# Direct C-Glycosylation of Guanine Analogues: The Synthesis and Antiviral Activity of Certain 7- and 9-Deazaguanine *C*-Nucleosides

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C-Glycosylation of two guanine analogues, 9-deaza- and 7-deazaguanine, has been achieved under Friedel–Crafts conditions, providing a direct synthetic route to 9-deazaguanosine (**4**; 2-amino-7-β-D-ribofuranosyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-4(3*H*)-one) and 8-β-D-ribofuranosyl-7-deazaguanine (**16**), respectively. This electrophilic C-glycosylation was applied successfully to six guanine and substituted-guanine analogues resulting in yields of approximately 50%. This represents the first reported C-ribosylation of preformed nitrogen heterocycles isosteric with guanine. These *C*-nucleosides were evaluated for their ability to provide protection against a lethal Semliki Forest virus infection in mice, relative to 7-thia-8-oxoguanosine which was used as a positive control. Two of the *C*-nucleosides, 2-amino-6-chloro-5-methyl-7-β-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**12**) and the corresponding 6-bromo derivative (**13**), showed good prophylactic activity in this virus model system.

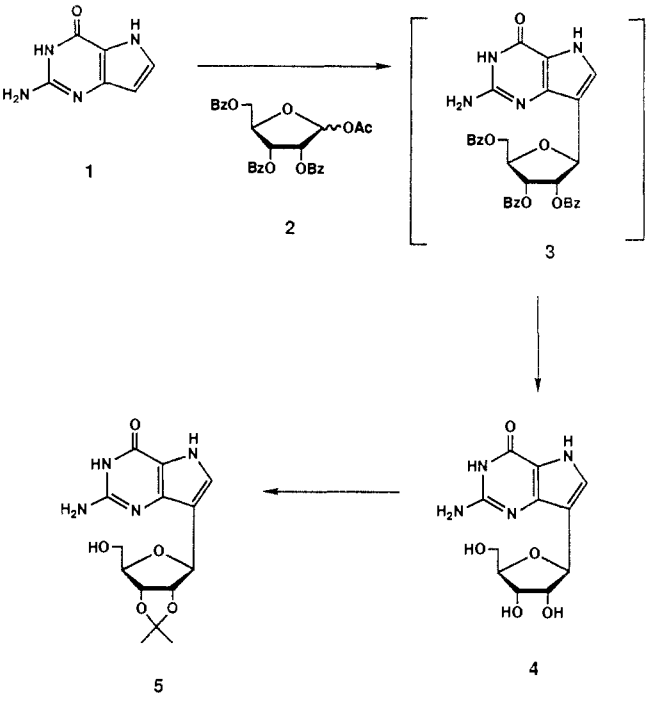
The biological importance of several naturally occurring *C*-nucleosides[1] such as the antibiotics formycin,[2] formycin B,[3] pseudouridine,[4] showdomycin,[5] pyrazomycin,[6] and oxazinomycin[7] has generated considerable interest over the years in the chemistry and biochemistry of this unusual class of nucleosides. This group of carbon-to-carbon linked nucleosides is stable to cleavage by purine nucleoside phosphorylase[8-11] and several exhibit anticancer[12] and broad-spectrum antiviral activity[13] in addition to their antibiotic activity. These biological properties have encouraged us to develop shorter and more versatile synthetic routes to purine-type *C*-nucleosides of this class.

To date, the synthetic approaches for the preparation of *C*-nucleosides fall into four categories:[14] (1) elaboration of the aglycon moiety on a suitable acyclic *C*-glycoside; (2) transformation of naturally occurring *C*-nucleosides; (3) total synthesis from non-carbohydrate materials; (4) direct attachment of a carbohydrate moiety to a preformed heterocycle. This latter method has been used to prepare naturally occurring pseudouridine[15] and some of its sugar analogues,[16] 5-ribosylcytosine[17] and 1-deazauridine,[18] by an organometallic procedure. However, the overall yields by this approach are extremely low (0.2–3%). A few simple heterocycles have been directly C-glycosylated under Friedel–Crafts conditions, such as furan[19] and thiophene.[20] Most rcently, Rao and co-workers reported the glycosylation of a functionalized thiophene which was subsequently converted to a novel inosine analogue.[21] However, there are no reports of any direct C-glycosylation studies using preformed fused nitrogen heterocycles, particularly those isosteric with guanine.

As part of our ongoing program to design and synthesize guanosine analogues as potential immunotherapeutic agents, we investigated a direct Lewis acid catalyzed C-glycosylation of preformed guanine-like heterocycles, namely, 9-deaza- and 7-deazaguanine and certain substituted derivatives thereof. We report here a much simplified synthesis of 9-deazaguanosine (**4**), the hydrochloride salt of which was first prepared by Klein and co-workers[22] by a multistep procedure beginning with an appropriate ribofuranose derivative which was ring closed to a substituted pyrrole *C*-nucleoside, subsequently cyclized to a pyrrolopyrimidine, and finally, deprotected to provide the desired guanosine analogue.

In addition, application of this C-glycosylation procedure to 7-deazaguanine resulted in the formation of 8-ribo-

**Scheme I**




furanosyl-7-deazaguanine. These deazaguanines constitute two examples in fused pyrrole ring systems in which there

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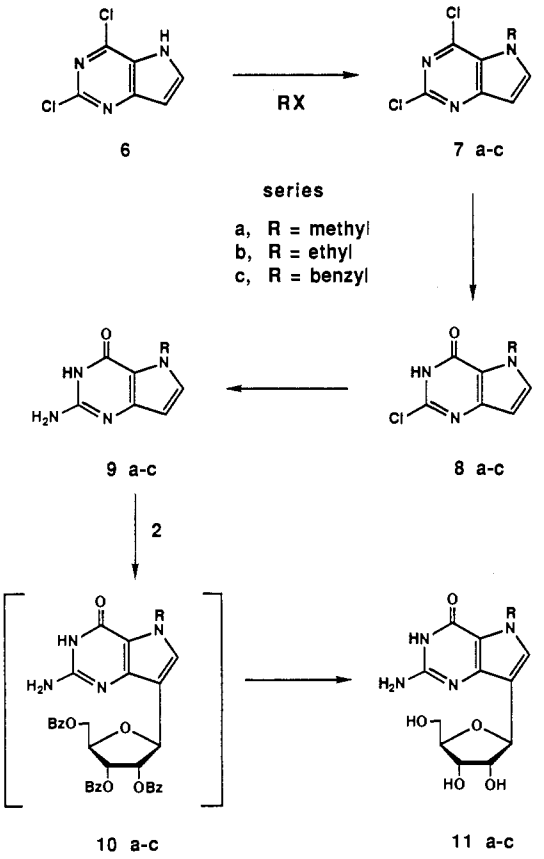
**Scheme II**



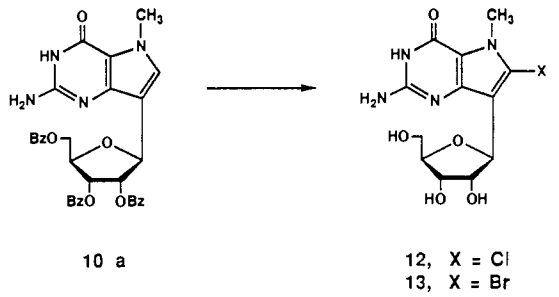

appears to be sufficient electron density at the pyrrole carbon atoms to allow the direct electrophilic C-glycosylation to occur.

Selected guanosine analogues were evaluated for their ability to protect mice against a lethal challenge of Semliki Forest virus.

## Chemistry

Treatment of 9-deazaguanine[23,24] (1) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (2) in nitromethane at 60 °C in the presence of $SnCl_4$ gave the desired $\beta$ anomer, 2-amino-7-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**3**), in 45% yield after chromatographic purification (Scheme I). Interestingly, no evidence of any $\alpha$ isomer was found in the reaction mixture. The remainder of the reaction products appeared to be non-nucleoside material as judged by proton NMR. Deprotection of **3** using sodium methoxide provided 9-deazaguanosine (2-amino-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo-[3,2-*d*]pyrimidin-4(3*H*)-one, 4) in 75% yield. The structure of 4 was assigned, in part, on the basis of $^1$H NMR spectroscopy in which there was an absence of the $C_7$H proton signal at 5.94 ppm (DMSO-$d_6$) of the heterocycle 1 (site of glycosylation) and the presence of a doublet ($C_{1'}$H) at 4.64 ppm, characteristic of the anomeric signal of a *C*-glycoside. In addition, the ultraviolet spectrum of 4 was essentially identical with the spectrum reported for the hydrochloride salt of 9-deazaguanosine.[22] Conversion of 4 to its isopropylidene derivative **5** using 2,2-dimethoxypropane allowed the configuration of this series to be assigned as $\beta$ by $^{13}$C NMR. The spectrum of **5** revealed two singlets at 25.49 and 27.52 ppm for the isopropylidene methyl groups, with a $\Delta\delta$ of 2.03 ppm. These data support the $\beta$ assignment, in agreement with observations derived from previous studies aimed at determining the anomeric configuration of *C*-nucleosides wherein the chemical shifts for $\beta$ anomers occur at 25.5 ± 0.2 and 27.5 ± 0.2 ppm while those of the $\alpha$ anomers occur at 24.9 ± 0.3 and 26.3 ± 0.2 ppm,[25] and the corresponding $\Delta\delta$ values for these same methyl groups have been found in almost all cases to be 1.90 ± 0.20 for the $\beta$ anomer and 1.25 ± 0.20 ppm for the $\alpha$ anomer.[26]

**Scheme III**




Several $N_7$-substituted 9-deazaguanosines were then prepared for which 2,4-dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine[24,27] (**6**) served as the starting material (Scheme II). Treatment of **6** with sodium hydride in acetonitrile and then with methyl iodide yielded 2,4-dichloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (**7a**) in 86% yield. Reaction of **7a** with sodium hydroxide provided 2-chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**8a**) in 94% yield. Amination of **8a** using methanolic ammonia at 160 °C gave the guanine analogue 2-amino-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**9a**) in 67% yield. This alkylguanine analogue was C-glycosylated, by the same procedure as that used to prepare **3**, to provide the benzoyl-protected *N*-7-methyl-9-deazaguanosine (**10a**, 2-amino-5-methyl-7-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one) in 47% yield after purification. Deprotection of **10a** using sodium

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**Table I.** Effects of Deazaguanine *C*-Nucleosides against a Semliki Forest Virus Infection in Mice

| compound | dose,[a] mg/kg | survivors/ total (%) | mean survival time,[b] days |
|---|---|---|---|
| | Experiment 1 | | |
| placebo[c] | | 2/12 (8) | 6.8 ± 2.1[d] |
| **4** | 100 | 1/12 (8) | 7.3 ± 1.6 |
| **11a** | 100 | 1/12 (8) | 8.1 ± 2.4 |
| 7-thia-8-oxoguanosine | 100 | 9/12 (75) | 8.3 ± 1.5 |
| | Experiment 2 | | |
| placebo | | 3/12 (25) | 7.7 ± 2.4 |
| **12** | 100 | 10/12 (83)[e] | 11.0 ± 1.4[f] |
| 7-thia-8-oxoguanosine | 100 | 11/12 (92)[e] | 10.0 ± 0.0 |
| | Experiment 3 | | |
| placebo | | 1/12 (8) | 7.5 ± 2.0 |
| **13** | 100 | 7/12 (58)[e] | 7.0 ± 1.9 |
| **19** | 100 | 0/12 (0) | 7.2 ± 1.7 |
| 7-thia-8-oxoguanosine | 100 | 11/12 (92) | 9.0 ± 0.0 |

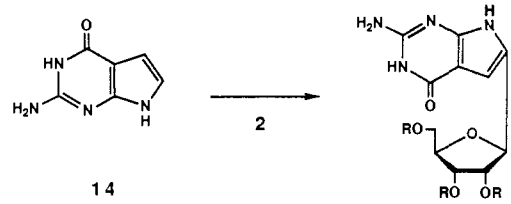
[a] Half-daily doses administered 24 and 18 h before virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] A 2% sodium bicarbonate solution served as the placebo in all experiments and as diluent for the compounds. [d] Standard deviation. [e] Statistically significant ($p < 0.02$), determined by the two-tailed Fisher exact test. [f] Statistically significant ($p < 0.05$), by two-tailed $t$ test.

methoxide furnished the alkylguanosine analogue 2-amino-5-methyl-7-β-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**11a**).

Two other alkylguanosine analogues were prepared in addition to the methyl derivative **11a**, namely the ethyl (**11b**) and benzyl (**11c**) derivatives. These compounds were synthesized by the same general procedure as that used to prepare **11a** in the five-step process starting with **6**, but with the appropriate alkyl halides (ethyl iodide or benzyl bromide) instead of methyl iodide. The critical step in this process, the C-glycosylation, was accomplished in remarkably good yield for both the ethyl (65%) and benzyl (66%) derivatives.

Because of our interest in immune potentiation[28] and the known immunoactivity of 8-bromoguanosine[29] and resultant antiviral activity of 7-methyl-8-oxoguanosine,[28,30] we considered it of great interest to prepare the 8-chloro and 8-bromo derivatives of **11a**, in which there is now the combination of a methyl at the 7-position and a halogen at the 8-position (purine numbering). Thus, chlorination of **10a** with sulfuryl chloride in dichloromethane followed by deprotection using sodium methoxide provided a 73% overall yield of 2-amino-6-chloro-5-methyl-7-β-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (**12**, see Scheme III). Likewise, bromination of **10a** using *N*-bromosuccinimide in dichloromethane followed by deprotection yielded the corresponding bromo derivative (**13**) in 48% overall yield.

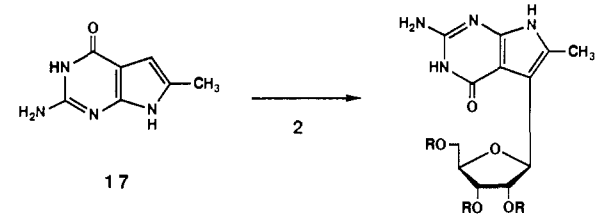
The other pyrrolopyrimidine isomer, 7-deazaguanine[31] (**14**), was glycosylated under the same conditions as described above to yield the 8-ribofuranosyl analogue 2-amino-6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**15**), in 56% yield. Treatment of **15** with sodium methoxide furnished 2-amino-6-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-(3*H*)-one (**16**) in 78% yield. The assignment of structure was again based on $^1$H NMR studies of **16**. It is interesting



**14** **15**, R = Bz; **16**, R = H

to note that, while one might predict that the electron density in this guanine analogue would favor the formation of the 7-glycoside rather than the observed 8-isomer, the presence of a 6-oxo function in the 7-deazapurines has been reported to direct electrophilic substitutions to the 8-position rather than the 7-position.[32]

It was of interest to determine if direct glycosylation would proceed in the 7-deazaguanine molecule if the 8-position were occupied by a group such as methyl. Thus, reaction of 8-methyl-7-deazaguanine[33] (**17**) with **2** under



**17** **18**, R = Bz; **19**, R = H

the C-glycosylation conditions provided benzoyl-protected nucleoside **18**, in 47% yield after purification. Deprotection of **18** furnished 8-methyl-7-deazaguanosine (**19**, 2-amino-6-methyl-5-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one. The structural assignment of **19** was based upon proton NMR and verified by single-crystal X-ray analysis.[34] It thus appears that, in the 7-deazaguanine molecule, electrophilic C-glycosylation under the conditions described here occurs preferentially at the 8-position, unless that position is unavailable, in which case the glycosylation will proceed at the 7-position (at least in the case where the group at the 8-position is methyl).

The reaction conditions reported here for the glycosylation of these guanine analogues are representative of conditions which have been optimized to some extent. For example, several solvents (1,2-dichloroethane, acetonitrile, nitromethane, etc.), Lewis acid catalysts ($BF_3$–$Et_2O$, $SnCl_4$, $TiCl_4$, etc.) and reaction temperatures (0–100 °C) were examined in various combinations and it was observed that $SnCl_4$ in nitromethane at 60 °C for about 3 h was most suitable in terms of greater yields and cleaner reaction products.

The utility of the direct C-glycosylation reaction to provide much shorter routes to complex *C*-nucleosides would appear to be limited to those heterocycles in which there is relatively high electron density on the ring carbon atoms. The guanine analogue in the pyrazolo[4,3-*d*]pyrimidine and the pyrazolo[1,5-*a*]-*s*-triazine ring systems could not be C-glycosylated under these reaction conditions. It is likely, however that certain thiophene and furan condensed systems will also undergo C-glycosylation.

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## Antiviral Studies

Selected guanosines were tested in vivo for their ability to provide protection against a lethal Semliki Forest virus infection in mice. The results of this study are shown in Table I and indicate that, at a dose of 100 mg/kg per day given intraperitoneally in half-daily doses for 1 day, only the halogenated 9-deazaguanosines **12** and **13** protected a significant number of mice (83 and 58%, respectively). The simple 9-deazaguanosine (**4**) and its 7-methyl derivative (**11a**) were inactive. Indeed, none of the compounds tested were as active against the Semliki Forest virus as 7-thia-8-oxoguanosine,[28] included in these studies as a positive control. In addition, none of the guanosines showed significant in vitro activity against this virus, suggesting that in the case of **12** and **13**, protection was conferred by immunopotentiation rather than by direct antiviral properties. It is interesting to note the structural features that appear to be required to potentiate the immune system sufficiently to provide protection against this viral infection. The simple substitution of a group such as halogen in the pyrrole ring at the 6-position (equivalent to purine 8-position) of the pyrrolo[3,2-*d*]pyrimidine results in such potentiation. Attachment of the carbohydrate to the equivalent 7-position with a methyl at position 8, such as in the case of **19** (a 7-ribosyl-7-deazaguanine), resulted in no immunopotentiation. Interestingly, evaluation of 7-deazaguanosine,[35] an *N*-nucleoside isomer of 9-deazaguanosine (**4**), in the same animal virus model revealed that it provides excellent protection for the mice and possesses potent interferon-inducing properties.[36] In this case, no substituent at the 8-position is required for this type of immunopotentiation. Other guanosines are being evaluated in order to clarify the structure–activity relationships of these guanosines.

While the detailed mechanism of action of these guanosines is not fully clear, it is likely that induction of interferon is a major factor in the observed antiviral prophylaxis. The induction of interferon and other factors may be the result of cellular activation via the interaction of these compounds with guanine nucleotide binding proteins (G proteins), as studies from our laboratories would suggest.[37,38]

## Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus or on a Haake-Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded with an IBM NR300AF spectrometer at 300.1 MHz for $^1$H and 75.46 MHz for $^{13}$C. All proton NMR were run in DMSO-$d_6$. The chemical shifts are expressed in $\delta$ values (parts per million) relative to the residual signal in the deuterated solvent as reference: DMSO proton signal set at 2.50 ppm and center resonance $^{13}$C signal for DMSO set at 39.50 ppm. Ultraviolet spectra (UV, sh = shoulder) were recorded on a Beckman DU-50 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Evaporations were carried out under reduced pressure with the bath temperature below 40 °C. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM reagents). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. HPLC purity determinations were done using a Waters 600 solvent delivery system equipped with a Waters 990 photodiode array detector and a Beckman Ultrasphere 5-$\mu$m reversed-phase column (4.6 × 250 mm).

**2-Amino-7-$\beta$-D-ribofuranosylpyrrolo[3,2-*d*]pyrimidin-4-(3*H*)-one (4; 9-Deazaguanosine).** To a suspension of 2-aminopyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one[23,24] (**1**, 9-deazaguanine; 1.5 g, 10 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**2**; 6.0 g, 12 mmol) in dry nitromethane (50 mL) at 60 °C was added stannic chloride (4.0 g, 1.8 mL, 15 mmol). The mixture was maintained at 60 °C for 3 h, cooled, and poured into cold, saturated, aqueous sodium bicarbonate solution (250 mL). The insoluble material was filtered off and washed with ethyl acetate (2 × 50 mL). The filtrate was extracted with ethyl acetate (2 × 150 mL), and the combined washings and extract were washed with water (1 × 200 mL), dried ($Na_2SO_4$), and evaporated to dryness. Purification by flash silica gel column chromatography (dichloromethane–methanol, 14:1) yielded benzoylated product **3** (2.6 g, 45%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 5.33 (d, $J$ = 4.7 Hz, 1 H, $C_{1'}H$), 5.96 (s, 2 H, $NH_2$ exchangeable), 7.27 (d, $J$ = 2.8 Hz, 1 H, $C_6H$), 7.41–8.04 (m, 15 H, benzoyls), 10.51 (s, 1 H, $N_3H$ exchangeable), 11.61 (s, 1 H, $N_5H$ exchangeable) and other sugar protons. To a suspension of **3** (1.2 g, 2 mmol) in dry methanol (50 mL) was added sodium methoxide until the pH reached about 13. The mixture was stirred overnight at room temperature, neutralized with glacial acetic acid, and evaporated to dryness. The residue was subjected to flash chromatography using dichloromethane–methanol (2:1) to give **4** as a colorless solid (0.42 g, 75%): mp >300 °C; UV $\lambda_{max}$ (pH 1) 232 nm ($\epsilon$ 14 540), 271 (12 380); UV $\lambda_{max}$ (pH 7) 229 nm ($\epsilon$ 19 020), 267 (7340); UV $\lambda_{max}$ (pH 11) 230 nm ($\epsilon$ 16 000), 259 (5500), 285 (5750); $^1$H NMR $\delta$ 4.64 (d, $J_{1'2'}$ = 6.9 Hz, 1 H, $C_{1'}H$), 5.73 (s, 2 H, $NH_2$ exchangeable), 7.13 (d, $J_{NH,C_6H}$ = 2.7, 1 H, $C_6H$), 10.51 (br s, 1 H, NH exchangeable), 11.41 (s, 1 H, NH exchangeable). Anal. ($C_{11}H_{14}N_4O_5$) C, H, N.

**2-Amino-7-(2,3-*O*-isopropylidene-$\beta$-D-ribofuranosyl)-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (5).** To an ice-cold suspension of **4** (0.1 g, 0.35 mmol) in a mixture of acetone (6 mL) and 2,2-dimethoxypropane (9 mL) was added perchloric acid (0.66 mL) dropwise. The resulting mixture was stirred at 0 °C for 30 min and then neutralized with 1 N NaOH while cold (less than 10 °C). The mixture was then evaporated to dryness and the residue was purified by flash chromatography on silica gel using dichloromethane–methanol (4:1) to give **5** (50 mg, 45%): $^1$H NMR $\delta$ 1.28 and 1.48 (2 s, 3 H each, isopropylidene), 4.82 (d, $J$ = 5.1 Hz, 1 H, $C_{1'}H$), 5.98 (br, 2 H, $NH_2$ exchangeable), 7.22 (d, $J$ = 2.7 Hz, $C_6H$), 11.47 (br, 1 H, NH exchangeable); $^{13}$C NMR (DMSO-$d_6$, 75.5 MHz) $\delta$ 25.49 and 27.52 (2 s, isopropylidene methyl carbons).

**2,4-Dichloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (7a).** To a solution of 2,4-dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine[24,27] (**6**, 3.76 g, 20 mmol) in dry acetonitrile (100 mL) was added sodium hydride (0.88 g, 22 mmol, 60% in oil) and the mixture was stirred at room temperature for 30 min. Methyl iodide (3.4 g, 1.5 mL, 24 mmol) was added to the mixture at 0–5 °C and the whole was stirred at this temperature for an additional 15 min and then allowed to warm to room temperature with stirring overnight. The resulting mixture was filtered through a Celite pad and evaporated to dryness. Ether was added to the residue and the sodium iodide which separated was filtered off. The filtrate was evaporated to dryness and the residue was crystallized from benzene–hexanes (5:1) to give 3.5 g (86%) of **7a**: mp 165–166 °C. Anal. ($C_7H_5Cl_2N_3$) C, H, N, Cl.

**2,4-Dichloro-5-ethyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (7b).** This compound was prepared as described above for methyl derivative **7a** with ethyl iodide instead of methyl iodide. After stirring overnight, the mixture was evaporated to dryness and water (200 mL) was added to the residue. The solid obtained was collected by filtration, washed with water, and dried to give 4.2 g (97%): mp 110–112 °C. Anal. ($C_8H_7Cl_2N_3$) C, H, N.

**5-Benzyl-2,4-dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine (7c).** This compound was prepared as in **7b** except that benzyl bromide was used to alkylate **6**. Workup as in **7b** yielded **7c** (5.1 g, 92%): mp 116–118 °C. Anal. ($C_{13}H_9Cl_2N_3$) C, H, N, Cl.

**2-Chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (8a).** A solution of the dichloro compound **7a** (3.03 g, 15 mmol)

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in dioxane (30 mL) was added to a boiling sodium hydroxide solution (30 mL, 2 N) and the mixture was stirred at reflux for 1 h. After cooling, the mixture was diluted with water and neutralized with glacial acetic acid. After cooling the mixture in ice, the precipitate obtained was collected by filtration and crystallized from ethyl acetate to give **8a** (2.6 g, 94%): mp 228–229 °C. Anal. ($C_7H_6ClN_3O$) C, H, N, Cl.

**2-Chloro-5-ethyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (8b).** This compound was prepared from **7b** as described for **8a** above to obtain **8b** after crystallization from ethanol (3.5 g, 88%): mp 221–222 °C; $^1H$ NMR $\delta$ 1.33 (t, 3 H, $CH_3$ of ethyl group), 4.34 (q, 2 H, $CH_2$ of ethyl), 6.30 (d, $J$ = 3.0 Hz, 1 H, $C_7H$), 7.49 (d, $J$ = 3.0 Hz, 1 H, $C_6H$), 12.82 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_8H_8ClN_3O$) C, H, N, Cl.

**5-Benzyl-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (8c).** Compound **8c** was prepared from **7c** as described above for **8a**. The resulting solid was crystallized from ethanol to yield 4.3 g (83%) of **8c** as a colorless solid: mp 234–235 °C; $^1H$ NMR $\delta$ 5.57 (s, 2 H, $CH_2$), 6.37 (d, $J$ = 2.9 Hz, 1 H, $C_7H$), 7.22–7.32 (m, 5 H, benzyl aromatics), 7.59 (d, $J$ = 2.9, 1 H, $C_6H$), 12.88 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_{13}H_{10}ClN_3O$) C, H, N, Cl.

**2-Amino-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (9a).** A mixture of **8a** (1.84 g, 10 mmol) and methanolic ammonia (50 mL, saturated at 0 °C) was heated in a steel bomb at 160 °C for 12 h. After cooling, the excess ammonia was allowed to evaporate in the fume hood. The resulting solid was filtered and crystallized from methanol to give 1.1 g (67%) of **9a**: mp >278 °C dec; $^1H$ NMR $\delta$ 3.85 (s, 3 H, $CH_3$), 5.87 (d, $J$ = 2.6 Hz, 3 H, $C_7H$ and $NH_2$ exchangeable), 7.10 (d, $J$ = 2.6 Hz, 1 H, $C_6H$), 10.40 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_7H_8N_4O$) C, H, N.

**2-Amino-5-ethyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (9b).** This compound was prepared from **8b** as described under **9a** above. Crystallization of the residue from ethanol gave 1.4 g (78%) of **9b** as a colorless solid: mp 262–264 °C; $^1H$ NMR $\delta$ 1.29 (t, 3 H, $CH_3$ of ethyl), 4.23 (q, 2 H, $CH_2$ of ethyl), 5.84 (s, 2 H, $NH_2$ exchangeable), 5.88 (d, $J$ = 2.8 Hz, 1 H, $C_7H$), 7.18 (d, $J$ = 2.8 Hz, 1 H, $C_6H$), 10.53 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_8H_{10}N_4O$) C, H, N.

**2-Amino-5-benzyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (9c).** The title compound was prepared from **8b** as described for **9a** above. The residue was crystallized from ethanol to yield 1.9 g (80%) of **9c**: mp 276–278 °C; $^1H$ NMR $\delta$ 5.46 (s, 2 H, $CH_2$), 5.86 (s, 2 H, $NH_2$ exchangeable), 5.95 (d, $J$ = 2.7 Hz, 1 H, $C_7H$), 7.19–7.32 (m, 6 H, $C_6H$ and benzyl aromatics), 10.53 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_{13}H_{12}N_4O$) C, H, N.

**2-Amino-5-methyl-7-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (10a).** The glycosylation of **9a** (1.65 g, 10 mmol) was performed according to the procedure described for the preparation of **3**. Following chromatographic purification using dichloromethane–acetone (4:1) and then dichloromethane–methanol (14:1), a 47% yield (2.6 g) of **10a** was obtained as a colorless foam. $^1H$ NMR $\delta$ 3.78 (s, 3 H, $CH_3$), 5.31 (d, $J$ = 5.4 Hz, 1 H, $C_{1'}H$), 5.90 (s, 2 H, $NH_2$ exchangeable), 7.21 (s, 1 H, $C_6H$), 7.31–8.01 (m, 15 H, benzoyl aromatics), 10.64 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_{33}H_{28}N_4O_8$) C, H, N.

**2-Amino-5-ethyl-7-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (10b).** This ethyl derivative was prepared from **9b** (1.78 g, 10 mmol), as in **3**, in 65% yield (4.0 g) after purification to a colorless foam. This material was used directly for deprotection.

**2-Amino-5-benzyl-7-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (10c).** This benzyl derivative was prepared from **9c** (2.4 g, 10 mmol) in 66% yield (4.5 g) according to the procedure for intermediate **3**. Intermediate **10c** was used directly for deblocking.

**2-Amino-5-methyl-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (11a).** Deprotection of **10a** (3.0 g, 5 mmol) in dry methanol (100 mL) was carried out as decribed for **4**. The crude product was purified by flash chromatography on silica gel using dichloromethane–methanol (4:1) to provide **11a** in 81% yield (1.2 g): mp >264 °C dec; $^1H$ NMR $\delta$ 3.83 (s, 3 H, $CH_3$), 4.62 (d, $J$ = 7.1 Hz, 1 H, $C_{1'}H$), 5.80 (s, 2 H, $NH_2$ exchangeable), 7.12 (s, 1 H, $C_6H$). Anal. ($C_{12}H_{16}N_4O_5$) C, H, N.

**2-Amino-5-ethyl-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]-pyrimidin-4(3*H*)-one** (11b). Compound **10b** (1.25 g, 2 mmol) was deblocked according to the general procedure employed to obtain **4**. The yield of colorless product **11b** was 80% (0.5 g) after chromatographic purification using dichloromethane–methanol (4:1): mp >262 °C dec; $^1H$ NMR $\delta$ 1.29 (t, 3 H, $CH_3$), 4.21 (q, 2 H, $CH_2$ of ethyl), 4.61 (d, $J$ = 7.2 Hz, 1 H, $C_{1'}H$), 5.72 (s, 2 H, $NH_2$ exchangeable), 7.22 (s, 1 H, $C_6H$), 10.53 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_{13}H_{18}N_4O_5$) C, H, N.

**2-Amino-5-benzyl-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (11c).** Intermediate **10c** (1.3 g, 2 mmol) was deprotected with sodium methoxide (see **4**). Yield after the usual purification was 79% (0.59 g): mp >184 °C dec (109 °C softens); $^1H$ NMR $\delta$ 4.61 (d, $J$ = 7.1 Hz, 1 H, $C_{1'}H$), 5.43 (s, 2 H, $CH_2$ of benzyl), 5.82 (s, 2 H, $NH_2$ exchangeable), 7.22–7.33 (m, 6 H, $C_6H$ and benzyl aromatics), 10.62 (br, 1 H, $N_3H$). Anal. ($C_{18}H_{20}N_4O_5$) C, H, N.

**2-Amino-6-chloro-5-methyl-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (12).** To a solution of **10a** (3.0 g, 5 mmol) in dry dichloromethane (50 mL) cooled to 0–5 °C was added sulfuryl chloride (5.5 mL, 5.5 mmol, 1 M solution in $CH_2Cl_2$). The stirred mixture was kept at 0–5 °C for 30 min and then allowed to warm to room temperature and stirred for an additional 6 h. The mixture was then poured in a saturated aqueous sodium bicarbonate solution (500 mL) and extracted with ethyl acetate (3 × 500 mL). The extract was washed with water (1 × 500 mL), dried ($Na_2SO_4$), and evaporated to dryness to provide 3.0 g (93%) of benzoylated product. This intermediate (2.6 g, 4 mmol) was deprotected as described in **4** and upon purification of the resulting crude product by flash chromatography using dichloromethane–methanol (5:1), provided 1.05 g (79%) of **12** as a colorless solid: mp 232–233 °C; $^1H$ NMR $\delta$ 3.85 (s, 3 H, $CH_3$), 4.65 (d, $J$ = 7.9 Hz, 1 H, $C_{1'}H$), 5.77 (s, 2 H, $NH_2$ exchangeable), 10.76 (br, 1 H, $N_3H$ exchangeable). Anal. ($C_{12}H_{15}ClN_4O_5$) C, H, N, Cl.

**2-Amino-6-bromo-5-methyl-7-$\beta$-D-ribofuranosyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-4(3*H*)-one (13).** To a solution of **10a** (5.6 g, 10 mmol) in 1,2-dichloroethane (200 mL) cooled to 0–5 °C was added with stirring *N*-bromosuccinimide (2.0 g, 11 mmol) and the temperature was allowed to warm to room temperature. After 2 h at room temperature the mixture was poured into water (250 mL) and extracted with ethyl acetate (2 × 300 mL). The extract was washed with warm water (2 × 250 mL) and dried ($Na_2SO_4$). Evaporation of the extract to dryness provided benzoylated **13** (6.2 g, 90%) as a colorless foam. This intermediate (3.4 g, 5 mmol) was deblocked as described in **4** and the resulting crude solid was purified by flash chromatography on silica gel using dichloromethane–methanol (4:1) to yield 1.0 g (53%) of **13** as a colorless solid: mp 191–193 °C; $^1H$ NMR $\delta$ 3.86 (s, 3 H, $CH_3$), 4.62 (d, $J$ = 8.1 Hz, 1 H, $C_{1'}H$), 5.84 (s, 2 H, $NH_2$ exchangeable), 10.80 (br, 1 H, $N_3H$). Anal. ($C_{12}H_{15}BrN_4O_5$) C, H, N, Br.

**2-Amino-6-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (15).** 7-Deazaguanine[31] (1.5 g, 10 mmol) was C-glycosylated according to the procedure outlined for **3**. Purification of the crude material by flash column chromatography provided 3.3 g (56%) of **15** as a colorless foam. Anal. ($C_{32}H_{26}N_4O_8$) C, H, N.

**2-Amino-6-$\beta$-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (16).** Compound **15** (3.0 g, 5 mmol) was deblocked as described for **4** and the crude product was purified by flash chromatography using dichloromethane–methanol (3:1) to yield 1.1 g (78%) of **16**: mp >300 °C; $^1H$ NMR $\delta$ 4.51 (d, $J$ = 6.3 Hz, 1 H, $C_{1'}H$), 6.10 (s, 2 H, $NH_2$ exchangeable), 6.16 (s, 1 H, $C_5H$), 10.29 (br, 1 H, NH exchangeable), 10.96 (s, 1 H, NH exchangeable). Anal. ($C_{11}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H, N.

**2-Amino-6-methyl-5-(2,3,5-tri-*O*-benzoyl-$\beta$-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (18).** Compound **17** (1.64 g, 10 mmol; 8-methyl-7-deazaguanine[35]) was reacted with the benzoyl sugar **2** under similar glycosylation conditions as described for obtaining compound **3**. The resulting residue was purified by flash column chromatography using dichloromethane–methanol (8:1) to give 2.85 g (47%) of **18** as a colorless foam: $^1H$ NMR $\delta$ 2.12 (s, 3 H, $CH_3$), 5.31 (d, $J$ = 6.9 Hz, 1 H, $C_{1'}H$), 6.12 (s, 2 H, $NH_2$ exchangeable), 7.35–8.04 (m, 15 H, benzoyl aromatics), 10.20 and 10.96 (2 br, 2 H, $N_3H$ and $N_7H$ both exchangeable). Anal. ($C_{33}H_{28}N_4O_8$) C, H, N.

**2-Amino-6-methyl-5-$\beta$-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (19).** Deprotection of 18 (2.0 g, 3.3

mmol) using sodium methoxide was performed as described to obtain 4. The crude product was purified by flash chromatography using dichloromethane–methanol (7:3) to yield 0.45 g (48%) of 19: mp >300 °C; $^1$H NMR $\delta$ 2.15 (s, 3 H, $CH_3$), 4.54 (d, $J$ = 8.1 Hz, 1 H, $C_1$H), 6.09 (s, 2 H, $NH_2$ exchangeable), 10.31 and 10.85 (2 br, 2 H, $N_3$H and $N_7$H both exchangeable). Anal. ($C_{12}H_{16}N_4O_5$) C, H, N.

**Antiviral Studies. Semliki Forest Virus Model.** Swiss Webster female mice (Charles River Labs, Wilmington, MA) weighing about 20 g each at the beginning of the experiment were inoculated intraperitoneally with test compounds (or placebo) in aqueous 2% sodium bicarbonate solution at −24 and −18 h relative to virus inoculation. The optimal dose of 7-thia-8-oxoguanosine (the positive control compound) was established in previous experiments.[28] The dosing schedule indicated here was also found to be optimum for all guanosines tested. A lethal dose (10 × $LD_{50}$) of the Semliki Forest virus (original strain) was administered by ip injection to groups of 12 mice. These procedures have been detailed elsewhere.[39]

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# Antimicrobial Properties of $N^3$-(Iodoacetyl)-L-2,3-diaminopropanoic Acid–Peptide Conjugates

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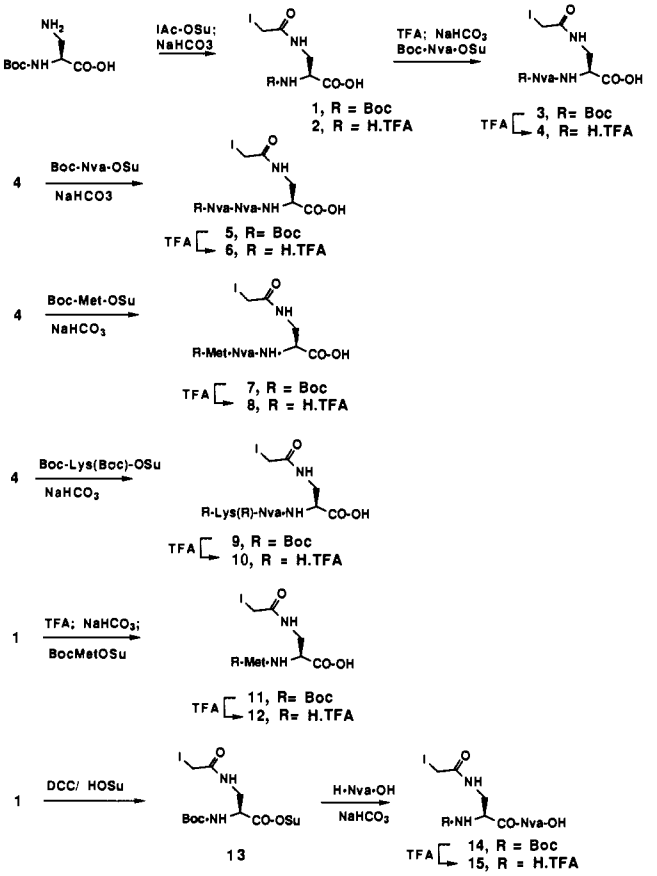
Six peptide conjugates consisting of either norvaline, methionine, or lysine and $N^3$-(iodoacetyl)-L-2,3-diaminopropanoic acid—a strong, irreversible inactivator of bacterial and fungal glucosamine-6-phosphate synthase—were synthesized and their antibacterial and antifungal activities were evaluated. Antimicrobial potencies of these peptides were correlated with their transport and cleavage rates inside the cells. Bacteriolysis of *Bacillus pumilus* cells and inhibition of [$^{14}$C]glucose incorporation into cell-wall polysaccharides of *Candida albicans* as a result of glucosamine 6-phosphate inactivation were also observed. Reversal of growth inhibitory effect of these peptides by *N*-acetylglucosamine in bacteria and fungi suggests the effective delivery of $N^3$-iodoacetyl-L-2,3-diaminopropanoic acid into the cell by a peptide-transport system.

Previous reports from our laboratory[1,2] have described the synthesis and biological properties of a series of peptides containing a new glucosamine-6-phosphate synthase inhibitor, i.e. $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid. Structure–activity relationship studies revealed that many of these peptides, beside antibacterial activity, also displayed remarkable anticandidal activity. It has been shown that inactivation and inhibition of glucosamine-6-phosphate synthase in fungi results in the fungistatic and fungicidal effects as a consequence of cell-wall mannoproteins and chitin biosynthesis inhibition.[3] Inactivation of glucosamine-6-phosphate synthase in bacteria leads to inhibition of peptidoglycan synthesis.[4] These peptides were found to be transported into microbial cells by peptide permeases and then cleaved by intracellular peptidases with the liberation of inhibitor inside the cells that may react with the target enzyme. Therefore glucosamine-6-phosphate synthase may be proposed as a promising target for the rational design of antimicrobial agents. As part of our continuing program aimed at the search for more active inhibitors of glucosamine-6-phosphate synthase, we have synthesized $N^3$-(haloacetyl) derivatives of L-2,3-diaminopropanoic acid[5,6] and found that

**Scheme I**




$N^3$-bromo- and especially $N^3$-(iodoacetyl)-L-2,3-diaminopropanoic acids were superior to $N^3$-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid as inhibitors of bacterial and fungal glucosamine-6-phosphate synthase.

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