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NUCLEOSIDE 5'-PHOSPHORDIAMIDATES

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Abstract—A one-flask method is reported for the preparation of the 5'-phosphordiamidate of adenosine (**3a**), cytidine (**3b**), guanosine (**3c**), uridine (**3d**) and thymidine (**3e**), and also for that of adenosine 5'-(N,N'-dimethyl) phosphordiamidate (**4a**), adenosine 5'-(N,N,N',N'-tetramethyl) phosphordiamidate (**5a**) and adenosine 5'-phosphordimorpholidate (**6a**). The method is based on the aminolysis of nucleoside 5'-phosphordichloridates performed *in situ* by NH_4OH, or aqueous amine solutions, respectively.

Nucleoside 5'-phosphordiamidates derived from ammonia (**3**) are useful synthetic intermediates. The selective substitution of one of their two amide groups under mild conditions rendered possible the elaboration of the synthesis of nucleoside 5'-phosphoramidates from nucleosides[1] and the preparation of P¹-(nucleoside 5'-)P¹-amino-triphosphates.[2] The synthesis of 5'-pyrophosphate and 5'-triphosphate derivatives of purine diribonucleoside monophosphates was accomplished also via phosphordiamidate intermediates.[3]

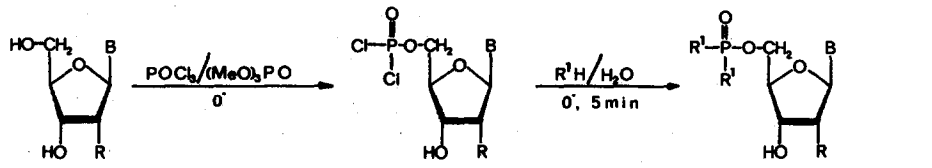
Hitherto, of the phosphordiamidates **3**, merely the synthesis of thymidine 5'-phosphordiamidate (**3e**) has been reported in detail.[1] This was performed according to the one-flask method summarised in Scheme 1. This method has now been improved and demonstrating its widespread applicability, been extended also to the preparation of some N(P)-alkylated adenosine 5'-phosphordiamidates. The present paper describes the synthesis of the 5'-phosphordiamidates of the four major ribonucleosides (**3a–3d**) and thymidine 5'-phosphordiamidate (**3e**) according to an improved procedure, as well as, that of adenosine 5'-(N,N'-dimethyl) phosphordiamidate (**4a**), adenosine 5'-(N,N,N',N'-tetrametil) phosphordiamidate (**5a**) and adenosine 5'-phosphordimorpholidate (**6a**).

RESULTS AND DISCUSSION

The one-flask method has been elaborated for the synthesis of the phosphordiamidates **3**, **4a**, **5a**, and **6a**, as follows. Nucleosides 5'-phosphordichloridates (**2**) were prepared from nucleosides (**1**) according to Yoshikawa's method[4] and were transformed *in situ* into the phosphordiamidates with an excess of 7.0 N NH_4OH (for **3**), aqueous CH_3NH_2 (for **4a**), $(CH_3)_2NH$ (for **5a**) and morpholine (for **6a**), respectively, at 0° for 5 min (Scheme 1).

Besides the main product, **3** and some unreacted starting material, **1**, the reaction mixtures always contained different by-products in small quantities (altogether <5%). For example, in the synthesis of the phosphordiamidate, **3a**, the following by-products were identified: adenosine 2',3'-(cyclic) phosphate (**7**), adenosine 2',3'-(cyclic) phosphate 5' phosphordiamidate (**8**), adenosine 5'-phosphoramidate (**9**), adenosine 5'-phosphate (**10**) and 1-methyladenosine 5'-phosphordiamidate (**11**) (Scheme 2).

The formation of by-products of similar types during the synthesis of the phosphordiamidates **3b**, **3c**, **3d**, **4a**, **5a** and **6a** might be suggested on the basis of a comparative tlc analysis of the appropriate peaks after column chromatographic separation of the mixtures. The formation of by-products may be ascribed partly to the non-selectivity of the Yoshikawa-reaction[5] (**7** and **8**), partly to the hydrolytic fission of P–Cl bonds of compound **2** (**9** and **10**) and, finally to the methylating ability of $(MeO)_3PO$, the solvent of the Yoshikawa-reaction in alkali (**11**).[6] To interpret the formation of the by-products **7** and **8**, the extreme lability of ribonucleoside 2'-(3')-phosphoramidates and ribonucleoside 2',3'-(cyclic)-phosphoramidates should also be taken into account. Instead of these derivatives 2',3'-(cyclic) phosphates could always be isolated.[1,7]



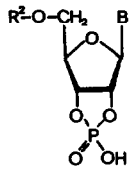
1

	B	R
a:	adeninyl-9	OH
b:	citosinyl-1	OH
c:	guaninyl-9	OH
d:	uracilyl-1	OH
e:	thyminyl-1	H

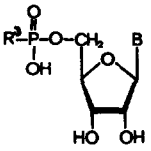
2

	R¹
3	NH_2
4a	$NH(CH_3)$
5a	$N(CH_3)_2$
6a	

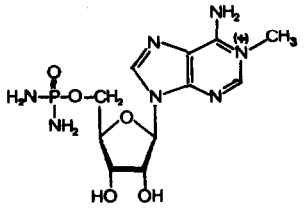
Scheme 1.



7, 8



9, 10



11

B	R^2
7: adeninyl-9	H
8: adeninyl-9	$P(O)(NH_2)_2$

B	R^3
9: adeninyl-9	NH_2
10: adeninyl-9	OH

Scheme 2.

Column chromatography was used for the isolation of the phosphordiamidates synthesised. The purine derivatives **3a** and **3c** were separated from the by-products and the staring materials on a Dowex 1×8 column {$HCOO^-$ form} with deionized H_2O as eluent. The compounds were eluted in the order of methylated derivative > phosphordiamidate > nucleoside, while, the by-products of anionic nature were retained by the column. This method could not be applied to the isolation of the pyrimidine derivatives **3b**, **3d** and **3e**, because of the overlapping of phosphordiamidate and nucleoside peaks. For this reason, the compounds **3b**, **3d** and **3e** were isolated by employing a silica gel column and $CHCl_3$–MeOH mixtures as eluents. In these cases an order of elution of methylated derivative > nucleoside > phosphordiamidate was observed. For the isolation of the compounds **4a**, **5a** and **6a** the method of Dekker[8] was adapted. An additional purification on a DEAE-cellulose column {HCO_3^- form} was essential in each case to remove last traces of Cl^- ions which hindered the crystallization of the products. Every phosphordiamidate was obtained in tlc pure, crystalline form. The column chromatographic methods used for isolation, yields, m.ps and mass spectrometric data are tabulated (Table 1).

The structural assignments of the compounds synthesised were based on the following observations. (1) The molecular weight of the TMS derivatives measured by mass spectrometry agreed well with the calculated one (Table 1). (2) The UV spectra at three different pH values closely resembled those of the parent nucleosides. (3) The behaviour on anion exchangers indicated the absence of phosphate dissociations. (4) All the phosphordiamidates were quantitatively converted to nucleoside 5′-phosphates upon hydrolysis with 1.0 N HCl, at room temperature as expected on the basis of the sensitivity of P–N bonds against acid.[10] The acid

Table 1. Methods of isolation, yields, m.ps and mass spectrometric data of nucleoside 5′-phosphordiamidates

Compound	Method of isolation[a]		Yield (%)	Melting point (°)	Mass spectrometric data of TMS derivatives[b]		
	Column chromatographic system	Peak position expressed in fraction number			Formula	Molecular weight Calculated	Found
3a	*C1*	15–25	85	186–9	$C_{25}H_{56}N_7O_5Si_5P$	705.2927	705.295
3b	*C2β*	46–66	42	115–7	$C_{24}H_{56}N_5O_6Si_5P$	681.2815	681.279
3c	*C1*[c]	28–41	68	250[d]	$C_{28}H_{58}N_7O_6Si_6P$	793.3272	793.325
3d	*C2α*	24–46	38	94–7	$C_{24}H_{55}N_4O_7Si_5P$	682.2655	682.267
3e	*C2γ*	40–50	63	100–3	$C_{22}H_{49}N_4O_6Si_4P$	608.2467	608.245
4a	*C3*	23–45	73	99–102	$C_{27}H_{60}N_7O_5Si_5P$	733.3240	733.325
5a	*C3*	20–33	89	78–9	$C_{23}H_{48}N_7O_5Si_3P$	617.2763	617.278
6a	*C3*	23–46	91	105–7	$C_{27}H_{52}N_7O_7Si_3P$	701.2974	701.294

[a] For details see Experimental, Column Chromatography

[b] The detailed mass spectrometric behaviour of the TMS derivatives will be published elsewhere[9].

[c] A column of 1.6x53 cm was used.

[d] With decomposition.

Table 2. ^{13}C Chemical shifts (ppm) and coupling constants (Hz)[a] of the phosphordiamidates **4a**, **5a** and **6a**

	4a	5a	6a
C-2	153.69	153.80	153.84
C-4	149.61	149.68	149.71
C-5	119.45	119.56	119.52
C-6	156.23	156.35	156.37
C-8	140.47	140.49	140.63
C-1'	88.77	89.03	89.09
C-2'	70.89	70.65	70.46
C-3'	75.00	74.77	74.29
C-4'	83.83	83.49	82.99
C-5'	65.14	64.84	64.99
P-N-CH_3	27.15	36.75 36.59	-
P-N-$\underline{C}H_2$	-	-	44.95 44.85
P-N-CH_2-$\underline{C}H_2$-O	-	-	67.55 67.48
$^3J_{PC4'}$	8.21	8.67	8.48
$^2J_{PC5'}$	5.02	4.74	4.67
$^2J_{PNC}$	<0.3	0.82	1.57
$^3J_{PNCC}$	-	-	3.75

[a]For details see Experimental

hydrolysis of the phosphordiamidates **3** was accompanied by the release of NH_3 as demonstrated by Nessler reagent. The substituents attaching to the amide nitrogens of the phosphordiamidates **4a**, **5a** and **6a** were identified by ^{13}C NMR spectroscopy (Table 2). (5) The phosphoridamidates **3** and **4a**, but not **5a** and **6a** could be hydrolysed to nucleosides by 0.1 N and 1.0 N NaOH respectively, at room temperature. The rapid basic hydrolysis of those phosphordiamidates bearing at least one ionisable (P)N–H groups is well-known.[11]

EXPERIMENTAL

Materials. Nucleosides (Sigma) were dried over P_2O_5 at 1 mmHg and 110° for 8 hr $POCl_3$, morpholine and $(MeO)_3PO$ (at 1 mmHg) were freshly distilled. 7.0 N aqueous solns of CH_3NH_2 and $(CH_3)_2NH$ were prepared from the commercial 40% ones. All other chemicals were of reagent grade. Compound **8** was prepared from adenosine by $P_2O_3Cl_4$ and subsequent aminolysis as reported in detail for the analogous guanosine derivatives.[1] The other adenosine derivatives used as control compounds, as well as *Escherichia coli* alkaline phosphatase were commercial products (Sigma).

Methods. M.ps are uncorrected and were determined on a Kofler apparatus. Evaporations were carried out using a rotary evaporator at 1 mmHg with a bath temp. of 30°. Absorption spectra were recorded on a Cary 15 spectrophotometer at pHs 1.0, 7.0 and 11.0. ^{13}C NMR spectra were obtained in D_2O at 25.16 MHz using a Varian XL-100/15 NMR spectrometer equipped with disk accessory. Dioxane was employed as internal reference, but the reported chemical shifts referred to TMS (dioxane 67.71 ppm). Mass spectral measurements were carried out with an accuracy of 3 ppm at a resolving power of 10,000 by an AEI MS-902 double focusing instrument operating at 70 eV and 200°. For the silylation of the phosphordiamidates the method of Miller *et al.* was applied.[12]

Column chromatography was performed in the following systems: Cl, column: Dowex 1×8 (AG, 200–400 mesh, Serva, $HCOO^-$ form, 2.0×64 cm), eluent: deionized H_2O, elution rate: 32 ml/hr, fraction volume: 8 ml; C2α, C2β and C2γ, column: silica gel (100–200μm, Serva, activated at 300° for 3 hr, 1.4×32 cm), stepwise elution with the eluents: α, $CHCl_3$–MeOH (9:1, 315 ml, 8:2, 360 ml), β, $CHCl_3$–MeOH (8:2, 450 ml, 6:4, 1065 ml) and γ, $CHCl_3$–EtOH (8:2, 300 ml, 6:4, 520 ml), elution rate: 120 ml/hr, fraction volume: 15 ml; C3, column: Dowex 1×2 (AG, 200–400 mesh, Serva, OH-form, 1.6×53 cm), eluent: H_2O–MeOH (7:3), elution rate: 32 ml/hr, fraction volume: 16 ml; C4α and C4β, column: DEAE-cellulose (DE 32, Whatman, HCO_3^- form, 1.4×32 cm), eluents: α, deionised H_2O, elution rate: 10 ml/hr, fraction volume: 5 ml, β, deionised H_2O (90 ml) then a linear gradient of H_2O – 0.3 M aqueous triethylammonium hydrogencarbonate, pH 7.5 (1000 ml), elution rate: 30 ml/hr, fraction volume: 10 ml.

Tlc was performed on precoated hptlc chromatoplates (Silica Gel 60 F_{254}, Merck) in the solvent systems: S1, 1-PrOH–conc NH_4OH–H_2O (11:7:2), S2, $CHCl_3$–MeOH (7:3); S3, $CHCl_3$–MeOH (4:6) and on precoated PEI-cellulose chromatosheets (Polygram Cel 300 PEI/UV_{254}, Macherey, Nagel + Co) in the soln S4, 0.1 M NaCl. Spots were detected by UV absorption, R_f values are listed in Table 3.

Hydrolytic degradations were performed at room temp. using 0.025 M solns of the phosphordiamidates in 1.0 N HCl, as well as, 0.1 N and 1.0 N NaOH, respectively. Enzymic hydrolysis was carried out with 1.0 A_{260} unit of the substrate and 0.3 unit of *Escherichia coli* alkaline phosphatase in 10μl of 0.1 M $NaHCO_3$ buffer, pH 10.6, at 37°, for 1 hr. Hydrolyses were followed by tlc.

General procedure for the synthesis of the phosphordiamidates **3**, **4a**, **5a** and **6a**. The whole procedure was performed at 0°. $POCl_3$ (200μl, 2.2 mmol) was added to a well-stirred suspension of the nucleoside (1.0 mmol) in $(MeO)_3PO$ (2.5 ml). Stirring was continued with the exclusion of atmospheric moisture for 1 hr (**1a** and **1b**), or 2 hr (**1c**), or 7 hr (**1d** and **1e**). The resulting soln was poured into 7.0 N NH_4OH (for **3**), or 7.0 N aqueous CH_3NH_2 (for **4a**), or $(CH_3)_2NH$ (for **5a**), or morpholine (for **6a**) {10 ml in each case} with stirring. After standing 5 min, the soln was evaporated to dryness. The residue was dissolved in deionised H_2O at 40–50° (7 ml for **3a**, 16 ml for **3c**) or H_2O–MeOH (7:3) (5 ml for **4a**, **5a** and **6a**), or was suspended in MeOH (5 ml for **3b**, **3d** and **3e**). Then each mixture was subjected to column chromatographic

Table 3: R_f values of compounds

Compound	Rf values in the systems			
	S1	*S2*	*S3*	*S4*
1a	0.72	0.57	0.65	0.57
3a	0.64	0.09	0.30	0.59
7	0.58		0.45	0.35
8	0.59		0.07	0.32
9	0.57		0.15	0.41
10	0.45			0.11
11	0.58			0.92
1-Methyladenosine	0.65	0.12	0.17	0.95
1b	0.59	0.26	0.60	0.75
3b	0.47		0.26	0.80
Cytidine 5'-phosphate	0.31			0.18
1c	0.62			0.52
3c	0.47			0.52
Guanosine 5'-phosphate	0.27			0.08
1d	0.51	0.64	0.80	0.81
3d	0.44	0.20	0.51	0.87
Uridine 5'-phosphate	0.48			0.15
1e	0.72	0.83	0.89	0.95
3e	0.64	0.18	0.58	0.85
Thymidine 5'-phosphate	0.44			0.19
4a	0.69	0.36	0.54	0.77
5a	0.73	0.56	0.68	0.83
6a	0.68	0.47	0.61	0.84

separation. For details of separation see Table 1. Appropriate fractions containing the product were pooled and evaporated to dryness. The residue was dissolved in deionised H_2O (15 ml for **3a** and **3c**, 2 ml for the other compounds) and the soln was chromatographed in the system C4*α*. Fractions containing the phosphordiamidate (8–12) were pooled and evaporated to dryness. Traces of hydrogencarbonates were removed by repeated co-evaporation with H_2O. The residues of the phosphordiamidates **3a** and **3c** were recrystallised from hot H_2O, while that of **3e** from MeOH. The phosphordiamidates **3b**, **3d**, **4a**, **5a** and **6a** crystallised upon standing under anhydrous ether. The acid hydrolysis of the phosphordiamidates—except that of **6a** was quantitative within 4 hr. For the quantitative hydrolysis of compound **6a** about 48 hr was necessary. The phosphordiamidates **3** were quantitatively hydrolyzed in 0.1 N NaOH for 5 min, but more than 3 days was required for the hydrolysis of phosphordiamidate **4a** in 1.0 N NaOH.

Identification of by-products formed during the synthesis of the phosphordiamidate **3a**. The first peak obtained during the column chromatographic separation of the mixture of the phosphordiamidate **3a** in the system C1 was pooled and evaporated. The UV spectra of the compound at pHs 1.0, 7.0 and 11.0 closely resembled those of 1-methyladenosine.[13] The compound was quantitatively converted to 1-methyladenosine by acid hydrolysis and subsequent incubation with *Escherichia coli* alkaline phosphatase as detected by tlc comparison with an authentic specimen.

An aliquot (about 20%) of the mixture was separated in the system C4*β*. The first peak eluted by the gradient contained, **7**, **8** and **9**, while the second one the phosphate **10** as detected by tlc comparison with authentic specimens.

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