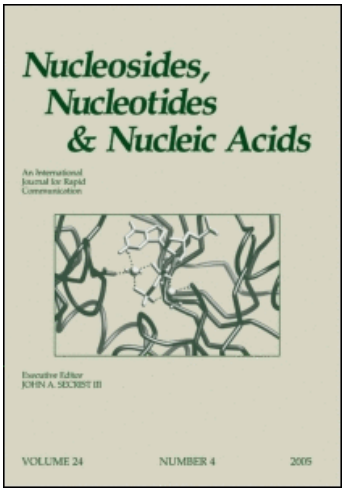

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Studies on the Alkylation of Derivatives of Guanines

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STUDIES ON THE ALKYLATION OF DERIVATIVES OF GUANINE

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Abstract

The synthesis of some N7- and N9-substituted guanine analogs was investigated. The influence of the base, the alkylating agent and of the type of derivatization of the purine moiety on the relative formation of the N7 and N9 isomers was studied.

Introduction

Some acyclic guanosine analogs show potent antiviral activity: acyclovir (9-(2-hydroxyethoxymethyl)guanine)[1 2], buciclovir ((R)-9-(3,4-dihydroxybutyl)guanine)[3] and gancyclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine)[4-6] possess antiherpes activity both *in vitro* and *in vivo*. They are open-chain analogs of guanosine and they are phosphorylated by viral thymidine kinase to the monophosphates[7 8 10]. Further phosphorylation by cellular enzymes[9] leads to the triphosphates[10] which inhibit viral DNA synthesis.

The compounds have been prepared by the condensation of a guanine precursor and the respective side chains, appropriately protected. In these as well as other alkylations, mixtures of N9 and N7 regioisomers are obtained.

In spite of their close resemblance to one another, the compounds cannot be synthesized by entirely analogous methods. In the case of

acyclovir (ACV) and gancyclovir (DHPG) the alkylating agents are strongly activated by the β-oxygen functions. This is also the case when guanosine or variants of guanosine are prepared[11-15].

ACV[16] and DHPG[17-20] have been prepared by heating 9,N^2-diacetylguanine with 2-oxa-1,4-butanediol diacetate and 2-O-(acetoxymethyl)-1,3-di-O-benzylglycerol, respectively, in the presence of a catalytic amount of p-toluenesulfonic acid in sulfolane. The driving force is the expulsion of acetic anhydride, a reaction which does not work with non-activated alkyl side-chains[21]. Martin et al.[19] obtained a 3:2 mixture of the 9 and 7 isomers when preparing DHPG.

Similarly silylated guanine derivatives have been used for condensations with activated halides. Tris(trimethylsilyl)guanine alkylated in the presence of a base (Et_3N, NaOMe) also gave the N9- and N7-alkylated products[22-25]. Lin et al.[26] noted that the reaction leading to N9 alkylation was favored by lower temperatures. Acyclovir has also been synthesized from silylated 2-amino-6-chloropurine and (2-acetoxyethoxy)methyl bromide in the presence of mercury(II) cyanide[27 11].

Guanine itself has been methylated with dimethylsulfate in dimethylacetamide to give 7,9-dimethylguanine[28]. Guanine has been alkylated at the N9-position in N,N-dimethylformamide (DMF) with the use of sodium hydride, by addition to an epoxide (1,2-anhydro-4-O-benzylbutane-1,2,4-triol)[29] and by substitution of p-toluene-sulfonate employed as a leaving group in 4-benzyloxy-3-benzyloxymethyl-1-butyl tosylate[30]. However, the yields were relatively low, 27 % and 8 %, respectively.

Alkylation of N^2-acetylguanine with 1-O-p-toluenesulfonyl-2(S),3-O-isopropylideneglycerol gave low and equivalent amounts of the two regio-isomeric alkylated products[31].

Halogenated purine derivatives have also been widely used in the synthesis of nucleosides. Schaeffer[1] employed the reaction between 2,6-dichloropurine and (2-benzyloxyethoxy)methyl chloride followed by hydrolysis, ammonolysis and hydrogenolytic debenzylation to obtain acyclovir. A similar condensation procedure using 2-chloro-6-iodopurine was described by Barrio et al.[32]. Condensation between 2-amino-6-chloropurine and 1-acetoxy-2-(chloromethoxy)ethane with potassium

carbonate used as a base followed by purification and deprotection gave acyclovir in fairly high yield[33 34]. Alkylation of 2-amino-6-chloropurine with halides in the presence of potassium carbonate in DMSO or DMF gives approximately a 4:1 mixture of the N9 isomer and the N7 isomer[35-38], respectively. The two isomers are separated by chromatography or chromatography in combination with recrystallization indicating some difficulties in isolating high yields of the desired product. MacCoss et al.[39] and Karkas et al.[40] alkylated the sodium salt of 2-amino-6-benzyloxypurine with chloromethylethers to obtain 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine. By using the 6-benzyl derivative of guanine no or very little of the N7 isomers were formed when the alkylating agents were activated by a β-oxygen function[41]. Several examples are given where the leaving group was either chlorine or acetate. However, when we employed non-activated halides, the regioselectivity was significantly decreased and the N7 isomer was formed in a relatively high amount

We initiated our studies on regioselective alkylation of guanine in order to find an alternative to 2-amino-6-chloropurine as a precursor for the synthesis of carboacyclic guanosine analogs with antiviral properties.

We have recently reported regioselective methods for synthesizing 7-(4-hydroxybutyl)guanine[42] and 9-(4-hydroxybutyl)guanine[43], the latter compound having antiviral properties[44].

Alkylation of N1,N^2-diacetylglyoxal-N^2-acetylguanine adduct (5-acetyl-6,7-diacetyloxy-5,6,7,9-tetrahydro-9-oxo-(3H-imidazo[1,2-a]-purine) (1) with 4-bromobutyl acetate in the presence of sodium hydride in DMF gave the N7 isomer and the N9 isomer in a ratio of 18:1. On the other hand, alkylation of 2-amino-6-(β-methoxyethoxy)-purine (4) with 4-bromobutyl acetate in the presence of lithium hydride in DMF at 80°C gave the N7 isomer and the N9 isomer in a ratio of 1:15. The blocking groups were removed by alkaline and acidic hydrolysis, respectively.

Different alkylating agents also influence the relative formation of the alkylated products. A remarkable example on the influence of the alkylating agent on the relative formation of the purine regio-isomers has been demonstrated by Rasmussen and Chan[45].

When the sodium salt of N^6-pivaloyladenine was alkylated with 1-chloro-3,3-dimethylbutane or chloromethyl pivalate the N7/N9 ratio changed from 1:13 to 40:1. When methyl iodide and benzyl chloride were used the N7/N9 ratios were 0.5 and 0.12, respectively. They tried to rationalize these divergent alkylation patterns by considering the looseness and tightness of the SN2 transition states involved for a particular alkylating agent. Nielsen and Pedersen[46] also showed the presence of a strong influence on the substitution pattern by the alkylating agent and the base in related purine systems.

Here we report results from condensations of 4-bromobutyl acetate with "keto-type" derivatives of guanine, N1-methyl- and N^2-palmitoyl-guanine, as well as condensations where the reactivity of various electrophiles with 2-amino-6-(β-methoxyethoxy)purine, 2-amino-6-chloropurine and 2-aminopurine was studied. Alkylations using silylation or phase-transfer procedures have also been investigated.

Results and discussion

When N1-methylguanine (6) and N^2-palmitoylguanine (7) were alkylated in the presence of a base with 4-bromobutyl acetate the N7- and N9-alkylated products were formed in equivalent amounts. The distribution between the two products was independent of the choice of the base used in the alkylation. Metal hydrides, metal carbonates and other bases were employed with no influence on the N9/N7 ratio. This observation is in analogy with our experiments on the alkylation of 7-methyl-10-oxo-9,10-dihydropyrimido-[1,2-a]purine (2)[47]. On the other hand, in the alkylation of N1,N^2-diacetyl-glyoxal-N^2-acetylguanine adduct (1) and the 6-protected guanine derivatives (3, 4 and 5) the choice of the base considerably affected the ratio between the two products.

These results are summarized in Table 1. The ratio between the two alkylated products was measured by ^{13}C and ^1H NMR[48] and the conversion was estimated by RP-HPLC and TLC. Alkylation with 4-bromobutyl acetate of N1-methyl-N^2-(methoxyacetyl)guanine or N^2-(p-methoxybenzoyl)guanine were according to ^1H NMR of the reaction mixture not influenced by the choice of the base. The two isomers were

TABLE 1

Alkylations of derivatives of guanine with 4-bromobutyl acetate. The reactions were performed in DMF at room temperature and the reaction time varied from 3 to 24 hours.

Ref[a]	Substrate	Base	Conversion %	Ratio N9/N7
42	1	NaH	50	0.06
42	1	DMP[b]	30	0.5
42	1	KH	65	0.2
42	1	TlEtO	55	0.2
47	2	NaH	88	1
47	2	K_2CO_3	98	1
III, 43	3	LiH	75	10
II, 43	3	NaH	80	2
II, 43	3	KH	70	1.5
I, 43	3	K_2CO_3	92	1
III, 43	4	LiH	82	10
II, 43	4	NaH	72	2
I, 43	4	K_2CO_3	87	2
I, 43	4	Na_2CO_3	50	1.5
III, 43	5	LiH	65	6
II, 43	5	NaH	90	4
I, 43	5	K_2CO_3	95	4
IV	6	NaH	89	1
IV	6	KH	52	1
V	6[c]	K_2CO_3	95	1
IV	6	TlEtO	39	1
V	6	DMP[b]	25	1
IV	7	LiH	15	1
IV	7	NaH	25	1
VI	7	K_2CO_3	80	1

[a] Roman numerals refer to the experimental section and the others refer to the list of references. [b] The base, 1,4-dimethylpiperazine, is abbreviated as DMP. [c] Performed in DMSO.

TABLE 2

Alkylation of 6-(β-methoxyethoxy)guanine (4) with some halides. Ratio N9/N7 isomers.

Halide	Base	
	K_2CO_3	LiH
Entry	VII	VIII
Hexyl chloride	2	3.5
Hexyl bromide	2.5	5
Hexyl iodide	2	2
Benzyl chloride	1.5	2
Benzyl bromide	1.5	2.5

found in equivalent amounts (not shown in Table 1). The alkylated products were not separated and identified.

We have also investigated the alkylation of 2-amino-6-(β-methoxyethoxy)purine with different alkylating agents. The reactions with benzyl- and hexyl halides in the presence of lithium hydride are summarized in Table 2. They show that even if the N9 isomer is the major product the selectivity is reduced, especially when more reactive electrophiles are employed.

As we have briefly reported[43] the alkylation of 6-methoxyethyl-substituted guanine, with 4-bromobutyl acetate, in the presence of lithium hydride gave almost selectively the N9 isomer. If sodium hydride was used instead the N9 isomer and the N7 isomer were formed in a ratio of 2:1. The effect of the higher temperature should be regarded as a preliminary result. The lithium salt of 6-methoxyethyl-guanine has since been used by Biggadike et al.[49] for regioselective synthesis of guanine N-9 carbocyclic nucleoside analogs.

We also used the commercial compounds 2-aminopurine (10) and 2-amino-6-chloropurine (9) as substrates for alkylations and varied

TABLE 3

Alkylation of 2-aminopurine (10). Ratio N9/N7 isomers.

Halide	Entry	Base K_2CO_3 VII	LiH VIII	NaH VIII
Hexyl chloride		5	10	
Hexyl bromide		5	8	7
Hexyl iodide		5	10	9
(R)-4-Bromo-1,2-O-iso-propylidene-1,2-butanediol		6	11	9

both the leaving groups of the alkylating agent and the base used in the reaction. For 2-aminopurine the leaving group of the alkyl halides does not influence the distribution between the alkylated products (Table 3).

Due to the lower reactivity of hexyl chloride the conversion was quite low (< 20 %). However, the choice of the base seemed to play an important role, especially in the alkylation of 2-aminopurine. The formation of the N9 isomer was increased by using lithium hydride or sodium hydride instead of potassium carbonate. The ratio between the N9 isomer and the N7 isomer was about 10:1. In the case of 2-amino-6-chloropurine the pattern was harder to interpret (Table 4). However, the N9 isomer was the dominating product in all cases.

The reaction times for the alkylation reactions described here have generally varied from 3 to 24 hours except for alkylation of tris-trimethylsilylated guanine (8) which was heated at 110 °C for 45 hours with hexyliodide to give 7,9-dihexylguanine (8a).

No attempt was made to optimize the reaction yields or to identify other possible minor reaction products beside the N9 and N7 isomers or to quantitate the mass balance of unreacted starting

TABLE 4

Alkylation of 2-amino-6-chloropurine (9).
Ratio N9/N7 isomers.

Halide	Entry	Base K_2CO_3 X,XI,XII	LiH VIII	NaH VIII
Hexyl bromide		6	8	4
Hexyl iodide		5	7.5	4.5
Benzyl chloride		5	5	
Benzyl bromide		3.5	4.5	
(R)-4-Bromo-1,2-O-isopropylidene-1,2-butanediol		5	7	

materials. A general observation from reactions using 2-amino-6-chloropurine with alkalimetal hydrides, especially with concentrated sodium hydride (97 %) is that unidentified byproducts were formed in addition to considerable amounts of starting material. The conversions, therefore, were lower in these cases. Also condensation reactions employing slow-reacting alkyl chlorides which gave low yields of products, leave unreacted starting materials in the crude reaction mixture.

The ratio between the N9 and the N7 isomers was estimated by comparison of the integral values of the different H-8 and NH_2 peaks in the ^1H NMR spectra. The error in the measurements of the ratio between the regio-isomers were larger when the N9/N7 ratio was increased. We estimated the error to be ±25 %. The approximate value for the conversions were estimated by ^1H NMR, TLC and in some cases RP-HPLC.

Summarizing the above results, it is obvious that with guanine in the "keto-form" (compounds 1, 2, 6 and 7) N7 alkylation is preferred and with guanine in the "enol-form" (compounds 3, 4, 5, 9 and 10) the

N9 position is preferentially alkylated. Guanosine in the "keto" and the "enol" forms have analogous properties. The N7 position of guanosine is a good nucleophile and is easily protonated as shown by a strong shielding of the ^{15}N NMR signal, whereas N7 of guanosine protected at the O^6 position is a poor nucleophile and is not protonated[50]. However, compare the results of Martin et al.[17] on the high-temperature alkylation of 9,N^2-diacetylguanine with 2-oxo-1,4-butanediol diaceate, which gave preferentially the 9-isomer.

The regioselectivity for alkylation of 1, 3 and 4 is greatly dependent on the metal cation and on the nature of the alkylating agent, both with regard to the presence of functional groups (cf. Hecht et al.[51]) and to the leaving group (shown for alkylation of compound 4). Also the solvation in different solvents is important for the reaction[42].

These data point to specific interaction between the metal ion, the heterocyclic compound and the alkylating agent (cf. Yamauchi et al.[52]), the nature of which is unknown but apparently important for regioselectivity. The involvement of ion-pairs in the alkylation of ambident carbon and oxygen nucleophiles have been extensively studied[53-55] but little is known about such complexes for ambident heterocyclic nucleophiles. N-versus O-alkylation of 2-hydroxy-pyrimidines, 4-hydroxypyrimidines and 2-pyridones have been studied in the laboratory of H. Tieckelmann and were found to be dependent on the metal cation, on steric factors of the alkylating agents and on the solvent[58-60]. In this context it is interesting that N-versus O-alkylation of α,ω-hydroxyalkylamines is greatly dependent on the alkali metal hydride used as a base[59]. Also a NMR study of alkali metal salts of guanosine monophosphate has shown the formation of metal cation specific complexes[60].

Evidence for migration of the alkyl group between N7 and N9, in analogy with the observations of Miyaki and Shimizu[61 62] and Ogilvie and Hanna[63], has not been confirmed.

Spectroscopic methods, primarily NMR and UV, have been used to distinguish the regio-isomers[64]. Independent unambiguous syntheses of 7-(4-hydroxybutyl)guanine[65] and 9-(4-hydroxybutyl)guanine[66] have been performed. The adducts protected in the six-membered moieties, 1a-b and 2a-b were readily transformed to these compounds by alkaline

hydrolysis[42 47]. The corresponding O^6-substituted analogs (3a-b, 4a-b) were hydrolyzed by aqueous HCl[43]. The alkylated 2-amino-6-chloropurine derivatives have also been hydrolyzed in acidic solution[36 38] and their ^{13}C NMR shifts have been unambiguously assigned[67].

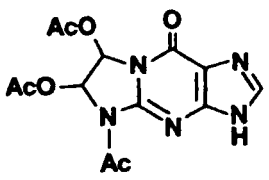
The N9 isomer, formed by alkylation of 1-methylguanine, was identified by comparison of its spectroscopic data with those of 1-methyl-9-hexylguanine and 1-methyl-9-(2-hydroxyethoxymethyl)guanine. These compounds were prepared by methylating 9-hexylguanine (1.6 eq. CH_3I, 1.4 eq K_2CO_3, 15 ml of DMF, RT, 3 h)[38] and 9-(2-hydroxyethoxymethyl)-guanine (1.9 eq. CH_3I, 1.0 eq. NaH, 40 ml of DMF, RT, 3 days)[21].

The two alkylated isomers of N^2-palmitoylguanine were correlated to literature data of the xylofuranoside regio-isomers[68].

The N7 and N9 isomers of 2-aminopurine were identified by independently preparing the compounds by catalytic hydrogenation of the corresponding 6-chloro derivatives (cf. Krenitsky et al.)[69]. The spectral data for the N7- or N9-substituted 2-aminopurines were consistent with those for 7- and 9-(2'-hydroxyethoxymethyl)-2-aminopurine[70].

Experimental Section

General Procedures. All solvents and starting materials were of the highest available purity. Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were stored over Linde molecular sieves (4Å). Melting points were determined on a Büchi 510 apparatus and are uncorrected. The NMR spectra were recorded on a Jeol JNM-FX 200 instrument. The mass spectra were obtained on a LKB 9000 (70 eV) mass spectrometer. Elemental analyses were performed by Novo Microanalytical Laboratory, Bagsvaerd, Denmark, by Analytische Laboratorien, Postfach 1249, 5250 Engelskirchen, Germany, and by Kemicentrum, Department of Analytical Chemistry, Lund, Sweden. Thin-layer chromatography (TLC) was performed on precoated glass plates of silica gel 60 F_{254} (Merck). Ultraviolet spectra were recorded on a Hewlett Packard 8450 A UV/VIS spectrophotometer. For reversed-phase high pressure liquid chromatography (RP-HPLC) analysis a Waters 440, RCM-100 system was used. All chromatographic purifications were carried out on silica gel. Hexa-



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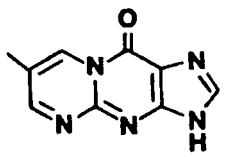

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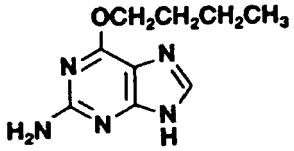

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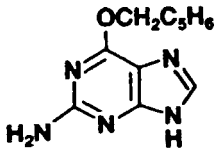

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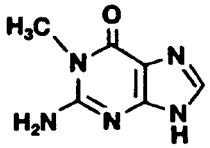

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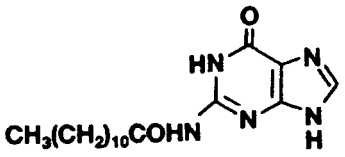

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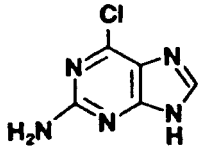

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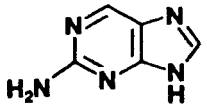

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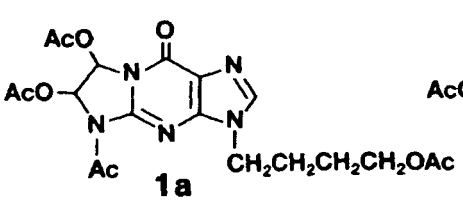

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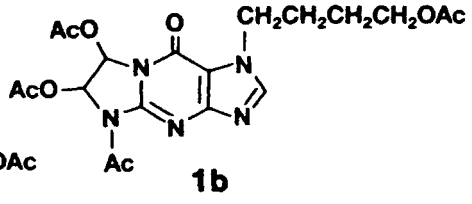

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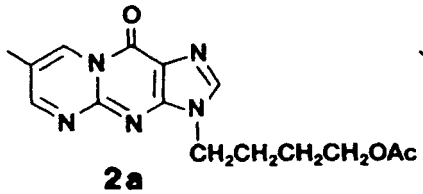

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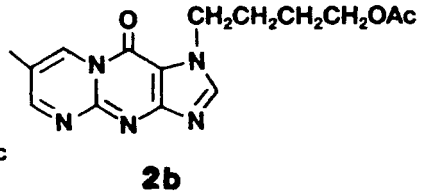

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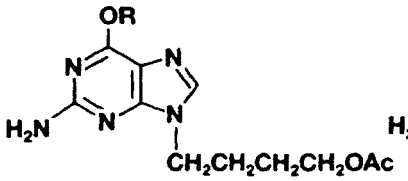

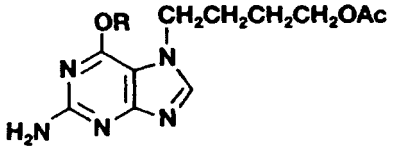

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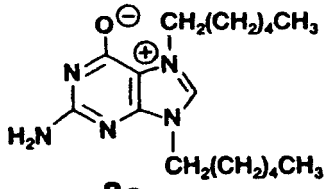

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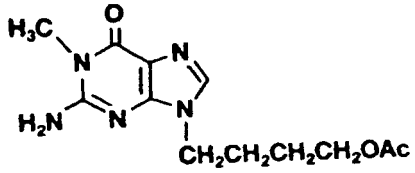

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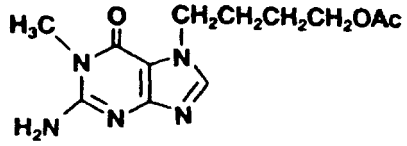

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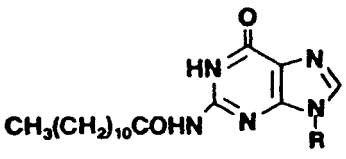
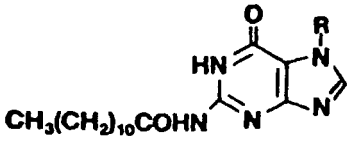
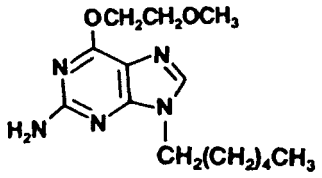


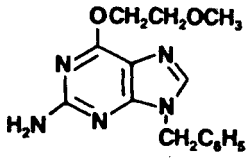


7a R= $CH_2CH_2CH_2CH_2OAc$ 7b



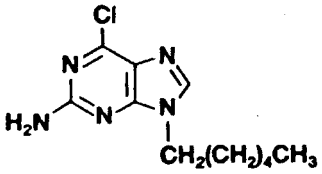
4c

4d



4e

4f



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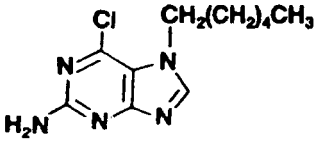

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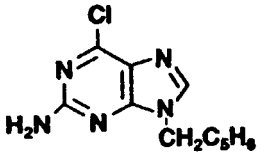

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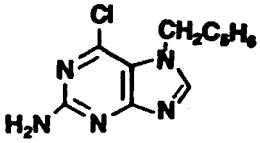

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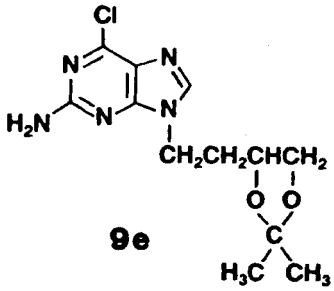

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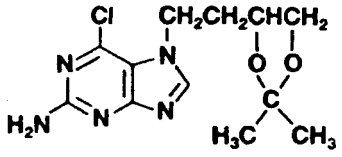

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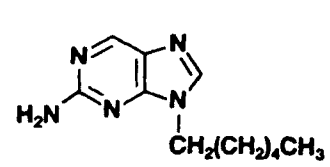
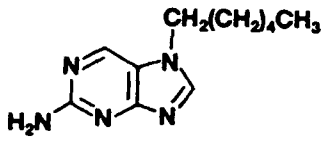
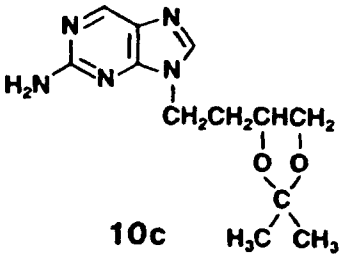


10a



10b



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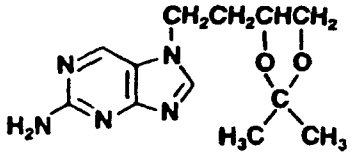

10d

deuteriodimethyl sulfoxide has been used as solvent for NMR analysis, unless otherwise stated.

The synthesis and the alkylation of ethanoic acid-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-3H-imidazo[1,2-a]-purine-6,7-diyl ester (1) and 7-methyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine (2) were described in previous papers[42 47]. The compounds 1a, 1b, 2a, 2b, 5a and 5b have also been characterized. The alkylations of 6-(β-methoxyethoxy)guanine (4) and 6-butoxyguanine (3) with 4-bromobutyl acetate have been briefly reported[43]. The analogous compound 2-amino-6-benzyloxypurine (5) was prepared according to described methods[39 71].

1-Methylguanine (6) was purchased from Fluka AG, CH-9470, Buchs. N^2-Palmitoylguanine (7) was synthesized by modification of the procedures of Runti et al.[72] and Furukawa et al.[73]. Methods for silylation followed by alkylation of guanine have been described[74].

Preparation of 2-amino-6-butoxypurine (3). Sodium (3.0 g, 0.13 mol) was added to 200 ml of n-butanol under anhydrous conditions. When the pieces of sodium had dissolved 2-amino-6-chloropurine (3.0 g, 0.018 mol) was added. The solution was refluxed for 18 h and the reaction was followed by RP-HPLC (MeOH/H_2O 50:50) and TLC ($CHCl_3$/MeOH 6:1). The solution was cooled and neutralized (pH 5) with 1 M HCl. Inorganic salts were removed by filtration. The resulting solution was evapo-

rated in vacuo and the residue was purified by flash chromatography (ethyl acetate with increasing amounts of ethanol) to give 3.0 g (83 %) of 3 as white crystals.

2-Amino-6-butoxypurine (3): m.p. 124-126 °C; UV (nm) λ_{max} = 286 (pH 1), λ_{max} = 239, 281 (pH 7), λ_{max} = 283 (pH 13); mass spectrum (m/e) = 207; ^{1}H NMR δ, 0.94 (t, 3H, CH_3), 1.45 (m, 2H, CH_2), 1.74 (m, 2H, CH_2), 4.39 (t, 2H, OCH_2), 6.18 (s, 2H, NH_2), 7.80 (s, 1H, H-8); ^{13}C NMR δ, 13.87 (C4''), 18.90 (C3''), 30.73 (C2''), 66.17 (C1''), 111.00 (C5), 139.65 (C8), 154.83 (C4), 159.09 (C6), 160.01 (C2); Anal. Calcd. for $C_9N_5H_{13}O \times 0.15\ H_2O$, C: 51.5 %; N: 33.3 %; H: 6.4 %, Found C: 51.5 %, N: 33.1 %, H: 6.3 %

Preparation of 2-amino-6-(β-methoxyethoxy)purine (4). The procedure for synthesizing 3 was modified by using ethylene glycol monomethyl ether instead of n-butanol. After purification 2.8 g (75 %) of 4 was isolated as white crystals.

2-Amino-6-(β-methoxyethoxy)purine (4): m.p. 203-204 °C; UV (nm) λ_{max} = 287 (pH 1), λ_{max} = 240, 281 (pH 7), λ_{max} = 289 (pH 13); mass spectrum (m/e) = 209; ^{1}H NMR δ, 3.31 (s, 3H, OCH_3), 3.69 (t, 2H, OCH_2), 4.52 (t, 2H, OCH_2), 6.63 (s, 2H, NH_2), 7.80 (s, 1H, H-8); ^{13}C NMR δ, 58.36 (C3''), 64.78 (C2''), 70.42 (C1''), 113.47 (C5), 138.02 (C8), 155.37 (C4), 159.92 (C6), 160.31 (C2).

Preparation of N^2-palmitoylguanine (7). Guanine (3.0 g, 20 mmol) was suspended in pyridine (25 ml) at 0°C for 10 min. Then palmitoyl chloride (5.5 g, 20 mmol) was added slowly and the suspension was kept below 5 °C. The mixture turned yellow. The suspension was vigorously stirred for 20 min and then refluxed for 2 h. The solvent was removed in vacuo by co-evaporating with toluene. The residue was suspended in ethanol (200 ml) and heated for 1 h. The white precipitate was filtered off and washed with hot ethanol and dried in an oven (100°C) for 2 h. The off-white product (3.3 g) was recrystallized twice from DMSO to give the compound 7 (1.3 g, 17%) as white crystals.

N^2-Palmitoylguanine (7): decomposes when heated; UV (nm) λ_{max} = 202, 260 (ethanol); mass spectrum (m/e) = 389; ^{1}H NMR δ, 0.85 (s, 3H, CH_3), 2.2-2.3 (broad, CH_2), 2.8 (t, 2H, $COCH_2$), 7.98 (s, 1H, H-8).

I. General procedure for the alkylation of the compounds 3, 4 and 5 with 4-bromobutyl acetate using potassium carbonate.
9-(4-Acetoxybutyl)-2-amino-6-butoxypurine (3a) and 7-(4-acetoxybutyl)-2-amino-6-butoxypurine (3b). A mixture of 2-amino-6-butoxypurine (3, 207 mg, 1.0 mmol), potassium carbonate (690 mg, 5.0 mmol) and dry DMF (25 ml) was stirred for 20 min at room temperature. The halide, 4-bromobutyl acetate (195 mg, 1.0 mmol), was added and the mixture was stirred over-night at room temperature. The inorganic salts were filtered off and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (10 ml) and filtered. After evaporation of the solvent the product was analyzed by ^{1}H NMR and the ratio of the N9 and N7 isomers was determined from the integral values of their respective NH_2 and H-8 signals. The two alkylated products were separated on a silica gel column by flash chromatography (gradient eluent 0-10 % MeOH in $CHCl_3$) to give 3a (121 mg, 38 %) and 3b (116 mg, 36 %).

9-(4-Acetoxybutyl)-2-amino-6-butoxypurine (3a): m.p. 82.5-84.5^{o}C; UV (nm) λ_{max} = 241, 290 (pH 1), λ_{max} = 250, 282 (pH 7), λ_{max} = 289 (pH 13); mass spectrum (m/e) = 321; ^{1}H NMR δ, 0.92 (t, 3H, CH_3), 1.35-1.51 (m, 4H, CH_2), 1.68-1.80 (m, 4H, CH_2), 1.98 (s, 3H, $COCH_3$), 3.99 (m, 4H, NCH_2 and CH_2OCO), 4.39 (t, 2H, OCH_2), 6.37 (s, 2H, NH_2), 7.87 (s, 1H, H-8); ^{13}C NMR δ, 13.77 (C4''), 18.88 (C3''), 20.80 ($\underline{C}H_3CO$), 25.54 (C3'), 26.06 (C2'), 30.77 (C2''), 42.45 (C1'), 63.44 (C4'), 65.54 (C1''), 114.09 (C5), 139.77 (C8), 152.42 (C4), 159.94 (C6), 160.72 (C2), 170.50 (CO)

7-(4-Acetoxybutyl)-2-amino-6-butoxypurine (3b): m.p. 99-101^{o}C; UV (nm) λ_{max} = 288 (pH 1), λ_{max} = 289 (pH 7), λ_{max} = 289 (pH 13); mass spectrum (m/e) = 321; ^{1}H NMR δ, 0.95 (t, 3H, CH_3), 1.45-1.60 (m, 4H, CH_2), 1.72-1.85 (m, 4H, CH_2), 1.98 (s, 3H, $COCH_3$), 4.00 (t, 2H, CH_2OCO), 4.22 (t, 2H, NCH_2), 4.44 (t, 2H, OCH_2), 6.10 (s, 2H, NH_2), 8.08 (s, 1H, H-8); ^{13}C NMR δ, 13.67 (C4''), 18.88 (C3''), 20.75 ($\underline{C}H_3CO$), 25.28 (C3'), 27.30 (C2'), 30.48 (C2''), 46.61 (C1'), 63.37 (C4'), 66.24 (C1''), 105.96 (C5), 145.32 (C8), 157.58 (C4), 158.80 (C6), 160.86 (C2), 170.40 (CO)

II. General procedure for the alkylation of the compounds 3, 4, and 5 with 4-bromobutyl acetate using sodium hydride.

9-(4-Acetoxybutyl)-2-amino-6-(β-methoxyethoxy)purine (4a) and 7-(4-acetoxybutyl)-2-amino-6-(β-methoxyethoxy)purine (4b). A mixture of 2-amino-6-(β-methoxyethoxy)purine (4, 209 mg, 1.0 mmol), sodium hydride (97 %, ~36 mg, ~1.5 mmol) and dry DMF (25 ml) was stirred under nitrogen. After 20 min at room temperature 4-bromobutyl acetate (195 mg, 1.0 mmol) was added and the reaction took place at room temperature over-night. No starting material was left according to TLC ($CHCl_3$/MeOH 9:1). Water (~3 ml) was added and the solvent was evaporated. The yellow residue was suspended in ethyl acetate (15 ml) and the insoluble material was filtered off. Ethyl acetate was removed in vacuo. The crude product (275 mg, 85 %) was analyzed by NMR and separated on a silica gel column by flash chromatography (gradient eluent 0-10 % MeOH in $CHCl_3$) to give 4a (150 mg, 46 %) and 4b (78 mg, 24 %).

9-(4-Acetoxybutyl)-2-amino-6-(β-methoxyethoxy)purine (4a): m.p. 103-105°C; UV (nm) λ_{max} = 242, 290 (pH 1), λ_{max} = 249, 281 (pH 7), λ_{max} = 249, 281 (pH 13); mass spectrum (m/e) = 323; ^{1}H NMR δ, 1.56-1.81 (m, 4H, CH_2CH_2), 1.99 (s, 3H, $COCH_3$), 3.31 (s, 3H, OCH_3), 3.69 (t, 2H, $\underline{CH_2}OCH_3$), 3.97-4.07 (m, 4H, NCH_2 and CH_2OCO), 4.53 (t, 2H, OCH_2), 6.45 (s, 2H, NH_2), 7.93 (s, 1H, H-8); ^{13}C NMR δ, 20.95 ($\underline{CH_3}CO$), 25.59 (C3'), 26.10 (C2'), 42.57 (C1'), 58.36 (C3''), 63.54 (C4'), 64.90 (C2''), 70.38 (C1''), 113.75 (C5), 140.16 (C8), 154.52 (C4), 159.96 (C6), 160.40 (C2), 170.64 (CO).

7-(4-Acetoxybutyl)-2-amino-6-(β-methoxyethoxy)purine (4b): m.p. = 95.5-98; UV (nm) λ_{max} = 287 (pH 1), λ_{max} = 288 (pH 7), λ_{max} = 289 (pH 13); mass spectrum (m/e) = 323; ^{1}H NMR δ, 1.55-1.90 (m, 4H, CH_2CH_2), 1.98 (s, 3H, $COCH_3$), 3.31 (s, 3H, OCH_3), 3.70 (t, 2H, $\underline{CH_2}OCH_3$), 3.99 (t, 2H, CH_2OCO), 4.18 (t, 2H, NCH_2), 4.53 (t, 2H, OCH_2), 6.12 (s, 2H, NH_2), 8.08 (s, 1H, H-8); ^{13}C NMR δ, 20.80 ($\underline{CH_3}CO$), 25.33 (C3'), 27.34 (C2'), 46.39 (C1'), 58.26 (C3''), 63.47 (C4'), 64.78 (C2''), 70.28 (C1''), 105.89 (C5), 145.56 (C8), 156.71 (C4), 159.72 (C6), 164.20 (C2), 170.47 (CO).

III. General procedure for the alkylation of the compounds 3, 4, and 5 with 4-bromobutyl acetate using lithium hydride.

9-(4-Acetoxybutyl)-2-amino-6-(β-methoxyethoxy)purine (4a). A mixture of 2-amino-6-(β-methoxyethoxy)purine (4,209 mg, 1.0 mmol), lithium

hydride (~15 mg, ~2 mmol) and dry DMF (25 ml) was stirred for 0.5 h at room temperature under dry nitrogen. Then 4-bromobutyl acetate (195 mg, 1.0 mmol) was added and the reaction was heated (80°C) for 2 h. Water (~3 ml) was added and the solvent was removed under reduced pressure. The residue was treated with ethyl acetate (10 ml) and insoluble material was removed. NMR showed that two products 4a and 4b were formed in a ratio 15:1. After chromatography (eluent 10 % ethanol in ethyl acetate) the yield of 4a was 198 mg (61 %).

IV. Alkylation of 1-methylguanine with 4-bromobutyl acetate using sodium hydride.

1-Methyl-9-(4-acetoxybutyl)guanine (6a) and 1-methyl-7-(4-acetoxybutyl)guanine (6b). A mixture of 1-methylguanine (165 mg, 1.0 mmol), sodium hydride (50 % oil dispersion, ~75 mg, ~1.5 mmol, washed with hexane before use), 4-bromobutyl acetate (293 mg, 1.5 mmol) and dry DMF (70 ml) was stirred at room temperature for 3 h. The reaction was followed by RP-HPLC (MeOH/H_2O 50:50). The mixture was quenched with water (~5 ml). The solvent was evaporated *in vacuo* and the residue was analyzed by NMR. The crude product was dissolved in ethanol (20 ml) and filtered. The two products were isolated by flash chromatography (eluent $CHCl_3$/MeOH 15:1) to give 6a (115 mg, 41 %) and 6b (112 mg, 40 %).

1-Methyl-9-(4-acetoxybutyl)guanine (6a): UV (nm) λ_{max} = 252, 280 (pH 1), λ_{max} = 255, 269 (pH 13); mass spectrum (m/e) = 279; ^{1}H NMR δ, 1.5-1.9 (m, CH_2), 1.97 (s, $COCH_3$), 3.34 (s, NCH_3), 3.92 (t, NCH_2), 4.22 (t, OCH_2), 6.85 (s, NH_2), 7.67 (s, H-8); ^{13}C NMR δ, 20.7 ($\underline{CH_3}CO$), 25.4 (C2'), 27.2 (C3'), 31.4 (NCH_3), 42.1 (C1'), 63.2 (C4'), 115.8 (C5), 137.7 (C8), 153.4 (C2), 157.4 (C6), 170.4 (CO).

1-Methyl-7-(4-acetoxybutyl)guanine (6b): UV (nm) λ_{max} = 252, 272 (pH 1), λ_{max} = 283 (pH 13); mass spectrum (m/e) = 279; ^{1}H NMR δ, 1.5-1.9 (m, CH_2), 1.97 (s, $COCH_3$), 3.34 (s, NCH_3), 3.98 (t, NCH_2), 4.22 (t, OCH_2), 6.53 (s, NH_2), 7.90 (s, H-8); ^{13}C NMR δ, 20.7 ($\underline{CH_3}CO$), 25.1 (C2'), 27.2 (C3'), 28.1 (NCH_3), 45.5 (C1'), 63.4 (C4'), 107.5 (C5), 143.6 (C8), 153.4 (C4), 154.3 (C2), 158.0 (C6), 170.4 (CO).

V. Alkylation of 1-methylguanine (6) with 4-bromobutyl acetate using potassium carbonate.

1-Methyl-9-(4-acetoxybutyl)guanine (6a) and 1-methyl-7-(4-acetoxybutyl)guanine (6b). A mixture of 1-methylguanine (165 mg, 1.0 mmol), potassium carbonate (828 mg, 6.0 mmol), 4-bromobutyl acetate (195 mg, 1.0 mmol) and dimethyl sulfoxide (40 ml) was stirred for 4 h at room temperature. No starting material was left according to TLC ($CHCl_3$/-MeOH 10:1). The inorganic material was filtered off and the solvent was evaporated in vacuo. The residue was suspended in ethyl acetate (50 ml) and filtered. The solvent was removed by evaporation and the white residue was analyzed by NMR. This showed that the isomers had been formed in equivalent amounts.

VI. Alkylation of N^2-palmitoylguanine with 4-bromobutyl acetate using potassium carbonate.

N^2-Palmitoyl-9-(4-acetoxybutyl)guanine (7a) and N^2-Palmitoyl-7-(4-acetoxybutyl)guanine (7b). A mixture of 7 (200 mg, 0.5 mmol), 4-bromobutyl acetate (100 mg, 0.5 mmol), potassium carbonate (102 mg, 0.75 mmol) and DMF (30 ml) was stirred for 4 days at room temperature. The reaction was monitored by TLC ($CHCl_3$/MeOH 20:1). After removal of insoluble material by filtration and evaporation of the solvent the two products were separated by flash chromatography (eluent $CHCl_3$/MeOH 20:1) followed by preparative thin-layer chromatography, using the same eluent. By extraction with chloroform (3x30 ml) 7a (50 mg, 20%) and 7b (50 mg, 20 %) were isolated.

N^2-Palmitoyl-9-(4-acetoxybutyl)guanine (7a): m.p. 79°C; UV (nm) λ_{max} = 261, 279 (ethanol); mass spectrum (m/e): 502; ^1H NMR ($CDCl_3$) δ 0.8 (t, 3H, CH_3), 1.0-1.8 (m, CH_2), 2.05 (s, 3H, CH_3CO), 2.65 (t, 2H, CH_2CO), 4.10 (t, 2H, OCH_2), 4.40 (t, 2H, NCH_2), 7.65 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ, 14.3 (CH_3), 21.1 ($\underline{C}H_3CO$), 25.1 (C3'), 25.8 (C2'), 27-32 (multiplet of CH_2), 37.6 ($\underline{C}H_2CO$), 43.3 (C1'), 63.6 (C4'), 121.8 (C5), 138.8 (C8), 147.4 (C2), 155.7 (C4), C6 was not detected, 171.3 ($\underline{C}OCH_3$), 174.7 (NCO).

N^2-Palmitoyl-7-(4-acetoxybutyl)guanine (7b): m.p. 105°C; UV (nm) λ_{max} = 223, 266 (ethanol); mass spectrum (m/e) = 502; ^1H NMR ($CDCl_3$) δ, 0.85 (t, 3H, CH_3), 1.2-1.9 (m, CH_2), 2.05 (s, 3H, CH_3CO), 2.50 (t, 2H, CH_2CON), 4.08 (t, 2H, OCH_2), 4.15 (t, 2H, NCH_2), 7.7 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ, 14.2 (CH_3), 21.0 ($\underline{C}H_3CO$), 25.0 (C3'), 28.0 (C2'), 25-32 multiplet of CH_2, 37.4 ($\underline{C}H_2CO$), 47.1 (C1'), 112.4 (C5), 143.0 (C8), 148.0 (C2), 153.4 (C4), 157.2 (C6), 171.0 ($\underline{C}OCH_3$), 176.1 (NCO).

VII. Alkylation of 2-amino-6-(β-methoxyethoxy)purine (4) with hexyl iodide using potassium carbonate.

2-Amino-9-hexyl-6-(β-methoxyethoxy)purine (4c) and 2-amino-7-hexyl--6-(β-methoxyethoxy)purine (4d). A mixture of 4 (105 mg, 0.5 mmol), potassium carbonate (345 mg, 2.8 mmol) and DMF (20 ml) was stirred at room temperature for 0.5 h. Hexyl iodide (106 mg, 0.5 mmol) was added. The suspension was stirred overnight and then the inorganic material was removed by filtration. The filtrate was evaporated and the residue suspended in ethyl acetate (10 ml) and filtered. After evaporation the crude product was applied to a silica gel column and the two products were separated by flash chromatography (eluent $CHCl_3$/MeOH 20:1) to give 4c (70 mg, 48 %) and 4d (30 mg, 21 %).

2-Amino-9-hexyl-6-(β-methoxyethoxy)purine (4c): m.p. 81-83°C; UV (nm) λ_{max} = 241, 290 (pH 1), λ_{max} = 249, 281 (pH 13), mass spectrum (m/e) = 293; ^{1}H NMR δ, 0.85 (t, 3H, CH_3), 1.1-1.3 (m, CH_2), 1.78 (q, 2H, CH_2CH_2N), 3.32 (s, 3H, OCH_3), 3.70 (t, 2H, CH_2OCH_3), 3.99 (t, 2H, NCH_2), 4.52 (t, 2H, OCH_2), 6.40 (s, 2H, NH_2), 7.87 (s, 1H, H-8); ^{13}C NMR δ, 13.92 (C6'), 22.07 (C5'), 25.79 (C4'), 29.27 (C3'), 30.82 (C2'), 42.79 (C1'), 58.26 (C3''), 64.76 (C2''), 70.33 (C1''), 113.80 (C5), 139.92 (C8), 154.52 (C4), 159.77 (C6), 160.00 (C2).

2-Amino-7-hexyl-6-(β-methoxyethoxy)purine (4d): m.p. 148-149°C; UV (nm) λ_{max} = 288 (pH 1), λ_{max} = 290 (pH 13), mass spectrum (m/e) = 293; ^{1}H NMR δ, 0.85 (t, 3H, CH_3), 1.1-1.3 (m, CH_2), 1.78 (q, 2H, CH_2CH_2N), 3.32 (s, 3H, OCH_3), 3.70 (t, 2H, CH_2OCH_3), 4.15 (t, 2H, NCH_2), 4.53 (t, 2H, OCH_2), 6.11 (s, 2H, NH_2), 8.08 (s, 1H, H-8); ^{13}C NMR δ, 14.04 (C6'), 22.21 (C5'), 25.69 (C4'), 30.73 (C3'), 30.90 (C2'), 46.85 (C1'), 58.36 (C3''), 64.85 (C2''), 70.38 (C1''), 105.89 (C5), 145.69 (C8), 156.73 (C4), 159.75 (C6), 164.20 (C2).

VIII. Alkylation of 2-amino-6-(β-methoxyethoxy)purine (4) with benzyl-bromide using lithium hydride.

2-Amino-9-benzyl-6-(β-methoxyethoxy)purine (4e) and 2-amino-7-benzyl--6-(β-methoxyethoxy)purine (4f). One half mmol of 4 was dissolved in dry DMF (15 ml) and lithium hydride (~10 mg, ~1.3 mmol) was added. The suspension was stirred for 3 h under a dry nitrogen atmosphere. Water (~3 ml) was added and the solvent was removed in vacuo. The residue was suspended in ethyl acetate (10 ml) and filtered. After evaporation

the two products were isolated by flash chromatography (eluent $CHCl_3$/MeOH 15:1) to give 4e (80 mg, 54 %) and 4f (25 mg, 17 %).

2-Amino-9-benzyl-6-(β-methoxyethoxy)purine (4e): m.p. 95.5-99°C; UV (nm) λ_{max} = 242, 291 (pH 1), λ_{max} = 249, 282 (pH 13); mass spectrum (m/e) = 299; ^1H NMR δ, 3.31 (s, 3H, OCH_3), 3.70 (t, 2H, $\underline{CH_2}OCH_3$), 4.54 (t, 2H, OCH_2), 5.26 (s, 2H, NCH_2), 6.46 (s, 2H, NH_2), 7.20-7.37 (m, 5H, C_6H_5), 7.98 (s, 1H, H-8); ^{13}C NMR δ, 45.95 (NCH_2), 58.26 (C4''), 64.85 (C2''), 70.30 (C1''), 113.87 (C5), 127.25 (2), 127.71 128.78 (2) benzylic; 137.39 ($NCH_2\underline{C}$), 139.92 (C8), 154.56 (C4), 160.01 (C6), 160.50 (C2). Anal. Calcd. for $C_{15}H_{17}N_5O_2$, C: 60.1 %; N: 23.4 %; H: 5.7 %, Found C: 59.9 %, N: 22.9 %, H: 5.7 %.

2-Amino-7-benzyl-6-(β-methoxyethoxy)purine (4f): m.p. 125-126°C; UV (nm) λ_{max} = 288 (pH 1), λ_{max} = 290 (pH 13); mass spectrum (m/e) = 299; ^1H NMR δ, 3.30 (s, 3H, OCH_3), 3.65 (t, 2H, $\underline{CH_2}OCH_3$), 4.48 (t, 2H, OCH_2), 5.38 (s, 2H, NCH_2), 6.14 (s, 2H, NH_2), 7.2-7.4 (m, 5H, C_6H_5), 8.32 (s, 1H, H-8); ^{13}C NMR δ, 49.99 (NCH_2), 58.28 (C3''), 64.80 (C2''), 70.18 (C1''), 104.50 (C5), 127.61 (2) 127.95 128.76 (2) benzylic, 137.61 ($NCH_2\underline{C}$), 145.73 (C8), 156.80 (C4), 159.84 (C6), 164.30 (C2).

IX. Alkylation of guanine with hexyl iodide using the silylation method[74].

7,9-Dihexylguanine (8a). Guanine (500 mg, 3.3 mmol), ammonium sulfate (350 mg, 2.7 mmol) and hexamethyldisilazane (20 ml) were refluxed under a nitrogen atmosphere for 6 days. NMR indicated that guanine was silylated with three silyl groups due to comparison between silyl groups and H-8. One mmol of the silylated base, hexyl iodide (1.2 mmol) and triethylamine (2.4 mmol) were dissolved in dry toluene (5 ml). The solution was refluxed for 45 h whereupon it turned brown. The solvent was evaporated in vacuo and the residue was suspended in ethanol (15 ml). The suspension was neutralized with concentrated ammonia.

The brown insoluble material was filtered off and washed with ethanol. The brown crystals were dissolved in hot water and recrystallized to give 8a (65 mg, 20%) as white crystals.

7,9-Dihexylguanine, zwitterion (8a): UV (nm) λ_{max} = 255, 282 (pH 1), λ_{max} = 283 (pH 13); mass spectrum (m/e) = 319; ^{1}H NMR δ, 0.9 (m, CH_3), 1.2-1.5 (m, CH_2), 1.7-1.9 (m, $\underline{CH_2}CH_2N$), 4.05 (t, 2H, 9-NCH_2), 4.18 (t, 2H, 7-NCH_2), 7.11 (s, 2H, NH_2), 9.18 (s, 1H, H-8).

X. General procedure for the alkylation of 2-amino-6-chloropurine with hexyl chloride, hexyl bromide or hexyl iodide using potassium carbonate

2-Amino-6-chloro-9-hexylpurine (9a) and 2-amino-6-chloro-7-hexylpurine (9b). A mixture of 2-amino-6-chloropurine (9.5 g, 29.3 mmol), hexyl iodide (6.3 g, 29.5 mmol), potassium carbonate (7 g, 50.7 mmol) and DMF (130 ml) was stirred over-night at room temperature. The inorganic salts were filtered off and the solvent was evaporated in vacuo. The residue was analyzed by NMR and the two products were separated on a silica gel column by flash chromatography (eluent $CHCl_3$/EtOH 95:5) to give 9a (2.9 g, 39 %) and 9b (0.75 g, 10 %).

2-Amino-6-chloro-9-hexylpurine (9a): m.p. 109-111°C; UV (nm) λ_{max} = 218, 240, 315 (pH 1), λ_{max} = 223, 246, 307 (pH 13); mass spectrum (m/e) = 253: ^{1}H NMR δ, 0.84 (t, 3H, CH_3), 1.15-1.35 (m, 6H, CH_2), 1.76 (q, 2H, $NCH_2\underline{CH_2}$), 4.04 (t, 2H, NCH_2), 6.92 (s, 2H, NH_2), 8.15 (s, 1H, H-8); ^{13}C NMR δ, 14.02 (C6'), 22.17 (C5'), 25.86 (C4'), 29.12 (C3'), 30.90 (C2'), 43.23 (C1'), 123.63 (C5), 143.47 (C8), 149.53 (C6), 154.30 (C4), 159.99 (C2).

2-Amino-6-chloro-7-hexylpurine (9b): m.p. 168-170°C, UV (nm) λ_{max} = 217, 320 (pH 1), λ_{max} = 222, 318 (pH 13); mass spectrum (m/e) = 253; ^{1}H NMR δ, 0.84 (t, 3H, CH_3), 1.1-1.4 (m, 6H, CH_2), 1.77 (q, 2H, $NCH_2\underline{CH_2}$), 4.28 (t, 2H, NCH_2), 6.63 (s, 2H, NH_2), 8.40 (s, 1H, H-8); ^{13}C NMR δ, 14.10 (C6'), 22.21 (C5'), 25.64 (C4'), 29.36 (C3'), 30.92 (C2'), 46.51 (C1'), 114.99 (C5), 142.53 (C6), 147.97 (C8), 151.19 (C2), 160.16 (C4).

XI. General procedure for the alkylation of 2-amino-6-chloropurine with benzyl chloride and benzyl bromide using potassium carbonate.

2-Amino-9-benzyl-6-chloropurine (9c) and 2-amino-7-benzyl-6-chloropurine (9d). A mixture of 2-amino-6-chloropurine (1 g, 5.9 mmol), benzyl chloride (0.75 g, 5.9 mmol), potassium carbonate (2.5 g, 18 mmol) and DMF (80 ml) was stirred over-night at room temperature.

After filtration and evaporation the two products were isolated by flash chromatography (eluent $CHCl_3$/MeOH 25:1) to give 9c (0.46 g, 30 %) and 9d (0.12 g, 8 %).

2-Amino-9-benzyl-6-chloropurine (9c): m.p. 202-203°C, UV (nm) λ_{max} = 245, 314, (pH 1), λ_{max} = 223, 248, 307 (pH 13); mass spectrum (m/e) = 259, ^{1}H NMR δ, 5.30 (s, 2H, CH_2), 6.96 (s, 2H, NH_2), 7.3 (m, 5H, C_6H_5), 8.25 (s, 1H, H-8); ^{13}C NMR δ, 46.37 (C1'), 123.53 (C5), 127.44, 128.00, 128.97 (benzylic), 136.88 (C2'), 143.47 (C8), 149.75 (C6), 154.32 (C4), 160.10 (C2); Anal. Calcd. for $C_{12}N_5H_{10}Cl$, C: 55.5 %, N: 27.0 %, H: 3.88 %, Found C: 54.9 %; N: 26.7 %; H: 3.81 %.

2-Amino-7-benzyl-6-chloropurine (9d): m.p. decomposes 190-210°C, UV (nm) λ_{max}= 215, 318 (pH 1), λ_{max} = 222, 317 (pH 13); mass spectrum (m/e) = 259; ^{1}H NMR δ, 5.57 (s, 2H, CH_2), 6.69 (s, 2H, NH_2), 7.3 (m, 5H, C_6H_5), 8.57 (s, 1H, H-8).

XII. Alkylation of 2-amino-6-chloropurine with (R)-4-bromo-1,2-O-isopropylidene-1,2-butanediol using potassium carbonate. (R)-2-Amino-6-chloro-9-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (9e) and (R)-2-amino-6-chloro-7-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (9f). A mixture of 2-amino-6-chloropurine (1 g, 5.9 mmol), (R)-4-bromo-1,2-O-isopropylidene-1,2-butanediol (1.2 g, 5.9 mmol), potassium carbonate (2.5 g, 18 mmol) and DMF (100 ml) was stirred over-night at room temperature. After filtration and evaporation the two alkylated products were isolated by flash chromatography (eluent $CHCl_3$/MeOH 20:1) and recrystallization from water to give 9e (0.88 g, 50 %) and 9f (0.21 g, 12 %).

(R)-2-Amino-6-chloro-9-(3,4-O-isopropylidene-3,4-dihydroxybutyl)-purine (9e): m.p. 135-136 °C; UV (nm) λ_{max} = 218, 241, 315 (pH 1), λ_{max} = 224, 246, 308 (pH 13); mass spectrum (m/e) = 297; ^{1}H NMR δ, 1.25 (d, 6H, $2CH_3$), 2.02 (m, 2H, CH_2), 3.48 (m, 1H, OCH), 4.0 (m, 2H, NCH_2), 4.1 (m, 2H, NCH_2), 6.82 (s, 2H, NH_2), 8.10 (s, 1H, H-8); ^{13}C NMR δ 25.55 27.00 ($2CH_3$), 33.10 (C2'), 40.25 (C1'), 68.10 (C4'), 73.35 (C3'), 108.20 ($\underline{C}(CH_3)_2$), 123.70 (C5), 143.80 (C8), 149.60 (C6), 154.20 (C4), 159.95 (C2); Anal. Calcd. for $C_{12}H_{16}N_5O_2Cl$, C: 48.4 %, N: 23.5 %, H: 5.42 %, Found C: 48.4 %, N: 23.5 %, H: 5.47 %.

(R)-2-Amino-6-chloro-7-(3,4-O-isopropylidene-3,4-dihydroxybutyl)-purine (9f): m.p. 180-181°C, UV (nm) λ_{max} = 216, 320 (pH 1), λ_{max} =

222, 319 (pH 13); mass spectrum (m/e) = 297; ^{1}H NMR δ, 1.25 (d, 6H, $2CH_3$), 2.0 (m, 2H, CH_2), 3.5 (m, 1H, OCH), 4.0 (m, 2H, OCH_2), 4.4 (m, 2H, NCH_2), 6.64 (s, 2H, NH_2), 8.36 (s, 1H, H-8); ^{13}C NMR δ, 25.60 27.00 ($2CH_3$), 34.80 (C2'), 43.80 (C1'), 68.20 (C4'), 73.10 (C3'), 108.25 ($\underline{C}(CH_3)_2$), 114.95 (C5), 142.15 (C6), 149.80 (C8), 159.95 (C2), 164.30 (C4); Anal. Calcd. for $C_{12}H_{16}N_5O_2Cl$, C: 48.4 %, N: 23.5 %, H: 5.42 %; Found C: 48.3 %, N: 23.4 %, H: 5.48 %.

XIII. Preparation of 2-amino-9-hexylpurine (10a) by catalytic hydrogenation of 2-amino-6-chloro-9-hexylpurine (9a). A mixture of 9a (253 mg, 1.0 mmol), sodium acetate (120 mg, 1.5 mmol), palladium on activated charcoal 5 % Pd (0.2 g) and ethanol (170 ml) was hydrogenated at 40 psi and at room temperature for 10 days in a Parr apparatus. After 2 days 0.1 g of the catalysator was added. After filtration and evaporation the residue was suspended in chloroform/methanol (10:1, 100 ml). After filtration the solvent was removed and the residue was washed with ethanol and ether to give 10a (61 mg, 28 %).

This method was used also for preparing 2-amino-7-hexylpurine (10b) from 2-amino-6-chloro-7-hexylpurine (9b), (R)-2-amino-9-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (10c) and (R)-2-amino-7-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (10d) from the corresponding 2-amino-6-chloropurine derivatives 9e and 9f. The hydrogenations in the Parr apparatus were very slow and the isolated yields were modest (~50%).

2-Amino-9-hexylpurine (10a): m.p. 78.5-81.0°C; UV (nm) λ_{max} = 228, 249, 314 (pH 1), λ_{max} = 221, 241, 304 (pH 13); mass spectrum (m/e) = 219; ^{1}H NMR δ, 0.85 (t, 3H, CH_3), 1.1-1.4 (m, 6H, CH_2), 1.7-1.8 (m, 2H, $NCH_2\underline{CH_2}$), 4.03 (t, 2H, NCH_2), 6.50 (s, 2H, NH_2), 8.07 (s, 1H, H-8), 8.56 (s, 1H, H-6)

2-Amino-7-hexylpurine (10b): m.p. decomposes; UV (nm) λ_{max}= 226, 267, 328 (pH 1), λ_{max} = 220, 256, 315 (pH 13); mass spectrum (m/e) = 219; ^{1}H NMR δ, 0.83 (t, 3H, CH_3), 1.1-1.4 (m, 6H, CH_2), 1.7-1.8 (m, 2H, $NCH_2\underline{CH_2}$), 4.18 (t, 2H, NCH_2), 6.19 (s, 2H, NH_2), 8.27 (s, 1H, H-8), 8.69 (s, 1H, H-6).

(R)-2-Amino-7-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (10d): m.p. decomposes when heated; UV (nm) λ_{max} = 220, 267, 327

(pH 1), λ_{max} = 220, 356, 315 (pH 13); mass spectrum (m/e) = 263; ^{1}H NMR δ, 1.28 (d, 6H, $2CH_3$), 2.0 (m, 2H, $NCH_2\underline{CH_2}$), 3.5 (m, OCH), 4.0 (m, 2H, OCH_2), 4.3 (m, 2H, NCH_2), 6.21 (s, 2H, NH_2), 8.27 (s, 1H, H-8), 8.68 (s, 1H, H-6); ^{13}C NMR δ, 25.74, 27.05 ($2CH_3$), 33.67 (C2'), 42.33 (C1'), 68.45 (C4'), 73.08 (C3'), 108.42 ($\underline{C}(CH_3)_2$), 119.12 (C5), 142.01 (C6), 147.73 (C8), 161.03 (C2), 162.54 (C4).

(R)-2-amino-9-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine (10c) 14 g (53 %) was obtained from hydrogenation of (R)-2-amino-6-chloro-9-(3,4-O-isopropylidene-3,4-dihydroxybutyl)purine 9e (30 g) at 40°C and 4 bar for 42 hours in the presence of sodium acetate (21 g), Pd/c (5 %, 6 g), water (20 ml) and ethanol (1500 ml): m.p. 143.5-144.5; UV (nm) λ_{max} = 224, 249 (weak), 314 (pH 1), λ_{max} = 221, 242, 304 (pH 13); mass spectrum (m/e) = 263; ^{1}H NMR δ, 1.27 (d, 6H, $2CH_3$), 2.0 (m, 2H, $NCH_2\underline{CH_2}$), 3.5 (m, 1H, OCH), 4.0 - 4.2 (m, 4H, OCH_2 and NCH_2), 6.51 (s, 2H, NH_2), 8.07 (s, 1H, H-8), 8.57 (s, 1H, H-6); ^{13}C NMR δ, 25.74, 27.03 ($2CH_3$), 33.13 (C2'), 40.48 (C1'), 68.45 (C4'), 73.27 (C3'), 108.42 ($\underline{C}(CH_3)_2$), 127.18 (C5), 142.92 (C8), 149.17 (C6), 153.20 (C4), 160.70 (C2); Anal. Calcd. for $C_{12}H_{17}N_5O_2$, C: 54.7 %, N: 26.6 %, H: 6.51 %; Found C: 54.4 %, N: 26.4 %, H: 6.48 %.

The C'1 peak is covered by DMSO peak and the shift is related to $CDCl_3$ as a solvent.

XIV. General procedure for the alkylation of 2-aminopurine, 10, with hexyl chloride, hexyl bromide, hexyl iodide and (R)-4-bromo-1,2-O-isopropylidene-1,2-butanediol using potassium carbonate. A mixture of 2-aminopurine (10.27 mg, 0.2 mmol), potassium carbonate (138 mg, 1.0 mmol), halide (0.2 mmol) and DMF (15 ml) was stirred over-night at room temperature. The inorganic salts were filtered off and the solvent was removed in vacuo. The crude product was analyzed by ^{1}H NMR and the ratio of the N9 and N7 isomers were determined from the integral values of their respective NH_2, H-6 and H-8 signals. These results are summarized in Table 3. The conversions using potassium carbonate as a base were ~80 % but significantly lower when hexyl chloride was used (~20 %).

XV. General procedure for the alkylation of 2-aminopurine, 10, with hexyl chloride, hexyl bromide, hexyl iodide and (R)-4-bromo-1,2-O-iso-

propylidene-1,2-butanediol using lithium hydride. To a solution of 2-aminopurine (27 mg, 0.2 mmol) in dry DMF (15 ml) lithium hydride (~10 mg, ~1.5 mmol) was added under nitrogen. After 20 minutes the halide (0.2 mmol) was added and the suspension was stirred for 2 hours. Water (~5 ml) was added and the solvent was removed in vacuo. The residue was suspended in ethyl acetate (15 ml) and the insoluble material was removed by filtration. Evaporation of the solvent gave a crude product, which was analyzed by ^{1}H NMR. The ratio between the N9 and the N7 isomers were determined from the integral values of their respective NH_2, H-6 and H-8 signals. The conversions using lithium hydride as a base were good (~90 %) but lower when hexyl chloride was used (~30 %).

The procedure using sodium hydride as a base was performed similarly.

XVI. Attempted alkylation of guanine using phase-transfer catalysis. Guanine (0.2 mmol, 30 mg) was dissolved in 50 % aqueous NaOH (6 g). Heptyl bromide (5 or 13.5 eq) and tetraheptylammonium bromide (1 or 5 eq) were dissolved in methylene chloride (20 ml). The solutions were mixed and the resulting suspension was refluxed for 14 h. The two phases were separated and the aqueous solution was neutralized with 1 M HCl. No alkylated product was found in the aqueous or in the organic phase. The attempts using tetrabutylammonium chloride and 4-bromobutyl acetate were also unsuccessful.

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