Regioselective Alkylation of Guanine *via* Diacyloxyglyoxal-N^2-acetylguanine Adduct to Obtain 7-Alkylguanine Derivatives. Studies on Alkylation of Guanine I

Jonas Kjellberg*

Department of Organic Chemistry, Royal Institute of Technology,
S-10044 Stockholm, Sweden

Nils Gunnar Johansson*

Department of Antiviral Chemotherapy, Research and Development Laboratories
Astra Läkemedel AB, S-151 85 Södertälje, Sweden

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The acylated guanine-glyoxal adduct (I) has been alkylated with 4-bromobutyl acetate and in the presence of sodium hydride the reaction was regioselective to give 7-alkylguanine.

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The antiviral activity of certain acyclic guanosine analogs has been the subject of intensive study. Acyclovir [1], Buciclovir ((*R*)-9-(3,4-dihydroxybutyl)guanine [2], and DHPG (9-(dihydroxypropoxymethyl)guanine) [3] show antiherpes activity both *in vitro* and *in vivo*.

In connection with our studies on buciclovir [2] and the analogous compound 9-(4-hydroxybutyl)guanine [5] we have investigated ways of alkylating guanine or guanine precursors. Alkylation of 6-chloroguanine gives predominantly the 9-isomer [6,7]. The results of alkylating guanine, N^2-acetylguanine, or silylated guanine were generally unsatisfactory [8].

The present work is a study on the reactivity and regioselectivity towards alkyl halides of a modified guanine base.

Diacetoxyglyoxal-N^2-acetylguanine adduct and diisobutyroxyglyoxal-N^2-acetylguanine guanine adducts (I and II respectively) have been prepared by a guanine-glyoxal addition, followed by acylation of the resulting diol [9, *cf.* 10 a-g]. The two compounds I (R = acetyl) and II (R = isobuturyl) differ notably in polarity and solubility.

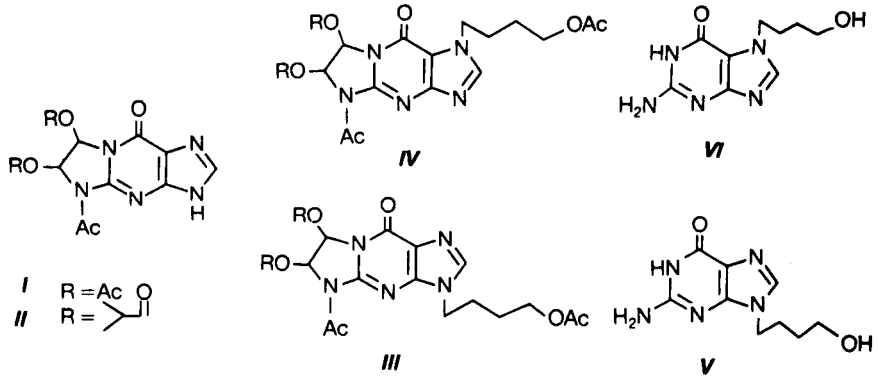
The compounds I and II were alkylated with 4-bromobutyl acetate under different conditions and the regioselectivity was found to be strongly dependent on the choice of the base. Alkylation was studied in the presence of metal hydrides, amines, alkoxides and quaternary ammonium hydroxides. The ratio between the 9-isomer (III) and the 7-isomer (IV) was determined by ^1H and ^{13}C nmr. The signals of 8-protons and the C8, C5, and C1' carbon atoms were distinctly different for the two isomers. The alkylated products were separated by flash column chromatography.

Deprotection readily occurred on alkaline hydrolysis and 7-(4-hydroxybutyl)guanine [11] and 9-(4-hydroxybutyl)guanine [12] were obtained and identified by comparison with authentic specimens.

The results are summarized in the table and show that the formation of the 7-isomer predominates. When sodium hydride was used as a base the 7-alkylguanine derivative was selectively formed. The 7-isomer was also formed predominantly in the presence of potassium hydride and calcium hydride, but the regioselectivity was lower which indicates that the sodium ion was involved in a specific interaction.

The reactions were performed in *N,N*-dimethylformamide. In the less polar solvents tetrahydrofuran and methylene chloride (CH_2Cl_2) no alkylation took place which may be explained by the S_N2 character of the reaction.

The yields have not been optimized and starting material and hydrolyzed products were present in the crude products.



Table

Alkylation of I, II to give III and IV

Substrate	Base	Solvent [a]	% Yield [b]	Ratio IV/III
I, II [j]	1,4-dimethylpiperazine	DMF	30	2 [c]
I	Et_3N	DMF	21 [d] [f] [h]	2
I, II [j]	$LiN(i\text{-}Pr)_2$	THF/DMF	35 [d] [e] [i]	2
II	$LiN(i\text{-}Pr)_2$	THF	—	
II	BuLi	THF	—	
I	NaH	DMF	50	>18 [c]
I	KH	DMF	65 [d] [i]	6
I	CaH_2	DMF	50 [g] [i]	4
I	TlEtO	DMF	55 [d] [f] [i]	5
I	t-BuOK	DMF	25 [d] [i]	6
II [j]	$N(Bu)_4OH$	CH_2Cl_2	2 [e] [h]	1
I	$N(Bu)_4OH$	DMF	18 [d] [e] [h]	2

[a] *N,N*-Dimethylformamide and tetrahydrofuran are abbreviated as DMF and THF, respectively. [b] Condensations performed at room temperature. [c] Experimental details are given. [d] 1 Equivalent (eq) of substrate, 1 eq of base and 1 eq of halide. [e] 1 Eq of substrate, 1 eq of base and 2 eq of halide. [f] 1 Eq of substrate, 3 eq of base and 3 eq of halide. [g] 1 Eq of substrate, 0.5 eq of base and 1 eq of halide. [h] Performed as for 1,4-dimethylpiperazine. [i] Performed as for sodium hydride. [j] The two alkylated products were not isolated for compound II and the ratio was estimated by ^1H nmr and ^{13}C nmr of the reaction mixture.

EXPERIMENTAL

The synthesis and the analysis (^1H and ^{13}C nmr, ms, uv, and elemental analysis) of the glyoxal-N^2-acetylguanine adduct is described in a previous paper [9]. The formation of the adduct is similar to previously described additions [10a-g]. The numbering system of purines has been used. The nmr spectra were recorded on a Jeol JNM-FX 200. The mass spectra were obtained on a LKB 9000 (70 eV). Elemental analyses were performed by Novo Microanalytical Laboratory, Bagsvaerd, Denmark and by Kemicentrum, Department of Analytical Chemistry, Lund, Sweden. For rp-hplc analysis a Waters, 440, RCM-100 was used. All bases, solvents, and starting materials were of highest purity available. The solvents were dried over molecular sieves before use. The ammonium hydroxides were co-evaporated with toluene to remove water.

Ethanoic Acid-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-3*H*-imidazo[1,2-*a*]-purine-6,7-diyl Ester (I).

Acetic anhydride (3.3 g, 32 mmoles) was added to a solution of 1,N^2-glyoxal-N^2-acetylguanine [9] (2.0 g, 8 mmoles) in dry pyridine (170 ml). The solution was stirred for 2 hours at room temperature and turned pale red. The solvent was removed by evaporation and the residue was washed with ethyl acetate and ether. The crude product was dissolved in 50% aqueous ethanol and kept at 60° for 0.5 hours. The clear solution was allowed to stand at 4° overnight. White needles (1.4 g, 52%) of compound I were collected by filtration and after drying the crystals were analyzed; ^1H nmr (hexadeuteriodimethyl sulfoxide): δ 2.08 and 2.12 (2s, 6H, 2 $OCOCH_3$), δ 2.68 (s, 3H, $NCOCH_3$), δ 6.73-6.85 (d, 2H, CHCH), δ 8.15 (s, 1H, H-8); ^{13}C nmr (hexadeuteriodimethyl sulfoxide): δ 20.6 20.7 (2 CH_3COOCH), δ 24.8 ($NCOCH_3$), δ 77.6 81.1 (CHCH), δ 115.0 (C5), δ 141.2 (C8), δ 147.6 (C4), δ 149.6 (C2), δ 152.1 (C6), δ 168.2 168.4 (3 CO); ms: m/e = 335; uv (nm) λ max = 256 in 0.1 *M* hydrochloric acid, λ max = 272 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_{13}H_{13}N_5O_6$: C, 46.57; H, 3.91; N, 20.89. Found: C, 46.50; H, 3.91; N, 20.83.

Propanoic Acid-2-methyl-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-3*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (II).

To a solution of 0.25 g (1 mmole) of glyoxal N^2-acetylguanine adduct in 150 ml of dry pyridine, 0.32 g (2 mmoles) of isobutyric anhydride was added. The solution was stirred for 20 hours at room temperature. After removal of the solvent, the residue was washed with ether and ethanol. The crude product was purified by flash chromatography (chloroform/-methanol 5:1) to give 0.20 g (51%) of diisobutyroxyglyoxal-N^2-acetylguanine adduct (II); ^1H nmr (hexadeuteriodimethyl sulfoxide): δ 1.06-1.13 (m, 12H, 4, CH_3), δ 2.60-2.66 (m, CH), δ 2.68 (s, $NCOCH_3$), δ 6.68-6.78 (d, 2H, CHCH), δ 8.16 (s, 1H, H-8); ^{13}C nmr (deuteriochloroform): δ 18.6, 18.7, 18.8, 18.9, (4,CH_3CH), δ 25.3 ($NCOCH_3$), 33.8, 33.9 (2, CH_3CH), δ 78.1, 81.6 (CHCH), δ 115 (C5), δ 142.1 (C8), δ 147.9 (C4), δ 149.6 (C2), δ 152.4 (C6), δ 168.7 (NCO), δ 174.1, 174.7 (2 OCO); ms: m/e = 391; uv (nm) λ max = 255 in 0.1 *M* hydrochloric acid, λ max = 274 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_{17}H_{21}N_5O_6$: C, 52.22; H, 5.31; N, 17.91. Found: C, 52.05; H, 5.33; N, 17.84.

Ethanoic Acid-3-(ethanoic acid-butur-4-yl Ester)-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-3*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (III) and Ethanoic Acid-3-(ethanoic acid-butur-4-yl Ester)-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-1*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (IV).

Three mmoles (1005 mg) of I were dissolved in DMF (80 ml). To the solution, 1,4-dimethylpiperazine (2 g, 18 mmoles) and 4-bromobutyl acetate (3.5 g, 18 mmoles) were added. The solution was stirred under nitrogen for 18 hours at room temperature. The reaction was followed by rp-hplc (methanol/water 50:50) and tlc (chloroform/methanol 20:1). The solution turned purple. The solvent was evaporated *in vacuo*. The residue was extracted wtih chloroform and the extract was flash chromatographed on a silica gel column (eluent chloroform/methanol 20:1). Fractions with the pure compounds III and IV were collected and 270 mg (20%) of the compound III and 135 mg (10%) of the compound IV were isolated.

Ethanoic Acid-3-(ethanoic acid-butur-4-yl Ester)-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-1*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (IV).

This compound had; ^1H nmr (deuteriochloroform): δ 1.71-2.00 (m, 4H, CH_2), δ 2.04 (s, 3H, $OCOCH_3$), δ 2.14 (s, 3H, $NCOCH_3$), δ 4.10 (m, 2H, OCH_2), δ 4.37 (m, 2H, NCH_2), δ 6.84-6.88 (d, 2H, CHCH), δ 7.80 (s, 1H, H-8); ^{13}C nmr (deuteriochloroform): δ 20.60, (2 CH_3COOCH), 20.99 (CH_3COOCH_2), 25.30 ($NCOCH_3$),25.69 (C2'), 27.90 (C3'), 47.24 (C1'), 63.47 (C4'), 77.79, 81.17 (CHCH), 111.48 (C5), 143.54 (C8), 147.75 (C4), 151.47 (C2), 158.36 (C6), 167.70 (NCO), 168.31, 168.82 (s $COOCH_2$), 171.05 ($COOCH_2$); ms: m/e = 449; uv (nm) λ max = 257 in 0.1 *M* hydrochloric acid, λ max = 274 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_{19}H_{23}N_5O_8 \cdot H_2O$: C, 48.8; H, 5.39; N, 15.0. Found: C, 48.7; H, 5.41; N, 15.1.

Ethanoic Acid-3-(ethanoic acid-butur-4-yl Ester)-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-3*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (III).

This compound had;^1H nmr (deuteriochloroform): δ 1.66-2.03 (m, 4H, CH_2), δ 2.03 (s, 3H, $OCOCH_3$), δ 2.10 (s, 3H, $OCOCH_3$), δ 2.11 (s, 3H, $OCOCH_3$), δ 2.73 (s, 3H, $NCOCH_3$), δ 4.06-4.17 (m, 4H, NCH_2 OCH_2), δ 6.83-6.84 (d, 2H, CHCH), δ 7.68 (s, 1H, H-8); ^{13}C nmr (deuteriochloroform): δ 20.63 (2 CH_3COOCH), 21.04 (CH_3COOCH_2), 25.18 ($NCOCH_3$), 26.00 (C2'), 26.93 (C3'), 43.93 (C1'), 63.93 (C4'), 78.30, 81.32 (CHCH), 120.80 (C5), 139.31 (C8), 148.60 (C4), 148.77 (C2), 153.66 (C6), 167.96 (NCO), 168.06 (2 COOCH), 171.05 (OCO); ms: m/e = 449; uv (nm) λ max = 257 in 0.1 *M* hydrochloric acid, λ max = 264 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_{19}H_{23}N_5O_8 \cdot H_2O$: C, 48.8; H, 5.39; N, 15.0. Found: C, 48.9; H, 5.40; N, 15.0.

Ethanoic Acid-3-(ethanoic acid-butur-4-yl Ester)-5,6,7,9-tetrahydro-5-(1-oxoethyl)-9-oxo-1*H*-imidazo[1,2-*a*]purine-6,7-diyl Ester (IV).

A selective method for the synthesis of III is described.

One mmole of I (335 mg) was dissolved in dry DMF (15 ml) under nitrogen at −60°. Sodium hydride (~1.2 mmoles) suspended in *n*-hexane (2 ml) was added to the solution. After the mixture had been stirred for 20 minutes at −60° 2 mmoles (390 mg) of 4-bromobutyl acetate was added. The solution was stirred at room temperature for 2 hours and turned brown. The reaction was followed by rp-hplc and tlc. The solvent was carefully evaporated *in vacuo*. The brown residue was suspended in ethyl acetate (15 ml) and the brown crystals were filtered off. Ethyl acetate was removed by evaporation and the crude product was analyzed with ^{1}H nmr and ^{13}C nmr. The yield of the 7-isomer (IV) was 50% and the 9-isomer (III) could not be detected (detection limit 3%).

7-(4-Hydroxybutyl)guanine (VI).

One tenth mmole of compound IV was treated overnight with a solution of potassium hydroxide (0.3 g) in absolute ethanol (10 ml). The solvent was removed under reduced pressure. The residue was dissolved in water (3 ml) and neutralized with 1 *M* hydrochloric acid. The resulting solution was kept overnight at 4° to deposit white crystals. The crystals were washed with cold water and analyzed with rp-hplc (methanol/water 30:70). The yield of VI was 20 mg (90%); ^{1}H nmr (hexadeuteriodimethyl sulfoxide): δ 1.34 (m, CH_2), δ 1.82 (m, CH_2), δ 3.39 (t, OCH_2), δ 4.19 (t, NCH_2), δ 6.05 (s, NH_2), δ 7.87 (s, H-8); ^{13}C nmr (hexadeuteriodimethyl sulfoxide): δ 27.5 (C2'), 29.4 (C3'), 46.2 (C1'), 60.4 (C4'), 108.3 (C5), 143.2 (C8), 152.9 (C2), 154.7 (C6), 160.0 (C4); ms: m/e = 223; uv (nm) λ max = 250 in 0.1 *M* hydrochloric acid, λ max = 279 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_{19}H_{13}N_5O_2 \cdot H_2O$: C, 44.8; H, 6.27; N, 29.0. Found: C, 44.8; H, 6.29; N, 29.1.

9-(4-Hydroxybutyl)guanine (V).

The method for synthesis of V above was followed and VI was isolated and analyzed in the same way. The yield was 17 mg (76%). The retention time (rp-hplc, methanol/water 20:80) of V was identical with an authentic sample; ^{1}H nmr (hexadeuteriodimethyl sulfoxide): δ 1.40 (m, CH_2), δ 1.80 (m, CH_2), δ 3.97 (t, OCH_2), δ 4.50 (t, NCH_2), δ 6.49 (s, NH_2), δ 7.72 (s, H-8); ^{13}C nmr (hexadeuteriodimethyl sulfoxide): δ 26.9 (C2'), 30.0 (C3'), 43.1 (C1'), 60.7 (C4'), 117.1 (C5), 138.0 (C8), 151.6 (C4), 153.9 (C2), 157.4 (C6); ms: m/e = 223; uv (nm) λ max = 253, 278 in 0.1 *M* hydrochloric acid, λ max = 268 in 0.1 *M* sodium hydroxide.

Anal. Calcd. for $C_9H_{13}N_5O_2 \cdot H_2O$: C, 44.8; H, 6.27; N, 29.0. Found: C, 44.9; H, 6.26; N, 29.1.

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