M+H-Me ₂ NCH) ⁺]
9	Benzoyl	2-O-Benzoyl-3,5-O-tetraiso-propyldisiloxanyl-β-D-ribofuranosyl	A	Dmam	2-O-Benzoyl-5-chloro-5-deoxy-3-O-(hydroxytetraiso-propyldisiloxanyl)-β-D-ribofuranosyl [ND]
Uracil starting compounds			Uracil products		
					
10	Benzoyl	2,3-Di-O-benzoyl-5-O-triphenylmethyl-β-D-ribofuranosyl	A	Benzoyl	2,3-Di-O-benzoyl-5-chloro-5-deoxy-β-D-ribofuranosyl [575 (M+H) ⁺]
11	Benzoyl	2,3-Di-O-benzoyl-5-O- <i>tert</i> -butyldimethylsilyl-β-D-ribofuranosyl			
12	Benzoyl	2,3,5-Tri-O-acetyl-β-D-ribofuranosyl	A	No change	

TABLE 3. ^1H NMR Spectral Shifts of the Hydrogen Atom of the $\text{N}=\text{CH}$ Group of Amidine Moiety (δ , ppm).

Amidine Group	N-protected Nucleoside Derivatives		
	Ado	Guo	Cyd
Dmam	8.91 ± 0.02	8.54	8.62 ± 0.01
Dbam	8.93 ± 0.02	8.58 8.61	8.63

**SCHEME 1.** Proposed mechanism of the exchange reaction in the series of adenine derivatives.

nucleosides remained unchanged under conditions given. Only in the case of N,O-peracetylated guanosine, the formation of traces of unknown product was observed. The resistance of N-acetylated nucleosides towards transprotection is still unclear and under study. Our preliminary hypotheses, that the resistance is due to the higher electron density on the carbonyl group of N-acetyl derivatives, was disproved by the use of N-(4-methoxybenzoyl) derivative (Entry 4), generally claimed as an electron rich system similar to that of the acetyl one. Treatment of diisopropyl 6-N-(4-methoxybenzoyl)-9-N-(2-phosphonmethoxyethyl)-

adenine with CDMC in CH_2Cl_2 , followed by methanol quenching afforded smoothly N-deacylated derivative (Entry 4, Method A-3).

As far as the stability of the O-protecting groups of ribonucleosides is concerned, the base-labile O-acetyl and O-benzoyl groups were stable under reaction conditions (Entry 1,3,6-12). The acid-labile O-trityl group was split off within 10 minutes whereby the 5'-chloro derivative was formed immediately (Entry 10). The O-silyl protecting groups were split off under action of CDMC as well, however, the 5'-O-*tert*-butyldimethylsilyl and 5'-O-*tert*-butyldiphenylsilyl groups remained intact during the reaction time needed for N-debenzoylation (10-15 minutes). Standing of the reaction mixture overnight caused almost complete splitting of the 5'-O-*tert*-butyldimethylsilyl group followed by chlorination of the free 5'-hydroxy group (Entry 11). Similarly, the 3',5'-O-tetraisopropylidisiloxanyl group underwent quickly disiloxanyl ring opening at the 5'-end, and it was also followed by chlorination of the 5'-hydroxy group (Entry 7,9). The presence of the 5'-chloro-5'-deoxy moiety (Entry 7, 9-11) was confirmed by ^{13}C NMR spectra (δ , ppm: 44.12 for CH_2Cl instead of 60.99 for CH_2OH).

Dilution of the reaction mixture with protic solvent (e.g., methanol) led, in the case of adenine or cytosine derivatives, to the complete removal of Dmam group in a few minutes (Entry 1, 8, Method A-2). Thus the replacement of N-benzoyl function by dimethylamino-methylene group under the action of CDMC in dichloromethane or DMF could be useful reaction in the cases when the free exocyclic amino groups of these nucleobases should be generated under nonbasic conditions. In the case of 2-N-dimethylaminomethyleneguanine derivatives, no splitting was observed³¹.

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