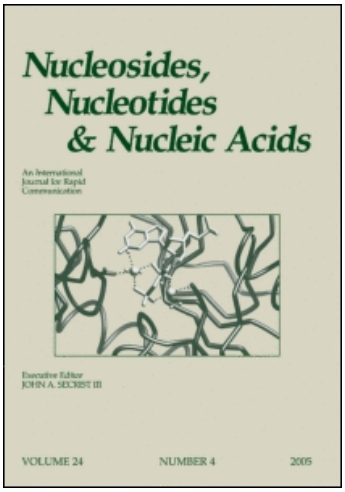

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Phosphonylalkoxyalkyl and Phosphonylalkyl Derivatives of Heterocyclic Bases

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PHOSPHONYLALKOXYALKYL AND PHOSPHONYLALKYL DERIVATIVES OF HETEROCYCLIC BASES

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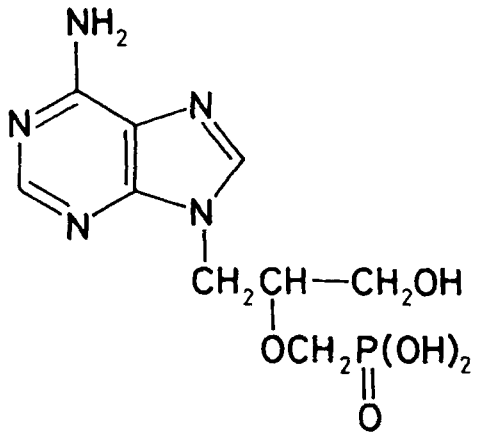
Abstract. This review describes chemical syntheses of acyclic nucleotide analogs containing various types of phosphonate grouping, with an emphasis on preparative methods for N-(3-hydroxy-2-phosphonylmethoxypropyl) and N-(2-phosphonylmethoxyethyl) derivatives of purine and pyrimidine bases.

The recently discovered antiviral activity of 9-(S)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA)(**1**)[1] prompted us to investigate in detail the structure - antiviral activity relationship in the series of acyclic nucleotide analogs containing a phosphonylalkoxy or phosphonyl group attached to the side chain of the acyclic nucleosides. These groups are investigated as isopolar substitutes of the phosphomonoester moiety in nucleotides, resistant to enzymatic dephosphorylation.



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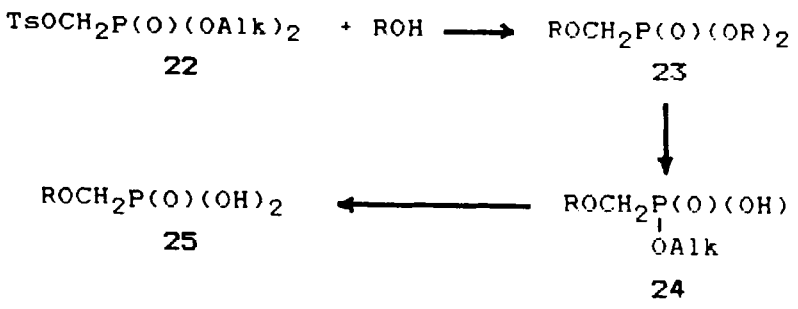
The first part of this investigation deals with the syntheses of HPMPA derivatives modified and/or substituted at the side-chain. This study is aimed at the elucidation of the role of mutual relation between the heterocyclic base and the phosphonylmethoxy group; the phosphonylalkyl analogs could help to ascertain the role of the oxygen atom in the vicinity of phosphorus in compound 1 . These compounds were synthesized systematically in the adenine series only (TABLE 1).

Alkyl ethers of HPMPA (2,3 were prepared by reaction of the corresponding 9-(3-alkoxy-2-hydroxypropyl)adenines (ref.[3,4]) with dimethyl (or diethyl) p-toluenesulfonyloxymethylphosphonate [5] (22) ; the reaction is best performed in DMF, in the presence of 2-3 equivs.of NaH at room temperature.The formation of N-substituted derivatives, arising as side-products in this reaction, can be prevented by protection of the 6-amino group in the starting compounds (N-benzoylation or transformation to an amidine derivative). Subsequent addition of aqueous methanol to the reaction mixture causes simultaneous cleavage of the N-protecting group and one of the alkyl ester groups. The alkyl esters

TABLE 1. 9-(Phosphonylmethoxyalkyl)adenines.

	Side Chain
2	$-CH_2CH(OCH_2P(O)(OH)_2)CH_2OCH_3$
3	$-CH_2CH(OCH_2P(O)(OH)_2)CH_2OC_8H_{17}$
4	$-CH_2CH(OCH_2P(O)(OH)_2)CH_3$
5	$-CH_2OCH_2P(O)(OH)_2$
6	$-CH_2CH_2OCH_2P(O)(OH)_2$
7	$-CH_2CH_2CH_2OCH_2P(O)(OH)_2$
8	$-CH_2(CH_2)_2CH_2OCH_2P(O)(OH)_2$
9	$-CH_2CH(OCH_2P(O)(OH)_2)CH(OH)CH_2OH$
10	$-CH_2C(CH_3)(OCH_2P(O)(OH)_2)CH_2OH$
11	$-CH_2CH(OCH_2P(O)(OH)_2)CH(OH)CH_3$
12	$-CH_2CH_2CH(OCH_2P(O)(OH)_2)CH_2OH$
13	$-CH_2CH(OCH_3)CH_2OCH_2P(O)(OH)_2$
14	$-CH_2CH_2CH(OH)CH_2OCH_2P(O)(OH)_2$
15	$-CH_2CH(OH)CH(OH)CH_2OCH_2P(O)(OH)_2$
16	$-CH_2CH(OH)CH(CH_3)OCH_2P(O)(OH)_2$
17	$-CH(CH_2OH)CH_2OCH_2P(O)(OH)_2$
18	$-CH(CH_3)CH(OH)CH_2OCH_2P(O)(OH)_2$
19	$-CH(C_6H_{11})CH(OH)CH_2OCH_2P(O)(OH)_2$
20	$-CH_2OCH_2CH_2OCH_2P(O)(OH)_2$
21	$-CH_2OCH_2CH_2P(O)(OH)2$

(23) were isolated by ion exchange chromatography and transformed to the free phosphonates 24 by the action of bromotrimethylsilane in acetonitrile followed by hydrolysis. This sequence is applicable to N-(monohydroxyalkyl)-adenines regardless of the character (primary,secondary) of their hydroxyl groups (SCHEME 1).

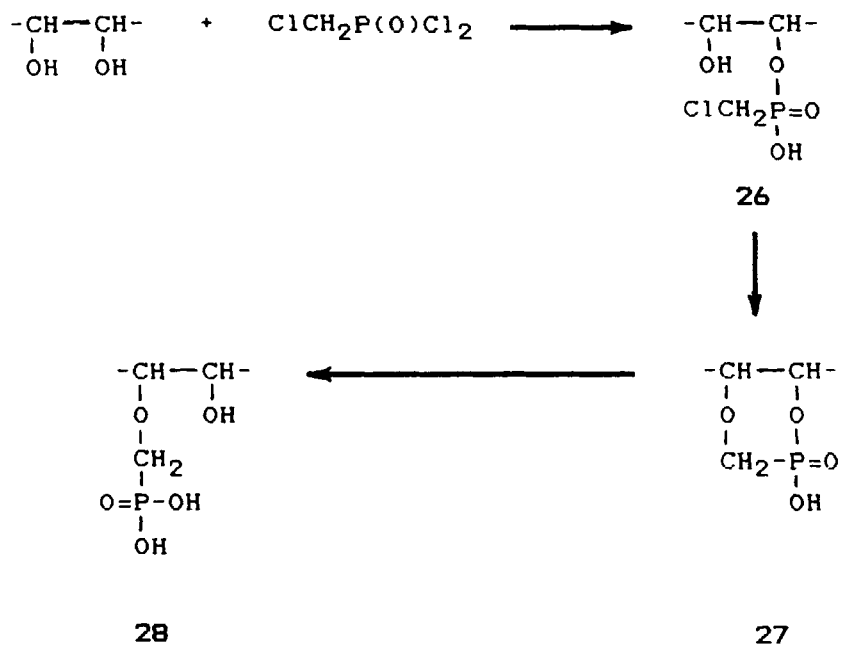


Alk=methyl,ethyl group;for other symbols,see Abbreviations

SCHEME 1

Thus, 9-(w-phosphonylmethoxyalkyl)adenines **6-8** and 9--(2-phosphonylmethoxypropyl)adenine **4** were obtained from the corresponding hydroxyalkyl derivatives [3,4,6]. Also 9--(2-phosphonylmethoxyethoxymethyl)adenine **20** and racemic compound **4** (an isomer of HPMPA methyl ether **2**) were prepared by this procedure from adenine analog of acyclovir[7] and 9-(3-hydroxy-2-methoxypropyl)adenine,respectively. The N-benzoylation of starting adenine derivatives can be easily performed by a consecutive treatment with chlorotrimethylsilane and benzoyl chloride[8].

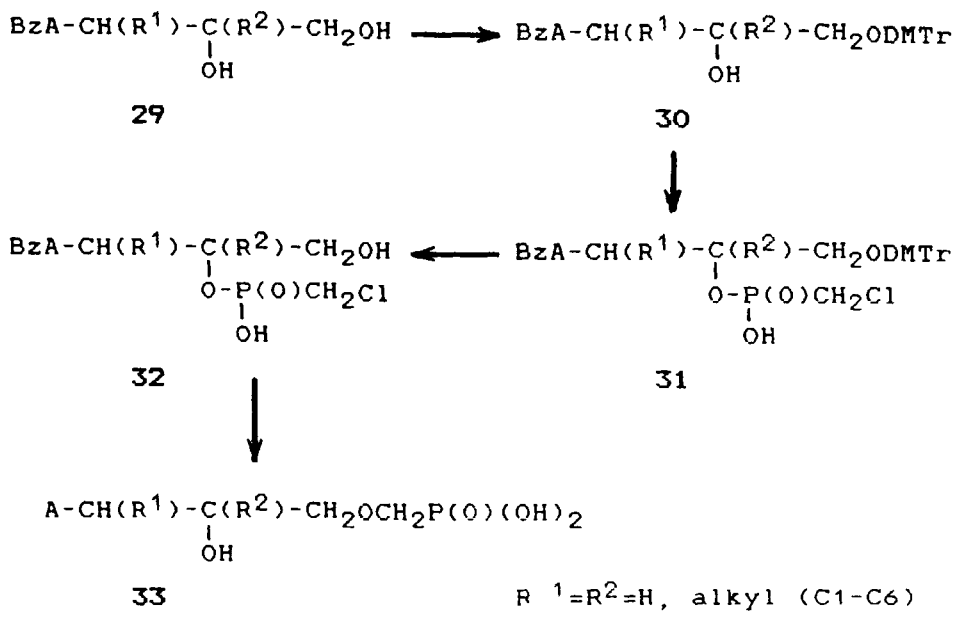
This procedure can also be applied to di- and trihydroxyalkyl derivatives; except for symmetrical 2-(adenin-9-yl)propan-1,3-diol which gives rise to the regioisomer of HPMPA [9-(1-hydroxy-3-phosphonylmethoxy-2-propyl)adenine] (**17**);the reaction in such cases always affords an isomeric mixture of phosphonylmethyl ethers. In order to obtain a single reaction product the remaining hydroxyl groups must be protected,preferably by alkali-stable protecting groups (cf.[9]),e.g.isopropylidene in 2,3,4-trihydroxybutyl derivatives,benzyl or trityl type groups in dihydroxyalkyl derivatives. This strategy was used for 3'-hydroxymethyl deri-



SCHEME 2

vative of HPMPA (**9**) as well as for its isomers, enantiomeric 9-threo-(2,3-dihydroxy-4-phosphonylmethoxybutyl)adenines(**15**). Also HPMPA (**1**) was prepared by this route from 9-(S)-(2-hydroxy-3-trityloxypropyl)-N^6-benzoyladenine[10] or -N^6-trityladen ine[11].

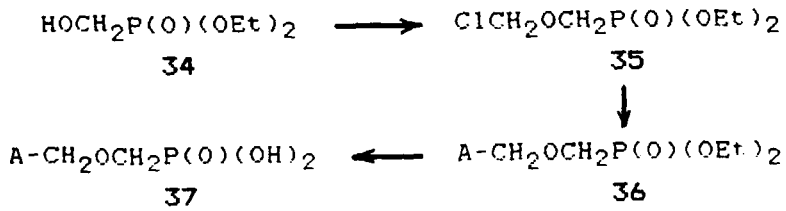
Another access to the synthesis of the phosphonylmethoxyalkyl derivatives is applicable exclusively to compounds containing vicinal diol grouping. It consists in an intramolecular etherification reaction of corresponding chloromethylphosphonic acid monoesters which takes place quantitatively in an aqueous alkali at elevated temperature. This method was first used for the preparation of 2'(3')-O-phosphonylmethyl ribonucleosides[9] (SCHEME 2). Thus, for the preparation of isomeric phosphonylmethyl ethers of 9-(2,3-or 3,4-dihydroxybutyl)adenine (**11,12**) or 9-(2,3-dihydroxy-2-methylpropyl)adenine (**10**) the unprotected star-



SCHEME 3

ting diols are treated with chloromethylphosphonic dichloride in triethyl phosphate. Hydrolysis and deionisation affords isomeric chloromethylphosphonates **26**which undergo reaction with aqueous sodium hydroxide solution to form the isomeric O-phosphonylmethyl derivatives **28** via the cyclic intermediates **27** (SCHEME 3).The separation of isomers can be mostly achieved by HPLC techniques.

The regiospecific alternative of this reaction is based on a selective protection of the primary hydroxyl group in N-benzoylated diol **29** by dimethoxytrityl group. The intermediate **30** is further transformed to the chloromethylphosphonate **31** ; in order to exclude deprotection of the trityl group by hydrogen chloride formed, the reaction with chloromethylphosphonic dichloride or its equimolar adduct with water must be performed in pyridine. The intermediates **31** obtained in a high yield can be deprotected under con-



SCHEME 4

trolled acidic conditions without migration of the ester group to form chloromethylphosphonates **32**. On treatment with aqueous sodium hydroxide, compounds **32** afford under simultaneous debenzoylation pure phosphonylmethyl ethers **33** (SCHEME 3).

This reaction was used to prepare the 3'-isomer of HPMPA[9] and its 1'-or 2'-alkyl derivatives **16,18,19**

The simplest member of this structural group is 9-(phosphonylmethoxymethyl)adenine (**5**). It was prepared by alkylation of adenine with preformed organophosphorus synthon **35** according to SCHEME 4.

Diethyl chloromethoxymethylphosphonate (**35**) was easily obtained by chloromethylation of hydroxymethylphosphonate **34**; reaction of this compound with sodium salt of adenine in DMF affords the intermediary diester **36** which is converted to the free phosphonate by treatment with bromotrimethylsilane under the usual conditions.

9-Phosphonylalkyladenines can be regarded as carba analogs of the above compounds. They are available by transformation of preformed 9-hydroxyalkyladenines or by alkylation of adenine with preformed organophosphorus synthons. Several compounds of this type which have been examined within the scope of this study are listed in TABLE 2.

The first route is based on Arbuzov reaction of 9-(w-bromoalkyl)-N^6-benzoyladenines **46** with triethyl phosphite

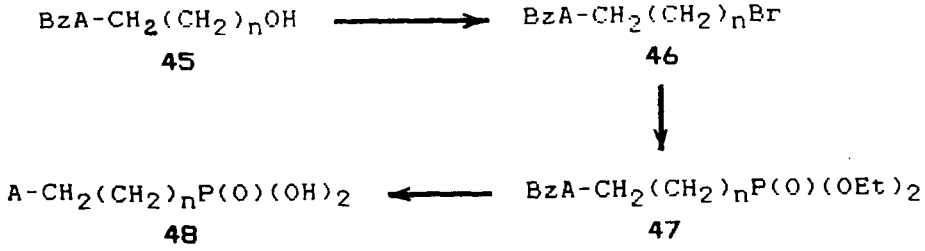
TABLE 2. 9-(Phosphonylalkyl)adenines.

No.	Side Chain
38	$-CH_2P(O)(OH)_2$
39	$-CH_2CH_2P(O)(OH)_2$
40	$-CH_2(CH_2)_2P(O)(OH)_2$
41	$-CH_2CH(OH)CH(OH)CH_2P(O)(OH)_2$
42	$-CH_2CH(OH)P(O)(OH)_2$
43	$-CH_2CH_2CH(OH)P(O)(OH)_2$
44	$-CH_2C(CH_3)(OH)P(O)(OH)_2$

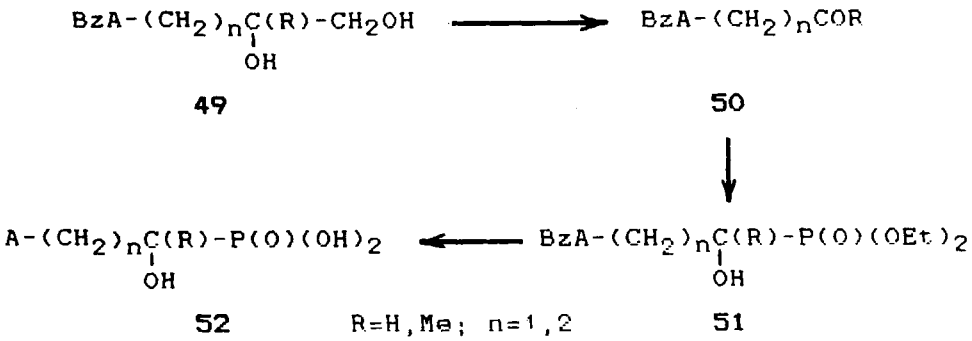
which affords the neutral diesters **47**. The starting compounds **46** can be obtained from the hydroxyalkyl derivatives **45** by the reaction with triphenylphosphane and carbon tetrabromide. The free phosphonates **48** are produced by cleavage of the diesters **47** under the usual conditions. This procedure (SCHEME 5) was used for compounds **39-41**; its application to 9-(2-hydroxyethoxymethyl)adenine afforded the oxa analog **21**(TABLE 1)which can be regarded as an isomer of 9-(2-phosphonylmethoxyethyl)adenine (**6**).

9-(Phosphonylmethoxymethyl)adenine (**38**)was synthesized by alkylation of adenine with the tosylate **22** followed by bromotrimethylsilane treatment of the diester intermediate.

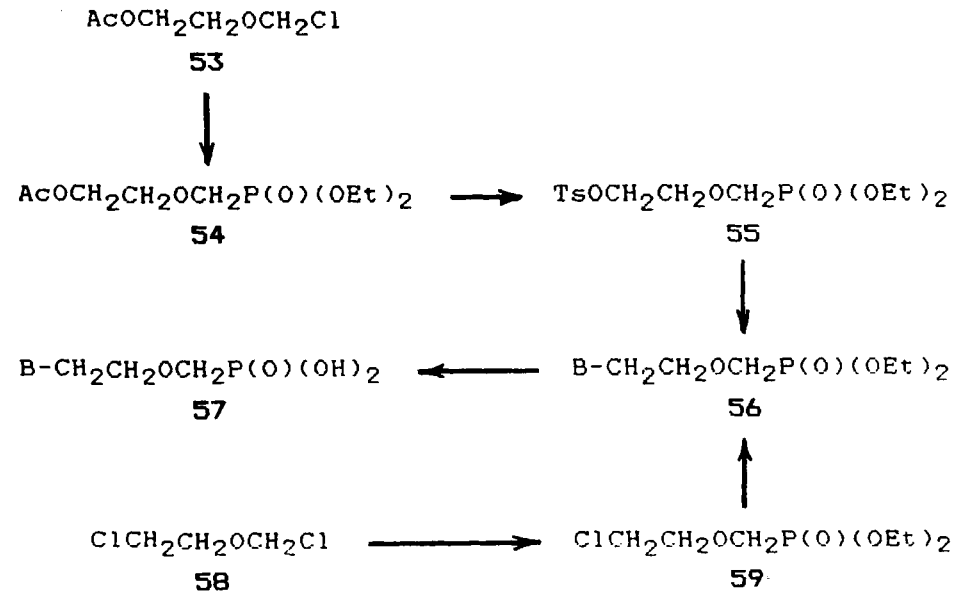
The last group of the side-chain modified HPMPA analogs, examined in our study, are isomers of phosphonylmethyl ethers - 9-(α-hydroxyphosphonylalkyl)adenines (**42-44**). Their synthesis was accomplished by condensation of 9-(oxoalkyl)-N^6-benzoyladenines(**50**)with diethyl phosphite in the presence of triethylamine, followed by methanolysis and removal of ester groups. The starting oxo derivatives are



SCHEME 5



SCHEME 6



SCHEME 7

obtained e.g.by periodate cleavage of suitable alkyladenines bearing a vicinal diol grouping (**49**) (SCHEME 6).

With the exception of 9-(2-phosphonylmethoxyethyl)adenine(PMEA)(**6**),none of the above side-chain modified phosphonylalkoxyalkyl, phosphonylalkyl or α-hydroxyphosphonylalkyl derivatives expressed any significant antiviral effect against DNA or RNA viruses[12]. This fact stresses the narrow margin for modifications of HPMPA at the side chain and the phosphonylmethyl group.On the other hand,compound **6** exhibited an antiviral activity against DNA viruses which is not only qualitatively and quantitatively comparable with that of HPMPA[1,13] but is directed also against retroviruses,including HIV[14,15]. Therefore, our study concentrated on structural alterations in the heterocyclic base moiety of HPMPA and PMEA.

For the preparation of PME-derivatives, the original procedure devised for PMEA (SCHEME 1)[16] was replaced by another method which is conveniently applicable to the synthesis of its base-modified analogs (SCHEME 7).This method consists in an alkylation of the corresponding heterocyclic base, its protected derivative or suitable precursor,with 2-substituted ethoxymethylphosphonic acid diesters **55,59**. Products of these alkylations, diesters **56**, can be easily isolated by crystallization or silica gel chromatography and converted to PME-derivatives **57** by bromotrimethylsilane cleavage reaction. The synthon **55** is accessible from 1,3-dioxolane *via* 2-acetoxyethoxymethylchloride(**53**);Arbuzov reaction of this compound results in 2-acetoxyethylphosphonate **54** which is transformed to the tosyl derivative **55** by an acid-catalyzed hydrolysis followed by tosylation. The synthon **59** is still better available: chloromethylation of 2-chloroethanol affords 2-chloroethoxymethyl chloride (**58**), which reacts smoothly with triethyl phosphite to form exclusively the diester **59** [17]. This reagent is superior to the tosyl derivative **55**:it is better avail-

able, distillable in vacuo and can be stored without special precautions. Alkylations with compounds **55,59** are performed with the sodium salts of bases prepared in situ by NaH treatment in DMF or, in case of the synthon **59**, simply with the base in the presence of potassium carbonate. The character of the base and directive rules of alkylations determine whether the base can be unprotected(adenine, cytosine and their derivatives, 6-methylthiopurine) or whether it must be protected in order to ensure site-specific alkylations.For example,uracil or thymine have to be transformed into their 4-O-methyl derivatives to exclude the N^3-alkylation. (In this case, the deprotection occurs during the bromotrimethylsilane reaction.) N^2-Acylguanines are poor substrates in these alkylations affording an equimolar mixture of 7- and 9-isomers. This difficulty is overcome by using of 2-amino-6-chloropurine which forms the required product **56** with a good yield and with a strong preference for the 9-isomer (8:1); this intermediate is transformed into the guanine derivative by acid hydrolysis (1 M HCl)[18].

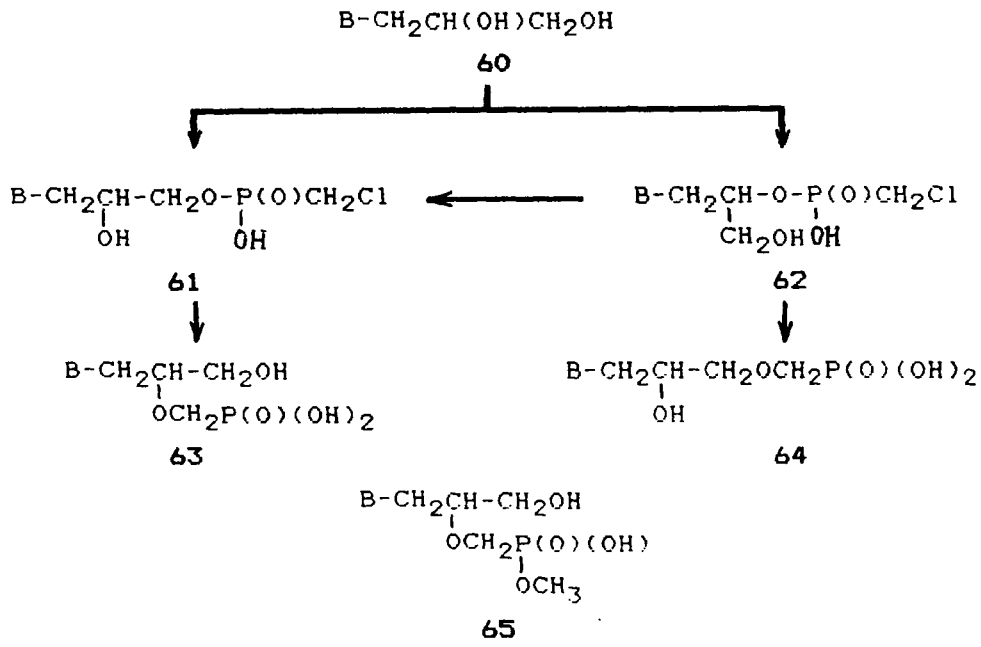
The method described in SCHEME 7 has been used to prepare compounds **57** comprised in TABLE 3. Since it does not utilize alkaline conditions, it is applicable also for preparation of alkali-sensitive derivatives(cytosine,6-methylthiopurine). Additional analogs not available by direct route can be made by subsequent conversion of the base in compounds **57** (e.g.hypoxanthine or isoguanine derivatives by deamination of adenine or 2-aminoadenine compounds). Free PME-derivatives **57** can be isolated mostly by ion exchange chromatography as crystalline free acids. In some cases (**57a,c,k**)they have a limited water-solubility; they can be conveniently stored as soluble sodium salts.

The availability of HPMP-derivatives is complicated by the need for isomeric homogeneity and enantiomeric purity of the products. One of the general procedures parallels the reaction sequence of SCHEME 2: the easily available[19]

TABLE 3. BASE-MODIFIED HPMP- AND PME-DERIVATIVES.

Base	HPMP	Abbrev.	PME	Abbrev.
Adenine	63a	HPMPA	57a	PMEA
2-Aminoadenine	63b	HPMPDAP	57b	PMEDAP
2-Methyladenine	63c		---	
2-Methylthioadenine	63d		57d	
N6-Dimethyladenine	63e		---	
6-Hydrazinopurine	63f		57f	
6-Hydroxylaminopurine	63g		---	
6-Methylthiopurine	---		57h	
Hypoxanthine	63i	HPMPHx	57i	PMEHx
2-Aminopurine	63j	HPMPMAP	57j	PMEMAP
Guanine	63k	HPMPG	57k	PMEG
Isoguanine	63l		57l	
lin-Benzoadenine	63m		57m	
1,6-Ethenoadenine	63n		57n	
Uracil	63o	HPMPU	57o	PMEU
Thymine	63p	HPMPT	57p	PMET
Cytosine	63q	HPMPC	57q	PMEC
5-Methylcytosine	63r		57r	
5-Fluorouracil	63s		---	

N-(2,3-dihydroxypropyl) derivatives **60** in a reaction with $ClCH_2P(O)Cl_2$ in triethyl phosphate afford a mixture (ca. 2:3) of chloromethylphosphonates **61,62**. In acidic conditions (e.g. 1M HCl), this equilibrium shifts in favor of the required isomer **61** (ca. 8:1) and the isomers can be separated by HPLC or ion exchange chromatography. On alkaline treatment, these compounds are quantitatively converted to the pure isomers **63,64**. No isomerisation or racemisation occurs under the above conditions (SCHEME 8).



SCHEME 8

Therefore,the reaction can be used for preparation of the (S)-enantiomers which are the exquisite carriers of biological activity[1]. The use of strongly alkaline conditions in the last step which is incompatible with the alkali-sensitive heterocyclic bases (see above) can be circumvented by the use of sodium methoxide in methanol. The methyl esters **65** which are formed under these conditions, can be converted into HPMP-derivatives **63** by bromotrimethylsilane.

General regiospecific synthesis of the pure 2'-isomers **63** is based on specific protection of the secondary 2'-hydroxyl group in compounds **60**(or in their N-benzoyl derivatives)(cf.[9])(SCHEME 9). Starting materials **60** were transformed to the acid-sensitive dimethoxytrityl derivatives **66** and subsequently treated with benzoyl cyanide [20] to form the fully protected intermediates **67**.Controlled acid hydrolysis converts compounds **67** to the 2'-O-benzoyl

derivatives **68**. This sequence does not require isolation of intermediates. The final conversion to the HPMP-derivatives **63** follows the above route(i.e.reaction with chloromethylphosphonyl dichloride followed by alkaline treatment or methanolysis) and affords, under simultaneous debenzoylation, pure compounds **63**.

These two methods enabled us to synthesize a series of base-modified HPMP-derivatives **63** (TABLE 3). Additional analogs were prepared by subsequent transformations of the heterocycle[21]. Free acid forms of these analogs were isolated by anion exchange chromatography; the products can be conveniently stored as sodium salts.

Antiviral screening in the both series revealed that, in addition to HPMPA and PMEA, 2-aminoadenine (57b, 63b) and guanine derivatives (57k, 63k) also exhibit high activity against DNA viruses[12-14]. Since, at the same time, these compounds show increased *in vitro* cytotoxicity, it remains to be established whether their pharmacological properties are better than those of the parent compounds. In the HPMP-series, also the cytosine derivative **63q** (HPMPC) has an excellent antiviral activity which seems to be in certain cases superior to that of HPMPA[22]. Other purine or pyrimidine derivatives listed in TABLE 3 are either totally inactive or substantially less active than the adenine derivatives[13].

The PME-derivatives of adenine, 2-aminoadenine and guanine (**57a,b,k**) exhibit significant cytostatic effects on L-1210 mouse leukemia cells *in vitro*[23]; preliminary experiments performed with several animal tumor models are rather promising. These results emphasize the importance of further biological evaluation in the series of phosphonate analogs of acyclic nucleotides, as well as further development of their chemistry.

Abbreviations used: A...adenin-9-yl, Ac...acetyl, B...pyrimidin-1-yl or purin-9-yl base moiety, Bz...benzoyl,

BzA.. N^6-benzoyladenin-9-yl, DMF...dimethylformamide, DMTr ...dimethoxytrityl, Et...ethyl, Me...methyl, Ts...p-toluenesulfonyl residue.

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