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PURINE NUCLEOSIDE ANALOGS. 12*. SYNTHESIS OF NOVEL 8,9-DISUBSTITUTED GUANINE DERIVATIVES BY S-ALKYLATION OF 2-ACETAMIDO-9-(2-ACETOXYETHOXY-METHYL)-6-OXO-8-THIOXOPURINE

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S-Alkylation of 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine was used to synthesize its novel S- and $N_{(7)}$-substituted derivatives. We have established the effect of the structure of the alkylating agent on the reaction conditions and its regioselectivity. We have shown that the synthesized guanine derivatives can be modified further.

Keywords: 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine, S-alkylation, regioselectivity.

Acyclic analogs of guanosine are a unique class of heterocyclic compounds, and are of interest as potentially biologically active substances [2] and also as models for investigation of molecular interactions in synthetic systems [3]. According to the data in [4, 5], the presence of an additional substituent at the position 8 of the guanine ring often improves both the pharmacokinetic and the biological characteristics of compounds in the indicated class. This work, a continuation of our research on synthesis and study of polyfunctional purine derivatives, is devoted to obtaining the poorly studied 9-alkoxyalkyl-8-alkylthioguanines; we focussed special attention on introducing, at the position 8 of the ring, long acyclic lyophilic substituents or else groups able to be lengthened further by subsequent chemical transformations. The presence of such substituents in guanine may promote improvement of the solubility of the compounds, which would facilitate their more detailed study and practical use.

Of all possible methods for synthesis of 9-alkoxyalkyl-8-alkylthio derivatives of guanine, as the most efficient method we chose S-alkylation of the previously synthesized 2-acetamido-9-(2-acetoxyethoxymethyl)-6-oxo-8-thioxopurine (**1**) [6]. Its N- and O-acetyl groups were introduced to facilitate isolation and purification of the reaction products. We know examples of S-alkylation of some 8-thioxo-9-substituted guanines, mainly by methyl iodide and propenyl bromide [7-9], but diacetate **1** was not used for this purpose.

We studied the possibilities for S-alkylation of compound **1**, considering as examples its reaction with 2-bromoethanol and benzyl bromide, ethyl chloroacetate, benzyl chloromethyl ether, octadecyl chloromethyl ether, octyl chloromethyl ether, and also vinyl butyl ether. The stability and reactivity of the alkylating agent determined the reaction conditions. S-alkylation of purine derivatives most often is carried out in aqueous

* For Communication 11, see [1].

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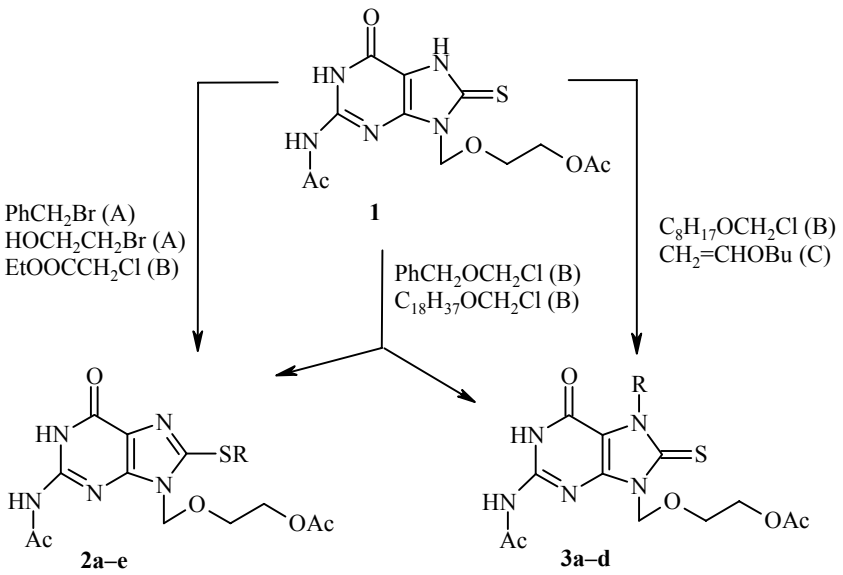


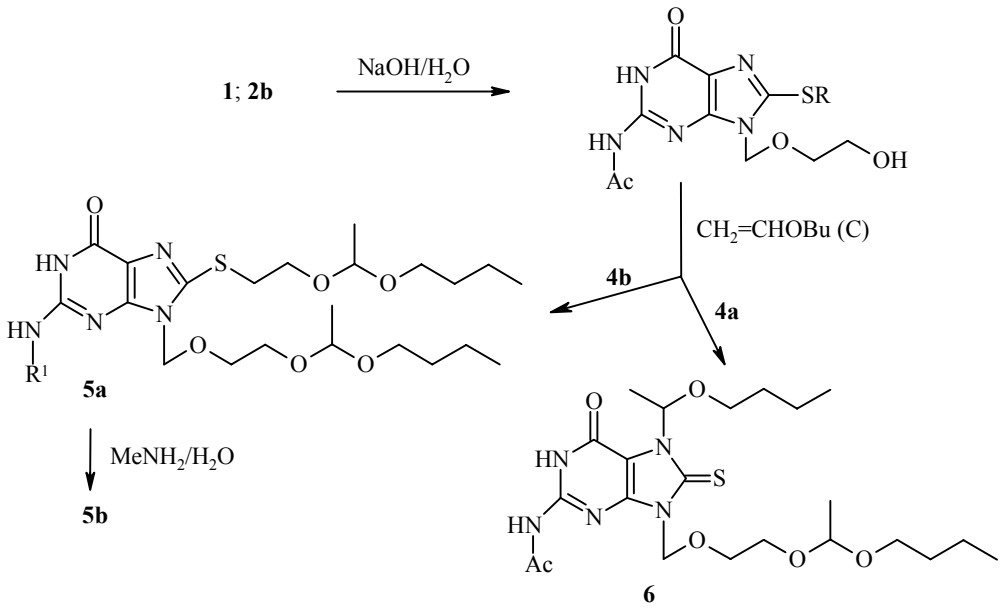
2 a R = CH_2Ph, **b** R = CH_2CH_2OH, **c** R = CH_2COOEt; **2d**, **3a** R = CH_2OCH_2Ph; **2e**, **3b** R = $CH_2OC_{18}H_{37}$; **3c** R = $CH_2OC_8H_{17}$; **3d** CHMeOBu

alkaline solutions. However, under these conditions (procedure A), we could carry out the reactions only with benzyl bromide and 2-bromoethanol. The processes occurred regioselectively, and in this case we obtained 2-acetamido-9-(2-acetoxyethoxymethyl)-8-benzylthio-6-oxopurine (**2a**) and 2-acetamido-9-(2-acetoxyethoxymethyl)-8-(2-hydroxyethyl)thio-6-oxopurine (**2b**) in 52% and 64% yields respectively. Despite the alkaline reaction medium, both the N- and O-acetyl groups were retained in the products **2a,b**. Since the rest of the alkylating agents proved to be unreactive in aqueous alkaline solution (octyl bromide, for example) or else unstable (chloromethyl ethers), alkylation using them was done in a DMF–K_2CO_3 system (procedure B). Under these conditions, the process often occurred nonselectively, and the target compounds **2** were obtained in low yields (11% to 28%). The regioselectivity of the process was determined by the structure of the alkylating agent. Thus in the case of ethyl chloroacetate, only the product of reaction at the sulfur atom was formed: 2-acetamido-9-(2-acetoxyethoxymethyl)-8-(ethoxycarbonylmethylthio)-6-oxopurine (**2c**). From benzyl and octadecyl chloromethyl ethers, we obtained both the products of S-alkylation: 2-acetamido-9-(2-acetoxyethoxymethyl)-8-(benzyloxymethyl)thio-6-oxopurine (**2d**) and 2-acetamido-9-(2-acetoxyethoxymethyl)-8-(octadecyloxymethyl)thio-6-oxopurine (**2e**), and the products of N-alkylation: 2-acetamido-9-(2-acetoxyethoxymethyl)-7-benzyloxymethyl-6-oxo-8-thioxopurine (**3a**) and 2-acetamido-9-(2-acetoxyethoxymethyl)-7-octadecyloxymethyl-6-oxo-8-thioxopurine (**3b**). The reaction with octyl chloromethyl ether occurred mainly at the $N_{(7)}$ atom, and 2-acetamido-9-(2-acetoxyethoxymethyl)-7-octyloxymethyl-6-oxo-8-thioxopurine (**3c**) was formed with a fairly small admixture of 2-acetamido-9-(2-acetoxyethoxymethyl)-1,7-di(octyloxymethyl)-6-oxo-8-thioxopurine, the structure of which is proposed on the basis of its ^1H NMR spectrum.

Based on data in [7, 8], we can hypothesize that in the case of chloromethyl ethers, initial alkylation at the sulfur atom was accompanied by partial or complete S → $N_{(7)}$ migration of the alkoxyalkyl substituent. In contrast to the analogous shift of the allyl group (DMF, 130°C, 6-9 days [7, 8]), in our case the process occurred readily, since the reaction temperature and the temperature at which the products were isolated did not exceed 40°C. The possibility of migration was confirmed experimentally by conversion of the S-alkylated product **2d** to the corresponding 7-substituted derivative **3a** (82% yield) when it was heated in DMF.

The reaction of guanine **1** with vinyl butyl ether was carried out in the presence of the acid catalyst TsOH (*p*-toluenesulfonic acid) in DMF [10] (procedure B), and in this case we obtained 2-acetamido-9-(2-acetoxyethoxymethyl)-7-(1-butoxy-1-ethyl)-6-oxo-8-thioxopurine (**3d**). We did not observe the S-alkylation product in the reaction mixture.

We found it was more successful to use vinyl butyl ether for further modification of some of the synthesized derivatives of guanine **1**. Thus reaction of 2-acetamido-9-(2-hydroxyethoxymethyl)-8-(2-hydroxyethyl)thio-6-oxopurine (**4b**) (obtained by O-deacetylation of derivative **2b**) with excess vinyl butyl ether led to 2-acetamido-8-[2-(1-butoxyethoxy)ethyl]thio-9-[2-(1-butoxyethoxy)ethoxymethyl]-6-oxopurine (**5a**). By subsequent removal of the acetyl protecting group, we synthesized 2-amino-8-[2-(1-butoxyethoxy)ethyl]thio-9-[2-(1-butoxyethoxy)ethoxymethyl]-6-oxopurine (**5b**): a novel derivative containing long lipophilic substituents at the positions 8 and 9 of the purine ring.



4 a R = H, **b** R = CH_2CH_2OH; **5 a** R^1 = COMe, **b** R^1 = H

When 2-acetamido-9-(2-hydroxyethoxymethyl)-6-oxo-8-thioxopurine (**4a**) obtained from compound **1** was reacted with vinyl butyl ether, alkylation of the purine ring and lengthening of the acyclic substituent occurred simultaneously, leading to 2-acetamido-7-(1-butoxy-1-ethyl)-9-[2-(1-butoxyethoxy)ethoxymethyl]-8-thioxopurine (**6**). Formation of some secondary derivatives during the reaction in this case reduced the yield of the target product **6**.

The composition and structure of the synthesized guanine derivatives were confirmed by the spectral characteristics and also by elemental analysis (Tables 1 and 2). Thus in the ^{1}H NMR spectra of products **2, 3, 5, 6** (Table 1), along with signals from the protons of the starting compound **1** there are additional signals from protons of the corresponding introduced alkyl or alkoxyalkyl substituents. The sites of their addition to the guanine ring were determined based on ^{1}H NMR spectra. According to rules established previously [1, 11], two-proton singlets, shifted downfield from the signal for the $N_{(9)}CH_2$ group (coinciding with the signal for the analogous group of the starting compound **1**), were assigned to the $N_{(7)}CH_2$ moieties, while the signals shifted upfield were assigned to the SCH_2 moieties. A possible alternative substitution at the position 1 of the guanine ring was contradicted by the absence of a signal from protons of the $N_{(1)}CH_2$ group in the ^{1}H NMR spectra of the synthesized compounds [1]. We also did not observe a singlet from the proton of the exocyclic functional group NHAc in the 10.4 ppm region, characteristic of $O_{(6)}$-alkylated guanine derivatives [1].

Thus as a result of our investigations, we have shown that it is possible to use S-alkylation of 8-thio-substituted derivatives of guanine for synthesis of novel polyfunctional acyclic analogs of guanosine. The reaction conditions and also the regiochemical orientation of the reaction (S- or N-alkylation) were determined by the structure of the alkylating agent. When compounds containing additional functional groups were used as the alkylating agents, guanine derivatives were synthesized that were suitable for further modification.

TABLE 1. ^{1}H NMR Spectral Data for Synthesized Compounds **2-6**

Com-pound	Chemical shifts, DMSO-d_6, δ, ppm (spin–spin coupling constant, *J*, Hz)*					
	NH (1H, s)	SCH_2 (2H, s) [$N_{(7)}CH_2$]	$N_{(9)}CH_2$ (2H, s)	OCH_2CH_2O (4H, two m)	$NCOCH_3$ $OCOCH_3$ (3H, s)	Other protons
2a	12.21, 11.81	4.49	5.31	4.05; 3.63	2.16, 1.94	7.36 (5H, m, C_6H_5)
2b	12.12, 11.81	3.33	5.41	4.12; 3.69*[2] (6H, two m)	2.16, 1.94	5.05 (1H, t, *J* = 5.0, OH)
2c	12.09, 11.81		5.43	4.16*[3]; 3.70 (8H, m)	2.16, 1.95	1.17 (3H, t, *J* = 7.0, $CH_2C\underline{H}_3$)
2d	12.12, 11.81	5.43	5.52	4.06; 3.72	2.17, 1.93	7.32 (5H, m, C_6H_5); 4.67 (2H, s, $C\underline{H}_2C_6H_5$)
2e*	11.95, 8.61	5.44	5.51	4.21; 3.72	2.29, 2.03	3.57 (2H, t, *J* = 6.2, $OC\underline{H}_2(CH_2)_{16}$); 1.22 (32H, m, $OCH_2(C\underline{H}_2)_{16}$); 0.87 (3H, t, *J* = 4.9, $(CH_2)_6C\underline{H}_3$)
3a	12.29, 12.03	[5.83 (2H, s)]	5.56	4.11; 3.85	2.16, 1.94	7.21 (5H, m, C_6H_5); 4.74 (2H, s, $C\underline{H}_2C_6H_5$)
3b*	12.07, 8.98	[5.85 (2H, s)]	5.67	4.24; 3.85	2.27, 2.05	3.67 (2H, t, *J* = 6.2, $OC\underline{H}_2(CH_2)_{16}$); 1.27 (32H, m, $OCH_2(C\underline{H}_2)_{16}$); 0.85 (3H, t, *J* = 4.8, $(CH_2)_{16}C\underline{H}_3$)
3c	12.34, 12.05	[5.72 (2H, s)]	5.58	4.07; 3.85	2.17, 1.94	3.61 (2H, t, *J* = 5.8, $OC\underline{H}_2(CH_2)_6$); 1.16 (12H, m, $OCH_2(C\underline{H}_2)_6CH_3$); 0.83 (3H, t, *J* = 4.6, $(CH_2)_6C\underline{H}_3$)
3d	12.23, 11.98	[6.52 (q, 1H, *J* = 6.0, CH)]	5.60	4.09; 3.87	2.19, 1.95	3.36 (2H, m, $OC\underline{H}_2(CH_2)_2$); 1.74 (3H, d, *J* = 6.0, $CHC\underline{H}_3$); 1.34 (4H, m, $OCH_2(C\underline{H}_2)_2$); 0.77 (3H, t, *J* = 6.4, $(CH_2)_2C\underline{H}_3$)
4a	12.32, 11.98		5.56	3.63; 3.52	2.18	13.46 (1H, s, SH); 4.61 (1H, t, *J* = 4.8, OH)
4b	12.05, 11.80	3.32	5.41	3.51 (4H, s)	2.16	5.04 (1H, t, *J* = 4.8, OH); 4.65 (1H, t, *J* = 5.0, OH); 3.69 (2H, m, $SCH_2C\underline{H}_2OH$)
5a	11.96 (2H, br. s)		5.39	3.60-3.29*[4] (12H, m)	2.17	4.66 (2H, m, $2OC\underline{H}$); 1.36 (8H, m, $2CH_2(C\underline{H}_2)_2$); 1.12 (3H, d, *J* = 5.0, $CHC\underline{H}_3$); 1.19 (3H, d, *J* = 5.0, $CHC\underline{H}_3$); 0.83 (6H, m, $2CH_3$)
5b*	11.74		5.44	3.69-3.42*[4] (12H, m)	—	6.68 (2H, s, NH_2); 4.76 (2H, m, 2OCH); 1.49 (8H, m, $2CH_2(C\underline{H}_2)_2$); 1.27 (6H, m, $2CHC\underline{H}_3$); 0.87 (6H, m, $2CH_3$)
6	12.25, 12.05	[6.54 (1H, q, *J* = 5.4, CH)]	5.61	3.78; 3.52	2.19	4.61 (1H, q, *J* = 5.2, OCH); 3.43 (4H, m, $2C\underline{H}_2(CH_2)_2$); 1.72 (3H, d, *J* = 5.4, $NCHC\underline{H}_3$); 1.34 (8H, m, $2(C\underline{H}_2)_2CH_3$); 1.15 (3H, d, *J* = 5.2, $OCHC\underline{H}_3$); 0.82 (6H, m, $2(CH_2)_2C\underline{H}_3$)

* ^{1}H NMR spectra of compounds **2e, 3b,** and **5b** were obtained in $CDCl_3$; the spectra for the rest of the compounds were obtained in DMSO-d_6.

*[2] The signal is overlapped by the $C\underline{H}_2OH$ signal.

*[3] Overlapping SCH_2, $C\underline{H}_2CH_3$, and $OC\underline{H}_2CH_2O$ signals.

*[4] Overlapping SCH_2CH_2, OCH_2CH_2O, and $2\times OC\underline{H}_2$ $(CH_2)_2$ signals.

TABLE 2. Characteristics of Synthesized Compounds **2-6**

Compound	Empirical formula	Found, % / Calculated, %			mp, °C	Yield, %
		C	H	N		
2a	$C_{19}H_{21}N_5O_5S$	52.73 / 52.89	4.78 / 4.91	16.59 / 16.23	143-145	63
2b	$C_{14}H_{19}N_5O_6S$	43.37 / 43.63	4.94 / 4.97	18.00 / 18.17	156-158	54
2c	$C_{16}H_{21}N_5O_7S \cdot H_2O$	43.19 / 43.14	5.12 / 5.20	15.69 / 15.72	169-171	11
2d	$C_{20}H_{23}N_5O_6S$	52.10 / 52.05	5.03 / 5.02	14.99 / 15.18	135-138	21
2e	$C_{31}H_{53}N_5O_6S$	59.47 / 59.68	8.61 / 8.56	11.14 / 11.23	105-109	28
3a	$C_{20}H_{23}N_5O_6S$	52.33 / 52.05	4.93 / 5.02	14.82 / 15.18	142-143	12
3b	$C_{31}H_{53}N_5O_6S \cdot H_2O$	58.64 / 58.61	8.75 / 8.64	10.64 / 10.91	98-100	19
3c	$C_{21}H_{33}N_5O_6S$	52.18 / 52.16	6.87 / 6.88	14.52 / 14.48	109-110	30
3d	$C_{18}H_{27}N_5O_6S$	49.40 / 48.97	6.14 / 6.16	15.67 / 15.86	148-151	33
4a	$C_{10}H_{13}N_5O_4S$	40.10 / 40.13	4.24 / 4.38	23.53 / 23.40	>250 (dec.)	95
4b	$C_{12}H_{17}N_5O_5S$	42.00 / 41.98	4.94 / 4.99	20.37 / 20.40	216-217	69
5a	$C_{24}H_{41}N_5O_7S$				Oil*	30
5b	$C_{22}H_{39}N_5O_6S$	52.25 / 52.68	7.89 / 7.84	13.62 / 13.96	43-46	72
6	$C_{22}H_{37}N_5O_6S$	52.71 / 52.89	7.39 / 7.46	14.02 / 14.02	148-150	30

* The compound was used later without crystallization.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker WH-90/DS (90 MHz) spectrometer, internal standard TMS. The melting points were determined on a Boetius apparatus and were uncorrected. Elemental analyses were done using a Carlo Erba 1108 analyzer. The products were purified on a chromatographic column (25 × 300 mm) packed with Merck silica gel G 60 (0.063-0.200 mm), the eluent was chloroform (for compounds **2a,d,e** and **3a,c,d**), 40:0.5 chloroform–ethanol (for compound **3b**), and 40:1 chloroform–ethanol (for **2c**). The course of the reactions was monitored by TLC on silica gel 60 F254 plates in the system 10:1 chloroform–ethanol.

The characteristics of the compounds obtained are given in Tables 1 and 2.

2-Acetamido-9-(2-acetoxyethoxymethyl)-8-benzylthio-6-oxopurine (2a). A. Benzyl bromide (0.22 ml, 1.7 mmol) was added dropwise to suspension of compound **1** (0.61 g, 1.7 mmol) in water (13 ml) and 0.4 N NaOH (5 ml). The reaction mixture was stirred at room temperature for 36 h. The precipitate of product **2a** was filtered out, washed on the filter with a moderate amount of water, and recrystallized from ethanol.

Product 2b was obtained similarly from compound **1** and β-hydroxyethyl bromide, but the reaction mixture was stirred for 36 h at 40°C.

2-Acetamido-9-(2-acetoxyethoxymethyl)-8-(ethoxycarbonylmethylthio)-6-oxopurine (2c). B. Ethyl chloroacetate (0.13 g, 1.3 mmol) was added dropwise to solution of compound **1** (0.40 g, 1.1 mmol) and anhydrous K_2CO_3 (0.15 g, 1.1 mmol) in DMF (20 ml). The reaction mixture was stirred at room temperature for

72 h and then was filtered; the filtrate was evaporated down under vacuum at a temperature below 40°C. Product **2c** was separated from the residue by column chromatography and recrystallized from ethanol.

A mixture of products **2d** and **3a** was obtained similarly by reaction of compound **1** with benzyl chloromethyl ether; a mixture of products **2e** and **3b** was obtained by reaction with octadecyl chloromethyl ether; and product **3c** was obtained by reaction with octyl chloromethyl ether. The individual compounds **2d,e, 3a-c** were separated by column chromatography.

Conversion of the 8-Benzyloxymethyl)thio Derivative 2d to the 7-Benzyloxymethyl-8-thioxo Derivative 3a. Solution of compound **2d** (0.10 g, 0.2 mmol) in DMF (15 ml) was stirred for 3 h at a temperature of 90°C. The solvent was evaporated off under vacuum and the residue was recrystallized from ethanol. Obtained 0.08 g (82%) of compound **3a**, identical to the sample synthesized by procedure B (no depression of the melting point for a mixed sample).

2-Acetamido-9-(2-acetoxyethoxymethyl)-7-(1-butoxy-1-ethyl)-6-oxo-8-thioxopurine (3d). B. Vinyl butyl ether (1.52 ml, 11.7 mmol) was added dropwise to solution containing compound **1** (1.0 g, 2.9 mmol) and TsOH (0.11 g, 0.6 mmol) in DMF (12 ml). The reaction mixture was stirred at room temperature for 3 h. Then $NaHCO_3$ (0.08 g, 0.6 mmol) was added; this was stirred for 20 min and the residue was filtered off. The filtrate was evaporated down under vacuum at a temperature below 40°C. The product **3d** was separated from the residue by column chromatography and recrystallized from ethanol.

2-Acetamido-9-(2-hydroxyethoxymethyl)-6-oxo-8-thioxopurine (4a) and 2-Acetamido-9-(2-hydroxyethoxymethyl)-8-(2-hydroxyethyl)thio-6-oxopurine (4b) were obtained by deacetylation of compounds **1** and **2b** respectively, by the procedure in [10].

2-Acetamido-8-[2(1-butoxyethoxy)ethyl]thio-9-[2-(1-butoxyethoxy)ethoxymethyl]-6-oxopurine (5a) and 2-Acetamido-7-(1-butoxy-1-ethyl)-9-[2-(1-butoxyethoxy)ethoxymethyl]-8-thioxopurine (6) were obtained similarly to derivative **3d** from compounds **4b** and **4a** respectively, using 8 equivalents of vinyl butyl ether.

2-Amino-8-[2-(1-butoxyethoxy)ethyl]thio-9-[2-(1-butoxyethoxy)ethoxymethyl]-6-oxopurine (5b) was obtained by deacetylation of compound **5a** by the procedure in [10].

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