# Synthesis of antiviral compounds. Preparation and rearrangement of 6-methoxyglyceropurines

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The synthesis of 6-methoxypurine (**4**) and 6-methoxyguanine (**11** and **12**) derivatives of the glyceropurine series is described. The N-7 guanine derivatives rearrange to the N-9 isomers under certain conditions. The 6-methoxyguanine derivative **12** was found to have a remarkably specific activity against equine herpesvirus.

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On décrit les synthèses des méthoxy-6 purine (**4**) et méthoxy-6 guanines (**11** et **12**) dérivées de la série de la glycéropurine. Sous certaines conditions, les dérivés de la N-7 guanine se transposent en isomères N-9. On a trouvé que le dérivé **12** de la méthoxy-6 guanine possède une activité spécifique remarquable contre le virus herpès de cheval.

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## Introduction

In our development of the "glycerosides" as a novel class of nucleoside analogues (1–5) having antiviral activity, we have found that some of the glyceropurines have powerful antiherpes activity. In particular, the guanine derivative 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (BIOLF-62) has perhaps the strongest and widest activity against herpesviruses of any reported compound (6–8). The 6-chloroguanine derivative also has significant activity against herpesviruses (4). Because substitution at C-6 of other purine nucleosides has led to a range of biological activity (4,9,10), we sought selective changes at C-6 in the glyceropurine series. In this report we wish to describe the preparation of the 6-methoxypurine and 6-methoxyguanine compounds **4**, **11**, and **12**. In the latter case we have found that the N-7 isomer rapidly rearranges to the more stable N-9 isomer under specific conditions. The 6-methoxy compounds **4**, **11**, and **12** have been tested for activity against a range of herpesviruses with only the N-9 guanine derivative **12** having activity and, in this case, only against equine HSV-2.

The 6-chloropurine (**1**) and 6-chloroguanine (**6**, **7**) derivatives were chosen as starting materials. Alkaline displacement of chlorine at C-6 of purines has led to mixed results, according to the literature. In some reports, ring opening has been the major result (11–14) while in others smooth displacement of chlorine has been observed (15–17). The general trend indicates that 9-alkyl-6-chloropurines are smoothly converted under alkaline conditions, while the nucleosides of 6-chloropurines often lead to ring opening (18). Conversion of 6-methoxy to 6-hydroxypurines with aqueous base has been established (11).

The 6-chloropurine **1** (4) was converted to the 6-methoxypurine **2** in 93% yield using sodium methoxide in methanol for 30 min at room temperature. Interestingly, the same result is obtained when **1** is treated with sodium hydroxide in methanol–water at room temperature for 15 min. However, if the latter reaction is carried out at reflux temperature the hypoxanthine derivative **3** is produced. Compound **2** can be converted to **3** on refluxing with sodium hydroxide in methanol–water. Both compounds, **2** and **3**, are easily deprotected to **4** and **5** respectively using boron trichloride solution.

In the preparation of the 6-chloroguanine derivative a mixture of the N-7 (**6**) and N-9 (**7**) isomers is obtained (ref. 4, ratio 2:3 by nmr). The mixture can be separated at this stage or (and generally more easily) after conversion of the chlorine. The 6-chlorine of the guanine derivative (2-amino-6-chloropurine derivative) is more difficult to displace than in the 6-chloropurine **1**. However, after reflux with sodium methoxide in methanol for 1.5 h, the 6-methoxy products **8** and **9** were obtained in virtually the same ratio as the starting mixture.

When the mixture of **6** and **7** was heated at reflux with sodium hydroxide in methanol–water (1.5 h) only the N-9 guanine derivative **10** was obtained. Furthermore, pure **6** (the N-7 isomer) yielded only the N-9 isomer **10** when subjected to the same conditions. Pure compound **8** and pure compound **9** are each completely converted to the guanine derivative **10** under reflux with sodium hydroxide in methanol–water.

The pure 6-methoxy derivative **9** was smoothly deprotected using boron trichloride (19) giving only the guanine derivative **12**. The N-7 isomer **8** was more sensitive to the conditions used. While tlc indicated that only the N-7 isomer **11** was produced during the actual reaction with boron trichloride, the product was affected by work-up conditions. With heating or during concentration of the reaction mixture, **11** was converted to **12** to varying degrees. Once isolated, **11** was quite stable and was unaffected by most solvents including hot ethanol. However, if after the removal of solvents during the work-up of the reaction the residue was heated in ethanol, significant conversion of **11** to the N-9 isomer occurred. Complete conversion of **11** to **12** could be achieved in this manner. It has previously been reported that the N-7 isomers of purine derivatives can be converted to the more stable N-9 isomers on heating in the presence of salts (20, 21). Pure compound **11** is stable in ethanolic sodium hydroxide (0.05 *N*) for at least 1.5 h at room temperature. However, pure **11** is rapidly converted to the N-9 isomer **12** in 0.05 *N* HCl in ethanol (aqueous) at room temperature.

The new compounds described in this manuscript were tested against a number of herpesviruses including human HSV-1, HSV-2, and CMV, canine herpesvirus, feline herpesvirus, bovine herpesvirus type 4, and equine herpesvirus type 1. In general, **4**, **11**, and **12** were inactive against all of these viruses at a level less than 100 μg/mL. However, the exception was compound **12** which was uniquely active against equine herpesvirus with an ED-50 of 2.8 μg/mL.

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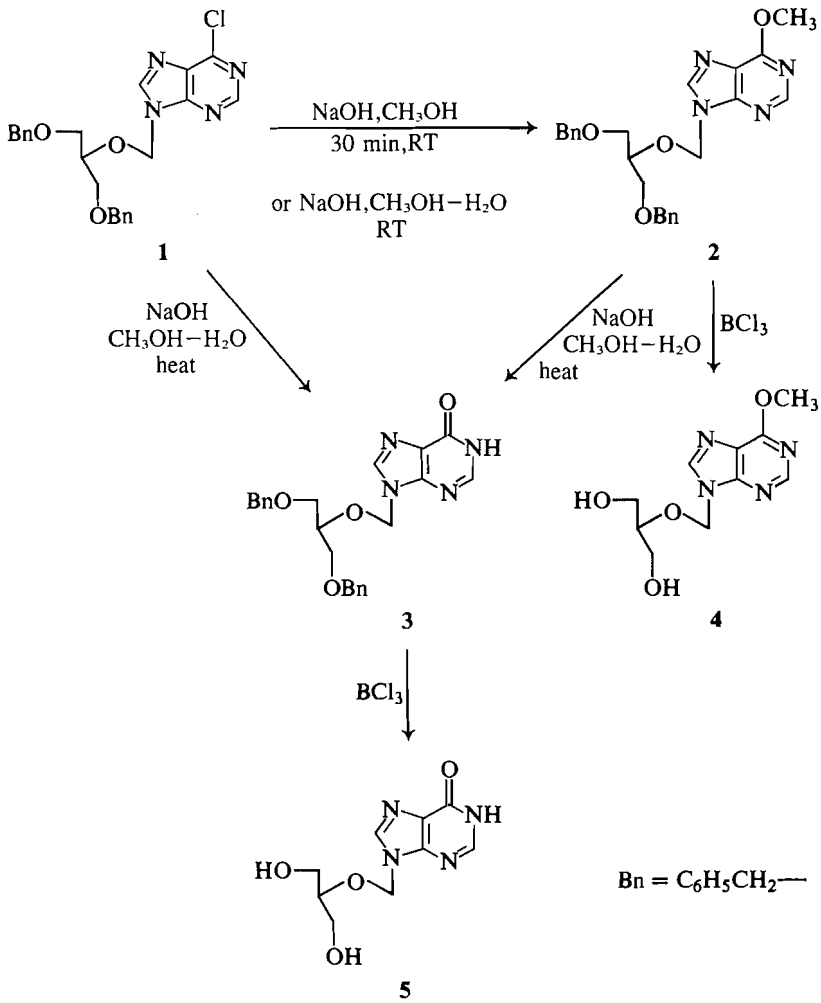



SCHEME 1

## Experimental

*General methods*

Products from preparative scale reactions were isolated by column chromatography using Merck silica gel 60 (230–400 mesh) packed in glass columns (15 g of silica per gram of crude material). Thin-layer chromatographic data ($R_f$ values) are recorded from Merck Kieselgel 60F254 analytical sheets. Purine bases were purchased from Sigma Chemical Co. The uv spectra were recorded on a Cary 17 spectrometer. Nuclear magnetic resonance spectra were recorded using a Bruker WH-90, a Varian XL-200, and a Varian T60A spectrometer.

The 6-chloropurine derivatives 9-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-chloropurine **1** and the N-7 and 9-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-2-amino-6-chloropurines **6** and **7** were prepared as previously described (4). Compounds **6** and **7** were obtained as a mixture in a ratio of ~2:3 as judged by nmr (4).

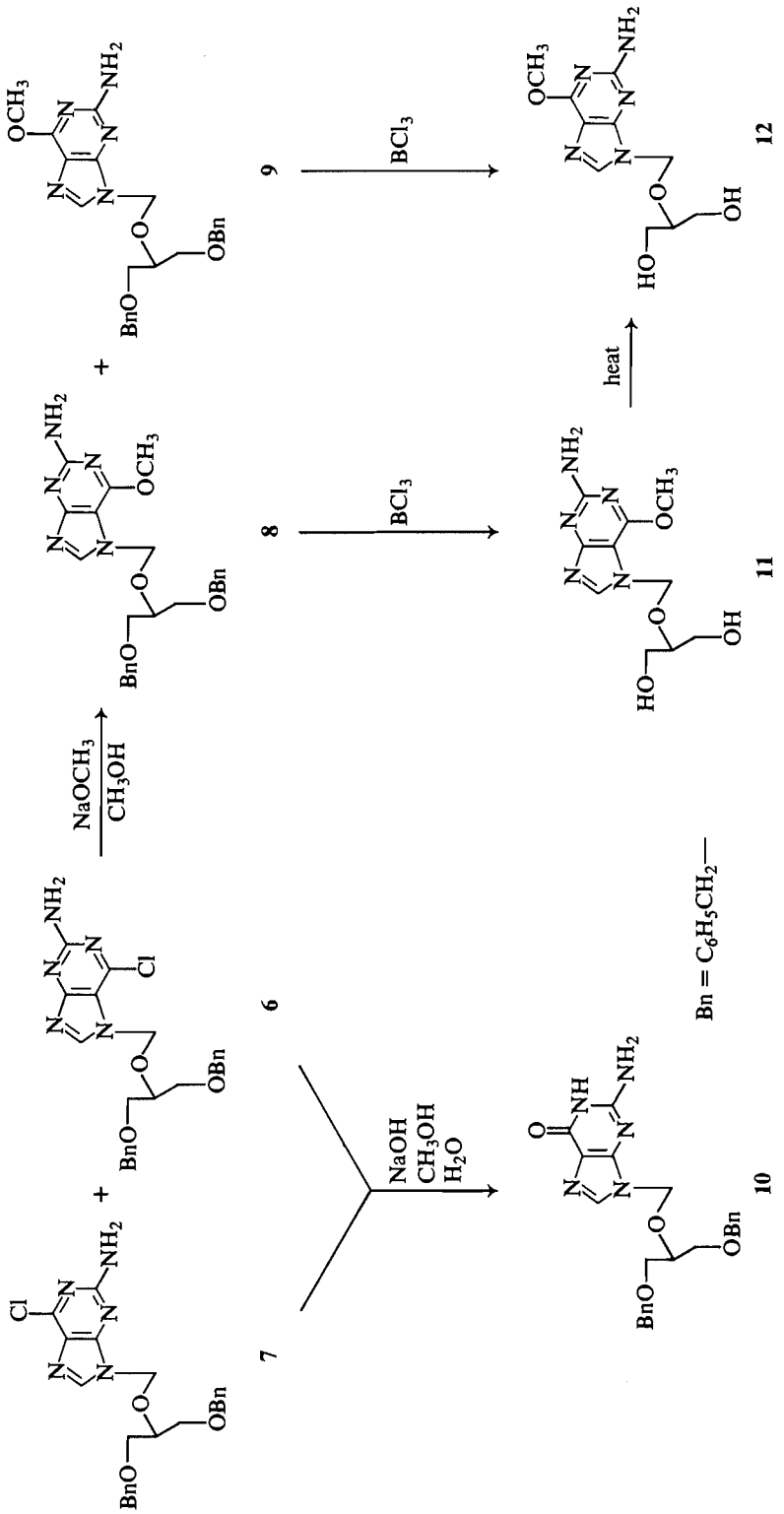
*9-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-methoxypurine 2*

A. Compound **1** (1 g) was dissolved in methanol (25 mL) containing sodium (0.3 g). The solution was stirred at room temperature for 30 min at which point tlc (EtOAc–hexane, 5:2) indicated complete conversion of **1** ($R_f$ 0.5) to a new compound of $R_f$ 0.2. The solvent was removed at reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed twice with water followed by drying over magnesium sulfate. The solution was collected by filtration and concentrated. The residue was chromatographed over silica gel eluted with ethyl acetate – hexane (5:7). The product **2** was obtained in 93% yield (0.92 g). The $^1$Hpmr spectrum of **2** in DMSO-$d_6$ showed signals (δ, ppm) at 3.49 (m, 4H), 4.0 (m, 1H), 4.17 (s, 3H, $OCH_3$), 4.43 (s, 4H), 5.8 (s, 1H), 7.28 (10H, aromatic), 8.05 (s, 1H), and 8.56 (s, 1H). The uv characteristics for **2** were $\lambda_{max}$ in nm(ε) at pH 1: 247 (8200); pH 7: 247 (8800); pH 13: 247 (8500).

B. Compound **1** (0.5 g) was dissolved in methanol (38 mL) to which water (4 mL) and sodium hydroxide (10.5 g) were added. After 5 min at room temperature tlc showed complete conversion of **1** to **2**. After a total of 15 min the solution was diluted with cold water (20 mL) and the solution was carefully neutralized (with cooling) with HCl (3*N* solution). The product was extracted into ether which was dried over magnesium sulphate. Evaporation of the ether gave 0.48 g (97%) of remarkably pure **2**.

*9-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-6-hydroxypurine 3*

A. Compound **1** (1 g, 2.3 mmol) was treated with sodium hydroxide (21 g) in methanol (75 mL) to which water (9 mL) had been added. A tlc taken immediately showed only a compound having the same $R_f$ as **2**. The solution was heated at reflux for 1 h at which point tlc indicated the presence of a single spot of $R_f$ 0.06 (ethyl acetate – methanol, 10:0.1). The solution was concentrated at reduced pressure, the residue was dissolved in water, and the solution was neutralized with acetic acid whereupon a precipitate formed. The precipitate was collected by filtration and was allowed to crystallize from ethyl acetate to yield **3** (0.57 g, 60%), mp 131–132°C. Compound **3** had uv properties ($\lambda_{max}$ in nm (ε)) as follows: pH 1, 247 (9100); pH 7, 247 (10 100); pH 13, 253 (10 700). The $^1$Hpmr spectrum in DMSO-$d_6$ showed signals at δ(ppm) of 3.42 (m, 4H), 4.03 (m, 1H), 4.38 (s, 4H), 5.64 (s, 1H), 7.24 (10H, aromatic), 8.06 (s, 1H), and 8.21




SCHEME 2

(s, 1H). The $^{13}C$ spectrum of **3** (DMSO-$d_6$) showed signals at δ(ppm) of 148.47 (C-2), 145.99 (C-4), 124.14 (C-5), 156.73 (C-6), 140.6 (C-8), 72.25 (C-1′), 76.94 (C-3′), 69.71 (C-4′), and 72.25 (—$CH_2\phi$). *Anal.* calcd. for $C_{23}H_{24}N_4O_4$: C 65.70, H 5.75, N 13.32; found: C 65.52, H 5.64, N 13.34.

Compound **3** was further characterized by treatment with boron trichloride in the standard way (ref. 4, see below) to give the known 6-hydroxypurine derivative **5** in 60% isolated yield. The properties of **5** were identical to those of an authentic sample (4).

B. Compound **2** (1.3 g) was heated in methanol (75 mL) containing water (9 mL) and sodium hydroxide (21 g) at reflux for 30 min. The reaction mixture was worked up as above to yield **3** in 60% yield. No other uv-absorbing material was detected.

*9-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-6-methoxypurine **4***

Compound **2** (0.9 g) was dissolved in dichloromethane (50 mL, spectrograde) and boron trichloride (8 mL, 1 *N* in dichloromethane) was added to the solution maintained at −78°C under a nitrogen atmosphere. A tlc showed that after 1 h all of **2** ($R_f$ 0.7, methanol − ethyl acetate (1:3)) had been converted to a new compound of $R_f$ 0.3. The solution was then neutralized with triethyl amine in methanol and the pH was raised to between 8 and 9. The solvents were removed at reduced pressure and the residue was chromatographed on silica gel (eluting first with hexane (500 mL), ethyl acetate (1 L), and ethyl acetate − methanol (18:1, 2 L). The product **4** was collected and after crystallization from methanol−dichloromethane pure crystals of **4** were obtained (70%, mp 135−136°C). The uv properties showed $\lambda_{max}$ (nm, (ε)) at pH 1: 247 (11 100); pH 7: 247 (12 000); pH 13: 247 (12 100). The $^1$Hpmr spectrum of **4** in DMSO-$d_6$ showed signals at δ(ppm) of 3.31 (m, 4H), 3.58 (m, 1H), 4.08 (s, 3H, —$OCH_3$), 4.58 (t, 2H, —OH), 5.72 (s, 1H), 8.48 (s, 1H), and 8.55 (s, 1H). The $^{13}C$ spectrum (DMSO-$d_6$) showed signals at δ(ppm) of 152.19 (C-2), 151.87 (C-4), 120.53 (C-5), 160.34 (C-6), 144.05 (C-8), 72.25 (C-1′), 80.88 (C-3′), 60.97 (C-4′), and 53.96 (—$OCH_3$). *Anal.* calcd. for $C_{10}H_{14}N_4O_4$: C 47.24, H 5.55, N 22.04; found: C 47.17, H 5.64, N 22.01.

*Conversion of **6** and **7** to the guanine derivative **10***

A. A mixture of **6** and **7** (5.5 g, 2:3 by $^1$Hpmr) was dissolved in methanol (300 mL) to which water (21 mL) and sodium hydroxide (84 g) were added. The solution was heated at reflux and after 1.5 h, tlc (methanol − ethyl acetate − dichloromethane, 1:3:3) showed that the starting material ($R_f$ 0.75) had been completely converted to material having $R_f$ 0.3. The solution was concentrated at reduced pressure and the residue was dissolved in water. The solution was neutralized with acetic acid whereupon a precipitate formed that was collected by filtration. The material gave, after crystallization from ethanol−water, 3.8 g (70%) of **10**, which was identical in all respects (mp, $^{13}C$, $^1$Hpmr, $R_f$, uv) to authentic material previously described (2,4)).

B. Pure N-7 isomer **6** (280 mg) was subjected to the above conditions. The sole product was compound **10** isolated in 95% yield (256 mg).

*Conversion of the mixture of **6** and **7** into the 6-methoxyguanine derivatives **8** and **9***

A mixture of **6** and **7** (2.5 g, 2:3 by $^1$Hpmr) was added to a solution of sodium (0.7 g) in methanol (50 mL). The solution was heated at reflux for 1.5 h at which point tlc (ethyl acetate − hexane, 3:1) showed the starting material ($R_f$ 0.5) had been completely converted to two materials having $R_f$ 0.3 and 0.1. The solvent was removed at reduced pressure and water (50 mL) was added. The pH was adjusted to ~5.5 with acetic acid. The product was extracted into dichloromethane. After drying over magnesium sulfate the solvent was evaporated to leave a dark syrup (2.56 g) which was chromatographed on silica gel. The column was eluted with ethyl acetate − hexane (1:1, 1 L; 2:1, until the first product was completely eluted; 3:1, to collect the second product). The first product, **9**, had $R_f$ 0.3 in ethyl acetate − hexane (3:1) and was obtained as a viscous oil. The material crystallized from ethyl acetate − hexane (mp 83−84°C, 0.87 g, 44%). Compound **9** had $\lambda_{max}$ (nm (ε)) at pH 1: 289 (8600), 244 (8600); pH 7: 282 (10 100), 247 (11 300); pH 13: 282 (9700), 247 (11 300). The $^1$Hpmr spectrum in $CDCl_3$ showed signals at 3.49 (m, 4H), 3.97 (m, 1H), 4.05 (s, 3H), 4.44 (s, 4H), 5.60 (s, 2H), 4.92 ($NH_2$), 7.26 (10H, aromatic), 7.7 (s, 1H). The $^{13}C$ spectrum (DMSO-$d_6$) showed signals at δ(ppm) of 160.72 (C-2), 154.24 (C-4), 113.78 (C-5), 160.08 (C-6), 139.79 (C-8), 71.60 (C-1′), 76.56 (C-3′), 69.66 (C-4′), 53.09 (—$OCH_3$), and 72.19 (—$CH_2\phi$). *Anal.* calcd. for $C_{24}H_{27}N_5O_4$: C 64.13, H 6.05, N 15.58; found: C 64.42, H 6.18, N 15.32.

The second fraction ($R_f$ 0.1) was obtained as a viscous oil (**8**, 0.53 g, 27%). Compound **8** had $\lambda_{max}$ (nm (ε)) at pH 1: 288 (6600), 247 (7200); pH 7: 283 (7200), 253 (9900); pH 13: 283 (6800), 253 (9700). The $^1$Hpmr spectrum in $CDCl_3$ showed signals at 3.5 (m, 4H), 3.96 (s, 3H), 4.05 (m, 1H), 4.44 (s, 4H), 5.65 (s, 2H), 6.27 ($NH_2$), 7.23 (10H, aromatic), 7.7 (s, 1H). *Anal.* calcd. for $C_{24}H_{27}N_5O_4$: C 64.13, H 6.05, N 15.58; found: C 63.96, H 5.95, N 15.41.

*Conversion of **9** to **10***

Compound **9** (0.5 g) was dissolved in methanol (30 mL) and water (3 mL) and sodium hydroxide (10.5 g) were added. The solution was heated at reflux for 1.5 h whereupon tlc showed complete conversion to **10**. The solvents were removed at reduced pressure and the residue was dissolved in water (80 mL). The solution was acidified with acetic acid whereupon a precipitate formed. The solid was collected by filtration, washed with water, and dried to yield 0.41 g (85%) of pure **10**.

*Conversion of **8** to **10***

The above procedure was repeated using 400 mg of **8**. Compound **8** was converted to **10** in 88% isolated yield.

*Conversion of **8** to 7-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-2-amino-6-methoxypurine (**11**)*

Compound **8** (500 mg) was treated with boron trichloride in the usual manner. After 1 h, tlc (methanol − ethyl acetate, 1:2) showed complete conversion of **8**, $R_f$ 0.7, to a new material of $R_f$ 0.2. The solution was neutralized with triethyl amine in methanol and the pH was adjusted to 8. The solvent was removed at reduced pressure to give a solid that was dissolved in methanol. Dichloromethane was added and a fine precipitate was obtained. This material was identified as compound **11** (0.17 g, 57%, mp 211−212°C). The mother liquor contained both **11** and **12**.

In a repeat of the above reaction the solid obtained directly from the reaction was dissolved in ethanol and allowed to stand; tlc showed that **11** was completely converted to **12** with time and the process was hastened by heating the solution. However, once isolated pure, **11** is stable on heating in ethanol.

Compound **11** showed λ max (nm (ε)) at pH 1: 287 (5900), 246 (6800); pH 7: 283 (7200), 253 (9700); pH 13: 283 (7100), 253 (9700). The $^1$Hpmr spectrum of **11** in DMSO-$d_6$ showed signals at 3.33 (m, 4H), 3.6 (m, 1H), 3.98 (s, 3H), 4.62 (OH), 5.58 (s, 2H), 7.40 ($NH_2$), and 8.01 (s, 1H). *Anal.* calcd. for $C_{10}H_{15}N_5O_4 \cdot \frac{1}{2}H_2O$: C 43.16, H 5.79, N 25.17; found C 43.37, H 5.66, N 24.91.

*Stability of **11** in acid and base*

Compound **11** was dissolved in (a) 0.05 *N* HCl in ethanol (aqueous) and (b) 0.05 *N* NaOH in ethanol (aqueous). Both solutions were stirred at room temperature. After 1.5 h compound **11** had been completely converted to **12** under the acid conditions (a) but was unaffected by the basic conditions (b).

*Conversion of **9** to **12***

Compound **9** (3 g) was treated with boron trichloride solution in the normal manner. The product was isolated pure following chromatography on silica gel using methanol − ethyl acetate (1:6). Compound **12** (mp 167−168°C) was obtained in 63% yield (1.1 g). Compound **9** showed λ max (nm (ε)) at pH 1: 288 (5800), 245 (6100); pH 7: 282 (6600), 247 (7500); pH 13: 282 (6500), 247 (7700). The $^1$Hpmr spectrum in DMSO-$d_6$ showed signals δ(ppm) at 3.35 (m, 4H), 3.54 (m, 1H), 3.97 (s, 3H), 4.62 (OH), 5.52 (s, 2H), 6.48 ($NH_2$), and 7.98 (s, 1H). The $^{13}C$ spectrum (DMSO-$d_6$) showed signals at δ (ppm)

of 160.88 (C-2), 154.35 (C-4), 113.78 (C-5), 160.18 (C-6), 140.11 (C-8), 71.65 (C-1′), 80.28 (C-3′), 61.02 (C-4′), and 53.31 (—$OCH_3$). *Anal.* calcd. for $C_{10}H_{15}N_5O_4 \cdot \frac{1}{2}H_2O$: C 43.16, H 5.79, N 25.17; found C 43.65, H 5.71, N 25.34.

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