

# REACTION OF GLYCIDAMIDE WITH 2'-DEOXYADENOSINE AND 2'-DEOXYGUANOSINE – MECHANISM FOR THE AMIDE HYDROLYSIS

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 $\Box$  2'-Deoxyadenosine (dA) and 2'-deoxyguanosine (dG) were reacted with the mutagenic epoxide glycidamide (GA, Scheme 1). The reactions yielded three GA-dA adducts (N1-GA-dA, N<sup>6</sup>-GA-dA and N1-GA-dI) and two GA-dG adducts (N1-GA-dG I and N1-GA-dG II) (Scheme 2). The structures of the adducts were characterized by spectroscopic and spectrometric methods (<sup>1</sup>H-, <sup>13</sup>C, and 2D NMR, MS, UV). The mechanism of the amide hydrolysis taking place during formation of the adducts N1-GA-dA and N1-GA-dG I was studied. We propose a mechanism where a transamidation is the key step in the hydrolysis of the amide function of GA.

Keywords Glycidamide; nucleosides; adducts; amide hydrolysis

## INTRODUCTION

Acrylamide (AM) (Scheme 1) is a high production volume chemical with a wide variety of industrial applications. AM is neurotoxic, clastogenic, and carcinogenic in animal experiments, and it is classified as a probable human carcinogen.<sup>[1]</sup> Recently, it was discovered that AM is present in a wide range of different starchy foodstuffs.<sup>[2]</sup> This has led to extensive investigations worldwide to determine the genotoxic mechanisms of AM. Previously, it has been shown that AM reacts with the bases in DNA, but the reactivity seems to be quite low.<sup>[3]</sup> It now has been concluded that the mutagenicity of acrylamide in human and mouse cells is due to the AM epoxide metabolite glycidamide (GA) (Scheme 2) and its enhanced reactivity with DNA.<sup>[4–6]</sup>

The first GA adduct that was structurally characterized, was the N7-GA-Gua adduct.<sup>[7]</sup> This adduct also is formed in DNA of mice and rats treated

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SCHEME 1 Structures of AM and GA.

with AM and GA.<sup>[8]</sup> More recently, Gamboa da Costa et al. reported on two adducts, N3-GA-Ade and N1-GA-dA, formed in the reaction of GA with 2'-deoxyadenosine (dA).<sup>[8]</sup> In addition, it was proposed that the N<sup>6</sup>-GA-adduct along with a cyclic unidentified adduct were formed in the reactions of dA and GA. The N3-GA-Ade and N1-GA-dA also were found in salmon testis DNA modified with GA.<sup>[8]</sup>

We previously have reported that GA is reactive toward cytidine and thymidine.<sup>[9]</sup>

Thymidine is considered to be the least reactive of the nucleosides, but we found that in the reaction with GA a surprisingly high yield (12.3 mol% isolated yield) of a thymidine adduct could be obtained.

In this work, we further explored the reactions of GA with 2'deoxyadenosine and 2'-deoxyguanosine (dG). The work resulted in identification of one dA adduct (N1-GA-dI) (Scheme 2) and two dG adducts (N1-GA-dG I, N1-GA-dG II) (Scheme 2) not previously described in



N1-GA-dA

N1-GA-dI

N<sup>6</sup>-GA-dA



SCHEME 2 Structures of the GA-derived nucleoside adducts identified in this study.

the literature. In addition, the  $N^6$ -dA adduct is for the first time fully characterized. Furthermore, we wanted to explore the mechanism for the facile hydrolysis of the amide function in the GA tail.

# MATERIALS AND METHODS

(Caution: GA has been found to be mutagenic on Salmonella typhimurium. Caution should therefore be exercised in the handling of the compound.)

#### **Chemicals and Enzymes**

GA was purchased from Toronto Research Chemicals (North York, ON, Canada). 2'-deoxyadenosine and 2'-deoxyguanosine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile (p.a.) was from Labscan Ltd. (Dublin, Ireland). The water used was distilled water purified with a Millipore system (Simplicity 185, Billerica, MA, USA).

### **Chromatographic Methods**

Liquid chromatography-diode array detector (LC-DAD) analyses were performed on an Agilent 1100 Series liquid chromatographic system (Agilent Technologies, Esbo, Finland) consisting of a quaternary pump, a vacuum degasser, an autosampler, a thermostated column compartment, and a diode array detector (DAD). The reaction mixtures were chromatographed on a 5  $\mu$ m, 4 mm  $\times$  125 mm reversed phase C18 analytical column (Hypersil BDS-C18, Agilent Technologies, Esbo, Finland). The column was eluted isocratically for 3 minutes with 2% acetonitrile in ammonium acetate buffer (0.01 M, pH 7) and then with a gradient from 2 to 30% acetonitrile over the course of 17 minutes at a flow rate of 1 mL/minute. The products were purified on a semipreparative 5  $\mu$ m, 10 mm  $\times$  250 mm reversed phase C18 column (BDS Hypersil C18, Thermo Hypersil-Keystone, Krotek Oy, Tammerfors, Finland). When the 2'-deoxyadenosine adducts were purified the semipreparative column was coupled to a Varian 5000 Liquid Chromatograph (Varian Aerograph, Walnut Creek, CA, USA) and a variablewavelength Shimadzu SPD-6 A UV spectrophotometric detector (Shimadzu Europe, Duisburg, Germany). When the 2'-deoxyguanosine adducts were purified the semipreparative column was coupled to the above mentioned Agilent 1100 Series liquid chromatographic system, with a fraction collector (Agilent 1100) added to the system.

#### **Spectroscopic and Spectrometric Methods**

The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the adducts were recorded at 25°C on a Bruker Avance 600 NMR spectrometer at 600 and 150 MHz, respectively (Bruker, Rheinstetten, Germany). The samples were dissolved in Me<sub>2</sub>SO- $d_{6}$ , except for one of the compounds (N<sup>6</sup>-GA-dA), which was dissolved in D<sub>2</sub>O. The <sup>1</sup>H NMR signal assignments were based on chemical shifts and H-H and C-H correlation data. The assignment of carbon signals was based on chemical shifts and C-H correlations.

The LC-ESI-MS/MS analyses were performed on an Agilent 1100 Series LC/MSD SL Trap instrument (Agilent Technologies, Esbo, Finland) consisting of a binary pump, a vacuum degasser, an autosampler, a thermostated column compartment, and a UV detector. The ion trap mass spectrometer was equipped with an electrospray source and operated in the positive ion mode. Operation parameters of the ESI ion source were as follows: drying gas temperature was 350°C, drying gas flow 12 L/minute, nebulizer gas pressure 40 psi, end plate voltage -3500 V, end plate offset -500 V, capillary exit 115 V and skim 1 was set at 40 V. Ion trap parameters were as follows: accumulation time was 20 ms, averages 5, rolling averaging on and ion charge control on. Nitrogen gas was used as drying and nebulizing gas. Collision induced dissociation (CID) experiments coupled with multiple tandem mass spectrometry (MS<sup>n</sup>) employed helium as collision gas. The fragmentation amplitude was varied between 0.7 and 1.0 V. The samples from the reaction mixtures were chromatographed on a 5  $\mu$ m, 4 mm  $\times$ 125 mm reversed phase C18 analytical column (Hypersil BDS-C18, Agilent Technologies, Esbo, Finland). The column was eluted with a gradient consisting of 0.01 M aqueous ammonium acetate and acetonitrile. First, the column was eluted isocratically for 5 minutes with 1% acetonitrile and then with a gradient from 1 to 30% acetonitrile over the course of 14 minutes at a flow rate 0.5 mL/minute.

The pure compounds also were analyzed by direct inlet infusion to the source by a syringe pump at a flow rate of 5  $\mu$ L/minute and at a concentration of about 1  $\mu$ g/mL. The compounds were dissolved in a 1:1 v/v mixture of 0.01 M aqueous ammonium acetate/acetonitrile. The operation parameters were as follows: drying gas temperature was 325°C, drying gas flow 5 L/minute, and nebulizer gas pressure 15 psi.

The UV spectra of the isolated compounds were recorded with the diode array detector as the peaks eluted from the HPLC column.

# Preparative-Scale Reaction of GA with 2'-Deoxyadenosine at pH 7. Preparation of N1-(2-Carboxy-2-hydroxyethyl)-2'-Deoxyadenosine (N1-GA-dA), N<sup>6</sup>-(2-Carboxy-2-hydroxyethyl)-2'-Deoxyadenosine (N<sup>6</sup>-GA-dA) and N1-(2-Carboxy-2-hydroxyethyl)-2'-Deoxyinosine (N1-GA-dI)

GA (690 mg, 7.96 mmol) was reacted with 2'-deoxyadenosine (100 mg, 0.39 mmol) in 20 mL of 0.5 M phosphate buffer solution (pH 7.0). The reaction was performed at 37°C. The progress of the reaction was followed by LC-DAD and LC-ESI-MS/MS analyses on the C18 analytical column. The reaction was stopped at optimum yield (4 days). The reaction mixture was concentrated by rotary evaporation to about 4 mL, and the precipitated phosphate salts were removed by filtration. The adducts were

isolated from the mixture by chromatography on the semi-preparative column. The column was first eluted isocratically with 2% acetonitrile in ammonium acetate buffer (0.01 M, pH 7) in 3 minutes, and then with a gradient from 2 to 30% over the course of 17 minutes. The flow rate was 3 mL/minute. One fraction contained the adducts N1-GA-dA and N1-GA-dI, and another fraction contained the adduct N<sup>6</sup>-GA-dA. N1-GA-dA and N1-GA-dI were separated from each other by re-injecting the fraction using the same conditions as during the initial fractionation, but the sample amount injected was lower, thus improving the separation. The solutions containing the pure compounds were then combined and rotary evaporated to dryness and further dried under vacuum. The residues were subjected to spectroscopic and spectrometric studies. The isolated amounts of the compounds were as follows: N1-GA-dA, 8.02 mg; N<sup>6</sup>-GA-dA, 6.8 mg; and N1-GA-dI, 2.25 mg. The yields were 9.5, 12.5, and 8.8 mol%.

N1-GA-dA had the following spectral characteristics: UV spectrum, UV<sub>max</sub> 260, 212 nm, UV<sub>min</sub> 234 nm (HPLC eluent: approximately 2% ACN in ammonium acetate buffer; pH 7). In the positive ion electrospray mass spectrum, the following ions were observed (m/z, relative abundance, formation): MS, 340 (100, MH<sup>+</sup>), 362 (5, MNa<sup>+</sup>), MS<sup>2</sup> of 340, 224 (100, MH<sup>+</sup>-deoxyribosyl + H), MS<sup>3</sup> of 340 $\rightarrow$ 224, 206 (21, MH<sup>+</sup>-deoxyribosyl-H<sub>2</sub>O + H), 178 (100, MH<sup>+</sup>-deoxyribosyl-CH<sub>2</sub>O<sub>2</sub> + H), 136 (37, MH<sup>+</sup>-deoxyribosyl-C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>), MS<sup>4</sup> of 340 $\rightarrow$ 224 $\rightarrow$ 178, 160 (100, MH<sup>+</sup>-deoxyribosyl-CH<sub>3</sub>O<sub>3</sub> + H). The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the compound are presented in Table 1.

| Proton                  | δ (ppm)      | Multiplicity | $J_{ m H,H}$ | Carbon            | δ (ppm)      | HMBC               |
|-------------------------|--------------|--------------|--------------|-------------------|--------------|--------------------|
| H-8 (1H) <sup>a</sup>   | 8.27; 8.28   | s            |              | C-8 <sup>a</sup>  | 139.0; 139.1 | H-1′               |
| H-2 (1H) <sup>a</sup>   | 8.08; 8.09   | s            |              | C-2               | 149.2        | H-10a; H-10b       |
| H-10a (1H)              | 4.59         | br dd        | 13.5; 2.2    | C-10 <sup>a</sup> | 52.48; 52.51 | H-2; H-11          |
| H-10b (1H) <sup>a</sup> | 3.79; 3.81   | dd           | 13.5; 7.8    |                   |              | ,                  |
| H-11 (1H)               | 3.97         | br d         | 7.7          | C-11              | 68.4         | H-10b              |
| ( )                     |              |              |              | C-12              | 174.2        | H-11; H-10a; H-10b |
|                         |              |              |              | C-4 <sup>a</sup>  | 142.6; 142.7 | H-8; H-1'; H-2     |
|                         |              |              |              | C-5               | 121.9        | H-8                |
|                         |              |              |              | C-6               | 153.3        | H-2; H-10a; H-10b  |
| H-1′ (1H)               | 6.25         | t            | 6.8          | C-1′              | 84.0         | H-8: H-2'          |
| H-2' (1H)               | 2.62         | m            |              | C-2′ <sup>b</sup> |              | H-4′               |
| H-2" (1H)               | 2.29         | m            |              |                   |              |                    |
| H-3′ (1H)               | 4.39         | dt           | 6.5: 3.3     | C-3'a             | 70.82: 70.84 | H-1′               |
| H-4' (1H)               | 3.86         | dt           | 4.5          | C-4′              | 88.2         | H-1′               |
| H-5' (1H) <sup>a</sup>  | 3.595; 3.588 | dd           | 11.7: 4.3    | C-5'a             | 61.80: 61.81 |                    |
| H-5" (1H) <sup>a</sup>  | 3.513; 3.506 | dd           | 11.8: 4.6    |                   | ,            |                    |

**TABLE 1** <sup>1</sup>H and <sup>13</sup>C chemical shifts ( $\delta$ ), spin-spin coupling constants,  $J_{H,H}$  (Hz) of protons, and long-range C-H correlations (HMBC) in the 2'-deoxyadenosine adduct N1-GA-dA (in DMSO- $d_6$ )

<sup>a</sup>Separate shifts due to mixture of diastereomers.

<sup>b</sup>Signal overlapped by solvent.

| Proton     | δ (ppm) | Multiplicity | $J_{ m H,H}$   | Carbon            | δ (ppm) | HMBC       |
|------------|---------|--------------|----------------|-------------------|---------|------------|
| H-8 (1H)   | 8.15    | s            |                | C-8               | 139.6   |            |
| H-2 (1H)   | 8.07    | s            |                | C-2               | 152.0   |            |
| H-11 (1H)  | 4.26    | br s         |                | C-11 <sup>a</sup> |         |            |
| H-10a (1H) | 3.83    | br s         |                | C-10              | 44.5    |            |
| H-10b (1H) | 3.68    | br s         |                |                   |         |            |
| · · · ·    |         |              |                | C-12              | 178.9   |            |
|            |         |              |                | C-4               | 146.9   | H-2; H-8   |
|            |         |              |                | C-5               | 119.0   | H-8        |
|            |         |              |                | C-6               | 154.3   | H-2        |
| H-1′ (1H)  | 6.31    | t            | 6.7            | C-1′              | 84.5    | H-2′       |
| H-2' (1H)  | 2.70    | ddd          | 13.9; 6.7      | C-2'              | 39.1    |            |
| H-2" (1H)  | 2.48    | ddd          | 13.9; 6.1; 3.3 |                   |         |            |
| H-3′ (1H)  | 4.56    | dt           | 6.1; 2.9       | C-3′              | 71.2    | H-4'; H-5' |
| H-4' (1H)  | 4.11    | dt           | 3.4; 3.2       | C-4'              | 87.3    |            |
| H-5′ (1H)  | 3.78    | dd           | 12.7; 3.1      | C-5′              | 61.6    |            |
| H-5" (1H)  | 3.71    | dd           | 12.7; 4.2      |                   |         |            |

**TABLE 2** <sup>1</sup>H and <sup>13</sup>C chemical shifts ( $\delta$ ) and spin-spin coupling constants,  $J_{\rm H,H}$  (Hz) of protons, and long-range C-H correlations (HMBC) in the 2'-deoxyadenosine adduct N<sup>6</sup>-GA-dA (in D<sub>2</sub>O)

<sup>a</sup>Signal overlapped by C-3'.

N<sup>6</sup>-GA-dA had the following spectral characteristics: UV spectrum, UV<sub>max</sub> 270, 210 nm, UV<sub>min</sub> 230 nm (HPLC eluent: approximately 8% ACN in ammonium acetate buffer, pH 7). In the positive ion electrospray mass spectrum, the following ions were observed (m/z, relative abundance, formation): MS, 340 (100, MH<sup>+</sup>), MS<sup>2</sup> of 340, 224 (100, MH<sup>+</sup>-deoxyribosyl + H), MS<sup>3</sup> of 340→224, 136 (36, MH<sup>+</sup>-deoxyribosyl-C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>), 178 (100, MH<sup>+</sup>-deoxyribosyl-CH<sub>2</sub>O<sub>2</sub> + H), 207 (9, MH<sup>+</sup>-deoxyribosyl-H<sub>2</sub>O + H), MS<sup>4</sup> of 340→224→207, 178 (100, MH<sup>+</sup>-deoxyribosyl-CH<sub>3</sub>O<sub>2</sub> + H). The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the compound are presented in Table 2.

N1-GA-dI had the following spectral characteristics: UV spectrum, UV<sub>max</sub> 250, 206, UV<sub>min</sub> 226, with a shoulder between 266 and 294 nm (HPLC eluent: approximately 3% ACN in ammonium acetate buffer, pH 7). In the positive ion electrospray mass spectrum, the following ions were observed (m/z, relative abundance, formation): MS, 341 (20, MH<sup>+</sup>), 225 (100, MH<sup>+</sup>-deoxyribosyl + H), MS<sup>2</sup> of 225, 137 (100, MH<sup>+</sup>-deoxyribosyl-C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>), 179 (65, MH<sup>+</sup>-deoxyribosyl-CH<sub>2</sub>O<sub>2</sub>). The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the compound are presented in Table 3.

# Preparative-Scale Reaction of GA with 2'-Deoxyguanosine at pH 9. Preparation of N1-(2- Carboxy -2-hydroxyethyl)-2'-deoxyguanosine (N1-GA-dG I) and N1-(2-Carbamoyl-2-hydroxyethyl)-2'-deoxyguanosine (N1-GA-dG II)

GA (652 mg, 7.49 mmol) was reacted with 2'-deoxyguanosine (100 mg, 0.37 mmol) in 30 mL of 0.5 M phosphate buffer solution (pH 9.0). The reaction was performed at  $37^{\circ}$ C. The progress of the reaction was followed

| Proton   | $\delta$ (ppm)          | Multiplicity | $J_{ m H,H}$   | Carbon            | $\delta$ (ppm)    | HMBC                      |
|--|-------------------------|--------------|----------------|-------------------|-------------------|---------------------------|
| H-8 (1H) <sup>a</sup>                              | 8.291; 8.294            | s            |                | C-8 <sup>a</sup>  | 139.0; 139.1      | H-1′                      |
| H-2 (1H)   | 8.23                    | s            |                | C-2 <sup>a</sup>  | 149.5; 149.6      | H-10 <sup>a</sup> ; H-10b |
| H-10a (1H) <sup>a</sup><br>H-10b (1H) <sup>b</sup> | 4.54; 4.55<br>app. 3.58 | dd           | 13.3; 3.6      | C-10 <sup>a</sup> | 50.5; 50.6        | H-2                       |
| H-11 (1H)  | 3.74                    | br d         | 9.3            | C-11              | 69.0              | H-10 <sup>a</sup>         |
|  |                         |              |                | C-12              | 173.6             | H-10b                     |
|  |                         |              |                | C-4 <sup>a</sup>  | 147.27;<br>147.31 | H-2; H-8; H-1'            |
|  |                         |              |                | C-5 <sup>a</sup>  | 123.81;<br>123.87 | H-8                       |
|  |                         |              |                | C-6               | 156.1             | H-2; H-8                  |
| H-1′ (1H)  | 6.29                    | dd           | 7.1; 6.6       | C-1′              | 83.8              | H-2'; H-4'                |
| H-2′ (1H) <sup>a</sup>                             | 2.62; 2.64              | ddd          | 13.3; 7.3; 5.8 | C-2'c             |                   |                           |
| H-2" (1H)  | 2.29                    | ddd          | 13.3; 6.4; 3.4 |                   |                   |                           |
| H-3' (1H) <sup>a</sup>                             | 4.38; 4.39              | dt           | 2.8            | C-3'a             | 70.83; 70.85      | H-4'; H-1';<br>H-2'       |
| H-4' (1H) <sup>a</sup>                             | 3.854; 3.858            | dt           | 4.5; 2.8       | C-4′              | 88.2              | H-5'; H-5'';<br>H-2''     |
| H-5′ (1H) <sup>a</sup>                             | 3.585; 3.593            | dd           | 11.7; 4.5      | C-5′              | 61.8              |                           |
| H-5" (1H) <sup>a</sup>                             | 3.50; 3.51              | dd           | 11.7; 4.5      |                   |                   |                           |

**TABLE 3** <sup>1</sup>H and <sup>13</sup>C chemical shifts ( $\delta$ ) and spin-spin coupling constants,  $J_{H,H}$  (Hz) of protons, and long-range C-H correlations (HMBC) in the 2'-deoxyadenosine adduct N1-GA-dI (in DMSO- $d_6$ )

<sup>a</sup>Separate shifts due to mixture of diastereomers.

<sup>b</sup>Signal overlapped by H-5'.

<sup>c</sup>Signal overlapped by solvent.

by LC-DAD and LC-ESI-MS/MS analyses on the C18 analytical column. The reaction was stopped at optimum yield (6 days). The reaction mixture was concentrated by rotary evaporation to about 5 mL. The adducts were isolated from the mixture by chromatography on the semi-preparative column. The column was first eluted isocratically with 2% acetonitrile in ammonium acetate buffer (0.01 M, pH 7) in 3 minutes, and then with a gradient from 2 to 30% over the course of 17 minutes. The flow rate was 3 mL/minute. Fractions containing the adducts N1-GA-dG I and N1-GA-dG II were collected. The solutions containing the pure compounds were then combined and rotary evaporated to dryness and further dried under vacuum. The residues were subjected to spectroscopic and spectrometric studies. The isolated amounts of N1-GA-dG I and of N1-GA-dG II were 3.78 mg and 0.55 mg, respectively, and the molar yields were 4.86 and 0.68 mol%, respectively.

N1-GA-dG I had the following spectral characteristics: UV spectrum, UV<sub>max</sub> 254, 204 nm, UV<sub>min</sub> 224 nm with a shoulder between 270 and 286 nm (HPLC eluent: approximately 12% ACN in ammonium acetate buffer, pH 7). In the positive ion electrospray mass spectrum the following ions were observed (m/z, relative abundance, formation): MS, 356 (32, MH<sup>+</sup>), MS<sup>2</sup> of 356, 240 (100, MH<sup>+</sup> -deoxyribosyl + H), MS<sup>3</sup> of 356 $\rightarrow$ 240, 194 (100,

| Proton                 | $\delta$ (ppm) | Multiplicity | $J_{ m H,H}$   | Carbon            | $\delta$ (ppm) | HMBC                    |
|------------------------|----------------|--------------|----------------|-------------------|----------------|-------------------------|
| H-8 (1H)               | 7.93           | s            |                | C-8               | 135.5          | H-1′                    |
| H-10a (1H)             | 4.29           | broad        |                | C-10              | 47.5           |                         |
| H-10b (1H)             | 3.99           | broad        |                |                   |                |                         |
| H-11 (1H)              | 3.74           | broad        |                | C-11 <sup>b</sup> | 72.1           |                         |
|                        |                |              |                | C-12              | 171.5          |                         |
|                        |                |              |                | C-2               | 156.6          | H-8                     |
|                        |                |              |                | C-4               | 148.9          | H-8; H-1′               |
|                        |                |              |                | C-5               | 116.0          | H-8                     |
|                        |                |              |                | C-6               | 155.1          |                         |
| H-1′ (1H)              | 6.12           | dd           | 7.8; 6.1       | C-1′              | 82.3           | H-2';H-3'; H-4'         |
| H-2′ (1H) <sup>a</sup> |                |              | ,              | C-2′ a            |                |                         |
| H-2" (1H)              | 2.19           | ddd          | 13.0; 6.0; 3.2 |                   |                |                         |
| H-3' (1H)              | 4.34           |              |                | C-3′              | 71.2           | H-2'; H-4'; H-5'; H-5'' |
| H-4' (1H) <sup>c</sup> | 3.799; 3.804   |              | 4.7            | C-4'              | 87.5           |                         |
| H-5′ (1H)              | 3.55           | dd           | 11.7; 4.8      | C-5′              | 61.6           |                         |
| H-5" (1H)              | 3.49           | dd           | 11.7; 4.6      |                   |                |                         |

**TABLE 4** <sup>1</sup>H and <sup>13</sup>C chemical shifts ( $\delta$ ) and spin-spin coupling constants,  $J_{H,H}$  (Hz) of protons, and long-range C-H correlations (HMBC) in the 2'-deoxyguanosine adduct N1-GA-dG I (in DMSO- $d_6$ )

<sup>a</sup>Signal overlapped by solvent.

<sup>b</sup>Carbon signal from the HSQC spectrum.

<sup>c</sup>Separate shifts due to mixture of diastereomers.

 $MH^+$ -deoxyribosyl- $CH_2O_2$ ), 152 (100,  $MH^+$ -deoxyribosyl- $C_3H_5O_3$ ). The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the compound are presented in Table 4.

N1-GA-dG II had the following spectral characteristics: UV spectrum, UV<sub>max</sub> 254, 204 nm, UV<sub>min</sub> 222 nm with a shoulder between 270 and 286 nm (HPLC eluent: approximately 16% ACN in ammonium acetate buffer, pH 7). In the positive ion electrospray mass spectrum the following ions were observed (m/z, relative abundance, formation): MS, 355 (10, MH<sup>+</sup>), 239 (13, MH<sup>+</sup>-deoxyribosyl + H), MS<sup>2</sup> of 355, 239 (100, MH<sup>+</sup>-deoxyribosyl + H), MS<sup>3</sup> of 355 $\rightarrow$ 239, 222 (100, MH<sup>+</sup>-deoxyribosyl-NH<sub>3</sub> + H), 194 (100, MH<sup>+</sup>-deoxyribosyl-CH<sub>3</sub>NO), 152 (100, MH<sup>+</sup>-deoxyribosyl-C<sub>3</sub>H<sub>5</sub>NO<sub>2</sub>). The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the compound are presented in Table 5.

# Small-Scale Reactions of GA with 2'-deoxyadenosine and 2'-deoxyguanosine

Small-scale reactions were performed to find the reaction conditions giving the highest possible yield of the adducts. These conditions were then applied in the large scale synthesis. GA (69.6 mg, 0.80 mmol was reacted with 2'-deoxyadenosine (10 mg, 0.04 mmol) in 3 mL of 0.5 M phosphate buffer solutions at pH 4.6 and 7.0. The reactions were performed at 37°C. The

| Proton                  | $\delta$ (ppm) | Multiplicity | $J_{ m H,H}$   | Carbon <sup>a</sup> | δ (ppm) | COSY                        |
|-------------------------|----------------|--------------|----------------|---------------------|---------|-----------------------------|
| H-8 (1H)                | 7.90           | s            |                | C-8                 | 136.0   |                             |
| H-10a (1H)              | 3.63           | m            |                | C-10                | 44.6    | H-10b; H-11; OH-11          |
| H-10b (1H)              | 3.33           | m            |                |                     |         | H-10a; H-11; O <i>H-</i> 11 |
| H-11 <sup>b</sup>       | 4.02; 4.03     | dd           | 7.4; 4.2       | C-11                | 69.9    | H-10a; H-10b                |
| $NH_a$ (1H)             | 7.34           | s            |                |                     |         |                             |
| $NH_b$ (1H)             | 7.29           | s            |                |                     |         |                             |
| OH-11 (1H)              | 7.15           | br s         |                |                     |         | H-10a; H-10b                |
|                         |                |              |                | C-12                |         |                             |
|                         |                |              |                | C-2                 |         |                             |
|                         |                |              |                | C-4                 |         |                             |
|                         |                |              |                | C-5                 |         |                             |
|                         |                |              |                | C-6                 |         |                             |
| H-1′ (1H)               | 6.15           | t            | 6.9            | C-1′                | 82.7    | H-2'; H-2''                 |
| H-2′ (1H)               | 2.60           | m            |                |                     |         | H-1'; H-3'                  |
| H-2'' (1H)              | 2.21           | ddd          | 13.2, 6.7; 3.2 | C-2′                | 39.4    | H-1'; H-3'                  |
| H-3′ (1H)               | 4.36           | dt           | 2.9            | C-3'                | 71.0    | H-4′                        |
| H-4′ (1H) <sup>b</sup>  | 3.808; 3.811   | dt           | 4.8; 2.8       | C-4′                | 87.9    | H-5'; H-5''                 |
| H-5′ (1H) <sup>b</sup>  | 3.55; 3.56     | dd           | 11.6; 5.03     | C-5'                | 62.0    |                             |
| H-5'' (1H) <sup>b</sup> | 3.49; 3.48     | dd           | 11.6; 4.6      |                     |         |                             |
| OH (1H)                 | 6.67           | br s         |                |                     |         |                             |

**TABLE 5** <sup>1</sup>H and <sup>13</sup>C chemical shifts ( $\delta$ ) and spin-spin coupling constants,  $J_{\text{H,H}}$  (Hz) of protons, and H-H correlations (COSY) in the 2-deoxyguanosine adduct N1-GA-dG II (in DMSO- $d_6$ )

 $^{a}$ All carbon chemical shifts are based on C-H correlations, due to insufficient sample amount for recording a  $^{13}$ C spectrum.

<sup>b</sup>Separate shifts due to mixture of diastereomers.

progress of the reaction was followed daily by LC-DAD and LC-ESI-MS/MS analyses of aliquots of the reaction mixture using the C18 analytical column.

GA (65 mg, 0.75 mmol) was reacted with 2'-deoxyguanosine (10 mg, 0.037 mmol) in 3 mL of 0.5 M phosphate buffer solutions at pH 7.0 and 9.0. The reactions were performed at 37°C. The progress of the reactions was followed daily by LC-DAD and LC-ESI-MS/MS analyses of aliquots of the reaction mixtures using the C18 analytical column.

#### Rearrangement of N1-GA-dA to N<sup>6</sup>-GA-dA and to N1-GA-dI

A sample of N1-GA-dA (130  $\mu$ g) in 250  $\mu$ L of 0.5 M phosphate buffer (pH 7.0 and 9.0) was incubated at 37°C, and aliquots were analyzed at various times by LC-DAD and LC-ESI-MS/MS.

#### **Determination of Nucleoside Adduct Yields**

Quantitative <sup>1</sup>H NMR analysis, using chloroform as an internal standard was performed on the purified adducts. Standard solutions were prepared for HPLC by taking an exact volume of NMR sample and diluting with an appropriate volume of water. The quantitative determination of adducts in

the preparative scale reaction mixtures was made by comparing the peak area of the adducts in HPLC standard solutions with the peak area of the adducts in the reaction mixtures. The molar yields were calculated from the original amount of deoxynucleoside in the reaction mixture.

#### **RESULTS AND DISCUSSION**

### Reaction of GA with 2'-deoxyadenosine

A small-scale reaction was performed at various pH conditions to find out the conditions that give the optimal yields of the adducts. The highest yields of the compounds were obtained in the reaction carried out for 4 days at pH 7.0. At these conditions three major products were formed. In the LC-DAD chromatogram shown in Figure 1, the product peaks are marked N1-GA-dA, N1-GA-dI, and N<sup>6</sup>-GA-dA. When the reaction was performed at pH 4.6, only the adduct N1-GA-dA was formed.

For the determination of the structure of the compounds, a large-scale reaction was performed at pH 7.0. After 4 days of reaction, the compounds were isolated from the reaction mixture by semipreparative C18 column chromatography. The isolated yields of N1-GA-dA, N1-GA-dI, and N<sup>6</sup>-GA-dA were 8.0, 6.8, and 2.3 mol%, respectively. On the basis of data from NMR and UV spectroscopy and mass spectrometry, the structures of the adducts were assigned as N1-GA-dA, N1-GA-dI, and N<sup>6</sup>-GA-dA, respectively (Scheme 2).

The mass spectrometric and UV and <sup>1</sup>H NMR spectroscopic data recorded for N1-GA-dA were in all essential features identical to those presented by Gamboa da Costa et al.<sup>[8]</sup> In the work of Gamboa da Costa,



**FIGURE 1** C18 analytical column HPLC chromatogram of the reaction mixture of glycidamide and 2'-deoxyadenosine held at 37°C and pH 7.0 for 4 days. The chromatogram was recorded at 254 nm by the UV diode-array detector.

the <sup>1</sup>H NMR signals were not fully assigned, but in Table 1 all <sup>1</sup>H and <sup>13</sup>C NMR signals are assigned.

In the paper of Gamboa da Costa et al. it was found that the N1-GA-dA adduct was unstable when stored at pH = 13 and yielded a chromophore which was proposed to be N<sup>6</sup>-GA-dA (8). However, no further proof was provided for the structural assignment of the adduct. In our hands, the N<sup>6</sup>-dA adduct was found to be formed in the reaction of GA and dA (Figure 1). The compound was isolated from the reaction mixture and was structurally characterized by UV and NMR spectroscopy and mass spectrometry.

The UV spectrum of  $N^6$ -GA-dA exhibited absorption maxima at 270 and 210 nm and an absorption minimum at 230 nm (Figure 2). These values



**FIGURE 2** C18 analytical column HPLC chromatogram of the reaction mixture of glycidamide and 2'-deoxyguanosine held at 37°C and pH 9.0 for 6 days. The chromatogram was recorded at 254 nm by the UV diode-array detector.

are similar to the absorption maxima and minima for other reported N<sup>6</sup>-deoxyadenosine adducts.<sup>[10].</sup>

In the positive ion electrospray mass spectrum of N<sup>6</sup>-GA-dA, the protonated molecular ion peak was observed at m/z = 340 and was the most abundant ion. In the MS<sup>2</sup> spectrum, the ion peak recorded at m/z = 224corresponds to the cleavage of the deoxyribosyl moiety from the protonated molecular ion (followed by the attachment of a proton to N-9). Upon isolation and fragmentation of m/z = 224 (MS<sup>3</sup>), ion peaks were observed at m/z = 206, m/z = 178, and m/z = 136. These correspond to the loss of water, the loss of formic acid and to protonated adenine, respectively.

The NMR spectra of N<sup>6</sup>-GA-dA were recorded in D<sub>2</sub>O, since the resonance peaks did not resolve as well in Me<sub>2</sub>SO- $d_6$  as in D<sub>2</sub>O. The <sup>1</sup>H NMR spectrum of N<sup>6</sup>-GA-dA displayed, besides the signals from the protons of the deoxyribosyl moiety, two one-proton singlets at  $\delta = 8.15$  ppm and 8.07 ppm (Table 2). The protons were assigned to H-8 and H-2, respectively. The one-bond C-H correlation spectrum (heteronuclear multiple quantum coherence, HMQC) showed the carbon signals at  $\delta = 139.6$  and 152 ppm to be due to C-8 and C-2, respectively. Moreover, the spectrum showed one-proton broad singlets at  $\delta = 4.26$  ppm, 3.83 ppm and 3.68 ppm. The signals were assigned to H-11, H-10a, and H-10b, respectively. In the COSY spectrum, correlations between these signals could be observed. The HMQC spectrum showed the carbon signal at  $\delta = 44.5$  ppm to be due to C-10. A correlation between H-11 and C-11 also could be observed, but the carbon signal was overlapped by C-3'. When the N<sup>6</sup>-GA-dA spectrum was recorded in DMSO- $d_6$ , the signals for the H-8 and H-2 protons appeared at  $\delta = 8.31$  ppm and 8.20 ppm, respectively. These chemical shifts are in accordance with those reported for the H-2 and H-8 protons in N<sup>6</sup>-adducts recorded in  $Me_{2}SO-d_{6}$ .<sup>[11,12]</sup>

The UV spectrum of N1-GA-dI exhibited absorption maxima at 250 and 206 nm and an absorption minimum at 226 nm, with a shoulder between 266 and 290 nm (Figure 2). The UV spectrum of N1-GA-dI is consistent with the spectral shape maxima of other N1-inosine adducts.<sup>[13]</sup>

In the positive ion electrospray mass spectrum of N1-GA-dI, the protonated molecular ion peak was observed at m/z = 341. The product ion peak of m/z = 341 was observed at m/z = 225 and was due to the loss of the deoxyribosyl unit. This ion peak produced fragment peaks (MS<sup>3</sup>) at m/z =179 and m/z = 137, which correspond to the loss of formic acid and to protonated hypoxanthine, respectively (Scheme 3).

In the NMR spectra of N1-GA-dI (Table 3) some of the proton and carbon atoms were represented by two signals with only slight differences in chemical shifts, which show that two diastereomers of the compound were present in the sample. In the <sup>1</sup>H NMR spectrum, the resonance signals were observed at chemical shifts close to those of N1-GA-dA. The only exception was the one-proton signal for H-2 which was observed at  $\delta = 8.23$  ppm,



SCHEME 3 The ions observed in the positive electrospray mass spectrum and proposed fragment structures.

that is, 0.15 ppm more deshielded than in N1-GA-dA. This low field shift can be explained by the more deshielding effect of the carbonyl group in N1-GA-dI, compared with the exocyclic imino group in N1-GA-dA. The MS fragmentation pattern was identical to that of N1-GA-dA, but the fragment masses were one unit higher for the inosine adduct (Scheme 3).

Gamboa da Costa et al. reported that the N3-adenine adduct is formed in the reaction of deoxyadenosine and GA.<sup>[8]</sup> The LC-MS/MS analyses of our reaction mixtures showed the formation of a compound with the mass of the N3-adenine adduct (m/z = 223), but in our hands the compound was formed in yields too low for preparative isolation and subsequent structural characterization. However, when DNA is reacted with GA, N3-GA-Ade is one of the major adducts found in the reaction.<sup>[8]</sup> The reason for the difference in the reactivity of N3 of the adenine base between the site in the nucleoside and in DNA can be that the N3-position of adenine is not involved in hydrogen bonding in DNA and is sterically exposed in DNA. As a free nucleoside however, the N3-position may be blocked by the sugar group or by intra- or intermolecular bonding.<sup>[14]</sup>

It is well known that N1-substituted adenosines rearrange to N<sup>6</sup>-substituted adenosines through the Dimroth rearrangement. Simple N1alkyladenosines require an alkaline pH to undergo the rearrangement,<sup>[15]</sup> while various N1-(2'-hydroxy)alkyladenines may undergo Dimroth rearrangement at neutral pH.<sup>[16]</sup> This is consistent with our findings; the reaction of GA with 2'-deoxyadenosine was performed at pH 7 and the N<sup>6</sup>-GA-dA adduct was detected already after 1 day of reaction. As further proof for correct identification of the N<sup>6</sup>-adduct, we incubated the N1-GAdA adduct in a buffer solution at pH = 9.0, and the adduct was found to be completely transformed to the N<sup>6</sup>-adduct within 24 hours. This finding is consistent with other reports for N1-dA adducts of simple epoxides<sup>[17,18]</sup> and of the findings of Gamboa da Costa et al.<sup>[8]</sup>

The deamination of N1-GA-dA yielding the N1-inosine adduct seems to occur readily since the inosine adduct could be detected already after 1 day of reaction. To further study the conversion of N1-GA-dA to N1-GA-dI, N1-GA-dA was incubated in neutral buffer solution (pH 7.0). After 2 days of incubation, both the N1-GA-dI and N<sup>6</sup>-GA-dA could be observed. The deamination of N1-GA-dA could proceed through a similar 5-membered intermediate as was proposed by Barlow et al. when studying the deamination of the styrene oxide adduct 1-(2-hydroxy-1-phenylethyl)adenosine.<sup>[19]</sup> But in the case of N1-GA-dA the deamination also could proceed through a 6-membered lactone ring intermediate formed through displacement of the amino group by the carboxyl group and subsequently the lactone ring intermediate is opened by attack of water at the 6-position in the purine ring. Which of the mechanisms are responsible for the formation of the inosine adduct was not further explored.

## Reaction of GA with 2'-deoxyguanosine

A small-scale reaction was performed at various pH conditions to find out the conditions giving the optimal yields of the adducts. LC-DAD and LC-ESI-MS/MS analyses of the reaction carried out at pH 7.0 showed the formation of a single adduct which on the basis of the mass spectral data was identified as the previously known N7-GA-Gua adduct.<sup>[7,8]</sup> Analyses of the reaction performed at pH 9.0 revealed the formation of two major adducts, N1-GA-dG I and N1-GA-dG II (Figure 3). In this reaction the N7 adduct could not be detected.

For the purpose of determining the structure of the compounds, a large-scale reaction was performed at pH 9.0. After 6 days of reaction, the compounds were isolated from the reaction mixture by semipreparative C18 column chromatography. On the basis of data from NMR and UV spectroscopy and mass spectrometry, the structures of the adducts were assigned as N1-GA-dG I and N1-GA-dG II, respectively (Scheme 2). The isolated yields of the compounds were 3.8 and 0.6 mol%, respectively.

Identical UV spectra were recorded for the two adducts (Figure 4). Absorption maxima at 254 and 204 nm with a shoulder between 270 nm and 286 nm are features consistent with other N1-substituted guanosine adducts.<sup>[11]</sup>

In the positive ion electrospray mass spectrum of N1-GA-dG I, the protonated molecular ion peak was observed at m/z = 356. The product ion peak of m/z = 356 was observed at m/z = 240 and was due to the loss of the deoxyribosyl unit. This ion peak produced fragment peaks (MS<sup>3</sup>) at



FIGURE 3 UV absorbance spectra of N1-GA-dA, N<sup>6</sup>-GA-dA, and N1-GA-dI. The UV spectra were recorded with the diode-array detector as the compounds eluted from the column (N1-GA-dA: app. 2% ACN; N<sup>6</sup>-GA-dA: app. 3% ACN and N1-GA-dI: app. 8% ACN).

m/z = 194 and m/z = 152, which correspond to the loss of formic acid and to protonated guanine, respectively (Scheme 3).

The <sup>1</sup>H NMR spectrum of N1-GA-dG I displayed, besides the signals from the deoxyribosyl moiety, a one-proton singlet at  $\delta = 7.93$  ppm (Table 4). This signal was assigned to the H-8 of the guanosine unit. A strong correlation between this signal and a signal at  $\delta = 135.5$  ppm was observed in the HMQC spectrum. Consequently, this signal was assigned to C-8. Moreover, the spectrum showed broad singlets at  $\delta = 4.29$  ppm, 3.99 ppm, and 3.74 ppm. These signals were assigned as H-10a, H-10b, and H-11, respectively. Strong correlations between all these signals were observed in the COSY spectrum. On the basis of C-H correlations, the carbon signals at  $\delta = 47.5$  ppm and 72.1 ppm were assigned to C-10 and C-11. In the carbon spectrum a weak signal was observed at  $\delta = 171.5$  ppm. This was assigned to C-12. This low field chemical shift was similar to the chemical shifts of C-12 observed in the spectra of N1-GA-dA and N1-GA-dI. On the basis of the spectral data, the compound was assigned to N1-GA-dG I where the "GA tail" had been hydrolyzed to the corresponding carboxylic acid.

In the positive ion electrospray mass spectrum of N1-GA-dG II, the protonated molecular ion peak was observed at m/z = 355. In the MS<sup>2</sup> spectrum, the ion peak recorded at m/z = 239 corresponds to the cleavage of the deoxyribosyl moiety from the protonated molecular ion. Upon isolation and fragmentation of m/z = 239 (MS<sup>3</sup>), ion peaks were observed at m/z = 222, m/z = 194 and m/z = 152. These correspond to the loss of ammonia, the loss of formamide and to protonated guanine.



**FIGURE 4** UV absorbance spectra of N1-GA-dG I and N1-GA-dG II. The UV spectra were recorded with the diode-array detector as the compounds eluted from the column (N1-GA-dG I: app. 12% CAN and N1-GA-dG II: app.16% ACN).

Since some of the protons gave rise to two resonance peaks close to each other in the <sup>1</sup>H NMR spectrum, N1-GA-dG II was isolated as a mixture of two diastereomers (Table 5). The HPLC profile shows two close chromatographic peaks of similar size for the adduct, also an indication of the presence of two diastereomers. The <sup>1</sup>H NMR spectrum of N1-GA-dG II displayed, besides the signals from the deoxyribosyl moiety, a one-proton singlet at  $\delta = 7.90$  ppm. This signal was assigned to H-8 of the guanosine unit. Moreover, the spectrum showed two multiplets at  $\delta = 3.63$  and 3.33 ppm, and a doublet of doublets at  $\delta = 4.02$  ppm. All these signals correlated strongly in the COSY spectrum. The HSQC spectrum revealed that the multiplets were attached to the same carbon, and the carbon signal was at  $\delta = 44.6$  ppm. Consequently, the multiplet signals were assigned to H-10a and H-10b, respectively, and the doublet of doublets signal was assigned to H-11. A C-H correlation in the HSQC spectrum showed that the resonance signal at  $\delta = 69.9$  ppm was due to C-11. The proton signals at  $\delta =$ 7.29 and 7.34 ppm were assigned to the amide group.

The mass spectral data and the observation of the signals due to the amide protons in the <sup>1</sup>H NMR proved that the original amide function in the "GA tail" was present and the structure of the compound was N1-GA-dG II (Scheme 2).

Selzer and Elfarra have previously reported on an N1 adduct formed in the reaction of butadiene monoxide and guanosine.<sup>[20]</sup> In this reaction, the nucleophilic N1 reacted with the carbon adjacent to the double bond in butadiene monoxide. This gives an adduct with a branched butadiene monoxide tail on N1 of guanosine, instead of a straight butadiene monoxide tail. We wanted to rule out this possible branched structure for our N1-GA-dG adducts. Since no correlations could be observed between the H-10 protons and C-2 in the HMBC spectra for the two N1-GA-dG adducts (this correlation could be observed for N1-GA-dA and N1-GA-dI), we instead focused on the chemical shifts for C-10 and C-11. By comparing the chemical shifts for C-10 ( $\delta = 47.5$  ppm for N1-GA-dG I and  $\delta = 44.6$  ppm for N1-GA-dG II) and C-11 ( $\delta$  = 72.1 ppm for N1-GA-dG I and  $\delta$  = 69.9 ppm for N1-GA-dG II) with chemical shifts found in the literature for similar structures.<sup>[11]</sup> it was clear that our N1-GA-dG adducts had straight "GA-tails" and not branched "GA-tails." In a branched structure, C-10 adjacent to a hydroxyl group could not have such a low field chemical shift as  $\delta = 47.5$ ppm or  $\delta = 44.6$  ppm. Instead, this C-10 must be attached to N1 of dG, thus giving a straight "GA-tail."

#### The Hydrolysis of the Amide Function

We previously have reported on a cytidine adduct of GA where the amide function had been transformed to a carboxyl function.<sup>[9]</sup> In the current work we found that the major adducts formed, that is, the N1-GA-dA, the N<sup>6</sup>-GA-dA and the N1-GA-dG I adducts have the carboxyl function instead of the amide function. The formation of the adducts with a carboxyl function is surprising, since the amide group should not easily undergo hydrolysis.

In the reaction with dG, we found both the amide and the carboxyl adduct. Any conversion of the amide adduct to the carboxyl adduct could not be observed upon storage of the pure amide adduct for several days at pH 7 and pH 9. This observation shows that the hydrolysis of the amide function takes place before the epoxide ring of GA is attacked by N1 of guanosine.

In the work of Golding et al.<sup>[21]</sup> concerning the reaction of glycidaldehyde and guanosine, it was found that at pH 10 the initial reaction is an attack of the exocyclic amino group of guanosine on the carbonyl function in glycidaldehyde followed by a ring closure by attack of N1 on the epoxide ring. A similar sequence of reactions may also be valid for the GA reaction (Scheme 4), but in this case we end up with the N1 carboxyl adduct. The amide function is attacked by the exocyclic nitrogen and the resulting



**SCHEME 4** The proposed reaction mechanisms for the formation of the carboxyl function in adducts NI-GA-dG I and NI-GA-dA.

intermediate A loses ammonia and B is obtained. The N1 anion in the intermediate B (the  $pK_a$  of guanosine is approximately 9.4) attacks the less substituted carbon in the epoxide ring forming the cyclic intermediate C. This ring intermediate is hydrolysed and the ring is opened at N<sup>2</sup>, and the carboxyl adduct N1-GA-dG is formed. The cyclization is favored by basic conditions and seems not to take place at neutral conditions (only the N7 adduct was obtained at pH 7).

Upon LC-MS analyses of the dG reaction mixture, we could not find any evidence for the presence of the intermediates A, B or C. However, in the reaction mixture of dA and GA, a compound was present with  $[M+H]^+ = 322$  mass units (D in Scheme 4). An intermediate with this mass has previously been detected by Gamboa da Costa et al.<sup>[8]</sup> and Solomon<sup>[17]</sup> and has been tentatively assigned as a cyclic structure (E). We isolated the compound by collecting the peak when it eluted from the UV-detector and found it to be unstable in water (pH = 7, 25 and  $37^{\circ}C$ ) yielding dA and N1-GA-dA. The formation of dA shows that this intermediate cannot possess the cyclic structure depicted as E, since the bond between N1 and the exocyclic carbon should be stable. This argument is based on the fact that no dA was formed upon storage of N1-GA-dA in solution (pH 7) for several days, only Dimroth rearrangement yielding N<sup>6</sup>-GA-dA was observed. On the other hand, structure D for the intermediate explains our observations. D may be hydrolysed to dA or it can form the intermediate E, which in turn is hydrolysed to N1-GA-dA. The conclusion of this discussion is that the hydrolysis of the amide seems to take place through a transamidation mechanism originally proposed by us in the paper dealing with the identification of cytidine and thymidine adducts of GA.<sup>[9]</sup> Further support for the

transamidation was the observation that the <sup>1</sup>H NMR spectrum recorded of a  $D_2O$  solution of GA and *n*-propylamine showed that the amino function attacked the carbonyl group of GA and not the epoxide ring (data not shown). Although it could be expected that the exocyclic aminogroup in dA should be less reactive than the amino group in *n*-propylamine, it is obvious that the dA amino group attacks GA and that the attack is on the amide function.

The amide adducts, N1-GA-dG II, N7-GA-Gua,<sup>[7]</sup> and N3-GA-Ade<sup>[8]</sup> are most likely formed by direct attack of the nucleophilic endocyclic nitrogens on the oxirane ring of GA.

## CONCLUSIONS

In this study, we fully characterized the major adducts formed in the reaction of GA with 2'-deoxyadenosine at physiological conditions, and the adducts formed in the reaction of GA with 2'-deoxyguanosine at pH 9.0 and 37°C.

We also propose a plausible mechanism for the hydrolysis of the amide function. The mechanism is supported by the identification of the degradation products of the intermediate in the dA reaction and by NMR studies of the reaction product of *n*-propylamine and GA. The adducts N7-GA-Gua, N3-GA-Ade and N1-GA-dG II are most likely formed through a direct attack of the endocyclic N1 in the purine on the  $\beta$ -carbon in the oxirane ring, in the case of N1-GA-dA and N1-GA-dG I the exocyclic aminogroups of dA and dG attack the carbonyl carbon in GA and the attack is followed by deamination and ring closure through reaction of the oxirane ring with the nucleophilic ring nitrogen of dA and/or dG. N<sup>6</sup>-GA-dA and N1-GA-dI are formed from N1-GA-dA through Dimroth rearrangement and a succeeding deamination, respectively.

The adducts characterized in this work may be used as reference compounds in studies aiming at the identification of adducts in laboratory animals exposed to GA.

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