SYNTHESIS OF *N*-(3-AZIDO-2-HYDROXYPROPYL), *N*-(3-PHTHALIMIDO-2-HYDROXYPROPYL) AND *N*-(3-AMINO-2-HYDROXYPROPYL) DERIVATIVES OF HETEROCYCLIC BASES*

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Alkylation of heterocyclic bases with azidomethyloxirane (*I*) under basic catalysis with potassium or cesium carbonate afforded *N*-(3-azido-2-hydroxypropyl) derivatives *II*. Hydrogenation of these compounds over palladium on carbon gave the corresponding 3-amino-2-hydroxypropyl derivatives *III*. The same compounds *III* were prepared by alkylation of heterocyclic bases with phthalimidomethyloxirane (*VII*) in the presence of cesium carbonate and subsequent reaction of the formed *N*-(3-phthalimido-2-hydroxypropyl) derivatives *VIII* with hydrazine. The phthalimido derivatives *VIII* are easily hydrolyzed already in weakly alkaline aqueous medium to give 9-[3-(*o*-carboxybenzoylamino)-2-hydroxypropyl] derivatives *IX* and *X*.

Within the framework of investigation on preparation and biological properties of nucleoside analogs we studied the preparation and properties of compounds bearing in the side-chain amino groups in addition to the hydroxyl functionalities. Some time ago we prepared the isomeric 9-azidohydroxypropyl derivatives of adenine and converted them into the corresponding aminohydroxypropyl derivatives by catalytic hydrogenation[1,2] (Scheme 1). It appeared that already 9-(3-azido-2-hydroxypropyl)adenine (*IIa*) exhibited marked mutagenic effect on *Salmonella typhimurium*[3].

In the light of this finding and of the already known mutagenicity of related 1-azidopropane-2,3-diol[4] we decided to study how the heterocyclic base affects the mutagenic activity of the 3-azido-2-hydroxypropyl derivatives *II*. However, the main stimulus for seeking a general approach to these compounds was the prospect of their further syn-

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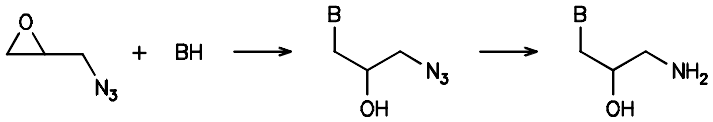
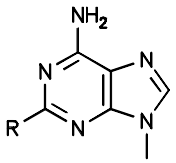


I *II* *III*

In formulae *II* and *III* for B :



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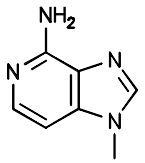

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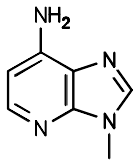

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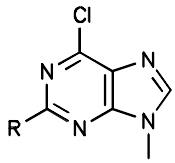

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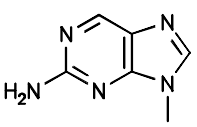

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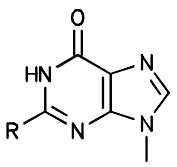
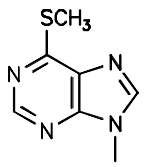


h, R = H
i, R = NH_2



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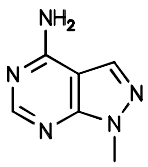

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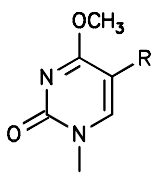

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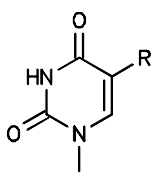

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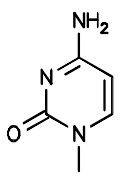

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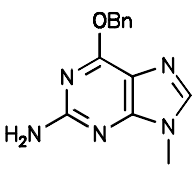

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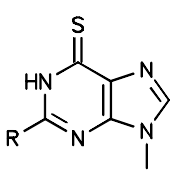
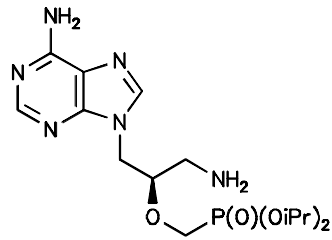


r, R = H
s, R = NH_2

SCHEME 1

thetic utilization, e.g. in the synthesis of 3-amino-2-hydroxypropyl derivatives *III* or 3-amino-2-phosphonomethoxypropyl derivatives[2] *IV*, analogs of antivirals of the HPMP series (ref.[5]) or NAD analogs[6].



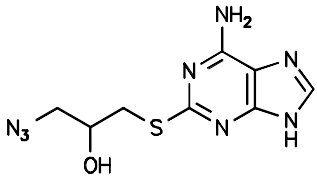
IV

Until now, synthetic methods leading to azido derivatives of acyclic nucleoside analogs of the type *II* consisted either in nucleophilic substitution of the tosyl group on the alkyl chain[1,7] or in alkylation of the heterocyclic base with a protected derivative of 1-azido-2-hydroxy-3-propyl tosylate[2]. The former method requires conversion of hydroxyl into a sulfonate group; in spite of this it appears to be more advantageous than the direct conversion of the hydroxyl into the azide functionality by reaction with triphenylphosphine, lithium azide and carbon tetrabromide[8,9] or tetraiodide[10], as described for syntheses of acyclic azidothymidine analogs, or by the Mitsunobu reaction of the hydroxyl with triphenyl phosphine, DEAD and azoimide[11]. The use of protected 3-azido-2-hydroxypropyl tosylate[4] for the alkylation of bases finds its application mainly in the preparation of optically active forms of 3-amino-2-hydroxypropyl derivatives[6] *III*. *In* the present study we make use of alkylation of the heterocyclic base with azidomethyloxirane (*I*) which is readily accessible from epichlorohydrin[6]. The reaction takes place under catalysis with potassium or cesium carbonate in dimethylformamide as solvent.

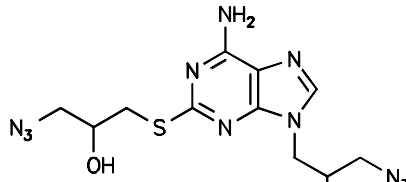
As expected, the reaction proceeded smoothly with adenine, its 3-deaza analog, 6-chloropurine, 6-methylthiopurine and 2,6-diaminopurine, affording predominantly the 9-isomers *IIa*, *IIc*, *IIe*, *IIj* and *IIb*, respectively. High regiospecificity in the alkylation of the base with oxirane *I* was also found for 4-aminopyrazolo[5,6-*d*]pyrimidine: in this case the alkylation takes place predominantly on N^7, leading to compound *IIk*. Also 4-methoxy-2-pyrimidone and 4-methoxy-5-methyl-2-pyrimidone afforded the expected N^1-isomers *IIl* and *IIm*; the former was ammonolyzed to give the cytosine derivative *IIp* which was also prepared by direct alkylation of cytosine with compound *I*.

A less unequivocal reaction course under the conditions described was observed with 2-amino-6-chloropurine which afforded, in addition to the N^9-isomer *IIf*, some N^7-alkylation product. As could be expected, alkylation of 2-mercaptoadenine with oxirane *I*

afforded compound *V* as the primary product of alkylation on the sulfur atom, together with the N^9,*S*-disubstituted derivative *VI*.



V



VI

Acid hydrolysis of 6-chloro derivatives *IIe* and *IIf* afforded 9-(3-azido-2-hydroxypropyl) derivatives of hypoxanthine and guanine *IIh* and *IIi*, respectively. They were reacted with thiourea to give the respective 6-mercaptopurine and 6-thioguanine derivatives *IIr* and *IIs*. In the same manner, the corresponding derivatives of 4-methoxy-2-pyrimidone and 4-methoxy-5-methyl-2-pyrimidone *IIl* and *IIm* were converted into the uracil and thymine derivatives *IIn* and *IIo*.

The azido compounds *II* were easily and in good yields converted into 3-amino-2-hydroxypropyl derivatives *III* by hydrogenation, e.g. over a palladium catalyst in ethanol. The only exception was the preparation of 2-amino-6-chloropurine derivative *IIIf* in which the reduction was accompanied by reductive dehalogenation and the resulting product was 9-(3-amino-2-hydroxypropyl)-2-aminopurine (*IIIg*).

The structure of azido derivatives *II* and amino derivatives *III* was proved by the usual techniques, including UV and ^{1}H NMR spectroscopy. In the determination of structures of the bases in the derivatives *II*, *III*, *V* and *VI* the limited information from the ^{1}H NMR spectra (sharp signals of heteroaromatic protons and broad signals of NH or OH protons, see Table I) was extended by that from the ^{13}C NMR data, particularly as concerns quaternary carbon atoms (Table II). The structural assignment of carbon atom signals was based on the experimental distinction of tertiary and quaternary atoms and their comparison with the spectra of the free bases. In contrast to the very broad carbon signals (halfwidths 10 – 100 Hz) in the spectra of free purine bases (particularly of C-4 and C-5), the corresponding signals in our azido and amino derivatives *II* and *III* were narrow (1 – 2 Hz), reflecting evidently the absence of the N-7 ↔ N-9 tautomerism in the substituted compounds. The only exception was compound *V* which, however, is the product of *S*-alkylation in position 2 (vide infra) and in which the mentioned tautomerism can exist. The presence of 3-azido- or 3-amino-2-hydroxypropyl groups was clearly demonstrated by signals in the region of aliphatic carbon atoms in ^{13}C NMR spectra (Table II). Whereas the signals of C-2′ and C-3′ reflect first of all character of substituents in these positions and differ only little within the series of azido or amino derivatives, signals due to C-1′ are markedly influenced by the

TABLE I
Proton NMR data of compounds *II*, *III*, *V* and *VI*

Compound	Chemical shifts (coupling constants)							
	H-2	H-8	H-1′a	H-1′b	H-2′	H-3′a	H-3′b	Other protons
IIa[a]	8.05 s	8.14 s	4.20 m (13.8; 4.2)	4.10 m (13.8; 7.7)	4.08 m	3.35 dd (12.7; 3.5)	3.24 dd (12.7; 6.2)	7.21 bs (NH_2); 5.65 d (5.1) (OH)
IIb	–	7.63 s	4.02 dd (13.8; 3.5)	3.91 dd (13.8; 8.0)	4.04 m	3.31 dd (13.0; 2.6)	3.20 dd (13.0; 6.0)	6.74 bs and 5.84 bs ($2 \times NH_2$); 5.72 b (OH)
IIc	7.68 d (5.9)	8.02 s	4.24 dd (13.8; 3.9)	4.11 dd (13.8; 7.3)	4.00 m	3.37 dd (12.7; 3.7)	3.24 dd (12.7; 6.1)	6.85 d (5.9) (H-3); 6.30 bs (NH_2); 5.70 bs (OH)
IId[a]	7.81 d (5.5)	8.00 s	4.22 (14.8; 4.7)	4.11 (14.8; 7.8)	4.09	3.35 dd (12.8; 3.5)	3.23 dd (12.8; 6.4)	6.38 d (5.5) (H-1); 6.35 bs (NH_2); 5.78 bs (OH)
IIe	8.77 s	8.62 s	4.37 dd (13.6; 4.1)	4.25 dd (13.6; 7.6)	4.13 m	3.42 dd (12.8; 4.0)	3.30 dd (12.8; 6.1)	5.63 bd (5.3) (OH)
IIf	–	8.04 s	4.09 dd (13.6; 3.0)	3.99 dd (13.6; 9.0)	4.07 m	3.37 dd (12.8; 3.6)	3.25 dd (12.8; 5.8)	6.87 bs (NH_2); 5.63 bd (5.2) (OH)
IIi	–	7.61 s	3.98 dd (13.6; 3.9)	3.88 dd (13.6; 8.2)	4.01 m	3.31 dd (12.6; 3.7)	3.20 dd (12.6; 5.9)	6.43 bs (NH_2); 10.56 s (OH(6)); 5.64 d (5.3) (OH(2′))
IIj	8.71 s	8.37 s	4.32 dd (13.3; 3.9)	4.20 dd (13.3; 7.5)	4.12 m	3.39 dd (12.8; 3.9)	3.27 dd (12.8; 6.0)	5.64 d (5.2) (OH); 2.67 s (SCH_3)
IIk	8.17 s	–	4.24 dd (13.4; 5.6)	4.35 dd (13.4; 6.8)	4.16 m	3.30 dd (12.9; 3.9)	3.22 dd (12.9; 5.8)	8.09 s (H-7); 7.68 bs (NH_2); 5.53 d (5.4) (OH)

TABLE I
(*Continued*)

Compound	Chemical shifts (coupling constants)							
	H-2	H-8	H-1′a	H-1′b	H-2′	H-3′a	H-3′b	Other protons
IIl	–	–	3.95 dd (13.8; 3.5)	3.56 dd (13.8; 9.2)	3.96 m	3.33 dd (12.9; 3.8)	3.21 dd (12.9; 6.2)	5.96 d (7.2) (H-5); 7.82 d (7.2) (H-6); 3.80 s (OCH_3)
IIm	–	–	3.91 dd (13.4; 3.7)	3.54 dd (13.4; 8.5)	3.97 m	3.33 dd (12.9; 3.7)	3.21 dd (12.8; 6.3)	7.67 q (0.9) (H-6); 1.87 d (0.9) (CH_3); 3.84 s (OCH_3); 5.52 d (5.7) (OH);
IIn	–	–	3.79 dd (13.7; 3.7)	3.50 dd (13.7; 8.3)	3.89 m	3.32 dd (12.8; 4.0)	3.21 dd (12.8; 6.3)	5.51 d (7.9) (H-5); 7.50 d (7.9) (H-6); 11.10 bs (NH); 5.56 bs (OH)
IIo	–	–	3.75 dd (13.7; 4.0)	3.48 dd (13.7; 8.3)	3.90 m	3.31 dd (12.8; 3.9)	3.20 dd (12.8; 6.4)	7.39 q (1.1) (H-6); 1.74 d (1.1) (CH_3); 5.54 bd (4.5) (OH); 11.15 bs (NH)
IIp	–	–	3.80 dd (13.2; 3.8)	3.42 dd (13.2; 7.9)	3.90 m	3.27 dd (12.8; 3.9)	3.16 dd (12.8; 6.2)	5.62 d (7.2) (H-5); 7.44 d (7.2) (H-6); 7.00 bs (NH_2); 5.52 bd (5.2) (OH);
IIr	8.18 d (3.4)	8.19 s	4.21 dd (13.9; 3.9)	4.12 dd (13.9; 8.1)	4.05 m	3.37 dd (13.0; 3.8)	3.26 dd (13.0; 6.1)	13.70 bs (NH); 5.70 bs (OH)
IIs	–	7.81 s	4.00 dd (13.6; 3.2)	3.91 dd (13.6; 8.4)	4.01 m	3.34 dd (12.7; 3.8)	3.23 dd (12.7; 5.9)	11.75 bs (NH); 6.78 bs (NH_2); 5.64 d (5.1) (OH)
IIIa	8.04 s	8.13 s	4.21 dd (14.0; 4.1)	4.05 dd (14.0; 7.2)	3.77 m	2.50 dd (13.0; 5.4)	2.46 dd (13.0; 6.4)	7.20 bs (NH_2); 3.20 b (NH_2 + OH);
IIIb	–	7.69 s	4.06 dd (13.9; 3.7)	3.97 dd (13.9; 7.6)	4.02 m	2.88 dd (12.9; 3.5)	2.68 dd (12.9; 8.5)	6.81 bs ($2 \times NH_2$); 5.83 b (OH)

TABLE I
(*Continued*)

Compound	Chemical shifts (coupling constants)							
	H-2	H-8	H-1′a	H-1′b	H-2′	H-3′a	H-3′b	Other protons
IIIc	7.68 d (6.1)	8.22 s	4.43 dd (14.2; 3.4)	4.24 dd (14.2; 7.4)	4.08 m	3.06 dd (12.7; 3.3)	2.71 dd (12.7; 8.8)	7.10 d (6.1) (H-3)
IIId	7.81 d (5.4)	7.94 s	4.22 dd (14.0; 4.3)	4.07 dd (14.0; 6.9)	3.78 m	2.46 dd (12.8; 5.5)	2.44 dd (12.8; 6.0)	6.34 d (5.4) (H-1); 6.32 bs (2 × NH_2)
IIIg	–	8.47 s	4.18 m (3 H)			3.00 bd	2.75 um	8.94 s (H-6); 6.00 b (OH); 8.22 b and 7.90 b (2 × NH_2)
IIIi	–	7.62 s	4.02 dd (14.9; 4.4)	3.95 dd (14.9; 7.9)	4.01 m	2.86 dd (13.0; 3.2)	2.67 dd (13.0; 8.5)	6.47 s (NH_2)
IIIo	–	–	3.76 d (2 H)		3.46 m	2.68 dd (13.0; 3.5)	2.51 dd (13.0; 7.5)	7.42 p (1.0) (H-6); 1.74 t (1.0) (CH_3)
IIIp	–	–	3.84 dd (13.5; 4.0)	3.55 dd (13.5; 6.6)	3.97 m	2.85 dd (13.0; 3.5)	2.65 dd (13.0; 8.6)	5.70 d (7.2) (H-5); 7.50 d (7.2) (H-6)
V	–	7.97 s	3.18 dd (13.6; 5.9)	3.13 dd (13.6; 6.6)	3.91 m	3.34 dd (12.7; 3.7)	3.30 dd (12.6; 6.6)	5.65 bs (OH); 7.19 s (NH_2); 12.55 bs (NH)
VI	–	7.94 s	[b]	[b]	[b]	[b]	[b]	7.33 bs (NH_2); 5.69 d (5.0) and 5.61 d (5.0) (2 × OH)

[a] Chemical shifts and coupling constants of strongly coupled protons in the alkyl substituent were determined by simulation of spectrum. [b] Not analyzable 2nd order multiplets of two $–CH_2–CH–CH_2–$ spin systems.

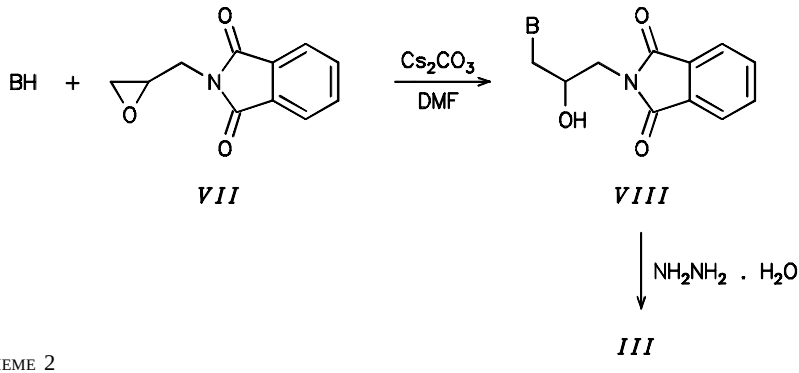
TABLE II
^{13}C NMR data (δ, ppm) of compounds *II*, *III*, *V* and *VI*

Compound	C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	Other carbons
IIa	152.49	149.82	118.78	156.10	141.63	46.74	68.28	53.97	–
IIb	160.33	152.01	113.24	156.28	138.39	46.49	68.37	54.11	–
IIc	140.02	138.92	126.71	152.41	142.51	48.11	68.86	53.85	97.02 (C-3)
IId	144.60	147.56	122.94	147.01	141.29	46.80	68.45	54.12	102.19 (C-1)
IIe	151.57	152.39	130.98	149.00	148.27	47.49	68.03	53.74	–
IIf	159.91	154.49	123.51	149.43	144.05	46.82	67.93	53.97	–
IIi	153.59	151.43	116.59	156.95	138.19	46.47	68.28	54.05	–
IIj	151.50	148.61	130.84	159.76	145.32	47.00	68.14	53.86	11.36 (CH_3)
IIk	156.02	153.66	100.22	158.25	–	49.96	68.82	54.04	132.29 (C-7)
IIl	155.74	171.37	93.77	150.45	–	53.04	67.33	54.01	–
IIm	155.76	170.20	102.02	147.42	–	52.89	67.50	54.09	11.67 (CH_3)
IIn	151.28	164.00	100.49	146.90	–	51.29	67.68	53.80	–
IIo	151.27	164.59	107.95	142.72	–	51.16	67.81	53.87	12.15 (CH_3)
IIp	156.30	166.25	93.07	147.27	–	52.70	68.05	54.12	–
IIr	144.92	144.47	135.06	175.88	143.76	47.09	68.27	53.82	–
IIs	153.03	148.20	128.33	174.87	141.31	46.53	68.12	53.97	–
IIIa	152.44	149.89	118.71	156.11	141.79	46.77	70.39	45.25	–
IIIb	160.31	152.08	113.11	156.27	138.44	46.22	70.58	45.23	–
IIIc	140.22	138.92	126.72	152.48	142.41	48.27	67.48	42.88	97.08 (C-3)
IIId	144.48	147.66	122.83	146.95	141.36	46.64	70.61	45.29	102.02 (C-1)
IIIg	155.67	156.61	126.31	149.03	140.59	46.64	65.60	42.17	–
IIIi	153.65	151.49	116.47	157.03	138.40	46.43	70.07	44.96	–
IIIo	151.33	164.57	107.82	142.87	–	51.15	68.05	44.04	12.17 (CH_3)
IIIp	156.44	166.16	92.80	147.36	–	52.35	70.34	45.32	–
V	163.35	151.40	116.65	155.82	138.65	34.88	69.91	55.54	–
VI	163.05	150.49	116.55	155.57	140.80	34.87	69.73	55.22	–

character of the base: for purine derivatives they appear in the region δ 46.2 – 48.3, for pyrimidine derivatives they range from δ 51.1 to 53.1. The considerable upfield shifts of C-1′ signals in the spectra of *V* and *VI* (δ 34.9) indicate bonding to the sulfur atom. The position of the alkyl substituent – N-9 in the purine and N-1 in the pyrimidine derivatives – was determined by comparison with ^{13}C NMR data of the corresponding free bases obtained under identical conditions. For most of the studied compounds *II*, *III* and *VI* we observed a marked alkylation shift of the C-8 signal (about 2 ppm downfield) for purine derivatives (and their deaza analogs) and of the C-6 signal (about 4.5 ppm downfield) for pyrimidine derivatives as compared with the free bases, in accord with the N-9 alkylation of purines[12] and N-1 alkylation of of pyrimidines[13]. Opposite alkylation effects – downfield shifts of the C-5 and C-6 signals (about 7.5 and 5.5 ppm) and upfield shifts of C-4 and C-8 signals (about −7 and −1.5 ppm) – were found for hypoxanthine and 6-mercaptopurine derivatives. Similar effects connected with N-9 alkylation may be also expected with guanine and 6-thioguanine derivatives for which, however, reference data for the free bases are lacking (they were not found in the literature and also our attempted measurements failed). The reason is apparently the very low solubility and extremely broad signals due to lactam–lactim or thione–thiol tautomerism[11]. The five-spin system of hydrogen atoms in the 3-azido- or 3-amino-2-hydroxypropyl substituent leads to more or less complex ^1H NMR spectra (2nd order effects). In some cases the chemical shifts and coupling constants had to be determined by simulation of the spectra and in the case of compounds *IIIg* and *VI* a complete analysis was not possible. The different vicinal coupling constants for both methylene groups in the whole series of the compounds studied (Table I) indicate a limited flexibility of the three-carbon alkyl which is more expressive around the C-1′–C-2′ bond (average J(H-1′,H-2′) = 3.7 and 8.0 Hz and J(H-2′,H-3′) = 3.3 and 6.6 Hz). Under the usual simplifying assumption of staggered rotamers, the J values show a markedly different population of the two possible rotamers with an *anti*-relation of hydrogen atoms. Since the stereospecific assignment of protons of the methylene groups is not possible, we cannot determine the predominant *anti*-rotamer from the J-values. Nevertheless, we may assume that, for steric reasons, the most populated rotamer will be that with *anti*-arrangement of the bulkiest substituents, i.e. the base moiety and the C(3′)H_2NR group attached to the C-1′–C-2′ bond and the groups C(1′)H_2–B and NR on the C-2′–C-3′ bond.

As an alternative synthetic approach to amino derivatives *III* we also studied the possible utilization of the easily accessible *N*-(3-phthalimido-2-hydroxypropyl) derivatives *VIII* which could be converted into amino derivatives *III*, e.g. by hydrazinolysis. They were synthesized in quantitative yields during several hours by reaction of the corresponding bases with the commercially accessible *N*-(2,3-epoxypropyl)phthalimide (*VII*) in the presence of catalytic amount of cesium carbonate in dimethylformamide; in the case of purine derivatives (adenine, 2,6-diaminopurine and 2-amino-6-chloropurine)

principal amounts of the pure N^9-isomers (*VIIIa*, *VIIIb*, *VIIIf*) were obtained by boiling the crude reaction mixture with methanol and filtration while hot (Scheme 2). Analogously, alkylation of cytosine with oxirane *VII* afforded predominantly the N^1-isomer *VIIIp* without any noticeable O^2-alkylation.



SCHEME 2

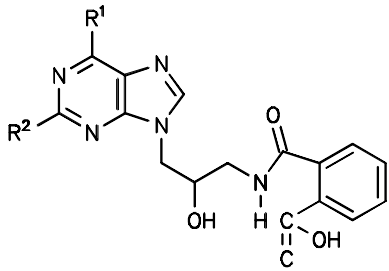
Phthalimido compounds *VIII* were smoothly converted to amino derivatives *III* by reaction with 98% hydrazine hydrate in boiling ethanol. (The recommended use of *N*-methylhydrazine[14] was not successful.) In this manner we prepared derivatives of adenine *IIIa* and 2,6-diaminopurine *IIIb* which were identical with samples synthesized from the azides *II*. Since the reaction of phthalimido derivatives with hydrazine is sufficiently rapid, even in the case of the cytosine derivative *VIIIp* there was no undesired transformation of the cytosine ring[15] and the synthesized compound *IIIp* was identical (HPLC) with that obtained by the former synthetic way.

In this context, synthesis of the guanine derivative *IIIi* represented a difficult methodological problem. For known reasons, direct alkylation of guanine with oxirane *VII* was of no advantage; on the other hand, use of the azide alternative for 2-amino-6-chloropurine with subsequent acid hydrolysis of the C–Cl bond and reduction also did not represent the best solution.

Therefore, our synthetic goal was also to utilize the 2-amino-6-chloropurine phthalimido derivative *VIIIf* for synthesis of the guanine analog *IIIi*. Attempted hydrolysis of the C–Cl bond by heating compound *VIIIf* with 0.4 M triethylammonium hydrogen carbonate in dimethylformamide resulted, however, in opening the phthalimide grouping under formation of phthalamic acid derivative *IX* whereas the base remained unchanged. The same result was obtained with the adenine derivative *VIIIa*. Structure of the compounds *IX* and *X* was unequivocally proved by ^{13}C NMR spectroscopy (see Table IV).

The failure of an easy conversion of chloro derivative *VIIIf* into the guanine derivative led us to try the phthalimide method with another guanine precursor, 2-amino-6-

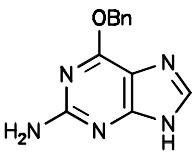
benzyloxypurine (*XI*). Although this known base[16] was used in studies of regiospecificity of alkylation with other reagents[17], no particular attention has been paid to its preparation. As the method of choice we have found the reaction of 2-amino-6-chloropurine with sodium benzylate in an aprotic solvent, toluene. As a side-product, the



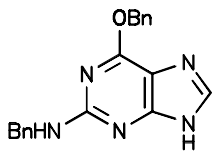
IX, $R^1 = Cl$; $R^2 = NH_2$

X, $R^1 = NH_2$; $R^2 = H$

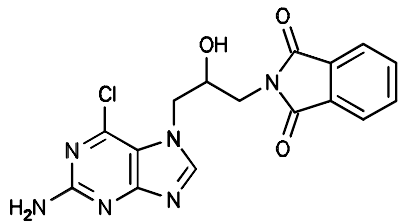
reaction afforded 2-benzylamino-6-benzyloxypurine (*XII*); this compound was, however, practically the sole product when the reaction was conducted in dimethylformamide. The alkylation of 2-amino-6-benzyloxypurine (*XI*) with oxirane *VII* proceeded similarly as in the case of compounds *VIIIa*, *VIIIb* and *VIIIf* giving the product *VIIIq* in a relatively high yield during 4 h. Compound *VIIIq* may serve as a suitable precursor for the preparation of other compounds of the guanine series.



XI



XII



XIII

Both the described methods leading to 3-amino-2-hydroxypropyl derivatives of nucleic bases are sufficiently general and for the given purpose they appear to be more suitable than the originally used syntheses based on transformation of azides obtained from the corresponding tosyl or mesyl derivatives. The phthalimide alternative in combination with hydrazinolysis is thus comparable and has its substantiation, particularly in cases where the amino group in the side-chain has to be protected in further synthetic steps, e.g. introduction of the phosphonomethylether functionality[5]. The high sensitiv-

ity of *N*-(phthalimido-2-hydroxypropyl) derivatives *VIII* to hydrolysis should, of course, be kept in mind.

The structure of compounds *VIII – XII* was determined by ^{1}H and ^{13}C NMR spectroscopy (Tables III and IV). The proton and carbon signals, assigned similarly as in the above-discussed compounds *II* and *III*, enabled us to prove the structure of the base. The *N*-phthalimido-2-hydroxypropyl substituent was confirmed by signals of the three-membered aliphatic fragment, a multiplet of four aromatic protons and particularly four signals of eight sp^2-carbon atoms of the symmetrical phthalimide moiety (Table IV). Whereas compounds *VIIIa*, *VIIIb*, *VIIIf*, *VIIIp* and *VIIIq* exhibited alkylation effects characteristic for N-9 substitution on the purine, or N-1 substitution on the pyrimidine nuclei (see the discussion above), for the minor product *XIII* we observed markedly different alkylation effects (for C-4 and C-8 downfield shifts of about 9 ppm and for C-5 and C-6 upfield shifts of about −8.5 and −7 ppm) indicating N-7 alkylation[18]. The number of signals in ^{13}C NMR spectra of compounds *IX* and *X* proves a loss of symmetry of the phthalimide part resulting from opening the phthalimide to the phthalamic acid. The vicinal coupling constants of the three-carbon alkyl in compounds *VIII – X* indicate similar conformational properties as in the compounds *II* and *III* discussed above.

Biological Activity

The azidohydroxypropyl and aminohydroxypropyl derivatives *II* and *III* were tested for activity against a standard choice of DNA and RNA viruses and retroviruses in the Laboratory of Professor E. De Clercq (Rega Institute, Catholic University, Leuven, Belgium). Neither of them showed any marked antiviral effect. The mutagenic effect of azido derivatives *II* was already reported elsewhere[3].

EXPERIMENTAL

Methods. The melting points were determined on a Kofler block and are uncorrected. Solvents were evaporated on a rotatory evaporator at 40 °C/2 kPa. Analytical samples were dried at 25 °C and 6.5 Pa for 8 h. NMR spectra were measured on Varian UNITY-200 (^{1}H at 200 MHz and ^{13}C at 50.3 MHz) or UNITY-500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz) instruments in CD_3SOCD_3. Chemical shifts of protons were referenced to TMS as internal standard, chemical shifts of carbon atoms against the solvent signal using the relationship $\delta(CD_3SOCD_3) = 39.7$ ppm. In addition to the common proton-decoupled ^{13}C NMR spectra were measured the *J*-modulated spectra ("attached proton test pulse sequence", ref.[19]) or proton-coupled spectra, enabling distinction of carbon signals of the type C, CH, CH_2 and CH_3. UV spectra were measured on a Pye Unicam 8800 UV/VIS spectrometer; the wavelengths of the extrema are given in nm. Thin-layer chromatography (TLC) was performed on Silufol UV_{254}, column chromatography on Silpearl (both Kavalier, Votice, The Czech Republic). Solvent systems for TLC: S1 chloroform–methanol (3 : 1), S2 chloroform–methanol (4 : 1), S3 chloroform–methanol (9 : 1), S4 chloroform–methanol (95 : 5), S5 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2). High performance liquid chromatography was performed on columns

TABLE III
^{1}H NMR data (δ, ppm; J, Hz) of compounds *VIII – XIII*

Compound	δ (*J*)							
	H-2	H-8	H--1′a	H-1′b	H-2′	H-3′a	H-3′b	Other protons
VIIIa	8.11 s[a]	8.08 s[a]	4.29 dd (13.7; 3.6)	4.11 dd (13.7; 8.0)	4.22 m	3.65 dd (13.9; 7.8)	3.59 dd (13.9; 4.8)	7.19 bs (NH_2); 5.47 d (5.6) (OH); 7.85 m (C_6H_4)
VIIIb	–	7.67 s	4.10 dd (13.9; 3.9)	3.94 dd (13.9; 7.8)	4.13 m	3.63 dd (13.9; 8.3)	3.54 dd (13.9; 4.2)	6.68 bs, 5.80 bs (2 × NH_2); 5.51 d (5.4) (OH); 7.82 – 7.90 m (C_6H_4)
VIIIf	–	8.07 s	4.19 dd (13.9; 3.4)	4.02 dd (13.9; 9.5)	4.16 m	3.65 dd (13.9; 7.8)	3.57 dd (13.9; 4.4)	6.92 bs (NH_2); 5.45 d (5.6) (OH); 7.80 – 7.90 m (C_6H_4)
VIIIp	–	–	3.95 dd (13.1; 3.0)	3.38 dd (13.1; 8.2)	4.02 m	3.60 dd (13.9; 7.6)	3.50 dd (13.9; 5.5)	7.85 m (C_6H_4); 5.62 d (7.3) (H-5); 7.51 d (7.3) (H-6); 7.05 bs (NH_2); 5.34 d (5.4) (OH)
VIIIs	–	7.82 s	4.19 m	4.00 dd (13.7; 9.3)	4.15 m	3.64 dd (13.9; 7.8)	3.55 dd (13.9; 4.2)	6.47 bs (NH_2); 5.44 d (5.4) (OH); 5.49 s (CH_2O); 7.50 (2 H), 7.39 (2 H), 7.34 (1 H) (C_6H_5)
IX	–	8.09 s	3.94 – 4.07 m		4.23 m	3.29 m (2 H)		6.90 bs (NH_2); 8.42 t (5.0) (NH); 13.10 bs and 5.15 b (2 × OH); 7.90 (1 H), 7.45 – 7.65 (3 H) (C_6H_4)
X	8.15 s[a]	8.11 s[a]	4.35 dd (13.7; 3.4)	4.08 dd (13.7; 8.2)	4.01 m	3.30 dt (13.4;5.8;5.8)	3.22 dt (13.4;6.1;6.1)	7.78 d, 7.55 t, 7.50 t, 7.49 d (C_6H_4); 7.28 bs (NH_2); 8.57 t (6.1; 5.8) (NH)
XI	–	7.85 s	–	–	–	–	–	12.45 bs (NH); 6.31 bs (NH_2); 7.50 (2 H), 7.39 (2 H), 7.33 (1 H) (C_6H_5); 5.49 s (CH_2O)
XII	–	7.85 s	–	–	–	–	–	12.58 bs, 8.00 bs (2 × NH); 5.49 s (CH_2O); 4.52 d (6.1) (CH_2N); 7.18 – 7.52 m (2 × C_6H_5)
XIII	–	8.81 s	4.46 dd (12.9; 2.2)	4.19 dd (12.9; 8.8)	4.14 m	3.67 dd (13.9; 6.4)	3.63 dd (13.9; 5.9)	7.50 bs (NH_2); 5.60 bs (OH); 7.82 – 7.90 m (C_6H_4)

[a] The assignment of signals may be interchanged.

TABLE IV
^{13}C NMR data (δ, ppm) of compounds *VIII – XIII*

Compound	C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	CO	C-1″ C-6″	C-2″ C-5″	C-3″ C-4″
VIIIa	152.51	149.80	118.74	156.10	141.74	47.14	66.41	42.03	168.17	131.94	123.18	134.51
VIIIb	160.40	152.03	113.14	156.29	138.46	46.72	66.54	42.15	168.22	131.97	123.20	134.54
VIIIf	159.95	154.47	123.41	149.45	144.09	47.13	66.18	42.02	168.17	131.90	123.19	134.53
VIIIp	156.21	166.29	92.95	147.43	–	52.89	66.26	42.06	168.17	131.92	123.19	134.54
VIIIs[a]	159.81	154.72	113.66	160.16	140.76	46.82	66.29	42.08	168.14	131.92	123.15	134.49
IX	160.12	154.84	123.88	149.90	144.52	47.48	68.13	43.01	169.65	139.03	128.26	131.97
									168.45	130.73	129.72	129.84
X	152.26	149.60	118.80	156.23	142.03	47.30	68.07	43.32	169.45	138.24	127.86	130.79
									168.80	132.63	129.27	129.34
XI[b]	159.85	155.41	113.72	160.06	138.02	–	–	–	–	–	–	–
XII[c]	158.88	155.00	113.70	159.68	138.19	–	–	–	–	–	–	–
XIII	160.05	164.55	115.03	142.20	150.55	50.32	67.39	41.61	168.12	131.81	123.25	134.65

Benzyl carbon signals: [a] 66.99; 128.19; 128.57 (2 C); 128.62 (2 C); 136.88. [b] 66.90; 128.19; 128.58 (2 C); 128.63 (2 C); 136.96. [c] OBn: 66.92; 128.18; 128.29 (2 C); 128.57 (2 C); 136.94; NBn: 44.69; 126.55; 127.15 (2 C); 128.64 (2 C); 141.02.

(250 × 4 mm or 250 × 17 mm) packed with Separon SGX C18 (5 μm, Laboratorni Pristroje, Prague, The Czech Republic); isocratic elution (1 ml/min) with 0.05 M triethylammonium hydrogen carbonate, pH 7.5 (S6) containing 5% (v/v) (S7) or 7% (S8) of acetonitrile, detection at 254 nm. Preparative chromatography on C18 silica gel was performed on a column of octadecyl silica gel (30 μm, 260 ml) in water and water–methanol mixtures. Paper electrophoresis was carried out on a Whatman 3 MM paper at 40V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate, pH 7.5 (S9).

Materials and reagents. Adenine, dimethylformamide, sodium azide, cesium carbonate, epichlorohydrin and bromotrimethylsilane were Janssen (Belgium) products, 2-amino-6-chloropurine was obtained from Mack (Germany), cytosine and phthalimidomethyloxirane from Fluka (Switzerland), 6-chloropurine from Sigma, 2, 6-diaminopurine from Kasyo (Japan), 6-methylthiopurine from Loba-Chemie (Austria) and 4-aminopyrazolo[5,6-*d*]pyrimidine from Lachema (The Czech Republic). 4-Methoxy-2-pyrimidone was prepared according to a described procedure[20]. Dimethylformamide, dichloromethane and acetonitrile were dried by distillation from phosphorus pentoxide and kept over molecular sieves.

Azidomethyloxirane (*I*)

A solution of sodium azide (19.5 g, 0.3 mol) in water (70 ml) was added dropwise during 90 min to a stirred solution of epichlorohydrine (25 g, 0.27 mol) in water (50 ml). After stirring for further 20 min, the bottom layer was separated and the upper one extracted with dichloromethane (3 × 40 ml). The combined extracts and the bottom layer were dried over sodium sulfate, the solvent was evaporated under diminished pressure and the residue was distilled in vacuo (the receiver cooled to –60 °C); b.p. 22 – 24 °C/13 Pa. This product was used in the alkylation reactions without further purification.

Preparation of *N*-(3-Azido-2-hydroxypropyl) Derivatives *II*. General Procedure

A mixture of the heterocyclic base (10 mmol), potassium carbonate (0.1 g), azidomethyloxirane (*I*, 1.44 g, 15 mmol) and dimethylformamide (25 ml) was heated under stirring at 80 – 140 °C for 5 – 8 h (calcium chloride protecting tube), filtered while hot and the precipitate was washed with dimethylformamide. The filtrate was concentrated in vacuo, the residue codistilled with toluene and extracted with boiling chloroform. The extract was filtered, the filtrate concentrated in vacuo and the residue chromatographed on a column of silica gel in chloroform with increasing concentration of methanol. The product-containing fractions were combined, the solvent was evaporated in vacuo and the residue was crystallized. Table V gives the reaction conditions and some characteristics of the prepared compounds *II*.

9-(3-Azido-2-hydroxypropyl)hypoxanthine (*IIh*) and 9-(3-Azido-2-hydroxypropyl)guanine (*IIi*)

A solution of compound *IIe* (0.47 g, 2 mmol) in 80% acetic acid (50 ml) was heated at 85 °C for 6 h. After evaporation in vacuo, the residue was codistilled with water (4 × 20 ml) and column chromatographed on C18 silica gel; elution with a water–methanol gradient. The product fraction was evaporated and the product crystallized from ethanol. Yield 70% of compound *IIh* (for analysis see Table V).

Similarly, compound *IIf* (0.42 g, 1.5 mmol) vas converted into derivative *IIi*; yield 0.36 g (87%). For analysis see Table V.

9-(3-Azido-2-hydroxypropyl)uracil (*IIn*) and 9-(3-Azido-2-hydroxypropyl)thymine (*IIo*)

A solution of compound *IIl* (0.255 g, 1 mmol) in 80% acetic acid (50 ml) was heated at 80 °C for 3 h. After evaporation of the solvent in vacuo, the residue was codistilled with water (2 × 20 ml) and

TABLE V
Preparation and properties of *N*-(3-azido-2-hydroxypropyl) derivatives of heterocyclic bases *II*

Compound	Base residue	*T*, °C	M.p., °C Yield, %	R_F (S)[a]	Formula M.w.	Calculated/Found		
						% C	% H	% N
IIa	Adenin-9-yl	140	189 – 190 50	0.39 (S1)	$C_8H_{10}N_8O$ 234.2	41.02 40.95	4.30 4.20	47.85 47.53
IIb	2,6-Diaminopurin-9-yl	140	171 – 172 50	0.22 (S2)	$C_8H_{11}N_9O$ 249.2	38.55 38.79	4.45 4.45	50.58 50.58
IIc	3-Deazaadenin-9-yl	75	189 56	0.77 (S5)	$C_9H_{11}N_7O$ 233.2	46.34 46.15	4.75 4.30	42.04 41.91
IId	1-Deazaadenin-9-yl	80	139 39	0.81 (S5)	$C_9H_{11}N_7O$ 233.2	46.34 45.96	4.75 4.76	42.04 42.03
IIe	6-Chloropurin-9-yl	80	126 – 129 44	0.71 (S3)	$C_8H_8ClN_7O$ 253.7	37.87 38.09	3.18 3.16	38.65 38.57
IIf	2-Amino-6-chloropurin-9-yl	80	163 – 164 63	0.59 (S2)	$C_8H_9ClN_8O$ 268.7	35.76 35.44	3.38 3.70	41.70 41.80
IIh	Hypoxanthin-9-yl	–	231 – 233 –	0.04 (S2)	$C_8H_9N_7O_2$ 235.2	40.85 41.15	3.86 4.12	41.69 42.00
IIi	Guanin-9-yl	–	238 – 242 –	0.17 (S2)	$C_8H_{10}N_8O_2$ 250.2	38.40 38.07	4.03 3.90	44.78 44.61
IIj	6-Methylmercaptopurin-9-yl	80	123 – 124 42	0.57 (S4)	$C_8H_{11}N_7OS$ 265.2	40.75 40.34	4.18 4.35	36.97 36.80

TABLE V
(*Continued*)

Compound	Base residue	*T*, °C	M.p., °C Yield, %	R_F (S)[a]	Formula M.w.	Calculated/Found		
						% C	% H	% N
IIk	6-Aminopyrazolo[3,4]pyrimidin-9-yl	80	118 – 120 64	0.40 (S3)	$C_8H_{10}N_8O$ 234.2	41.02 41.32	4.30 4.46	47.84 48.25
IIl	4-Methoxy-2-pyrimidon-1-yl	80	122 – 124 51	0.12 (S4)	$C_8H_{11}N_5O_4$ 225.2	42.66 42.45	4.92 5.12	31.10 31.35
IIm	4-Methoxy-5-methyl-2-pyrimidon-1-yl	80	95 – 96 70	0.36 (S4)	$C_9H_{13}N_5O_3$ 239.2	45.18 45.41	5.48 5.20	29.28 29.45
IIn	Uracil-1-yl	–	133 – 134 –	0.12 (S4)	$C_7H_9N_5O_3$ 211.8	39.81 40.24	4.29 4.43	23.17 23.24
IIo	Thymin-1-yl	–	141 – 143 –	0.14 (S4)	$C_8H_{11}N_5O_3$ 225.2	42.66 42.58	4.92 4.69	31.10 31.32
IIp	Cytosin-1-yl	–	172 – 174 –	0.18 (S2)	$C_7H_{10}N_6O_2$ 250.2	40.00 40.09	4.79 4.77	39.99 39.64
IIr	6-Mercaptopurin-9-yl	–	226 – 228 –	0.33 (S3)	$C_8H_9N_7OS$ 251.2	38.25 38.17	3.61 3.48	39.03 38.65
IIs	6-Mercaptoguanin-9-yl	–	233 – 235 –	0.15 (S3)	$C_8H_{10}N_8OS$ 266.2	36.09 35.87	3.79 3.60	42.09 41.99

[a] Systems: S1, ethyl acetate–methanol, 3 : 1; S2, ethyl acetate–methanol, 4 : 1; S3, ethyl acetate–methanol, 9 : 1; S4, ethyl acetate–methanol, 95 : 5; S5, 2-propanol–concentrated aqueous ammonia–water, 7 : 1 : 2.

crystallized from tetrahydrofuran–light petroleum to give compound *IIn* in the yield of 70%. For analysis see Table V.

Similarly, compound *IIm* was converted into derivative *IIo* in 80% yield. For analysis see Table V.

9-(3-Azido-2-hydroxypropyl)cytosine (*IIp*)

A solution of compound *IIl* (1.0 g, 4.4 mmol) in 30% methanolic ammonia (35 ml) was heated in a pressure vessel at 110 °C for 4 h. The mixture was concentrated to dryness in vacuo and the residue was chromatographed on a column of C18 silica gel; elution with a gradient of water–methanol. The product-containing fraction gave, after evaporation in vacuo and crystallization from methanol–ether, compound *IIp* (0.74 g, 80%). For analysis see Table V.

9-(3-Azido-2-hydroxypropyl)-6-mercaptopurine (*IIr*) and 9-(3-Azido-2-hydroxypropyl)-6-thioguanine (*IIs*)

A solution of compound *IIe* (0.47 g, 2 mmol) and thiourea (0.30 g, 4 mmol) in ethanol (25 ml) was refluxed for 5 h. After evaporation of the solvent in vacuo, the residue was chromatographed on a preparative loose layer of silica gel (50 × 18 × 0.4 cm) in chloroform–methanol (9 : 1). The product band was eluted with methanol and the residue crystallized from ethanol to give 0.36 g (72%) of compound *IIr*. For m.p. and analysis see Table V.

Similarly, reaction of compound *IIf* (0.84 g, 3 mmol) with thiourea (0.45 g, 6 mmol) in ethanol (50 ml) afforded compound *IIs*; yield 0.63 g (79%). For m.p. and analysis see Table V.

2-(3-Azido-2-hydroxypropyl)thioadenine (*V*) and 2,9-Bis(3-azido-2-hydroxypropyl)thioadenine (*VI*)

Azidomethyloxirane (*I*; 1.98 g, 22.5 mmol) was added at 100 °C in portions to a stirred suspension of 2-mercaptoadenine (2.51 g, 15 mmol) and potassium carbonate (0.15 g) in dimethylformamide (60 ml). The mixture was refluxed for 10 h (calcium chloride protecting tube) and filtered while hot. The filtrate was concentrated in vacuo and the residue extracted with chloroform (200 ml). The chloroform extract was filtered through Celite and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel in chloroform (elution with chloroform–methanol 9 : 1) to give a mixture of both products which were further separated on a column of octadecyl silica gel (200 ml) by elution with a gradient of water–methanol (0 – 100%). Crystallization from tetrahydrofuran–methanol (1 : 5) afforded 1.43 g (36%) of compound *V*, m.p. 218 °C, R_F 0.51 (S3). For $C_8H_{10}N_8OS$ (266.2) calculated: 36.09% C, 3.79% H, 42.09% N; found: 36.05% C, 3.69% H, 41.42% N. Further elution afforded 1.73 g (32%) of compound *VI*, m.p. 145 °C (tetrahydrofuran–methanol 1 : 5). For $C_{11}H_{15}N_{11}O_2S_2$ (365.3) calculated: 36.16% C, 4.13% H, 42.18% N; found: 36.04% C, 4.01% H, 42.04% N.

Preparation of *N*-(3-Amino-2-hydroxypropyl) Derivatives *III* by Hydrogenation of Azido Compounds *II*. General Procedure

Compound *II* (5 mmol) was hydrogenated in 50% aqueous ethanol (120 ml) over 10% palladium on carbon (0.67 g) at room temperature overnight. After filtration through Celite and washing the solid with 50% aqueous ethanol (25 ml), the filtrate was made alkaline with ammonia, concentrated in vacuo and the residue was crystallized from ethanol–ether. Compound *IIIg* was isolated by chromatography on C18 silica gel in 50% aqueous methanol. The yields and properties of the thus-obtained compounds *III* are given in Table VI.

General Procedure for Preparation of Phthalimido Derivatives *VIII*

N-(2,3-Epoxypropyl)phthalimide (*VII*; 4.8 g, 24 mmol) was added to a mixture of the corresponding base (20 mmol), cesium carbonate (0.4 g, 1.2 mmol) and dimethylformamide (100 ml). The mixture was heated at 120 °C for 6 h under stirring and protection from moisture (calcium chloride tube) until the starting base disappeared (TLC, S2). After evaporation of the dimethylformamide and codistillation with toluene (3 × 50 ml), the residue was boiled with methanol (200 ml) and undissolved compound was collected by filtration of the hot mixture. This procedure afforded predominant amount of the pure N^9- (or N^1-) isomers whereas the mother liquors contained a mixture of both isomers. Further crops of products were obtained by chromatography of the mother liquors on silica gel.

9-(3-Phthalimido-2-hydroxypropyl)adenine (VIIIa); yield 3.97 g (60%), m.p. 250 °C, R_F 0.59 (S2). For $C_{16}H_{14}N_6O_3$ (338.3) calculated: 56.80% C, 4.17% H, 24.83% N; found: 56.85% C, 4.37% H, 24.51% N.

9-(3-Phthalimido-2-hydroxypropyl)-2,6-diaminopurine (VIIIb); yield 5.49 g (78%), m.p. 237 –239 °C, R_F 0.41 (S2). For $C_{16}H_{15}N_7O_3$ (353.3) calculated: 54.39% C, 4.28% H, 27.74% N; found: 54.44% C, 4.36% H, 27.68% N.

TABLE VI
N-(3-Amino-2-hydroxypropyl) derivatives *III*

Compound	M.p., °C Yield, %	R_F [a]	Formula M.w.	Calculated/Found		
				% C	% H	% N
IIIa	176 – 178	0.53	$C_8H_{12}N_6O$. $2H_2O$	39.33	6.60	34.44
	42		244.3	39.40	6.12	34.06
IIIb	205 – 206	0.50	$C_8H_{13}N_7O$. H_2O	39.82	5.43	40.61
	63		241.3	39.49	5.99	40.50
IIIc	192		$C_9H_{13}N_5O$. H_2O	47.98	6.72	31.10
	48		225.2	48.12	6.45	30.96
IIId	162 – 163		$C_9H_{13}N_5O$. H_2O	47.98	6.72	31.10
	76		225.2	47.77	6.82	31.34
IIIg	249 – 251	0.47	$C_8H_{12}N_6O$. H_2O	42.47	6.24	37.15
	55		226.2	42.81	5.89	37.47
IIIi	223 – 225	0.26	$C_8H_{12}N_6O_2$. H_2O	39.66	5.82	34.47
	66		242.2	39.40	5.62	34.06
IIIo	131 – 133	0.49	$C_8H_{13}N_3O_3$. H_2O	44.23	6.96	19.34
	54		217.2	44.25	6.81	19.25
IIIp	203 – 205	0.27	$C_7H_{12}N_4O_2$. H_2O	41.57	6.98	27.71
	55		202.2	41.75	6.42	27.86

[a] Solvent system S1.

9-(3-Phthalimido-2-hydroxypropyl)-2-amino-6-chloropurine (*VIIIf*) and 7-(3-Phthalimido-2-hydroxypropyl)-2-amino-6-chloropurine (*XIII*)

The residue obtained by concentration of the reaction mixture was boiled with a 1 : 1 mixture of methanol and chloroform and the crystalline material was collected by filtration of the hot mixture; yield 1.6 g of pure N^9-isomer *VIIIf*. The mother liquor was chromatographed on silica gel (50 g) in chloroform. The product *VIIIf* was eluted with chloroform–methanol (9 : 1). Total yield of the N^9-isomer was 4.46 g (60%), m.p. 248 – 250 °C, R_F 0.68 (S2). For $C_{16}H_{13}ClN_6O_3$ (372.7) calculated: 51.56% C, 3.52% H, 22.54% N, 9.51% Cl; found: 51.64% C, 3.69% H, 22.24% N, 9.05% Cl.

Further elution afforded 0.60 g (8%) of the N^7-isomer *XIII*, m.p. >250 °C, R_F 0.58 (S2). For $C_{16}H_{13}ClN_6O_3$ (372.7) calculated: 51.56% C, 3.52% H, 22.54% N, 9.51% Cl; found: 51.98% C, 3.57% H, 22.36% N, 10.28% Cl.

1-(3-Phthalimido-2-hydroxypropyl)cytosine (*VIIIp*)

Chromatography of the mother liquor on silica gel (125 g) in chloroform afforded 1.14 g of product *VIIIp* (elution with chloroform–methanol 4 : 1). Total yield 4.44 g (71%), m.p. >250 °C, R_F 0.25 (S2). For $C_{15}H_{14}N_4O_4$ (314.3) calculated: 57.33% C, 4.49% H, 17.82% N; found: 57.52% C, 4.46% H, 17.97% N.

9-(3-Phthalimido-2-hydroxypropyl)-2-amino-6-benzyloxypurine (*VIIIq*)

The reaction mixture after codistillation with toluene was chromatographed on silica gel (600 g) in chloroform. The product was eluted with chloroform–methanol (9 : 1). Crystallization from ethanol afforded 3.8 g (43%) of compound *VIIIq*, R_F 0.52 (S3). For $C_{23}H_{20}N_6O_4$ (444.4) calculated: 62.16% C, 4.54% H, 18.90% N; found: 61.80% C, 4.59% H, 18.98% N.

9-[3-(*o*-Carboxybenzoyl)amino-2-hydroxypropyl]-2-amino-6-chloropurine (*IX*)

A mixture of phthalimido derivative *VIIIf* (2.0 g, 6 mmol), 0.4 M triethylammonium hydrogen carbonate solution (50 ml) and dimethylformamide (50 ml) was kept at 50 °C for 30 h. After evaporation and codistillation with toluene (3 × 100 ml), the white crystalline residue was triturated with water, collected on filter and washed with acetone and ether. Yield 1.68 g (72%) of compound *IX*, m.p. 195 – 196 °C, R_F 0.36 (S2), E_{Up} 0.4. For $C_{16}H_{15}ClN_6O_4$ (390.7) calculated: 49.17% C, 4.39% H, 21.50% N, 9.07% Cl; found: 48.74% C, 4.01% H, 21.41% N, 8.81% Cl. Mass spectrum (*m*/*z*): 391 (M + H).

9-[3-(*o*-Carboxybenzoyl)amino-2-hydroxypropyl]adenine (*X*)

A mixture of compound *VIIIa* (1.0 g, 3 mmol), ammonia (10 ml) and water (20 ml) was stirred at room temperature for 24 h. After evaporation, the reaction mixture was separated by preparative HPLC (elution with water for 30 min, then with a gradient 0.5% methanol/min to 40% methanol). Yield 0.5 g (50%) of compound *X*, m.p. 200 – 202 °C, *k* 26.5 (0.05 M TEAB 30 min, gradient to 10% acetonitrile in 0.05 M TEAB/10 min), E_{Up} 0.4. For $C_{16}H_{16}N_6O_4 \cdot H_2O$ (374.3) calculated: 51.34% C, 4.85% H, 22.44% N; found: 51.29% C, 4.63% H, 22.05% N. Mass spectrum (*m*/*z*): 357.2 (M + H).

Preparation of *N*-(3-Amino-2-hydroxypropyl) Derivatives *III* by Hydrazinolysis of *N*-(Phthalimido-2-hydroxypropyl) Derivatives *VIII*. General Procedure

A mixture of phthalimido derivative *VIIIa*, *VIIIb* or *VIIIp* (1 mmol), ethanol and 98% hydrazine hydrate (0.055 ml) was refluxed for 8 h (calcium chloride protecting tube). The reaction mixture was concentrated and the amino derivative was separated from phthalazine on Dowex 1 (AcO^-, 50 ml) by elution with water. After deionization on Dowex 50 (H^+ form, 50 ml), evaporation and codistillation with ethanol (3 × 20 ml), the amino derivatives *III* were crystallized from ethanol. The obtained compounds *III* were identical with authentic samples (vide supra) according to HPLC (S6 for *IIIp*, S7 for *IIIb* and S8 for *IIIa*). Yields: compound *IIIa* 93%, compound *IIIb* 82% and compound *IIIp* 87%.

2-Amino-6-benzyloxypurine (*XI*)

Benzyl alcohol (50 ml, 460 mmol) was added dropwise at room temperature to a stirred mixture of sodium hydride (60% dispersion, 2.5 g, 62 mmol) and toluene (250 ml) and stirring was continued for 1 h. 2-Amino-6-chloropurine (5 g, 30 mmol) was added, the mixture was refluxed for 6 h and filtered while hot. The crystalline material collected on the filter represented the main portion of compound *XI* and was purified by crystallization from ethanol; yield 2.4 g. The mother liquor after filtration of the reaction mixture was chromatographed on silica gel (300 g) in chloroform. Elution with chloroform–methanol (98 : 2) afforded dibenzyl derivative *XII* (0.8 g), R_F 0.39 (S3), elution with chloroform–methanol (96 : 4) gave further amount (1.6 g) of the monobenzyl derivative *XI*. Total yield 4.0 g (56%) of compound *XI*, m.p. 190 – 192 °C, R_F 0.24 (S3). For $C_{12}H_{11}N_5O$ (241.2) calculated: 59.75% C, 4.60% H, 29.20% N; found: 59.21% C, 4.63% H, 28.83% N. Mass spectrum (*m*/*z*): 242.1 (M + H). UV spectrum (methanol): λ_{max} 284.0 nm (ε_{max} 7 420), λ_{max} 241.0 nm (ε_{max} 7 420).

2-Benzylamino-6-benzyloxypurine (*XII*)

Benzyl alcohol (13.5 g, 125 mmol) was added dropwise under stirring to an ice-cooled mixture of sodium hydride (60% dispersion, 5.0 g, 125 mmol) and dimethylformamide (250 ml) and the stirring was continued for 1 h. 2-Amino-6-chloropurine (4.25 g, 25 mmol) was added to the obtained solution and the mixture was stirred at 60 °C for 8 h (calcium chloride tube). After cooling, the reaction mixture was neutralized with acetic acid, the solvent was evaporated and the residue was codistilled with toluene. Chromatography on silica gel (500 g) in chloroform–methanol (97 : 3) afforded as the principal product the dibenzyl derivative which was then crystallized from ethyl acetate. Yield 3.1 g (37%), m.p. 165 – 167 °C, R_F 0.39 (S3). For $C_{19}H_{17}N_5O$ (331.3) calculated: 68.87% C, 5.17% H, 21.13% N; found: 68.69% C, 5.16% H, 20.44% N. Mass spectrum (*m*/*z*): 332.2 (M + H). UV spectrum (methanol): λ_{max} 284.0 nm (ε_{max} 7 420), λ_{max} 241.0 nm (ε_{max} 7 420).

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