PREPARATION OF 9-(2-PHOSPHONOMETHOXYETHYL)ADENINE ESTERS AS POTENTIAL PRODRUGS

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Esters of 9-(2-phosphonomethoxyethyl)adenine with substituted aliphatic alcohols were prepared as potential prodrugs. Activation of the phosphonate moiety with dimethylchloromethyleneammonium chloride, generated by reaction of thionyl chloride or triphosgene with dimethylformamide, proved to be the method of choice. The esters were also prepared by alkylation of the phosphonate group with dimethylformamide dialkyl acetals or a mixture of the appropriate alcohol with dimethylformamide dineopentylacetal.

Recently, a series of in vivo effective phosphonomethyl ethers of acyclic nucleoside analogs have been described[1]. One of the most important members of this group, 9-(2-phosphonomethoxyethyl)adenine[2] (PMEA, *I*), exhibits high antiviral effect against a broad spectrum of retroviruses[3] as well as DNA viruses[4] and is now undergoing clinical trials against AIDS, caused by HIV. However, its very low oral absorption (e.g. in rats 11% but in monkeys less than 1%) represents evidently a limitation of its chemotherapeutical use[5].

Transport studies on nucleotides and some of their analogs have shown that these molecules are not capable of permeating through the cell membrane and, if these compounds are detected inside the cells, it is the result of intracellular phosphorylation of the nucleoside that arose by dephosphorylation of the nucleotide on the membrane[6]. In phosphonate nucleotide analogs, the dephosphorylation by phosphomonoesterases is not possible and therefore these analogs cannot penetrate into the cells by mechanisms common for natural nucleosides. So far, only one paper dealing with this problem appeared[7]. The published results show the existence of an active transport in the penetration of phosphonates into cells whose mechanism, however, is different from that for nucleosides (it is not inhibited by classical nucleoside transport inhibitors). The active character of the transport is confirmed by the fact that at very low extracellular concentrations the intracellular concentration of the compound exceeds that in the medium, the transport exhibits saturation characteristics at increasing concentrations, and is tempera-

ture-dependent. The mentioned facts are in accord with the characteristics of receptor-mediated endocytosis.

Recently, an attempted transport of PMEA bound to a polymeric carrier[8] has been reported. The authors made use of mannosylated poly-L-lysine for the transport of PMEA to HIV-infected macrophages in which endocytosis of the glycosylated macromolecules takes place. After appropriate corrections, the conjugate is reported to have ten times higher antiviral effect than PMEA itself.

Preparation and biological activities of substituted aryl esters, diesters, amides and other derivatives of PMEA have recently been the subject of a patent[9]. Aryl esters do not show any significant antiviral activity in vitro and the amides are very unstable, particularly in acidic media. The same group of scientists described the preparation of acyloxymethyl esters of PMEA, especially the pivaloyloxymethyl ester which allegedly has an outstanding capability of cell penetration and exhibits enhanced biological activity[10]. Also this compound is undergoing clinical trial.

Our aim has been to prepare derivatives of reduced polarity that could improve the pharmacological properties of the phosphonate analogs. We were interested in the effect of esterification of the phosphonate group with lipophilic as well as hydrophilic chains bearing other functionalities that could participate in the ester cleavage to give the active compound. The preparation of such compounds required elaboration of methods that could be generally used for further phosphonate derivatives.

Alkylphosphonates can be esterified by methods employed for activation of phosphates, recently investigated in detail e.g. in the chemistry of oligonucleotides. In order to compare the effectivity of individual methods in the case of 9-(2-phosphonomethoxyethyl)adenine (*I*), we studied the reaction of this compound with propanol. We tried to activate PMEA under comparable conditions with various types of activation and condensation reagents: *N*,*N*′-dicyclohexylcarbodiimide[11] (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride[12] (EDC), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate[8] (BOP), 1,1′-carbonyldiimidazole (CDI), 2,4,6-triisopropylbenzenesulfonyl chloride[13] (TIPS-Cl), trichloroacetonitrile[14], phosgene[15], triphosgene, and thionyl chloride in DMF[16]. In addition to these methods, we also made use of direct alkylation of PMEA with propyl bromide[17] and of a modified Mitsunobu alkylation. In the last-mentioned reaction, the corresponding alcohol is activated with triphenylphosphine and diethyl azodicarboxylate[18] (DEAD). Comparison of the results obtained by individual methods (Table I) shows that, in the case of compound *I*, classical condensation methods of nucleotide chemistry afford only low yields of the mono ester *VIII*. One of the reasons is the low solubility of compound *I* in most organic solvents due to its zwitterionic character[19]. For most of the above-mentioned reactions, dimethylformamide is the solvent of choice; at least in some cases (in reactions of trialkyl- or tetraalkylammonium salts) this solvent gives homogeneous reaction mixtures.

Since higher yields of the esterification reaction were achieved only after conversion of compound *I* into chloride or dichloride using the reagents given in the second part of Table I, we studied these reactions in more detail.

Reaction of dimethylformamide with reagents such as phosgene, thionyl chloride, PCl_5 etc. gives rise to dimethylchloromethyleneammonium chloride[16] (*II*). This strongly electrophilic reagent reacts with the phosphate or phosphonate oxygen atom with formation of activated ester *III* which is further converted by chloride anion into chloride of the acid and, analogously, into phosphonodichloride *IV* (Scheme 1). As follows from ^{31}P NMR spectra, the reaction of compound *I* with $SOCl_2$ or triphosgene in dimethylformamide gives almost instantaneously the corresponding dichloride *IV* which is stable under the aprotic conditions in the reaction mixture. Only on longer heating above 90 °C, the dichloride undergoes destruction: ^{31}P NMR spectrum exhibits a signal, whose chemical shift corresponds to a P–N bond, and a multiplet of characteristic shift and splitting, corresponding to a P–P bond. The P–N derivative can arise by reaction of the dichloride with dimethylformamide[20] or by substitution at the nitrogen atoms of the heterocyclic base. We neither isolated nor characterized these products. Anyway,

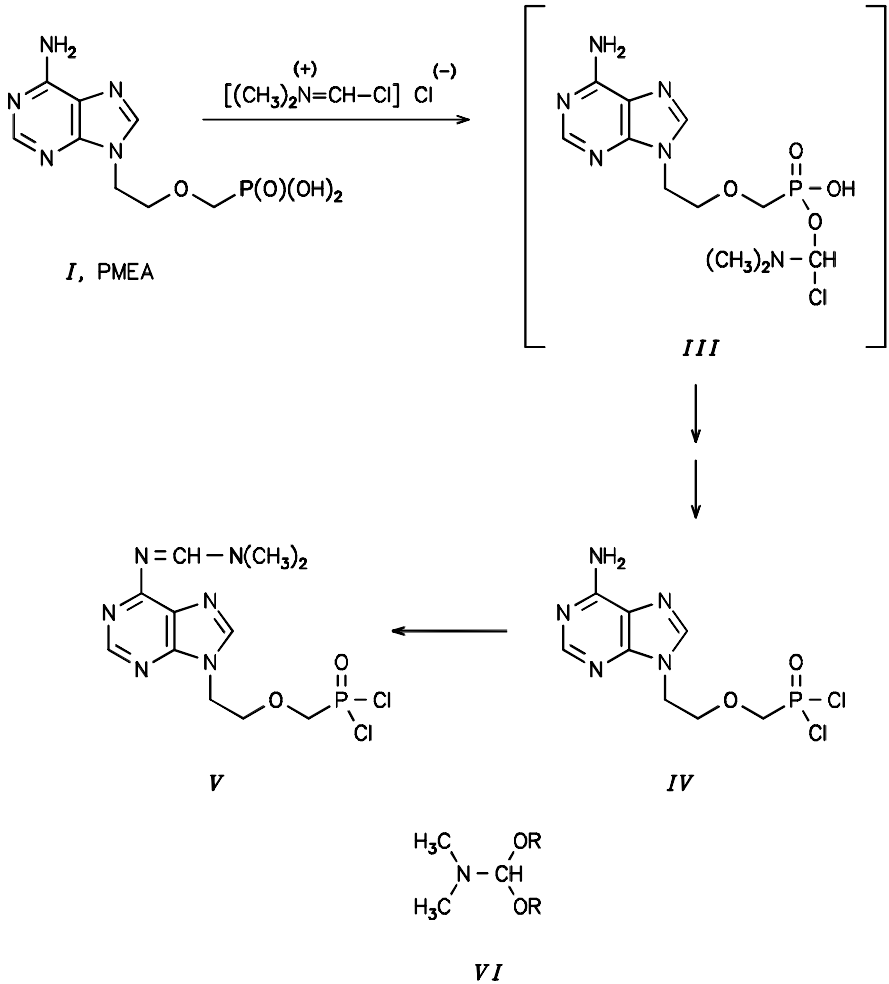
TABLE I
Reaction conditions and yields in the esterification reactions of PMEA (*I*) with propanol

Procedure	Reagent	Temperature[a] °C	Reaction time, h	Yield, %	
				monoester	diester
1	DCC	r.t.	72	5	0
1	EDC	r.t.	72	10	0
1	BOP	r.t.	72	14	0
1	TIPS-Cl, *N*-Me-imidazole	r.t.	72	5	0
2	CDI	80	8	55	0
3	$BrCH_2CH_2CH_3$	90	12	28	0
4	PPh_3, DEAD	r.t.	24	52	0
5	CCl_3CN, Py	75 – 80	16	8	0
6	$SOCl_2$, DMF	r.t.	24	85	0
6	$SOCl_2$, DMF	r.t.	24	87	0
6	Triphosgene, DMF	r.t.	24	90	0
7	$SOCl_2$	r.t.	24	78	0
7	$SOCl_2$	80	24	58	25
8	$SOCl_2$, DMF	r.t.	24	72	0

[a] r.t., room temperature.

at room or slightly elevated temperature, solutions of dichloride *IV* are sufficiently stable in an inert atmosphere and can be easily handled.

Under the conditions of activation in DMF, the iminium species *II* reacts with the phosphonate oxygen atom; however, even if less than two equivalents of the reagent are used, the reagent simultaneously reacts with the amino group of adenine under forma-



I, PMEA

III

IV

V

VI

SCHEME 1

tion of dimethylformamidinium salt which in the presence of base affords formamidine derivative[21] *V*. Experiments with dimethylchloromethyleneammonium chloride *II* under anhydrous conditions have shown that quantitative conversion of compound *I* into amidine derivative *V* requires at least three equivalents of the reagent. After the reaction of the phosphonate group with alcohol or amine, this group can be removed by alkaline hydrolysis in dilute ammonia or in an acid medium such as acetic acid. Table I shows that most of the procedures esterify the compound *I* only to the first degree. Higher temperature, longer reaction time, or use of harder nucleophiles (such as alkoxides), a tertiary base or 4-dimethylaminopyridine as catalyst, did not lead to diesters but only to mixtures of other products, probably due to a considerably reduced reactivity of the chlorine atom in the partially esterified phosphonochloridate. Also the effect of the heterocyclic base and its functional groups, and possibly the reactivity of dimethylformamide and the activated intermediates, may operate in this case. We have found that the presence of any base reduces the yields of the monoester and leads to mixtures of products.

The use of common alkylation reagents such as alkyl halides, sulfonyl esters or ortho esters affords the monoesters in very low yields and moreover at relatively high temperatures. These reactions are employed mainly for alkylation of phosphates[17] or phosphonates which contain no other functionalities susceptible to alkylation and in such cases they give satisfactory yields. However, the molecule of PMEA contains more groupings sensitive to alkylation and therefore, particularly at higher temperatures, the esterification of the phosphonate is also accompanied by alkylation of the heterocyclic base.

Substantially milder conditions can be employed in reactions with dialkyl acetals of dimethylformamide[22] *VI*. Alkylation of nucleotides at the heterocyclic base with these reagents was reported[23] to be accompanied with alkylation at the phosphate moiety as side reaction. Also when protecting the amino group in PMEA with dimethylaminomethylene group, introduced by treatment with dimethylformamide dimethyl acetal, we observed that at higher temperatures the monoester is formed as side-product. For this reason, we verified the possible use of these reagents for the preparation of other PMEA esters. The alkylations were performed in dimethylformamide at elevated temperature. The corresponding dialkyl acetals were prepared from dimethylformamide dimethyl acetal by transacetalization[24]. Under the reaction conditions, one equivalent of the reagent was consumed for the formation of N^6-dimethylaminomethylene derivative of adenine (during the work-up procedure, this protecting group is removed by treatment with dilute ammonia solution). In the case of reactive dialkyl acetals such as dimethylformamide dimethyl and dibenzyl acetals we achieved very good yields under very mild conditions. Good results were also obtained with cyclic acetals of dimethylformamide such as 2-dimethylamino-1,3-dioxolane, 2-dimethylamino-1,3-dioxane and 2-dimethylamino-4-methyl-1, 3-dioxane which afforded hydroxyalkyl esters of PMEA.

As expected, the latter reagent was attacked at the less substituted side of the 1,3-dioxane ring.

In order to circumvent the separate preparation of each acetal, we tried to generate the desired acetal in situ[25]. A suspension of phosphonate *I* in DMF was mixed with less reactive dimethylformamide dineopentyl acetal and a two-molar excess of the appropriate alcohol. Under the reaction conditions transacetalization took place and the phosphonate was alkylated with the more reactive alkyl. In some cases, the conversion into the ester could be increased by addition of a tertiary amine. The reaction conditions for a series of alcohols are given in Table II. The reaction can be successfully employed with more reactive alcohols which after transacetalization are capable of alkylation at temperatures to 80 °C. In the case of less reactive alcohols, which react only at higher temperatures, the expected product is accompanied by the neopentyl ester[25]. After six hours at 120 °C the formation reaches more than 35% of the neopentyl ester *XVIII* . At temperatures higher than 100 °C also the adenine moiety begins to be alkylated and the reaction mixtures are separable only with difficulty.

TABLE II
Reaction conditions and yields of alkyl esters of compound *I* in alkylations with dimethylformamide dialkyl acetals

Procedure	Dimethylformamide dialkyl acetal	Temperature °C	Reaction time, h	Yield, %
9	Dimethyl acetal	70	16	63
9	Dibenzyl acetal	70	16	60
9	Propylene acetal	70	16	65
9	1,3-Buthylene acetal	70	16	48[a]
9	Ethylene acetal	70	16	54
9	Dineopentyl acetal	110	16	38
9	Dineopentyl acetal	70	16	<3
10	Benzyl alcohol, dineopentyl acetal	70	16	55
10	Ethylene glycol, dineopentyl acetal	70	16	36
10	Isopropylidene glycerol, dineopentyl acetal	80	16	39
10	1-*O*-Benzyl glycerol, dineopentyl acetal	80	16	35[b]
10	Glycerol, dineopentyl acetal	80	16	21[c]
10	*N*-Hydroxyethylmorpholine, dineopentyl acetal	70	16	60
10	2,2,2-Trifluoroethanol, dineopentyl acetal	80	26	29
10	2,2,2-Tribromoethanol, dineopentyl acetal	80	16	24

[a] Mixture of 1′- and 3′-*O*-isomers in ratio 5 : 1. [b] Mixture of 1′- and 2′-*O*-isomers 8 : 1. [c] Mixture of 1′- and 2′-*O*-isomers 4 : 1.

The above-mentioned reactions were applied to the preparation of selected esters of PMEA which could influence the pharmacological behaviour of compound *I*. We prepared esters with aliphatic alcohols (*VII – XII*, *XVIII*; Table III) that may be considerably stable and resistant to cleavage inside the cell and thus devoid of biological activity. Esters whose cleavage is facilitated by a β-elimination reaction (halogeno esters *XIV – XVI* and the 2-cyanoethyl ester *XVII*) may behave differently.

Another target of our study was to investigate the effect of hydrophilic groups in the ester chain on the cleavage and biological activity of PMEA esters. A hydroxy or amino group, or generally a group with a lone electron pair, could participate in the ester bond cleavage, as e.g. in the case of hydroxyethyl and hydroxypropyl phosphates[26]. For this reason, we prepared a series of esters with ethylene glycol, glycerol and other polyols, ethers and saccharides (*XIX – XXVII*). In addition, we synthesized esters with further alcohols and amino alcohols which could be cleaved into the starting phosphonate (see Tables IV and V).

The preparation of esters with the ester group bonded to a primary hydroxyl was realized by reaction of an appropriately protected alcohol with compound *I* according to the above-mentioned procedures. During isolation of products by chromatography on ion-exchangers we observed migration of the phosphonate group to the neighbouring hydroxy group and the reaction afforded a mixture in which the product with esterified

TABLE III
Conditions of preparation and yields of alkyl esters of PMEA

Compound	Alkyl	Procedure	Temperature[a] °C	Reaction time, h	Yield, %
VII	CH_3	*A*	r.t.	16	85
VIII	$(CH_2)_2CH_3$	*A*	r.t.	16	73
IX	$(CH_2)_5CH_3$	*A*	r.t.	16	56
X	$(CH_2)_7CH_3$	*A*	r.t.	16	46
XI	$(CH_2)_{17}CH_3$	*A*	50	36	12
XII	Cholesteryl	*A*	50	48	20
XIII	$CH_2C_6H_5$	*B*	60	48	60
XIV	CH_2CF_3	*A*	r.t.	16	74
XV	CH_2CCl_3	*A*	r.t.	16	86
XVI	CH_2CBr_3	*A*	r.t.	48	64
XVII	CH_2CH_2CN	*A*	r.t.	4	51
XVIII	$CH_2C(CH_3)_3$	*A* (*B*)	110	48	42 (38)

[a] r.t., room temperature.

primary hydroxyl predominated. We have proved this isomerization for the 2,3-dihydroxypropyl ester by HPLC and NMR. In the preparation of this ester from 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane (2,3-*O*-isopropylideneglycerol) by procedure *A* the isopropylidene functionality was partly cleaved and therefore the reaction mixture was worked up by acid hydrolysis to give free 2,3-dihydroxypropyl ester *XXI*, isolated as a mixture of primary and secondary alkyl esters. As shown by HPLC, under the conditions of cleavage of the isopropylidene group the ester bond in the dihydroxypropyl ester was isomerized to the extent of ten per cent (from the position 1 to the position 2). This ester ratio was also confirmed by the ^{13}C NMR spectrum. In the reaction with

TABLE IV
Conditions of preparation and yields of hydroxyalkyl esters of PMEA

Compound	Alkyl	Procedure	Temperature[a], °C	Reaction time, h	Yield, %
XIX	CH_2CH_2OH	*A*	r.t.	16	57
XX	$CH_2CH_2CH_2OH$	*B*	70	16	46
XXI	$CH_2CH(OH)CH_2OH$	*A*	r.t.	16	53
XXII	$CH_2CH(OH)CH_2OCH_2C_6H_5$	*A*	r.t.	16	45
XXIII	$CH_2CH_2CH(OH)CH_3$	*B*	70	16	48
XXIV	$CH_2CH(OH)CH(OH)CH_2OH$	*A*	r.t.	16	47
XXV	$CH_2[CH(OH)]_4CH_2OH$	*A*	r.t.	16	35
XXVI	6-Mannosyl	*A*	r.t.	16	38
XXVII	2,2-Dimethyl-1,3-dioxolan-4-ylmethyl	*B*	r.t.	16	39

[a] r.t., room temperature.

TABLE V
Conditions of preparation and yields of aminoalkyl esters of PMEA

Compound	Alkyl	Procedure	Temperature[a]	Reaction time, h	Yield, %
XXVIII	$CH_2C(CH_2OH)_2NH_2$	*A*	r.t.	16	36
XXIX	$CH_2CH_2N(CH_3)_2$	*A*	r.t.	16	40
XXX	$CH_2CH_2N(CH_3)_3$	*A*	r.t.	16	38
XXXI	$CH_2CH_2NH_2$	*A*	r.t.	16	36
XXXII	CH_2CH_2(N-morpholinyl)	*A*	r.t.	16	68

[a] r.t., room temperature.

1,2-diacetoxypropanol, after esterification and deacetylation with dilute ammonia we detected by HPLC only the product of esterification at the primary hydroxyl. However, the isolation by chromatography on ion exchangers in a gradient of acetic acid was again accompanied by at least 5% of the isomerization product. In the preparation of other polyhydroxyalkyl esters we made use of alkali-labile acetyl protective groups. The protected alcohols with free primary hydroxyl were prepared via the corresponding trityl derivatives.

We also tried alkylation reactions under conditions of in situ transacetalization with dimethylformamide acetals. However, these reactions are not suitable for preparation of polyhydroxyalkyl esters because with partially protected alcohols they afford the desired esters in very low yields. With unprotected alcohols (glycerol) they gave, in low yields, mixtures in which the primary ester slightly predominated. Most of the esters could be prepared in higher yields by procedure *A*. The synthesized esters, together with the reaction conditions, are listed in Tables III – V.

In order to ascertain whether the synthesized esters act as prodrugs of PMEA, it was necessary to prove their hydrolysis to the parent compound. Therefore, we investigated the possible chemical as well as enzymatic cleavage. The chemical stability was tested both in alkaline and acidic medium (0.05 M NaOH and HCl, respectively). No hydrolysis was found by TLC and HPLC after 36 h. As expected, the only exception was 2-cyanoethyl ester *XVII* which in alkaline medium was completely hydrolyzed after 2 h. On the other hand, hydroxyalkyl and aminoalkyl esters of PMEA proved to be surprisingly stable.

Since the key question was whether the prepared compounds can be hydrolyzed in vivo, we followed their chemical stability in the presence of an inert protein. The compounds were incubated at 37 °C in a solution of thermally inactivated bovine serum albumin; this very approximately simulated the chemical conditions in the plasma and made it possible to assess stability of compounds in a medium of similar nucleophilicity. As expected, esters with unsubstituted alkyl chains were stable under the hydrolysis conditions. These results are in accord with the literature data[27] on phosphate esters, according to which alkyl esters are stable in organisms and do not undergo any chemical or enzymatic hydrolysis. Similarly, we did not observe any hydrolysis with compounds in which the ester-bonded alkyl contained an amino or hydroxy group in proximity of the ester bond.

We also tried enzymatic hydrolysis of the obtained esters by treatment with a cell-free extract. No PMEA was detected in any of the experiments; in the course of 24 h the ester concentrations remained unchanged. However, the value of these negative results is limited by the fact that the experiments were performed with disintegrated cells and thus cannot give information about in vitro behaviour of the compounds.

Save one exception, we did not prove chemical or enzymatic hydrolysis for any group of the synthesized esters. These results show that the ester bond in phosphonates

is more stable than in phosphates. In accord with these results, esterification with simple alkyls leads to loss of biological activity. In spite of this, some esters derived from substituted alkanols exhibit significant biological activity[28]. Esters of diols, triols and alditols show an antiviral activity about one order of magnitude lower than PMEA, but the activity of the 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl and 2,2,2-tribromoethyl esters is comparable with that of compound *I*. As shown by in vivo experiments in mice[29], compound *I* appears in the plasma very soon after i.v. application.

The cytostatic activity was tested on L-1210 leukemia cells but, contrary to compound *I*, neither of the compounds tested exhibited significant cytostatic activity.

As already mentioned above, the main problem of application of PMEA consists in its low oral absorption. In this respect, it cannot be excluded that some of the synthesized esters may have more advantageous pharmacological parameters. Further biological investigations are under way in order to clarify the mechanism of action and to study other biological properties of these compounds.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40 °C/2 kPa and the products were dried over phosphorus pentoxide at 13 Pa. Analytical samples were dried at 25 °C and 6.5 Pa for 8 h. Melting points were determined on a Kofler block and are uncorrected.

Thin-layer chromatography was performed on Silufol UV 254 sheets (Kavalier, The Czech Republic) in the systems: S1, 2-propanol–concentrated ammonia–water (7 : 1 : 2); S2, chloroform–methanol (9 : 1); S3, chloroform–methanol (4 : 1); S4, chloroform–methanol (20 : 1); S5, toluene–ethyl acetate (10 : 1); S6, toluene–ethyl acetate (3 : 1). Compounds were detected by (i) UV light at 254 nm, (ii) spraying with 2% solution of 4-(*p*-nitrobenzyl)pyridine in ethanol, followed by heating and exposure to ammonia vapours (for compounds capable of alkylation), and (iii) carbonization.

Analytical high performance liquid chromatography was carried out on 250 × 4 mm columns packed with Separon SGX C18 (5 μm or 10 μm; Laboratorni pristroje, Prague, The Czech Republic). Preparative reversed-phase chromatography was performed on a 20 × 500 mm octadecylsilica gel column (20 μm, Laboratorni pristroje, Prague, The Czech Republic); detection at 254 nm with a Uvicord 4 701 instrument (LKB, Sweden).

Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer by the EI (electron energy 70 eV) and FAB (ionization with Xe, accelerating voltage 8 kV) techniques.

^{1}H NMR spectra were taken on a Varian UNITY 200 (200.01 MHz) and Varian UNITY 500 (499.8 MHz) spectrometers in hexadeuteriodimethyl sulfoxide with tetramethylsilane (TMS) as internal standard. Free phosphonates and monoesters were measured in D_2O with sodium disilapentasulfonate (DSS) as internal standard. ^{13}C NMR spectra were obtained with a Varian UNITY 200 instrument (50.31 MHz); the signals were referenced to the solvent signal, $\delta(^{13}C)$ (DMSO) = 39.7, or to dioxane as external standard, $\delta(^{13}C)$ (dioxane) = 66.86, for solutions in D_2O.

Paper electrophoresis was performed on a Whatman No. 3 MM paper at 20 V/cm (1 h) in 0.1 M triethylammonium hydrogen carbonate (TEAB).

Solvents, compounds and reagents: DMF was dried by standing over P_2O_5 for 24 h and subsequent distillation in vacuo (water pump), bath temperature up to 50 °C. Thionyl chloride was freshly distilled. Pyridine, triethylamine, dichloromethane and toluene were dried over P_2O_5 and distilled.

Acetonitrile, ether, dioxane and tetrahydrofuran were dried by distillation from sodium hydride. Alcohols, used for the esterifications, were distilled and dried over activated molecular sieves.

Propyl Ester of 9-(2-Phosphonomethoxyethyl)adenine (*VIII*)

Procedure 1. Tributylamine (0.1 ml, 0.5 mmol) was added to a solution of compound *I* (55 mg, 0.2 mmol) in dry methanol (20 ml). The mixture was refluxed to dissolution of the starting compound, the solvent was evaporated and the residue was codistilled with DMF. A mixture of the residue, DMF (10 ml), 1-propanol (0.5 ml) and the condensation reagent (1 mmol) was stirred at room temperature for 72 h, decomposed with TEAB (1 ml) and after 1 h analyzed by reversed-phase HPLC. For the results see Table I.

Procedure 2. 1,1′-Carbonylimidazole (0.811 g, 5 mmol) was added to tributylammonium salt of compound *I* (1 mmol) in DMF (20 ml) (prepared as in Procedure *1*). After stirring for 24 h at room temperature, 1-propanol (10 ml) was added and the mixture was heated at 80 °C for 8 h. The mixture was decomposed with TEAB, concentrated, dissolved in water and applied onto a column of Dowex 1X2 (acetate form; 40 ml). Elution with a gradient 0 – 0.5 M acetic acid afforded 183 mg (58%) of compound *VIII*.

Procedure 3. A mixture of tributylammonium salt of compound *I* (27 mg, 0.1 mmol), 1-bromopropane (123 mg, 1 mmol) and acetonitrile (5 ml) was heated at 90 °C for 12 h in a sealed ampoule. According to HPLC analysis, the resulting mixture contained 28% of monoester *VIII*, beside other products.

Procedure 4. Diethyl azodicarboxylate (104 mg, 0.6 mmol) was added at 0 °C to triphenylphosphine (158 mg, 0.6 mmol) in dry tetrahydrofuran (3 ml). After standing at 0 °C for 20 min, the mixture was added at the same temperature to a suspension of tributylammonium salt of compound *I* (135 mg, 0.5 mmol) in dimethylformamide containing 0.03 ml (0.5 mmol) of 1-propanol. The mixture was stirred at room temperature for 24 h, decomposed with TEAB, concentrated to 1 ml and repeatedly extracted with ether–light petroleum (1 : 1; 20 ml). The residue was dissolved in water, deionized on Dowex 50 and the resulting monoester was isolated by HPLC; yield 82 mg (52%) of compound *VIII*.

Procedure 5. Trichloroacetonitrile (0.72 g, 5 mmol) was added to a mixture of compound *I* (135 mg, 0.5 mmol), 1-propanol (0.12 g, 2 mmol) and pyridine (5 ml). After heating at 75 – 80 °C for 16 h, the mixture contained 8% of monoester *VIII*.

Procedure 6. The activation reagent (3.3 equivalents; in the case of triphosgene 1.5 equivalent) was added at room temperature to a mixture of compound *I* (27 mg, 0.1 mmol) and dimethylformamide (5 ml). After the starting compound dissolved, the mixture was set aside at room temperature for 1 h. 1-Propanol (1 ml) was added, the mixture was allowed to stand for 24 h and neutralized with TEAB. After standing for 20 min, 20% aqueous ammonia solution (1 ml) was added. The reaction mixtures were analyzed by HPLC after 16 h. For results see Table I.

Procedure 7. A suspension of compound *I* (136 mg, 0.5 mmol) in thionyl chloride (5 ml) was refluxed for 2 h. The solvent was evaporated and the residue was dissolved in a mixture of acetonitrile–propanol (1 : 1; 20 ml). After standing for 24 h, the reaction mixture was neutralized with TEAB. The solvent was evaporated, the residue was dissolved in water and the solution was applied onto Dowex 50X8 (H^+ form; 20 ml). The column was washed with water (0.5 l) and then with dilute aqueous ammonia (1 : 10). The UV-absorbing fractions, containing the product, were combined, the solvent was evaporated and the residue was separated by preparative reversed-phase HPLC; yield 123 mg (78%) of compound *VIII*.

Procedure 8. Bromotrimethylsilane (0.24 ml, 1.5 mmol) was added to a solution of diethyl ester of compound *I* (165 mg, 0.5 mmol) in acetonitrile (20 ml). After standing at room temperature for

24 h, the mixture was evaporated, and the residue was codistilled with dry acetonitrile. The residue was dissolved in dimethylformamide (10 ml) and thionyl chloride (0.146 ml, 2 mmol) was added. The mixture was set aside for 5 h and then 1-propanol (1 ml) was added. After standing for 24 h, the mixture was worked up as described for Procedure *1*, and then applied onto a column of Dowex 1X2 (acetate form). The product was eluted with acetic acid (linear gradient 0 – 0.25 M, à 1 litre). The product-containing fractions were combined, the solvent was evaporated and the residual acetic acid was removed by repeated codistillation with 2-propanol. Yield 115 mg (72%) of monoester *VIII*.

Procedure 9. The pertinent dimethylformamide acetal (5 equivalents) was added to a solution of compound *I* (50 mg, 0.18 mmol) in dimethylformamide (5 ml) and the mixture was heated to the temperature given in Table II. After 16 h, the mixture was decomposed with an aqueous ammonia solution (5 ml) and after 24 h the solvent was evaporated and the residue was desalted on Dowex 50X8. The product was eluted with ammonia, the solvent was evaporated and the residue was separated by preparative HPLC with a gradient water–methanol. For results see Table II.

Procedure 10. Dimethylformamide dineopentyl acetal (230 µl, 1 mmol) and the corresponding alcohol (1 mmol) were added to a solution of compound *I* (50 mg, 0.18 mmol) in dimethylformamide (3 ml). Further work-up was the same as described in Procedure *9*. For results see Table II.

General Procedure for Preparation of Monoesters of Compound *I*

Procedure A. Compound *I* (273 mg, 1 mmol) was dried in vacuo (oil pump) at 90 °C for 1 h and dissolved in dimethylformamide (10 ml). Thionyl chloride (2.4 ml, 3.3 mmol) was added dropwise under ice-cooling and, after dissolution of the starting compound, the mixture was set aside for 1 h at room temperature. The hydroxylic component was added, the mixture was left at room temperature for 24 h, and 2 M TEAB was added (to alkaline reaction). After 30 min, the mixture was concentrated, the residue was dissolved in 2% aqueous ammonia and set aside at room temperature overnight. The solvent was evaporated, the residue was dissolved in water, applied onto a column of Dowex 50X8 (H^+ form; 25 ml). The column was washed with water to drop of UV absorption and the product was eluted with 2.5% ammonia solution. The UV-absorbing fractions were combined, evaporated and applied on a column of Dowex 1X2 (acetate form; 25 ml) and the product was eluted with acetic acid (gradient 0 – 0.5 M, à 1 liter). The product-containing fractions were concentrated and repeatedly codistilled with 2-propanol. When the starting compound was present, the mixture was separated by preparative HPLC (gradient of methanol in water). Hydrophilic esters were separated on DEAE Sephadex (Pharmacia) (gradient of triethylammonium hydrogen carbonate 0 – 0.2 M, à 1 liter). Fractions, containing pure product, were concentrated and the residue was several times codistilled with 2-propanol. The obtained mixture of the ester and its triethylammonium salt was converted into the free acid on Dowex 1X2 (10 ml) by elution with 0.1 M acetic acid. After evaporation of the solvent and codistillation with 2-propanol, the pure product was dissolved in a minimum amount of methanol or its mixture with minimum amount of water and crystallized by addition of 2-propanol to turbidity or precipitated with acetone. Most of the compounds were obtained as microcrystalline precipitates which were dried in vacuum of an oil pump at 30 °C. The purity and isomeric composition of the compounds were checked by HPLC.

Procedure B. The corresponding dimethylformamide acetal, or an equimolar mixture of dimethylformamide dineopentyl acetal with the corresponding alcohol (10 mmol), was added to a suspension of compound *I* (273 mg, 1 mmol; pre-dried in oil pump vacuum at 90 °C for 1 h). After stirring the reaction mixture under conditions given in Table II, 2.5% ammonia solution was added and the stirring was continued for further 16 h. The solvent was evaporated, the residue was dissolved in water and applied onto a column of Dowex 50X8 (H^+ form; 25 ml). The column was washed with water to drop of UV absorption of the eluate to the original value and the desalted mixture was eluted with

2.5% ammonia solution. The UV-absorbing fractions were combined, concentrated and applied onto a column of Dowex 1X2 (acetate form; 25 ml) and the product was eluted with a gradient of acetic acid (0 – 0.25 M, à 1 litre). The product-containing fractions were concentrated and codistilled several times with 2-propanol. When the starting compound was present, the mixture was separated by preparative HPLC in methanol–water (gradient). The product was again crystallized from methanol or, when insoluble, in aqueous methanol with addition of acetone or 2-propanol.

Methyl ester of 9-(2-phosphonomethoxyethyl)adenine (VII): For $C_9H_{14}N_5O_4P$. H_2O (305.2) calculated: 35.42% C, 5.28% H, 22.94% N, 10.15% P; found: 35.68% C, 5.55% H, 23.21% N, 10.41% P. R_F 0.68 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 288 (M + H, 100).

Propyl ester of 9-(2-phosphonomethoxyethyl)adenine (VIII): For $C_{11}H_{18}N_5O_4P$. H_2O (333.3) calculated: 39.64% C, 6.05% H, 21.01% N, 9.29% P; found: 39.47% C, 5.87% H, 20.84% N, 9.12% P. R_F 0.78 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 316 (M + H, 100).

Hexyl ester of 9-(2-(phosphonomethoxyethyl)adenine (IX): For $C_{14}H_{24}N_5O_4P$. H_2O (375.4) calculated: 44.80% C, 6.98% H, 18.66% N, 8.25% P; found: 44.95% C, 7.08% H, 18.48% N, 8.18% P. R_F 0.85 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 358 (M + H, 100). ^{1}H NMR spectrum (D_2O): 0.75 t, 3 H, $J(CH_3,CH_2)$ = 7.1 (CH_3); 0.85 – 1.20 m, 8 H (CCH_2); 3.53 brq, 2 H, $J(P,OCH_2)$ = 6.6, $J(CH_2,CH_2)$ = 6.1 ($POCH_2$); 3.63 d, 2 H, $J(P,CH_2)$ = 9.0 (PCH_2); 3.89 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (OCH_2); 4.42 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (NCH_2); 8.16 s, 1 H (H-base); 8.18 s, 1 H (H-base).

Octyl ester of 9-(2-phosphonomethoxyethyl)adenine (X): For $C_{16}H_{28}N_5O_4P$ (385.4) calculated: 49.86% C, 7.32% H, 18.17% N, 8.04% P; found: 50.11% C, 7.17% H, 18.02% N, 7.88% P. R_F 0.90 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 386 (M + H, 90). ^{1}H NMR spectrum (DMSO): 0.80 t, 3 H, $J(CH_3,CH_2$ = 7.1 (CH_3); 1.15 m, 10 H (CCH_2); 1.34 m, 2 H (CCH_2); 3.48 d, 2 H, $J(P,CH_2)$ = 8.5 (PCH_2); 3.59 q, 2 H, $J(CH_2,CH_2)$ = 6.6, $J(P,CH_2)$ = 6.6 ($POCH_2$); 3.79 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (OCH_2); 4.28 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (NCH_2); 7.16 bs, 2 H (NH_2); 8.12 s, 1 H (H-base); 8.20 s, 1 H (H-base).

Octadecyl ester of 9-(2-phosphonomethoxyethyl)adenine (XI) (*ammonium salt*): After decomposition with TEAB, the ammonolysis was performed in methanolic ammonia. The mixture was evaporated with silica gel (5 g) to dryness and the residue was applied onto a column of silica gel in ethyl acetate (40 ml). The compound was eluted with 2-propanol–ammonia–water (7 : 1 : 2). For $C_{26}H_{48}N_5O_4P$. NH_3 . H_2O (560.7) calculated: 55.69% C, 9.53% H, 14.99% N; found: 56.10% C, 9.83% H, 15.43% N. R_F 0.32 (S3).

Cholesteryl ester of 9-(2-phosphonomethoxyethyl)adenine (XII) (*ammonium salt*): The reaction mixture was processed as described for compound *XI*. For $C_{35}H_{54}N_5O_4P$. NH_3 (656.8) calculated: 64.00% C, 8.75% H, 12.79% N, 4.72% P; found: 64.34% C, 9.09% H, 13.13% N, 4.56% P. R_F 0.3 (S3).

Benzyl ester of 9-(2-phosphonomethoxyethyl)adenine (XIII): For $C_{15}H_{18}N_5O_4P$. H_2O (381.3) calculated: 47.25% C, 5.29% H, 18.37% N, 8.12% P; found: 47.08% C, 5.22% H, 18.20% N, 8.46% P. R_F 0.68 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 364 (M + H, 80). ^{1}H NMR spectrum (D_2O): 3.70 d, 2 H, $J(P,CH_2)$ = 9.2 (PCH_2); 3.86 t, 2 H, $J(CH_2,CH_2)$ = 5.1 (OCH_2); 4.38 t, 2 H, $J(CH_2,CH_2)$ = 5.1 (NCH_2); 4.57 d, 2 H, $J(P,CH_2)$ = 6.4 ($POCH_2$); 7.23 m, 5 H (C_6H_5); 8.05 s, 1 H (H-base); 8.09 s, 1 H (H-base).

2,2,2-Trifluoroethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XIV): M.p. 238 – 239 °C. For $C_{10}H_{13}F_3N_5O_4P$. 0.5 CH_3OH (371.2) calculated: 33.96% C, 4.07% H, 15.35% F, 18.86% N, 8.34% P; found: 34.28% C, 4.15% H, 14.99% F, 19.19% N, 8.27% P. R_F 0.82 (S1). Mass spectrum (FAB; *m*/*z*, rel.%): 356 (M + H, 60). ^{1}H NMR spectrum (D_2O): 3.70 d, 2 H, $J(P,CH_2)$ = 8.8 (PCH_2); 3.94 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (OCH_2); 3.98 dq, 2 H, $J(F,CH)$ = 8.8, $J(P,OCH)$ = 7.3 ($POCH_2$); 4.40 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (NCH_2); 8.125 s, 1 H (H-base); 8.13 s, 1 H (H-base).

2,2,2-Trichloroethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XV) (*ammonium salt*): M.p. 225 – 227 °C. For $C_{10}H_{13}Cl_3N_5O_4P$. NH_3 (421.6) calculated: 28.49% C, 3.82% H, 25.23% Cl, 19.93% N, 7.35% P; found: 28.22% C, 3.88% H, 24.93% Cl, 19.68% N, 7.40% P. R_F 0.85 (S1). Mass spectrum (FAB; *m/z*, rel.%): 405 (M + H, 100). ^{1}H NMR spectrum (D_2O): 3.73 d, 2 H, *J*(P,CH) = 8.8 (PCH_2); 3.96 t, 2 H, *J*(CH_2,CH_2) = 5.0 (OCH_2); 3.99 d, 2 H, *J*(P,OCH) = 5.9 ($POCH_2$); 4.43 t, 2 H, *J*(CH_2,CH_2) = 5.0 (NCH_2); 8.19 s, 2 H (2 × H-base).

2,2,2-Tribromoethyl ester of 9-(2-phosphonomethoxyethyl)adenine (*XVI*): M.p. 175 – 178 °C. For $C_{10}H_{13}Br_3N_5O_4P$. 0.5 H_2O (546.9) calculated: 21.96% C, 2.58% H, 43.83% Br, 12.80% N, 5.66% P; found: 22.15% C, 2.37% H, 43.48% Br, 13.08% N, 5.16% P. R_F 0.90 (S1). ^{1}H NMR spectrum (D_2O): 3.70 d, 2 H, *J*(P,CH) = 9.1 (PCH_2); 3.93 d, 2 H, *J*(P,OCH) = 5.1 (OCH_2); 3.97 t, 2 H, *J*(CH_2,CH_2) = 5.0 (OCH_2); 4.20 t, 2 H, *J*(CH_2,CH_2) = 5.0 (NCH_2); 8.215 s, 1 H (H-base); 8.23 s, 1 H (H-base).

2-Cyanoethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XVII): For $C_{11}H_{15}N_6O_4P$. H_2O (344.3) calculated: 38.38% C, 4.98% H, 24.41% N, 9.00% P; found: 38.16% C, 4.72% H, 24.10% N, 8.68% P. R_F 0.75 (S1). Mass spectrum (FAB; *m/z*, rel.%): 327 (M + H, 70). ^{1}H NMR spectrum (D_2O): 2.55 t, 2 H, *J*(CH_2,CH_2) = 6.1 (OCH_2); 3.67 d, 2 H, *J*(P,CH) = 9.2 (PCH_2); 3.81 brq, 2 H, *J*(P,OCH) = 6.7, *J*(CH_2,CH_2) = 6.1 ($POCH_2$); 3.92 t, 2 H, *J*(CH_2,CH_2) = 5.2 (OCH_2); 4.44 t, 2 H, *J*(CH_2,CH_2) = 5.2 (NCH_2); 8.20 s, 2 H (2 × H-base).

2,2-Dimethylpropyl ester of 9-(2-phosphonomethoxyethyl)adenine (XVIII): For $C_{13}H_{22}N_5O_4P$. H_2O (361.3) calculated: 43.21% C, 6.69% H, 19.38% N, 8.57% P; found: 43.02% C, 6.80% H, 19.09% N, 8.18% P. R_F 0.87 (S1). Mass spectrum (FAB; *m/z*, rel.%): 344 (M + H, 70). ^{1}H NMR spectrum (D_2O): 0.62 s, 9 H (CH_3); 3.14 d, 2 H, *J*(P,CH) = 5.4 ($POCH_2$); 3.62 d, 2 H, *J*(P,CH) = 9.0 (PCH_2); 3.93 t, 2 H, *J*(CH_2,CH_2) = 5.0 (OCH_2); 4.41 t, 2 H, *J*(CH_2,CH_2) = 5.0 (NCH_2); 8.16 s, 1 H (H-base); 8.17 s, 1 H (H-base).

2-Hydroxyethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XIX): M.p. 204 – 206 °C. For $C_{10}H_{16}N_5O_5P$. 2 H_2O (353.3) calculated: 34.00% C, 5.71% H, 19.82% N, 8.77% P; found: 33.16% C, 5.98% H, 20.05% N, 9.24% P. R_F 0.50. Mass spectrum (FAB; *m/z*, rel.%): 318 (M + H, 80). ^{1}H NMR spectrum (D_2O): 3.50 m, 2 H (OCH_2); 3.67 d, 2 H, *J*(P,CH_2) = 9.6 (PCH_2); 3.69 m, 2 H ($POCH_2$); 3.94 t, 2 H, *J*(CH_2,CH_2) = 5.0 (OCH_2); 4.40 t, 2 H, *J*(CH_2,CH_2) = 5.0 (NCH_2); 8.13 s, 1 H (H-base), 8.15 s, 1 H (H-base).

3-Hydroxypropyl ester of 9-(2-phosphonomethoxyethyl)adenine (XX): For $C_{11}H_{18}N_5O_5P$. H_2O (331.3) calculated: 37.83% C, 5.77% H, 20.05% N, 8.87% P; found: 38.03% C, 5.98% H, 19.75% N, 8.50% P. R_F 0.58. Mass spectrum (FAB; *m/z*, rel.%): 332 (M + H, 40). ^{1}H NMR spectrum (D_2O): 1.55 m, 2 H (CCH_2); 3.45 m, 4 H (2 × OCH_2); 3.60 d, 1 H, *J*(P,CH) = 9.0 (PCH_2); 3.65 d, 1 H, *J*(P,CH) = 5.0 (PCH_2); 4.41 t, 2 H, *J*(CH_2,CH_2) = 5.0 (NCH_2); 8.17 s, 1 H (H-base); 8.18 s, 1 H (H-base).

A Mixture of 2,3-Dihydroxypropyl and 1,3-Dihydroxy-2-propyl Esters of 9-(2-Phosphonomethoxyethyl)adenine (*XXI*)

After chromatography on Dowex 50, the mixture was separated by reversed-phase HPLC to give the isomeric esters on C-1 and C-2 carbon atoms of glycerol. As shown by analytical HPLC, the isolated fractions were more than 99% pure. However, isomerization took place already during evaporation of the solvent, giving rise again to a mixture of both isomers. The ^{13}C NMR spectrum distinguished and identified the isomers; in both cases about 20% of the second isomer was detected (which was also confirmed by HPLC). M.p. 125 – 128 °C. For $C_{11}H_{18}N_5O_6P$. 2 H_2O (383.29) calculated: 34.46% C, 5.78% H, 18.27% N, 8.08% P; found: 34.86% C, 5.19% H, 18.68% N, 8.64% P. R_F 0.45 (S1). Mass spectrum (FAB; *m/z*, rel.%): 348 (M + H, 50).

2,3-Dihydroxypropyl ester of 9-(2-phosphonomethoxyethyl)adenine: ^{1}H NMR spectrum (D_2O): 3.46 m, 2 H (OCH_2); 3.46 m, 3 H (OCH and OCH_2); 3.68 d, 2 H, J(P,CH_2) = 8.8 (PCH_2); 3.94 t, 2 H, J(CH_2,CH_2) = 5.0 (OCH_2); 4.00 t, 2 H, J(CH_2,CH_2) = 5.0 (NCH_2); 8.14 s, 1 H (H-base); 8.16 s, 1 H (H-base). ^{13}C NMR spectrum (D_2O): 44.62 s (NCH_2); 66.51 d, J(P,C) = 6.1 (POC-1′); 66.76 d, J(P,C) = 158.7 (PCH_2); 71.52 d, J(P,C) = 12.2 (OCH_2); 71.96 d, J(P,C) = 7.6 (C-2′); 119.22 s (C-5); 143.00 s (C-8); 149.78 s (C-4); 153.36 s (C-2); 156.42 s (C-6).

1,3-Dihydroxy-2-propyl ester of 9-(2-phosphonomethoxyethyl)adenine: ^{1}H NMR spectrum (D_2O): 3.45 m, 2 H (OCH_2); 3.64 m, 3 H (OCH and OCH_2); 3.67 d, 2 H, J(P,CH_2) = 8.8 (PCH_2); 3.95 t, 2 H, J(CH_2,CH_2) = 5.0 (OCH_2); 4.42 t, 2 H, J(CH_2,CH_2) = 5.0 (NCH_2); 8.17 s, 1 H (H-base); 8.18 s, 1 H (H-base). ^{13}C NMR spectrum (D_2O): 44.63 s (NCH_2); 62.64 d, J(P,C) = 7.5 (C-1′ and C-3′); 67.32 d, J(P,C) = 160.2 (PCH_2); 71.52 d, J(P,C) = 12.2 (OCH_2); 76.80 d, J(P,C) = 5.0 (PC-2′); 119.28 s (C-5); 143.00 s (C-8); 149.90 s (C-4); 153.40 s (C-2); 156.49 s (C-6).

3-Benzyloxy-2-hydroxypropyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXII): 1-Benzyloxy-2,3-propanediol was prepared according to ref.[30]. For $C_{18}H_{24}N_5O_6P$. H_2O (455.4) calculated: 47.47% C, 5.75% H, 15.38% N, 6.80% P; found: 47.28% C, 5.66% H, 15.58% N, 6.51% P. R_F 0.70 (S1). Mass spectrum (FAB; *m/z*, rel.%): 438 (M + H, 15), 347 (M – C_7H_7, 25), 91 (C_7H_7, 100).

3-Hydroxybutyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXIII): After separation on ion exchangers an 8 : 1 mixture of the 1- and 2-isomeric esters was isolated. Separation by HPLC afforded pure 1′-isomer, m.p. 135 – 140 °C. For $C_{12}H_{20}N_5O_5P$. H_2O (363.3) calculated: 39.67% C, 6.10% H, 19.28% N, 8.53% P; found: 39.44% C, 5.87% H, 19.60% N, 8.29% P. R_F 0.68 (S1). Mass spectrum (FAB; *m/z*, rel.%): 346 (M + H, 80). ^{1}H NMR spectrum (D_2O): 1.03 d, 3 H, J(CH_3,CH) = 6.1 (CH_3); 1.50 m, 2 H (CCH_2); 3.45 m, 2 H (OCH_2); 3.61 d, 1 H, J(P,CH) = 9.0 (PCH_2); 3.65 d, 1 H, J(P,CH) = 5.0 (PCH_2); 4.41 t, 2 H, J(CH_2,CH_2) = 5.0 (NCH_2); 8.16 s, 1 H (H-base); 8.17 s, 1 H (H-base).

2,3,4-Trihydroxy-1-butyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXIV): 1,2,3-Tri-*O*-acetylthreitol was prepared according to ref.[31]. For $C_{12}H_{20}N_5O_7P$. H_2O (395.3) calculated: 36.46% C, 5.61% H, 17.72% N, 7.84% P; found: 36.77% C, 5.72% H, 17.83% N, 7.45% P. R_F 0.42 (S1). Mass spectrum (FAB; *m/z*, rel.%): 378 (M + H, 35). ^{1}H NMR spectrum (D_2O): 3.45 – 3.75 m, 8 H (OCH); 3.68 d, 2 H, J(P,CH) = 8.8 (PCH_2); 3.95 t, 2 H, J(CH_2,CH_2) = 5.0 (OCH_2); 4.43 t, 2 H, J(CH_2,CH_2) = 5.0 (NCH_2); 8.20 s, 1 H (H-base); 8.21 s, 1 H (H-base).

2,3,4,5,6-Pentahydroxy-1-hexyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXV): 1,2,3,4,5-Penta-*O*-acetyl-D-mannitol was prepared according to ref.[31]. For $C_{14}H_{24}N_5O_9P$. H_2O (455.4) calculated: 36.93% C, 5.75% H, 15.38% N, 6.80% P; found: 37.37% C, 5.59% H, 14.62% N, 6.54% P. R_F 0.28 (S1).

6-O-(D-Mannosyl) ester of 9-(2-phosphonomethoxyethyl)adenine (XXVI): 1,2,3,4-Tetra-*O*-acetyl-D-mannopyranose was prepared according to ref.[31]. The product was isolated as a mixture of α- and β-anomers of pyranose and furanose. For $C_{14}H_{22}N_5O_9P$. 1.5 H_2O (462.34) calculated: 36.37% C, 5.45% H, 15.15% N, 6.70% P; found: 36.70% C, 5.38% H, 14.87% N, 6.45% P. R_F 0.38 (S1).

2,2-Dimethyl-1,3-dioxolan-4-ylmethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXVII): For $C_{14}H_{23}N_5O_6P$. H_2O (406.4) calculated: 41.38% C, 6.20% H, 17.23% N, 7.62% P; found: 40.95% C, 6.03% H, 17.06% N, 7.15% P. R_F 0.65 (S1). Mass spectrum (FAB; *m/z*, rel.%): 389 (M + H, 45).

2-Amino-3-hydroxy-2-hydroxymethylpropyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXVIII): For $C_{12}H_{21}N_6O_6P$ (376.3) calculated: 38.30% C, 5.62% H, 22.33% N, 8.23% P; found: 38.42% C, 5.54% H, 22.42% N, 8.12% P. R_F 0.42 (S1). Mass spectrum (FAB; *m/z*, rel.%): 377 (M + H, 20). ^{1}H NMR spectrum (D_2O): 3.28 d, 2 H and 3.30 d, 2 H, J_{gem} = 11.5 (2 × OCH_2); 3.49 d, 2 H, J(POCH) = 5.6 ($POCH_2$); 3.67 d, 2 H, J(P,CH) = 8.8 (PCH_2); 3.96 t, 2 H, J(CH_2,CH_2) = 5.0 (OCH_2); 4.43 t, 2 H, J(CH_2,CH_2) = 5.0 (NCH_2); 8.19 s, 1 H (H-base); 8.20 s, 1 H (H-base).

2-Dimethylaminoethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXIX): For $C_{12}H_{21}N_6O_4P$. 2 H_2O (380.3) calculated: 37.90% C, 6.62% H, 22.10% N, 8.14% P; found: 38.12% C, 6.85% H, 21.72% N,

7.92% P. R_F 0.45 (S1). Mass spectrum (FAB; *m/z*, rel.%): 345 (M + H, 85). ^{1}H NMR spectrum (D_2O): 2.93 s, 6 H (NCH_3); 3.30 t, 2 H, $J(CH_2,CH_2)$ = 5.1 ($POCH_2$); 3.63 d, 2 H, J(P,CH) = 8.1 (PCH_2); 3.9 and 4.3 m, 8 H (NCH_2 and OCH_2); 8.11 s, 1 H (H-base); 8.17 s, 1 H (H-base).

2-Trimethylammoniumethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXX): For $C_{13}H_{23}N_6O_4P$. 2 H_2O (394.4) calculated: 39.59% C, 6.90% H, 21.31% N, 7.85% P; found: 39.83% C, 7.11% H, 21.02% N, 7.57% P. R_F 0.10 (S1). Mass spectrum (FAB; *m/z*, rel.%): 359 (M + H, 70). ^{1}H NMR spectrum (DMSO): 3.11 s, 9 H (NCH_3); 3.36 m, 4 H (PCH_2 and $POCH_2$); 3.82 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (OCH_2); 3.98 brd, 2 H (CH_2N); 4.39 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (NCH_2); 8.38 s, 1 H (H-base); 8.41 s, 1 H (H-base).

2-Aminoethyl ester of 9-(2-phosphonomethoxyethyl)adenine (XXXI): For $C_{10}H_{17}N_6O_4P$. H_2O (334.3) calculated: 35.93% C, 5.73% H, 25.14% N, 9.27% P; found: 36.24% C, 5.98% H, 24.88% N, 9.02% P. Mass spectrum (FAB; *m/z*, rel.%): 317 (M + H, 60).

2-Morpholinoethyl ester of 9-(2-phosphononomethoxyethyl)adenine (XXXII): For $C_{14}H_{23}N_6O_5P$ (386.4) calculated: 43.52% C, 6.00% H, 21.75% N, 8.02% P; found: 43.45% C, 6.15% H, 21.57% N, 8.11% P. R_F 0.75 (S1). ^{1}H NMR spectrum (D_2O): 2.23 t, 2 H, $J(CH_2,CH_2)$ = 5.1 (NCH_2); 2.32 m, 4 H (2 × NCH_2); 3.64 – 3.68 m, 8 H (3 × OCH_2 and PCH_2); 3.93 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (OCH_2); 4.42 t, 2 H, $J(CH_2,CH_2)$ = 5.0 (NCH_2); 8.16 s, 1 H (H-base); 8.18 s, 1 H (H-base).

Stability of the Esters of PMEA

a) Chemical stability in acidic and alkaline medium. A solution of the monoester (1 mg) in 0.05 M HCl or 0.05 M NaOH (1 ml) was set aside at room temperature. The mixture was analyzed by HPLC after 2, 24 and 36 h. Under the given conditions, all the monoesters were stable and did not cleave to PMEA. The only exception was the 2-cyanoethyl ester *XVII* which in the alkaline medium was entirely hydrolyzed during 24 h. In the acidic medium this compound was completely stable.

b) Chemical stability in presence of inert protein. The compounds (1 mg/ml) were incubated in a solution of thermally inactivated (75 °C, 15 min) bovine albumin (fraction *IV*, 20 mg/ml) for 12 and 72 h at 37 °C. The albumin was removed by precipitation with 2-propanol (final concentration 80% (v/v)). After centrifugation (15 000 g, 15 min) and concentration (vacuum evaporator), the stability of the compounds was determined by TLC (S1) and HPLC. Free compound *I* was not detected in any experiment.

c) Enzymatic stability. Crude and purified cell extracts, as well as suspensions of cell debris prepared from mice lymphomas L-1210, were used. Cells, obtained from lymphomas of 25 female mice, were homogenized by sonication (4 × 15 s, 0 °C) in 0.1 M Sørensen buffer, pH 7.4 (10 ml). Centrifugation (30 000 g, 35 min) gave crude cell extract and a pellet, containing cell debris. A part of the extract was used directly in the experiment and another part only after purification on a PD 11 Pharmacia column. The cell debris were suspended by gentle sonication in 0.1 M Sørensen buffer, pH 7.4 (10 ml).

The incubation mixture (final volume 250 μl) contained 0.2 or 0.02 mg/ml of the compound, 0.1 M Sørensen buffer, pH 7.4, and 50 μl of cell extract or suspension of cell debris. After 2 and 12 h, the samples were analyzed by TLC (S1) and HPLC.

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